

Project Title:

**The Efficacy of Oxidative Coupling for Promoting In-Situ
Immobilization of Hydroxylated Aromatics in Contaminated Soil
and Sediment Systems**

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INTRODUCTION

The principal objective for Year 1 of this study has included sorbent collection, preparation and characterization, as well as investigation of the efficacy of abiotic/enzymatic coupling reactions on the irreversible binding of phenolic compounds on natural soils and sediments. In response to a budget reduction request specific modifications were made without compromising the integrity of the proposed work. The modified Phase 1 experimental matrix consists of four natural sorbents and three phenolic sorbates. Preliminary experiments with Chelsea soil indicated excessive release of soil organic matter (SOM) into solution, thereby complicating determination of aqueous phase phenol concentrations. It was therefore decided to substitute Lachine shale for the Chelsea soil. This shale is a well-characterized natural sorbent used previously in our laboratory. Additionally two field soils having similar soil morphology were identified based on their particle size distribution and organic matter content. These soils were located from US Department of Agriculture soil survey data and collected aseptically from a forested and a grassland site.

Another deviation from the proposed schedule of tasks was the initiation of work from Phase II and Phase III. In addition to experiments with natural systems, preliminary work with model and engineered systems was initiated earlier than scheduled in order to integrate and relate all three aspects of the study and provide a more robust perspective of field applications of this remediation technology.

WORK TO DATE

PHASE I: NATURAL SYSTEMS

Sorbent Preparation: Two field soils identified as belonging to the Fox series were collected aseptically from the A-horizon (surface soil) at adjacent forested and grassland areas. They were transported on ice and stored at 4°C to maintain intrinsic microbial populations and enzymatic activity. The soils were passed through a 2-mm sieve and split by coning and quartering at 4°C.

Wurtsmith soil and Lachine shale were air dried and ground to <250 µm and <180 µm particle size, respectively. The soils were then riffle split to workable sizes.

In sorption studies that required sorbent sterilization, the soils were treated by autoclaving or Co-60 irradiation.

Sorbent Characterization: The sorbents were analyzed for pH, moisture content, SOM content, surface area, particle size distribution, cation exchange capacity, iron and manganese contents, and specific phenol, o-cresol and 2,4,5-trichlorophenol (TCP) degrading microorganisms. This data is presented in Tables 1 and 2.

Table 1: Physical and chemical properties of the natural sorbents selected for this study.

	Fox Grassland	Fox Forest	Wurtsmith	Lachine Shale
Type of Soil¹	loam	sandy loam	sandy loam	silt loam
% Sand	47.1	53.1	65.1	13.1
% Silt	31.4	33.4	23.4	73.4
% Clay	21.4	13.4	11.4	13.4
Soil pH	6.9	7.2	9.2	7.0
CEC (meq/100 g)²	12.4	3.7	9.9	3.9
Total Iron (ppm)	16	9	147	840
Total Manganese (ppm)	24.2	44.8	19.0	23.2
% Organic Matter	2.5	5.9	0.6	12.6
% Carbon	1.46	3.43	0.36	7.31
BET Surface Area (m²/g)	8.8864	4.5201	0.7593	14.9851

¹based on particle size analysis

²cation exchange capacity

Table 2: Intrinsic substrate specific microbiological activity¹ of test sorbents.

	Fox Grassland	Fox Forest	Wurtsmith	Lachine Shale
Phenol	35	4.7	2.6	5.1
o-Cresol	24	63	20	no growth
2,4,5-Trichlorophenol	94	36	410	no growth

¹in colony forming units per gram equivalent dry weight of soil (CFU/g soil)

Experimental Protocol Development: Experimental protocols were developed using ¹⁴C-labeled and unlabeled sorbates (phenol, o-cresol and TCP). For the unlabeled phenols, analytical methods were developed utilizing a HPLC equipped with a UV detector. An HPLC equipped with a UV/scintillation detector was used for ¹⁴C-labeled phenols. Additionally, thin layer chromatography techniques were developed to quantify phenol degradation during sorption. Autoclaving, Co-60 irradiation and azide addition were investigated as sterilization methods. Preliminary experiments were conducted to select appropriate pH buffers and determine the correct soil loading for sorption studies.

