

# FINAL REPORT

U.S DEPARTMENT OF ENERGY

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THE EFFICACY OF OXIDATIVE COUPLING FOR PROMOTING IN-SITU  
IMMOBILIZATION OF HYDROXYLATED AROMATICS IN CONTAMINATED  
SOIL AND SEDIMENTS SYSTEMS

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## EXECUTIVE SUMMARY

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An urgent science need for DOE is to obtain a better understanding of processes controlling the fate and transport of contaminants in subsurface environments; this in order to improve conceptual modeling capabilities and current remediation technologies. Our research on the oxidative coupling reactions of hydroxylated aromatic compounds (HACs) in soil and sediment systems addresses an important but heretofore less explored category of interactions of these contaminants with soils. The overall objective of this research has been to elucidate the role of oxidative coupling reactions in HAC transport and evaluate the efficacy of inducing such reactions for remediation of subsurface systems contaminated by such organic compounds.

Hydroxylated aromatic compounds are ubiquitous contaminants of subsurface environments. Current remediation technologies, such as pump-and-treat and bioremediation, can be cost intensive for this class of compounds due to their relatively high water solubilities and associated environmental mobilities. Oxidative coupling processes are recognized as a category of potential transport attenuation mechanisms. In these processes hydroxylated aromatic compounds can actually become chemically incorporated into soil organic matter, but only if appropriate enzymes and mineral oxides, ones that are often naturally present in these environments, can be engaged as catalysts in the process. It has been our contention that an improved understanding of these coupling processes in subsurface systems can lead to innovative cost-effective treatment technologies; e.g., permeable reactive barriers in which the induction of coupling reactions can be engineered by addition of appropriate coupling catalysts and/or reactive sorbent materials and by maintenance of conditions that we learn are conducive to such reactions.

In the research described here, oxidative coupling reactions were examined using a large experimental matrix of different soil/sediment organic matter, a wide range of concentrations of

HACs and mineral and enzymatic catalysts, in both batch and continuous-flow systems. While most oxidative coupling research has been performed in aqueous systems with model chemicals, our project simultaneously studied oxidative coupling reactions in natural (soil and sediment), model (aqueous) and engineered (catalyst amended soil) systems. This is, to the best of our knowledge, the first research that: a) studies the effect of couplings in sorption/desorption processes in soil and sediment systems; b) demonstrates a direct association between types of soil organic matter and the capacity of natural materials to mediate oxidative coupling reactions; c) reveals the roles of catalyst activity on the type of oxidative coupling reaction (cross-coupling and self-coupling) induced and composition of the reaction products; d) develops a mechanistic model to describe the dynamics of enzymatic coupling of HACs in natural sorbents; and, e) investigates oxidative coupling reactions in continuous flow systems, thus simulating conditions encountered in natural subsurface systems.

The study clearly shows that the structure and composition of the organic matter of soils and sediments are essential considerations for the selection of materials for engineered applications of oxidative coupling processes. Soils containing geologically "young" soil organic matter (SOM), such as humic or peat types of materials, are more likely to promote oxidative coupling reactions than are those containing primarily "older" kerogen type SOM. Additions of horseradish peroxidase and manganese oxide catalysts to soils greatly increase rates and extents of coupling reactions and thus decrease HAC mobility. Further investigations in model and continuous flow systems reveal that increased non-extractability, and therefore decreased environmental mobility, may occur via: 1) oxidative coupling reactions between phenolic radicals and reactive components of SOMs (cross-coupling reactions); and/or 2) the formation of polymeric forms of the compounds (self-coupling reactions).

The quantification and characterization of both HAC disappearance and coupling product formation as a function of time, initial HAC concentrations, and catalyst type and activity resulting from this study constitutes a major contribution to the science of such systems and to appropriate engineering applications of that science. Improved overall and specific understandings of peroxidase enzyme-mediated oxidative coupling reactions of HACs and their relationships to the sequestration by natural sorbents of compounds comprising this broad category of common organic contaminants were gained in the course of and as a result of the investigations. It was found that both geologically young and old soils can increase the non-extractability of HACs over that which occurs in soil-free systems. It was observed that peroxidase inactivation is significantly reduced in the presence of each type of soil. As noted above, the extent of coupling was found to be significantly higher in the systems containing humic-type SOM than in those containing kerogen- type SOM. Importantly from an engineered applications perspective, a rate model was developed to facilitate quantitative evaluation and mechanistic interpretation of these fairly complex coupling processes. .

Our work on abiotic and enzymatic coupling reactions of hydroxylated aromatic compounds in natural soil and sediments not only provided us with the basic understanding of the processes involved, but also demonstrated that induction of oxidative coupling by addition of catalysts is a promising subsurface remediation option. It has been made evident in our work that, in order to bring oxidative coupling technology into practical application in soil decontamination, several aspects of these processes may be important to consider. This is particularly the case for those related to induction of cross and self-coupling reactions and the effects of the various types of SOM. For example, our investigations have shown that at low birnessite concentrations, cross-coupling of phenolic compounds to reactive sites on soil organic matter appear to be the predominant coupling reactions. At higher birnessite dosages, prevailing self-coupling results in phenolic polymers that associate with catalyst surfaces. For use in reactive barrier systems, then, the induction of cross-

coupling reactions of HACs onto highly reactive soils surfaces may be more desirable since polymeric products may negatively affect birnessite activity. This is but one example of the practical application insights that can be derived from the science described here.

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## RESEARCH OBJECTIVES

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Hydroxylated aromatic compounds (HACs) are ubiquitous contaminants in subsurface environments. Accidental spills or past waste disposal practices have resulted in the introduction of HACs to groundwaters at many DOE, DoD, and industrial sites. Different substituted phenols have been identified in groundwaters and soil/sediments at DOE facilities nationwide (Riley, 1992). HACs have been classified as priority pollutants because of their multiple toxic health effects at very low concentrations (Clean Water ACT, USEPA 1985).

Oxidative coupling processes of HACs are well recognized as being part of the soil natural attenuation mechanisms in which these compounds become incorporated into the soil organic (i.e., humification) (Orlov, 1992). Increasing evidence in the literature indicates that oxidative coupling reactions may be significant in controlling the fate of HACs in the subsurface (Wang et al., 1986; Voudrias and Reinhard, 1986; Nannipieri and Bollag, 1991; Bollag, 1992a, b; Gianfreda and Bollag, 1994; Burgos et al., 1996; Bhandari et al., 1996). A wide range of catalysts, commonly present in soil and sediments, can mediate oxidative coupling reactions including inorganic compounds and microbial or plant-produced enzymes (Bollag, 1983; Vaughn et al., 1994). Inorganic catalysts include oxides or hydroxides of manganese and iron. (Stone and Morgan, 1984a,b, Voudrias and Reinhard, 1986; McBride, 1989).

Current treatment technology of HACs in the subsurface, such as pump-and treat systems and bioremediation, can become very cost intensive for this class of compounds due to their relatively high water solubilities and consequent high environmental mobility. In recent years, novel in-situ treatment approaches for groundwater decontamination, such as reactive permeable barriers, have been given much deserved attention due to their benefits in cost reduction and the low environmental disturbance they offer. (Gavaskar, 1999; Puls et al., 1999). An attractive decontamination strategy for these mobile and yet reactive compounds is to induce these coupling reactions in the subsurface in engineered system configurations such as permeable reactive barriers by addition of coupling catalysts. Since innovative technologies for decontamination should be based upon sound scientific research, our research intended to investigate the mechanisms of oxidative coupling reactions in soil and sediment systems for application in engineered systems. Information gathered from this study was considered crucial for the selection of materials, catalysts, and conditions for the design of such in-situ remediation strategies. We proposed to investigate i) the role of abiotic/enzymatic coupling reactions on the sequestration of hydroxylated aromatic

compounds (HACs) by natural sorbents, ii) the effects of sorbent structure and chemical compositions on such processes; and iii) the conditions for the induction of these abiotic/enzymatic coupling reactions by addition of suitable catalysts and sorbents.

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## METHODS AND RESULTS

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### METHODS

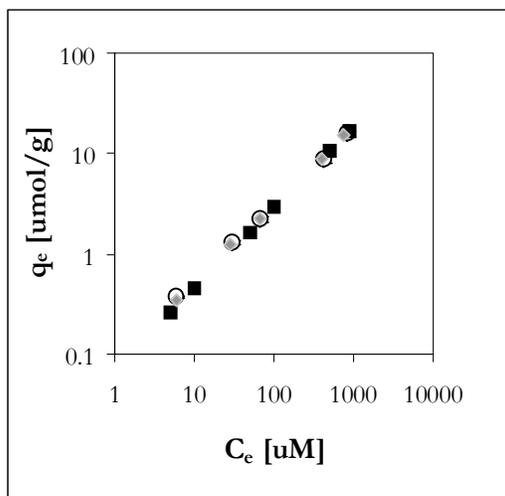
Different experimental and analytical methods were developed to evaluate the efficacy of inducing sequestration of HACs through oxidative coupling reactions by addition of various catalysts. The studies performed can be grouped in three broad categories:

- 1) Equilibrium sorption-desorption-extraction of HACs in natural and catalyst-amended systems. These studies were used to evaluate the sequestration potential of HACs in various soil types with and without catalyst additions and analyze equilibrium sorption/desorption parameters for mechanistic understanding of processes involved.
- 2) Coupling rate studies in soil and soil-free systems with enzyme and manganese oxide additions. These studies helped develop and validate mechanistic kinetic models that describe the coupling process in soil and sediment systems, and
- 3) Flow-through desorption studies in soil and/or manganese oxide packed columns. These studies were performed to evaluate induced sequestration of HACs in subsurface flow conditions in natural or engineered (e.g., reactive barrier configuration).

### EQUILIBRIUM SORPTION-DESORPTION-EXTRACTION STUDIES

The experimental method for equilibrium sorption/desorption/extraction in batch systems is a bottle-point, fixed sorbent dosage technique in CMBRs (completely mixed batched reactors). The reactors consisted on vigorously shaken 9-mL centrifuge tubes capped with Teflon-lined septa and equilibrated at room temperature. Samples were prepared in quadruplicates or triplicates; soil-free control samples were used to account for any losses. Abiotic conditions were maintained by gamma irradiation of the sorbent and addition of sodium azide. Sorbent dosage was pre-determined for each solute-sorbent system by conducting sorption experiments at a fixed mid-range solute concentration. Equilibration time was also pre-determined in separate experiments. This is the standard isotherm procedure that has been used in our laboratories for many years and proven to be highly precise and reproducible. For example, Figure 1 shows the high coincidence of three different sorption

isotherms of phenol on Lachine shale performed 1 1/2 years apart with different soil concentrations, different workers but same experimental protocol. Complete mass-balances were obtained in all systems studied. The target compounds were added as a mixture  $^{14}\text{C}$ - labeled and  $^{12}\text{C}$  forms of the target compound, scintillation counting techniques were used for assessing concentrations. Soil-



**Figure 1. Reproducibility of isotherm data**

liquid separation was obtained by centrifugation (35 min at 2500 rpm). A determined volume of the remaining supernatant was removed and replaced with equal amounts of organic-free carbonate buffer to obtain the desorption isotherm. Multiple extractions were performed, using similar desorption procedure, with water, methanol or a methanol-dichloromethane mixture (80:20 ratio) until total extractable reached background levels. Thereafter the sorbent was air-dried, and combusted in a biological oxidizer (Harvard Instruments) where the evolved  $^{14}\text{CO}_2$  from the sequestered HAC was captured in scintillation cocktail and measured. Experimental methods in

enzyme-amended studies followed same procedure described before with the exception that sodium azide was excluded due to its enzyme inhibition properties. Aseptic transfer of sterile solutions and reagents maintained microbial control.

#### RATE STUDIES IN ENZYME AND MINERAL CATALYTIC SYSTEMS

Two types of catalytic systems were investigated in this research: i) enzymatic (horseradish peroxidase) and ii) mineral ( $\delta$ -manganese oxide or birnessite). Studies were performed to evaluate both coupling and catalyst inactivation rates to develop and validate predictive coupling models for oxidative coupling in soil and sediment systems.

##### *Horseradish Peroxidase*

Horseradish peroxidase (HRP) inactivation rate experiments for the selected sorbents were carried out at variable sorbent/water ratio and initial HRP concentrations with excess  $\text{H}_2\text{O}_2$  cofactor in batch reactors. At predetermined time intervals, a 0.5-ml aliquot of sample was withdrawn to a 50

ml volumetric flask, and diluted by phosphate buffer. The enzyme activity in the diluted solution was measured. The rate of color change at 405 nm was used to determine the HRP activity by equilibrating the enzyme with 0.002M ABTS (2,2'-azinobis[3-ethylbenzothiazoline-6-sulphonic acid] in phosphate buffer (Bergmeyer, 1983).

Coupling rate experiments were carried out in identical batch reactors as those for the enzyme inactivation study. At predetermined time intervals, a 0.4ml aliquot of solution was sampled to a 2.0 ml Spin-X centrifugal filtering device that has a 1.0ml insert with 0.22  $\mu\text{m}$  membrane filter. After 0.4 ml methanol was added to the solution sample to stop the coupling reaction, the Spin-X was centrifuged at 10,000rpm for 10 min to separate the solid and liquid phases. Concentration was measured using liquid scintillation counting procedures. The fraction remaining in the Spin-X filter insert was transferred to a new 2.0 ml micro-centrifuge tube for extraction with methanol. Centrifugation was performed again to separate the phases. An assessment of the non-extractable product formation is obtained with this method.

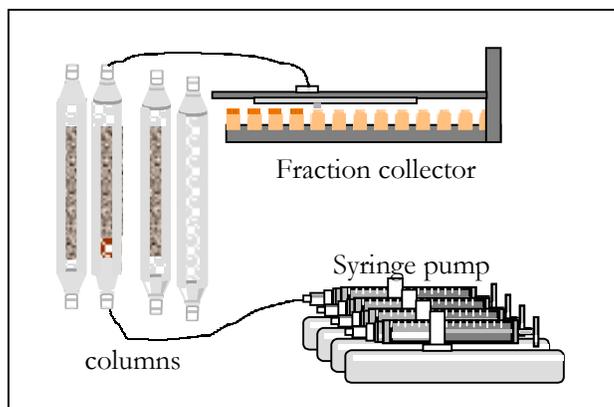
### *Manganese Oxide*

Rates of oxidative coupling were measured in birnessite/water systems. Formation of soluble and insoluble coupling products was performed by measuring phenol with HPLC techniques and total  $^{14}\text{C}$  in solution by scintillation counting techniques at a wide range of manganese oxide and phenol concentrations over time. Birnessite ( $\delta\text{-MnO}_2$ ) was synthesized using the method described by McKenzie (McKenzie 1971). CMBRs consisted of 30 mL serum bottles sealed with Teflon-lined rubber caps and crimped closed. Samples were mixed in shaker table at 190 revolutions per minute (rpm) and 22.4 °C. At set time intervals, 0.85 mL of the birnessite/solution mixture was drawn into a syringe and quickly dispensed into a centrifugal filtering Spin-X device and centrifuged for 10 minutes at 14,000 rpm. Methanol extraction of the filter insert was performed as before.