Experimental Results: A 6-day sorption kinetics study for TCP on Lachine shale showed that the fast sorption phase was complete in 4 days (Figure 1). A four-day soil/solution contact time was, therefore,

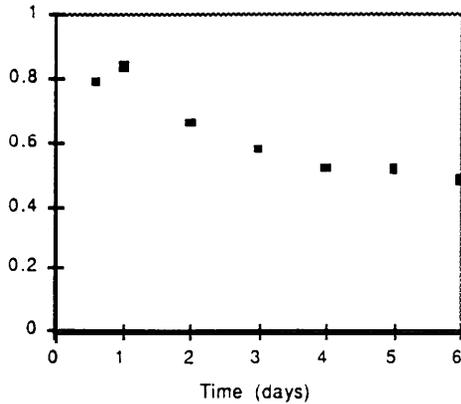


Figure 1. Sorption kinetics for TCP on Lachine Shale.

chosen as the equilibration period for all TCP/Lachine shale sorption experiments. Preliminary results for sorption-desorption of TCP on Lachine shale in oxic conditions indicates significant desorption hysteresis for the irradiated and nonirradiated shale. Sorption-desorption isotherms for the two cases are illustrated in Figures 2 and 3 below. The hysteresis indices defined as $(q_{e(des)} - q_{e(ads)})/q_{e(ads)}$, where $q_{e(ads)}$ and $q_{e(des)}$ are the solid phase TCP concentrations at equilibrium for the adsorption and desorption phases respectively, were calculated to be 1.01 and 0.76 for the irradiated and nonirradiated and

nonirradiated shale at $C_e=100\ \mu\text{M}$. Although irradiation doesn't appear to alter the Freundlich n value or isotherm linearity to a great extent, there is a significant increase in desorption hysteresis as defined by the Hysteresis Index (H.I.).

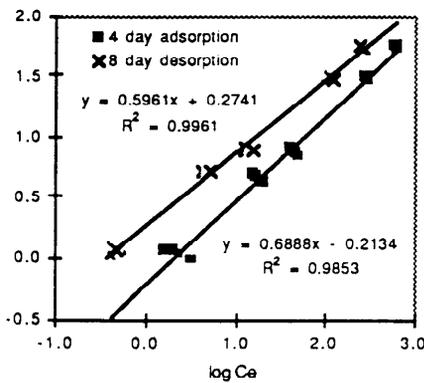


Figure 2. Sorption-desorption isotherms for TCP on irradiated Lachine shale under oxic conditions at pH 7.5. H.I.= 1.01.

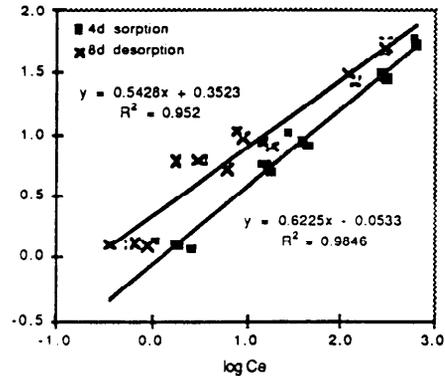


Figure 3. Sorption-desorption isotherms for TCP on nonirradiated Lachine shale under oxic conditions at pH 7.5. H.I.=0.76.

Lachine shale was also subjected to sequential desorption with fresh buffer containing no TCP. This was done for each of the five samples containing different amounts of sorbed TCP. Figure 4 shows the 4 day adsorption isotherm and the sequential desorption data. The sequential desorption data fall into a line which appears to be only slightly different from the single-point desorption isotherm shown in Figure 2. The sequential desorption data for the soil sample contacted with the highest initial aqueous concentration of TCP ($C_0=1000\ \mu\text{M}$) is presented in the normal scale in Figure 5. The soil/solution contact time between each sequential quadruplicate set of data points was 24

hours. This figure also illustrates the presence of a significant amount of desorption hysteresis in the case of TCP sorption on Lachine shale under oxic conditions.

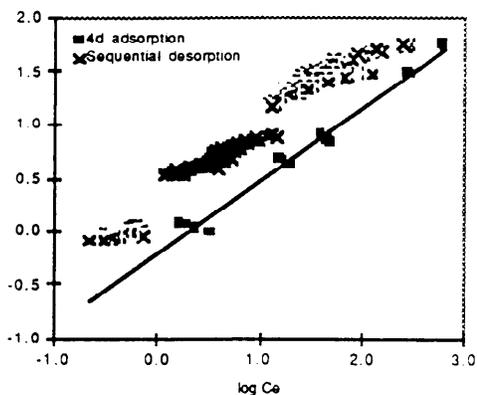


Figure 4. Sorption followed by sequential desorption for TCP on irradiated Lachine shale under oxic conditions at pH 7.5.