HPLC analyses were performed in HP model 1100 equipped with C18- reverse phase column. The mobile phase was 60% methanol and 40% of 0.1% (v/v) acetic acid in distilled water with a flow rate was 0.250 mL/min. Two UV signals were used to measure phenol, 254 and 280 nm, depending on concentration range.

## FLOW-THROUGH COLUMN STUDIES

Flow-through desorption-extraction studies were carried out in reactor columns consisting on 8 mm ID, 150 mm long glass columns (volume approx. 7.5 mL), equipped with Teflon tubing and fittings for in-flow and out-flow ports and lines (Figure 2). Blank runs (e.g., no column) with a set amount of phenol and glass bead-packed columns were performed to evaluate possible losses in the column and collection systems. A set of eight columns was operated simultaneously.



**Figure 2. Schematic representation of the flow-through columns experimental set-up.**

Columns were packed dry with selected soils amended with variable masses of birnessite, spiked with set amounts of  $^{14}\text{C}$ -labeled phenol solution, and incubated isothermally for 24 hours. Aqueous desorption followed by methanol extraction were performed until no  $^{14}\text{C}$  activity was detected in the effluent. Columns were then ultrasonicated for 1 hour to improve extractability, and methanol flush was

continued. Water and methanol were delivered through two four-channel syringe pumps with gas-tight glass-Teflon syringes. Flow conditions were maintained at 1 mL/hr (superficial velocity = 2 cm/hr). Effluent was collected automatically in two Fraction Collectors in scintillation vials containing scintillation cocktail.

## RESULTS

### EQUILIBRIUM SORPTION, DESORPTION AND EXTRACTABILITY OF HACs

The oxidative coupling of HACs in natural and model geosorbent systems, batch and continuous-flow reactors, with and without additions of enzyme and manganese oxide catalysts was investigated. Results derived from this study expanded our understanding of the processes involved, and demonstrated that induction of oxidative coupling by addition of catalysts is a subsurface remediation option worth exploring further.

## Natural Systems

This is the first study that demonstrates a direct association between the composition and structure of the soil organic matter and the capacity of natural materials to mediate oxidative coupling reactions. The equilibrium sorption, desorption and sequestration of phenol, o-cresol, and p-chlorophenol in five different natural sorbents with varying types of organic matter structure and composition were investigated (Table 1).

**Table 1. Relevant characteristics of the selected natural sorbents used in this study**

SORBENT	<sup>1</sup> S <sub>A</sub> , m <sup>2</sup> /g	% ORGANIC MATTER	TYPE OF ORGANIC MATTER	<sup>2</sup> CHEMISTRY OF ORGANIC MATTER	Mn, ppm	Fe, ppm
Lachine shale	15.0	12.6	Kerogen	Aliphatic, aromatic	23.2	840
Wagner III	1.5	0.15	Kerogen	n/d	n/d	n/d
Fox Forest	4.5	5.9	Humus	O-aliphatic, phenolic, aromatic	44.8	9
Fox Grassland	8.9	2.5	Humus	O-aliphatic, phenolic, aromatic	24.2	16
Canadian Peat	2.3	99.1	Peat	O-aliphatic	13	91

1) S<sub>A</sub> = BET surface area determined by N<sub>2</sub> adsorption, 2) predominant groups determined by solid state<sup>13</sup>C NMR spectra, n/d = not determined

Our results substantiate one of our original hypotheses that sequestration of HACs in abiotic and non-enzymatic conditions via oxidative coupling is more likely to take place in natural sorbents that have geologically young organic matter. Sorption-desorption hysteresis was significant for both humus- and kerogen- type SOMs (Table 2). However, the non-extractable fraction (% Seq.) of phenol, o-cresol and p-chlorophenol was only significant in the diagenetically young near-surface Fox Forest and Grassland soils (Table 2). The organic fraction of these soils contains relatively high concentrations of phenolics (Table 1), which are the reactive groups more likely to participate in oxidative coupling reactions. In contrast, almost total extractability of all three contaminants (% Seq. less than 5%) was observed on Lachine shale and Wagner III soil, comprised of geologically older kerogen SOMs. Absence of cross-coupling reactions in kerogen-type SOMs may be limited by the availability of reactive coupling sites in this chemically reduced and physically condensed SOM type. Absence of sequestration and hysteresis was also evident in Canadian Peat, a very young soil with little diagenetic alteration (Table 2). Although phenolic groups are not abundant in this young SOM, methoxy and other oxygen-substituted aliphatic moieties, which can also couple with phenolic radicals, were detected in <sup>13</sup>C NMR analysis of this soil (Table 1).

Addition of mineral catalysts to peat resulted in significant sequestration suggesting that this anoxic material with ultra low mineral content (99% organic matter) may not contain sufficient mineral oxides to catalyze oxidative coupling reactions. These results suggest that oxidative coupling induced sequestration depends on the availability of reactive sites, function of the SOM structure and composition (i.e., diagenesis) and the presence of oxidative coupling catalysts in the system.

**Table 2. Selected parameters that illustrate the sorption linearity (n), sorption-desorption hysteresis (H.I.<sub>250</sub>) and extractability (% Seq) of phenol, o-cresol, and p-chlorophenol in natural sorbents described in Table 1.**

	PHENOL			O-CRESOL			P-CHLOROPHENOL		
	<sup>1</sup> n	<sup>2</sup> H.I. <sub>250</sub>	<sup>3</sup> %Seq	<sup>1</sup> n	<sup>2</sup> H.I. <sub>250</sub>	<sup>3</sup> %Seq	<sup>1</sup> n	<sup>2</sup> H.I. <sub>250</sub>	<sup>3</sup> %Seq
Lachine Shale	0.80	0.18	4.5 ± 1.1	0.74	0.25	4.6 ± 1.1	0.54	0.31	2.1 ± 0.3
Canadian Peat	n/d	n/d	n/d	0.91	0.05	4.6 ± 1.6	0.85	0.001	1.0 ± 0.07
Fox Forest	0.76	0.67	74 ± 10	0.87	0.49	19 ± 3	0.61	0.24	61 ± 18
Fox Grassland	0.78	0.51	48 ± 6	n/d	n/d	n/d	0.65	0.49	n/d
Wagner III soil	n/d	n/d	n/d	0.82	0.22	4.7 ± 0.2	0.74	0.29	1.3 ± 0.2

1) n = Freundlich n ( $q_e = K_F C_e^n$ ), 2) H.I.<sub>250</sub> = hysteresis index at  $C_e = 250 \mu\text{M}$ .  $H.I. = (q_{e,des} - q_{e,ads}) / q_{e,ads}$ , 3) % Seq is the percentage of the sorbed fraction ( $q_e$ ) that remains in the solid after water/solvent extraction averaged for all organic concentrations tested  $10 \mu\text{M} \leq C_o \leq 1000 \mu\text{M}$ .

The non-linear character of HAC sorption in soils containing humus-like organic matter is another unique and significant finding of this research. Extensive studies in our laboratories on the sorption of phenanthrene and other organic compounds that do not undergo oxidative coupling reactions (e.g., Huang and Weber, 1997) have established that sorption of organics in younger soils tend to be more linear and reversible than those of diagenetically older materials such as shale, suggestive of a partition mechanism. Thus, non-linear sorption isotherms of HACs in young soils, such as Fox series soils, indicate that the sorption -desorption behavior of these compounds is controlled by oxidative coupling reactions. This is further confirmed by the evident sorption-desorption hysteresis and the significant non-extractable fraction observed (e.g., more 60% sequestration for phenol and chlorophenol in Fox Forest soil) (Table 2).

This study clearly shows that organic matter structure and composition is an essential parameter for the selection of materials for engineering applications of oxidative coupling processes (e.g., permeable reactive barriers).

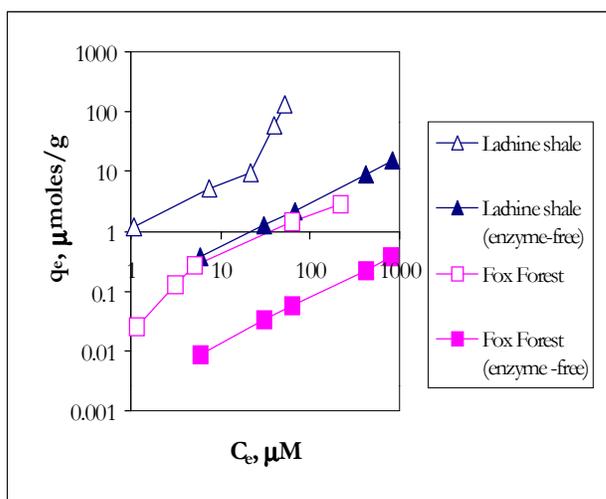
### *Catalyst Amended Systems*

Induction of oxidative coupling reactions by additions of horseradish peroxidase and manganese oxide to natural sorbents systems was performed to investigate its effect on HACs' equilibrium

sorption/desorption behavior and the consequent changes in the environmental mobility of these compounds. Reduced mobility of HACs was expected to occur: 1) by oxidative coupling reactions between phenolic radicals and reactive components of the natural sorbents organic fraction (cross-coupling reactions) and 2) by the formation of polymeric forms of the compounds with increased sorption tendency (self-coupling reactions).

#### Enzyme induced oxidative coupling

Sorption of phenol onto natural sorbents upon addition of horseradish peroxidase was markedly different in each sorbent studied and appears to be related to the unlike capacity of each material to



**Figure 3. Sorption of phenol on Lachine shale and Fox Forest in the presence and absence of horseradish peroxidase and hydrogen peroxide**

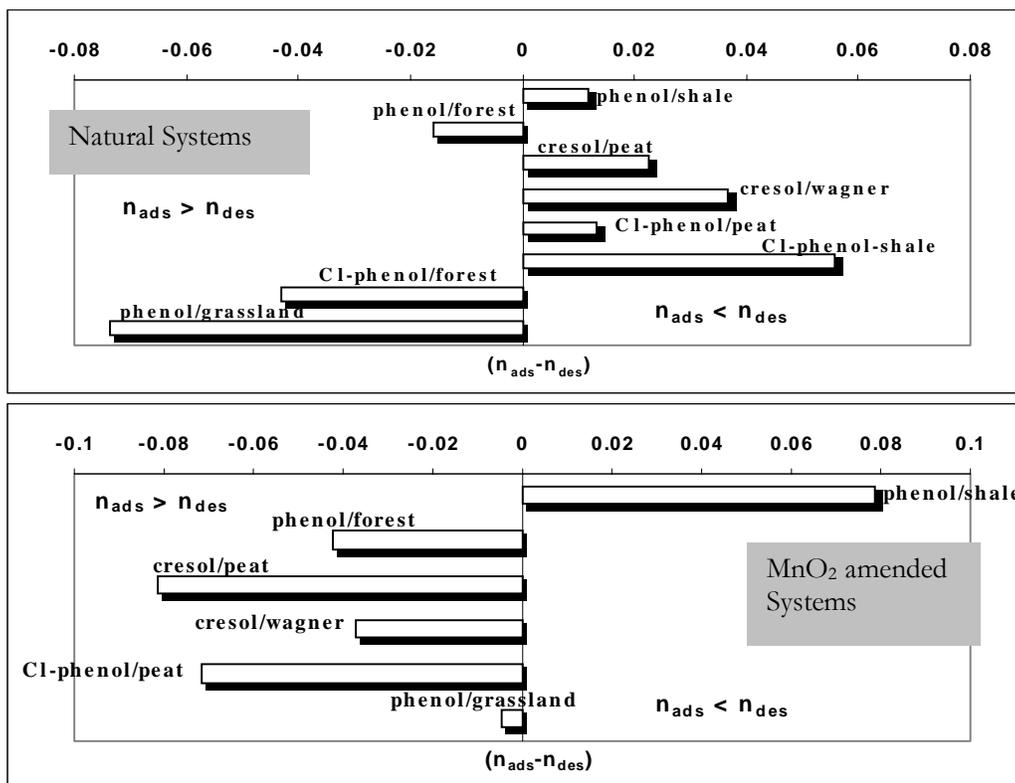
induce abiotic oxidative coupling reactions. Similar to the observed results in enzyme-free natural sorbent studies, sorption behavior of HACs was highly dependent on the diagenesis of soil organic matter but significantly different than that observed with the enzyme-free systems. Horseradish peroxidase (HRP) was added to two diagenetically different natural sorbents: a surface soil characterized by geologically younger organic material (Fox Forest) and an older diagenetically altered soil (Lachine Shale). Adsorption isotherms of phenol on both sorbents exhibited increased sorption when HRP and its cofactor, hydrogen peroxide, were added (Figure 3). However, contrary to enzyme-free systems, the Freundlich isotherm model cannot describe the results obtained over the entire phenol concentration range tested; different sorption behavior was exhibited at low and high initial phenol concentrations, particularly in the Lachine shale-containing systems. The unfavorable sorption behavior (Freundlich  $n > 1$ ) observed in the Lachine Shale system at high phenol concentrations may indicate the precipitation of large phenolic polymers, which were observed in soil-free controls (Figure 3). These polymers may exist as a separate phase or sorbed onto the natural sorbent.

#### Manganese Induce Oxidative Coupling

Additions of manganese oxide (birnessite) to five different natural sorbents (sorbent properties in Table 1) were performed to evaluate the equilibrium sorption, desorption and sequestration of phenol, o-cresol, and p-chlorophenol under these conditions. These experiments were expected to

identify sorption-desorption and extractability of phenol and the coupling reaction products under batch equilibrium conditions. Our results show that, similar to the enzyme-amended systems, addition of birnessite greatly increased sorption hysteresis and non-extractability of HACs from all geosorbents tested.

Increase linearity from sorption to desorption isotherms ( $n_{ads} < n_{des}$ ) have been reported for organic compounds that do not undergo oxidative coupling, particularly in kerogen type SOMs. This phenomenon has been related to differences in the displacement from different sorption microenvironments and non-Fickian diffusion processes within condensed SOM matrices. Similar results were obtained in our HACs systems where sequestration, and consequently coupling, was negligible in natural systems (Figure 4A) and in kerogen type SOM of materials in the manganese oxide amended sorbents (Figure 4B). Contrary to these observations, desorption isotherms became more nonlinear ( $n_{ads} > n_{des}$ ) in natural systems with significant sequestration (i.e., Fox series soils) compared to the sorption isotherms (Figure 4A) and, interestingly, in systems with humic or peat SOM in  $MnO_2$  amended systems (Figure 4B). Based on differences in the sorption/desorption