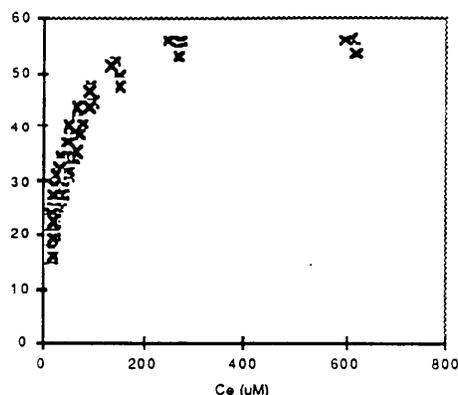


Figure 5. Sequential desorption data for TCP on Lachine shale after a 4 day soil/solution contact time.

PHASE II: MODEL SYSTEMS

Experimental Protocol Development: Experimental methods were developed to study the enzyme catalyzed oxidative polymerization of phenols in model aqueous systems. Known activities of the enzyme horseradish peroxidase were added to a phenol solution in the presence of the cofactor hydrogen peroxide (H_2O_2). A kinetics study showed that the polymerization reaction was complete within 2 minutes. The enzyme, substrate and cofactor were contacted for a period of 2 minutes at different solution pHs. The TCP/ H_2O_2 molar ratios were maintained at unity to minimize nonenzymatic removal of TCP by H_2O_2 . A HPLC equipped with a UV detector was used to analyze for unreacted

(or free) phenol. An acetate buffer was used for pHs of 4 and 5 while a phosphate buffer was utilized for the pH range of 6 to 8.

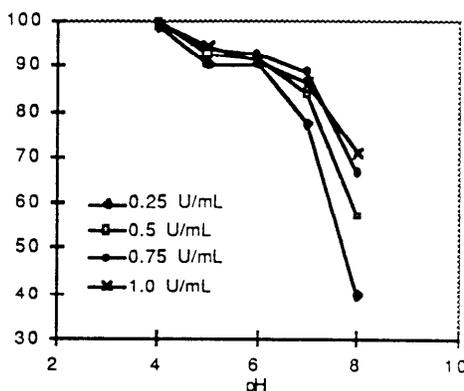


Figure 6. Horseradish peroxidase (HRP) catalyzed removal of TCP at different enzyme doses (units of activity per mL or U/mL) and a solution pH range of 4 to 8.

Experimental Results: It was observed that the removal of TCP from solution as a result of horse-radish peroxidase (HRP) catalyzed polymerization was affected by the solution pH and the activity of enzyme. Figure 6 illustrates the percent removal of TCP observed at different enzyme activities as a function of solution pH for an initial TCP concentration of $100\mu M$. A pH range of 4 to 8 was selected to closely reflect the range of pHs

most likely to be encountered in groundwater aquifers. The removal efficiencies of TCP increased with an increase in the added activity of HRP or a decrease in the solution pH. At more acidic pHs, much smaller enzyme additions were sufficient to achieve near complete removal of the TCP. Nearly 100% of the free TCP was polymerized at a pH of 4. Since 2,4,5-TCP has a pK_a of 6.95, it appears that the nonionized species of phenol more readily participates in the enzymatic polymerization. A significant drop in removal efficiency is seen as the pH increases from 7 to 8.

PHASE III: ENGINEERED SYSTEMS

Design of Column: To gain a better understanding of the practical considerations in applying oxidative coupling as an *in situ* remediation technology, a packed column system was design and constructed. An adjustable-bed liquid chromatography column was modified with sampling ports along its length (please see design schematic in Appendix). The column is intended to mimic natural behavior when a contaminated groundwater flows past 3 reactive catalyst wall. The adjustable-bed configuration and sampling ports were designed so that the effects of the catalyst wall can be observed as they travel further downgradient, either by monitoring phenol disappearance or polymer production.