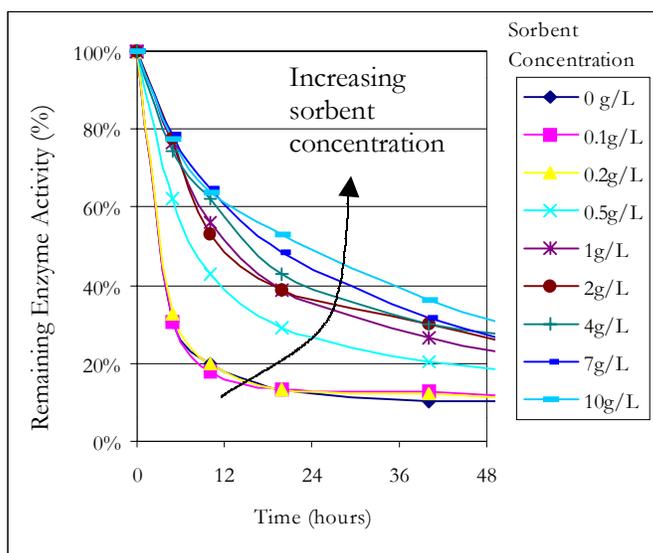
**Figure 4. Comparison of Freundlich constants for sorption ( $n_{ads}$ ) and desorption ( $n_{des}$ ) in natural (4A) and manganese oxide (4B) amended systems**

behavior on Lachine shale compared to the humic and peat SOM type sorbents in natural and MnO<sub>2</sub> amended systems, it appears that sorption/desorption hysteresis may indicate that oxidative coupling reactions are the processes controlling the movement of these chemicals in sorbents with peat and humus-type OM compared to those containing kerogen-type OM.

## OXIDATIVE COUPLING AND ENZYME INACTIVATION RATES

### *Enzyme Inactivation in Natural Sorbent Systems*

Increased understanding of enzyme-mediated oxidative coupling reactions of hydroxylated aromatic compounds (HACs) and their relationship with sequestration by natural sorbents was accomplished in the progress of our investigations. The oxidative coupling of phenol catalyzed by horseradish peroxidase (HRP) was investigated in systems containing two geosorbents with different organic matter composition: Chelsea soil, a humic-type near-surface young soil, and Lachine shale, a kerogen-type, diagenetically older natural material. A significant finding of this study was to show that in natural sorbent systems, the formation of non-extractable products is increased and the inactivation of the enzyme is reduced when compared to soil-free systems (Figure 3). This is a very significant finding since it makes enzyme-decontamination technologies in subsurface systems, such as permeable reactive barriers, a promising remediation strategy. Fully desorbable enzymes in

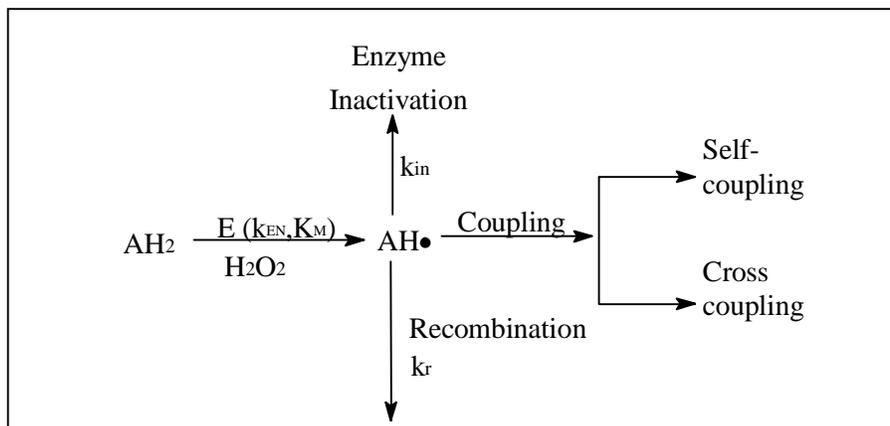


**Figure 3. Decrease HRP inactivation at different concentrations of Lachine shale. [Phenol] = 50  $\mu$ M, [HRP] = 0.2 U/ml, [H<sub>2</sub>O<sub>2</sub>] = 1 mM**

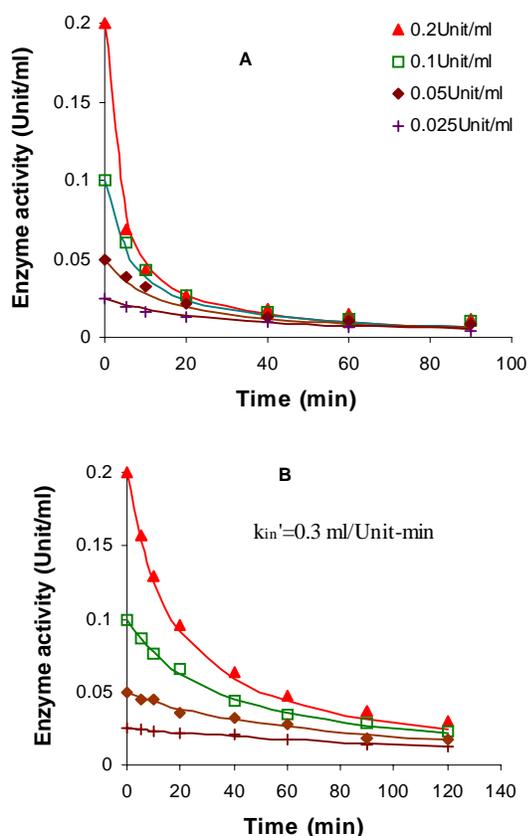
selected supports can be envisioned as a catalyst slow delivery system to be used in reactive barrier configurations. This option may be a more suitable catalyst delivery method compared to costly and involved enzyme immobilization techniques.

Advances on the development of a mechanistic model to describe the dynamics of enzymatic coupling of HACs in natural sorbents were accomplished. The conceptual relationships of the processes involved are summarized in Figure 4, which

illustrates how the substrate radicals (AH•), produced in the enzymatic step, can participate in several post-enzymatic reactions. These reactions include i) radical recombination; ii) attack and consequent inactivation of the enzyme; and iii) self-coupling of the phenol radicals resulting in polymer formation or cross-coupling of the radicals with reactive groups on the soil organic matter.



**Figure 4. Mechanistic model of phenol coupling and enzyme inactivation**



**Figure 5. Decrease HRP inactivation in the absence (A) and presence (B) of Lachine shale. Solid lines represent model predictions and symbols data.**

It was found that, under enzyme saturation conditions (substrate  $\gg$  Michaelis-Menten  $K_M$ ), the decrease of enzyme activity over time could be described by the second order relationship:

$$-\frac{d[E]}{dt} = k_{in}'[E]^2 \quad (1)$$

where 
$$k_{in}' = \frac{k_{in}k_E}{k_r}$$

and  $k_E$  and  $k_r$  are the rate constants of production and recombination of radicals, respectively,  $k_{in}'$  represents the intrinsic inactivation rate constant, and  $E_o$  is the initial enzyme activity.

Equation 1 describes very well the loss of HRP activity over time in both sorbent-containing and sorbent-free systems as exemplified in Figure 5.

Note that the rate of enzyme inactivation was reduced in Lachine shale-containing systems when compared with soil free systems.

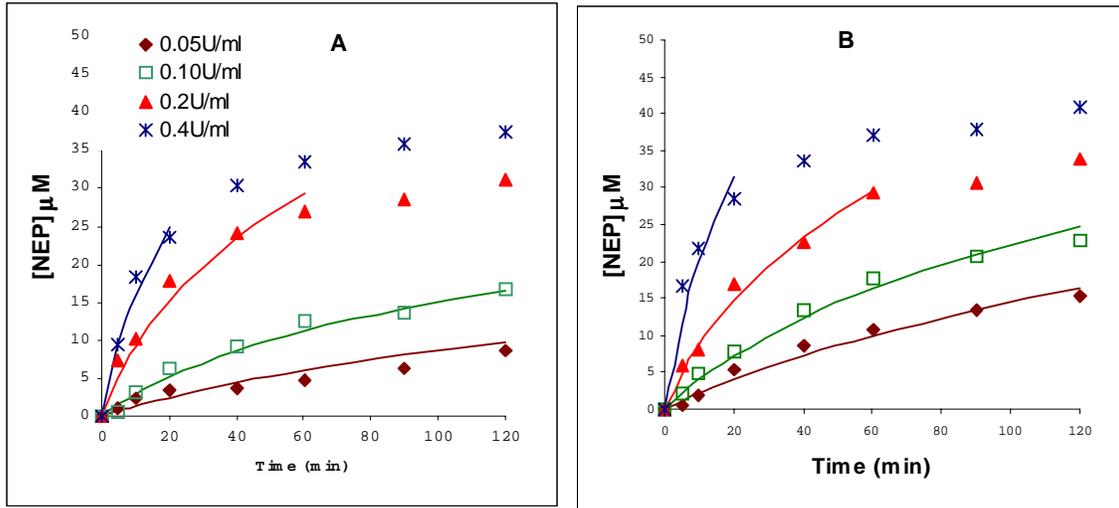
*Enzyme-Mediated Coupling in Natural Sorbent Systems.*

As schematically illustrated in Figure 4, only a fraction of phenoxy radicals produced in the enzymatic step may lead to the formation of non-extractable coupling products NEP. Non-extractable products in this context are comprised of cross-coupling products (i.e., binding to soil organic matter) and strongly sorbed large polymeric products of self-coupling reactions. By assuming that the coupling reaction is first order with respect to the free radical concentration with a rate constant of  $k_c$ , an expression for the rate of coupling is obtained, whose integral expression is.

$$[NEP]_t = \frac{k'_c}{k_{in}} [\ln(1 + [E]_0 k'_{in} t)] \quad (2)$$

where  $k'_c = \frac{k_c k_E}{k_r}$  is a lumped rate constant related to coupling rates. Figure 6 shows the

simulation results obtained using equation 2 for phenol coupling in Chelsea soil in the presence of HRP. The model describes very well the data at lower enzyme activities and at the initial stages of the higher range of enzyme activities, but deviates at later times. Similar results were obtained in



**Figure 6. Time courses of phenol coupling at varying initial enzyme concentrations in the presence of Lachine shale (A) and Chelsea Soil (B). Solid lines show model simulations and symbols are experimental data points**

Lachine shale systems. This deviation appears to be caused by the depletion of substrate, which makes the saturated enzyme assumption (i.e.,  $K_M \ll \text{substrate}$ ) not valid.

#### OXIDATIVE COUPLING RATES IN MANGANESE OXIDE AMENDED SYSTEMS

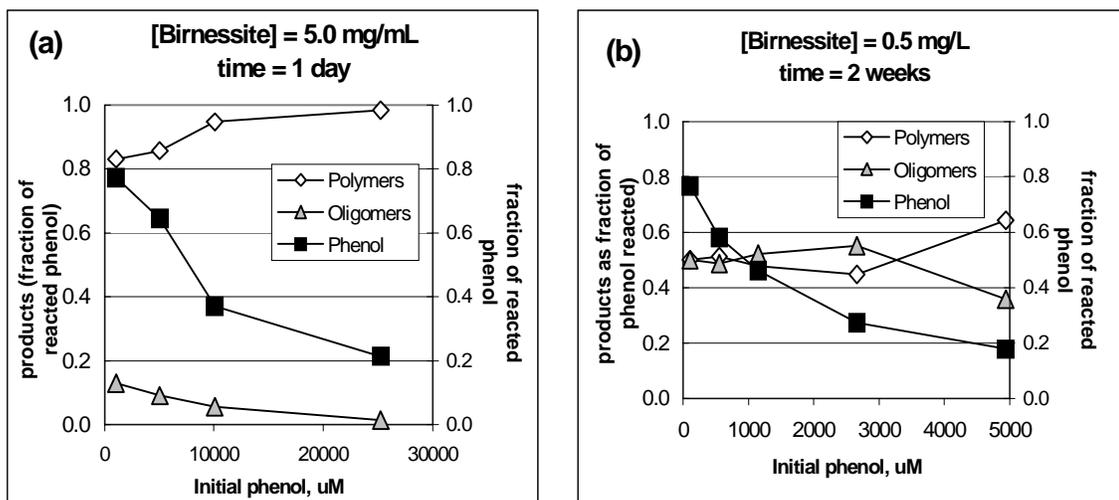
This study investigated metal oxide induced phenolic polymer formation in two separate phases of work. The first phase investigated the oxidative coupling phenomena with soil-free birnessite-phenol batch systems. The second phase investigated the feasibility of using birnessite-induced phenolic polymer formation as a reactive barrier remediation technology. This was accomplished using non-equilibrium sorption flow-through column systems of natural sorbents and birnessite amendments. Birnessite, an amorphous manganese oxide of mixed oxidation state (III/IV) was selected as the catalyst due to its abundance in natural materials and ease of preparation

The most significant and unique finding of this study is the unfolding of the role of catalyst activity on the type of oxidative coupling reaction (cross-coupling and self-coupling) induced and composition of the reaction products. The experimental results obtained indicate that manganese oxide concentration controls the nature and rate of the coupling reactions so that: i) at low birnessite concentrations phenol-phenol coupling is minimal and oxidative coupling onto soil organic matter is significant, ii) at increasing birnessite concentrations, polymer formation becomes predominant and coupling to SOM decreases, and iii) at high birnessite concentrations, larger and increasingly insoluble polymer that become strongly associated to the birnessite or natural sorbent particles are preferentially formed, resulting in low extractabilities.

#### *Rate of Formation and Composition of Coupling Products*

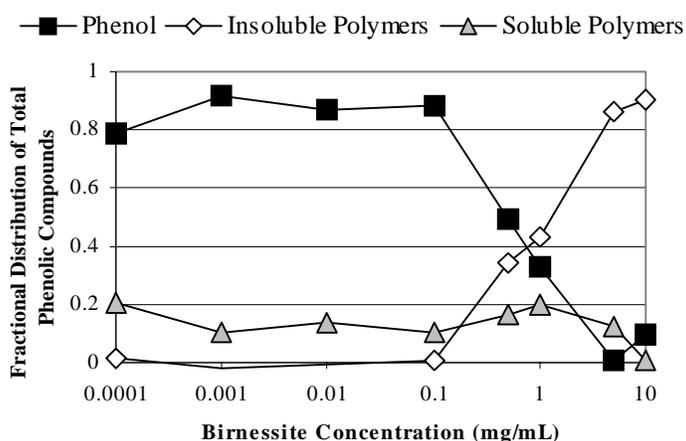
Soil-free birnessite-phenol systems were investigated to characterize the self-coupling (phenolic polymer formation) phenomena. A major contribution of this study is the quantification and characterization of both phenolic monomer disappearance and polymer formation as a function of time, initial phenol concentrations and catalyst activity (birnessite concentration). A wide range of phenol (50 mM to 10  $\mu\text{M}$ ) and birnessite concentrations (0.001 to 10 mg/mL) was included. The polymers formed were classified operationally as soluble if they were able to pass through a 0.22  $\mu\text{m}$  cellulose acetate filter or insoluble if they were retained on the filter. Differentiation between soluble polymers and phenol was achieved by direct detection of phenol with HPLC chromatography techniques.

Figures 7 a and b exemplify the major trends observed in this study. At high birnessite concentrations, as the initial concentration of phenol increases the concentration of soluble polymers decreases and the insoluble polymer formation increases ((Figure 7a). In contrast, at lower catalyst activities, an even distribution of polymers and oligomers are observed over almost the entire phenol range tested (Figure 7b)



**Figure 7. Coupling product distribution as a function of phenol concentration at two constant birnessite concentration and reaction times: a) 5 mg/mL/1 day and b) 0.5 mg/L/2 weeks. Products are expressed as fraction of total phenol reacted.**

Coupling product composition at a fixed phenol concentration (1000  $\mu\text{M}$ ) and at variable birnessite concentrations is shown in Figure 8. At low birnessite concentrations insoluble polymer production is negligible; and



**Figure 8. Distribution of coupling products as a function of birnessite concentrations. [Phenol] = 1000  $\mu\text{M}$**

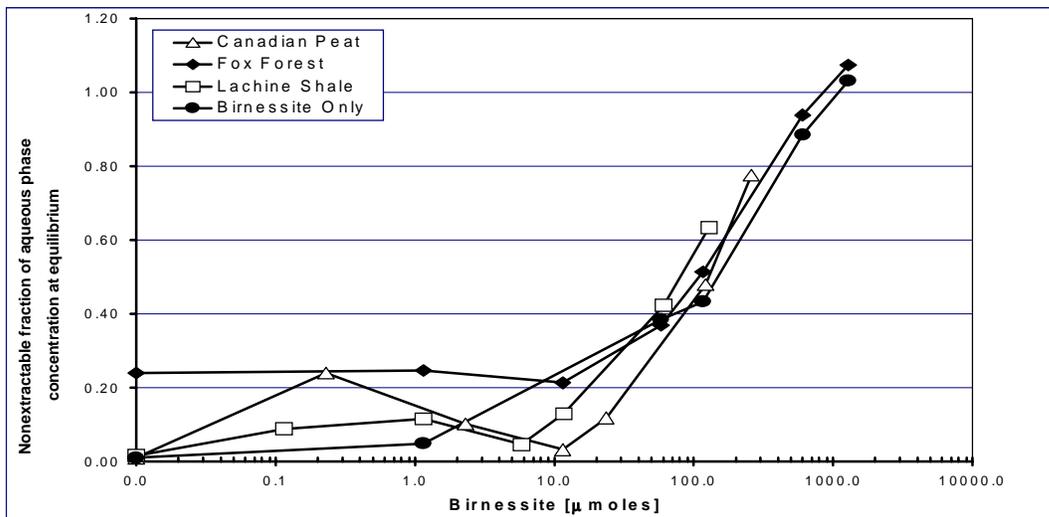
production is negligible; and coupling products are only oligomers (soluble polymers). At increasing birnessite concentrations, note that oligomer production remains the same but polymer production dramatically increases. Phenol removal at 24 hr also increases with increasing birnessite concentrations. The significant differences in coupling rates with

increasing birnessite concentrations can also be seen in Figures 7a and 7b. At similar birnessite to phenol ratio, samples containing 0.5 mg/L of birnessite required two weeks to achieve similar levels of phenol removal as those observed at 5 mg/L of birnessite in 1 day.

### *Oxidative Coupling in Flow-Through Systems*

The effect of HACs cross-coupling to the soil organic matter and self-coupling (polymer formation) was investigated in non-equilibrium sorption flow-through systems. Two different systems were analyzed: a) glass bead packed columns with variable doses of birnessite ( $\delta$ -MnO<sub>2</sub>), that were used to evaluate the extractability of coupling products in the absence of cross-coupling, and b) soil packed columns with variable doses of birnessite, to evaluate coupling in subsurface conditions. Short glass columns (7.5-mL capacity) equipped with Teflon fittings and tubing were packed with glass beads or three different soil types: a surface soil Fox Forest, the diagenetically older Lachine shale and the diagenetically young Canadian Peat (sorbent properties in Table 1) and varying birnessite doses.

The effect of birnessite amount on overall non-extractability and coupling to SOM was investigated in natural sorbent systems with birnessite amendments. At low birnessite amounts, equivalent to full extractability from glass beads flow-through and soil-free birnessite batch systems, increased non-extractability in peat, and in lesser extent in shale systems, were observed. This can be attributed to oxidative coupling of phenol to their soil organic matter (cross-coupling) (Figure 9). Note that in the absence of birnessite (the point of zero additions in Figure 9), non-extractable



**Figure 9. Non-extractability of phenol in both soil and soil-free birnessite amended systems as a function of mass of birnessite**

phenol was only evident in systems containing the surface soil Fox Forest, confirming our previous results obtained in batch equilibrium studies in natural systems. Note that in Fox Forest systems, the only systems where abiotic coupling naturally occurs, non-extractability is not affected in this birnessite range. This suggests that coupling in near-surface soils such as Fox Forest may be limited by reactive sites at the SOM but not by catalyst availability. The reduced coupling to SOM in the diagenetically older shale, as compared to the younger peat, may also result from limitations of reactive sites in the shale as discussed before in previous sections. At moderate birnessite loadings, coupling to SOM is reduced in both the shale and the peat, which most likely results from increase in self-coupling reactions at this birnessite range (Figure 9). Apparently, the formation of polymers becomes dominant, reducing coupling to SOM. These polymers may correspond to the soluble polymer (oligomers) fraction identified in the soil-free batch studies. At high birnessite loading, the non-extractable fraction is very similar for all four systems indicating that at these birnessite levels, non-extractability is independent of soil type and dependent only on birnessite amount. This indicates that large, insoluble polymers can strongly interact with the birnessite surface and form strongly bound polymeric aggregates.

Based on our experimental findings, there appear to be three distinct coupling reactive regions, corresponding to changes in birnessite concentration (i.e., catalyst activity): i) cross coupling controlled, ii) oligomer (dissolved/monodispersed polymer) controlled, and iii) polymer controlled. Evidence suggestive of oxidative coupling to SOM was only shown at the very low birnessite concentrations, where phenolic polymers were not detected in soil-free systems (monomer/dimer range) (Figure 9). At increasing birnessite concentrations, oxidative coupling of phenols to SOM appears to decrease, as shown by the reduced non-extractability of products (soluble or monodispersed polymers). Apparently, phenol-phenol polymerization decreases the degree of phenol-SOM coupling. Further increase in birnessite concentrations, showed a markedly increase in non-extractability, resulting not from SOM coupling but from polymer association with birnessite particles, which was clearly demonstrated in the soil-free column (birnessite-only) and batch systems.

Investigations from our project have shown that at low catalytic activities, cross-coupling of phenolic compounds to reactive sites on soil organic matter are the predominant coupling reactions. At higher catalytic activities, prevailing self-coupling results in phenolic polymers that associate with catalyst surfaces. While both phenomena have the net effect of temporarily or permanently immobilizing phenolic contaminants in the catalytic zone, for use as reactive barrier remediation

technology, inducing permanent immobilization of phenolic contaminants onto highly reactive soils surfaces at low catalytic activities is desired.

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#### RELEVANCE, IMPACT, AND TECHNOLOGY TRANSFER

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An urgent science need for DOE is to obtain a better understanding of processes controlling the fate and transport of contaminants in subsurface environments to improve conceptual modeling capabilities and current remediation technologies. Our research on the oxidative coupling reactions of hydroxylated aromatic compounds (HACs) in soil and sediment systems addresses an important but less category of interactions of these contaminants with soils. The overall objective of this research has been to elucidate the role in HAC transport and evaluate the efficacy of inducing such reactions for remediation of subsurface systems contaminated by such organic compounds.

Improved understanding of coupling processes in the subsurface could translate into innovative cost-effective treatment technologies such as permeable reactive barriers where the induction of these coupling reactions in the subsurface can be engineered by addition of coupling catalysts and/or reactive sorbent materials. While most oxidative coupling research has been performed in aqueous systems with model chemicals, our project simultaneously studied oxidative coupling reactions in natural (soil and sediment), model (aqueous) and engineered (catalyst amended soil) systems. By doing so basic phenomenological research could be directly driven by potential technological applications, helping to bridge the gap between them.

This research has greatly advanced our understanding of oxidative coupling reactions in soil and sediment systems. This is to the best of our knowledge the first research that: a) studies the effect of coupling in sorption/desorption processes in soil and sediment systems; b) demonstrates a direct association between type of soil organic matter and the capacity of natural materials to mediate oxidative coupling reactions; c) reveals the role of catalyst activity on the type of oxidative coupling reaction (cross-coupling and self-coupling) induced and composition of the reaction products; d) develops a mechanistic model to describe the dynamics of enzymatic coupling of HACs in natural sorbents; and, e) investigates oxidative coupling reactions in continuous flow systems, thus simulating conditions in natural subsurface systems.

In order to bring abiotic and enzymatic oxidative coupling reactions technology into application, several mechanistic aspects of these processes need to be resolved such as: a) elucidation of the role of sorbent structure and chemical composition on the oxidative coupling of HACs by expanding the experimental matrix to synthetic sorbents; b) acquisition of basic information required to establish selection criteria for sorbents and catalysts selection in engineered systems; and c) expansion of developed quantitative models for describing oxidative coupling processes in heterogeneous systems.

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**PROJECT PRODUCTIVITY**

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As described in detail in the body of this report, significant progress was made in developing a more comprehensive understanding of the role of oxidative coupling reactions in contaminated soil and sediments and the efficacy of inducing such reactions in engineered subsurface remediation strategies. Research personnel relocations caused some delays in the project timetable and in preparation of the resulting publications. However, an additional one-year no cost extension was requested and issued, thus ensuring the accomplishment of the principal objectives and goals of this work. The preparation and submission of manuscripts has been productive and rewarding, and a number of important papers are or will soon be in press in peer review journals. The work has clearly revealed, however, that additional investigations should be performed in order to facilitate the full incorporation of this new knowledge we have gained in practical remediation schemes. These activities were of course well beyond the scope of this project, but we intend to seek additional funding to pursue the investigations on catalyst and material selection for the induction of coupling reactions.

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**PERSONNEL SUPPORTED**

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Professional personnel supported/associated with the research effort:

Principal Investigator	Walter J. Weber, Jr	Undergraduate Students	Ray Malewitz
Postdoctoral Researchers	Alok Bhandari (Sep. 1996- Dec. 1997)		Kristy Jurey
	Hildegarde Selig (Sep. 1998 – Sep. 2000)		Tim Griffith
Doctoral Students:	T. Michael Keinath		Deborah Ross
	Alexander Orlov		Carl Lenker
	Qingguo Huang		
Master Students:	Jung Wook. Suh Crystal Y. Payne Michael Taylor		

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## PUBLICATIONS

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Huang, Q., H. Selig, and W.J. Weber Jr. 2001. Peroxidase-catalyzed oxidative coupling of phenols in the presence of geosorbents: rates of non-extractable product formation *submitted to Environmental Science and Technology*.

Manuscripts in preparation:

Selig, H., T.M. Keinath, A. Orlov, Q. Huang, A. Bandhari, and W.J. Weber Jr. 2001. Evaluating Phenol Decontamination in Soils by Induction of Oxidative Coupling Reactions. *To be submitted to the Water Environment Federation*

Selig, H., T.M Keinath, R. Pinto, and W.J. Weber, Jr. 2001 Understanding Natural and Induced Abiotic Oxidative Coupling Reactions of Hydroxylated Aromatic Compounds in Geosorbents I: The Role of Soil Organic Matter. *To be submitted to Environmental Science and Technology*

Keinath, T.M , H. Selig, C. Payne, and W.J. Weber, Jr. Understanding Natural and Induced Abiotic Oxidative Coupling Reactions of Hydroxylated Aromatic Compounds in Geosorbents II: The Role of Catalyst Activity. *To be submitted to Environmental Science and Technology*.

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## INTERACTIONS

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Some of the significant findings of the current project have been presented in national conferences and symposiums. We participated in all EMSP sponsored workshops and have contributed to the following events:

1997, November. 8-9. Orlov, A.; Bhandari, A.; Weber, W.J. Jr. Interactions of phenolic contaminants with shale materials: sorption, desorption and hysteresis. 20th Midwest Environmental Chemistry Workshop, University of Indiana, Bloomington, IN. Oral presentation and abstract.

1998, October 16 – 18. T. M. Keinath II, H. Selig, and W.J. Weber, Jr. Enzyme Catalyzed Oxidative Coupling: Effect of Horseradish Peroxidase additions on sorption of phenols on natural Organic Sorbents. 21st Midwest Environmental Chemistry Workshop. The University of Michigan. Ann Arbor, Michigan. Poster presentation and abstract.

1998, October 16 – 18. A. Orlov, A. Bhandari, H. Selig, and W. J. Weber, Jr. Sequestration of phenolic compounds in natural sorbents through abiotic oxidative coupling reactions: The role of organic matter composition. 21st Midwest Environmental Chemistry Workshop. The University of Michigan. Ann Arbor, Michigan. Poster presentation and abstract.

1999, August 21-25. T. M. Keinath II, H. Selig, C. Payne, J. W. Suh, and W.J. Weber, Jr. Induced Sequestration of phenolic compounds by natural sorbents via MnO<sub>2</sub> and oxidoreductase enzymes by coupling: Comparison of equilibrium and non-equilibrium systems. American Chemical Society, 217th National Meeting, New Orleans, LA. Poster presentation and abstract.

2000, March 5-9, T. M. I Keinath II, H. Selig, Michael Taylor, Crystal Payne and Walter J. Weber, Jr. Sequestration of Phenolic Compounds by Natural Sorbents via Birnessite Induced Oxidative Coupling. American Institute of Chemical Engineers (AIChE). 2000 Spring National Meeting. Atlanta, GA.

2000, March 26-31. Huang, Q., H. Selig, T.M. Keinath, and W.J. Weber Jr., Horseradish peroxidase-catalyzed phenol coupling in the presence of soil: Effects of enzyme inactivation, 219<sup>th</sup> ACS National Meeting, San Francisco, CA. Extended abstract and oral presentation.

2000, August 20-24. T. M. Keinath II, H. Selig, C. Y. Payne, and W. J. Weber Jr. Sequestration of phenolic compounds by natural sorbents via birnessite induced oxidative coupling 220<sup>th</sup> ACS National Meeting. Washington, D.C. Extended abstract and poster presentation.

2000, August 20-24. Q. Huang, H. Selig, and W. J. Weber Jr. Influence of soils on the horseradish peroxidase-catalyzed oxidative coupling of phenol. 220<sup>th</sup> ACS National Meeting. Washington, D.C. Extended abstract and poster presentation.

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#### **TRANSITIONS**

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This technology is not yet at a stage to be used in field applications, as described in the body of this report.

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#### **PATENTS**

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No patents were derived from this research

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#### **FUTURE WORK**

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In light of the significant findings of our project, we strongly believe that induction of oxidative coupling reactions for the immobilization of HACs in selected sorbents can be an effective remediation strategy for HACs contaminated groundwater. A natural course of action would be to continue exploring this area by determining optimal sorbent materials based on their surface chemistry, type and dosage of catalyst, and expanding our modeling efforts to describe coupling rates on engineered system configurations.

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