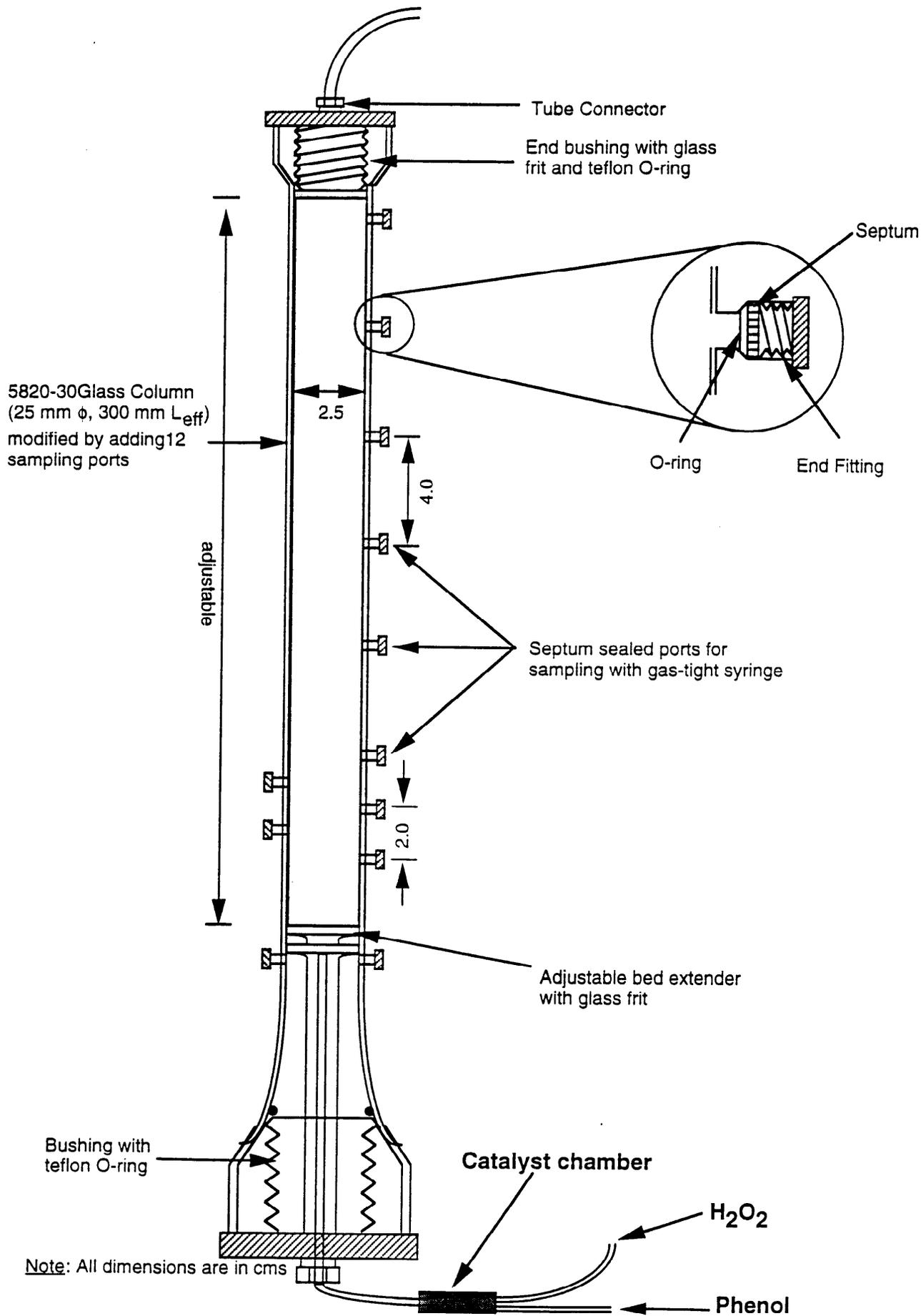
Initially, tracer tests were conducted to insure uniform flow throughout the column. The first phase of the column study will investigate the propagation of radical induced polymerization downgradient will all three of the test phenols. Non-reactive packing of glass beads will be used so that will be virtually no sorption to the packing material. Once Phase I of the study has led to a detailed understanding of the sorption phenomena of test phenols in natural sorbents, glass beads will be replaced with natural sorbents and the interaction between sorption and polymerization phenomena will be investigated.

Catalyst Preparation: In order to maintain catalyst activity in the column system, a procedure was developed to immobilize enzymes on four supports. Horseradish peroxidase was successfully immobilized on controlled pore glass, sand, glass beads and Wurtsmith soil. The bound activity was then determined using a calorimetric method.

FUTURE DIRECTIONS

It is estimated that all work associated with Phase I of the study will be completed during the second year of the project. This includes sorption-desorption experiments with the four natural sorbents and three sorbates in oxic and anoxic conditions and in the presence of enzymes such as horseradish peroxidase and mushroom tyrosinase. The extent of irreversible binding of the phenols will be compared for each phenol under the different conditions.

The second year of the project will also involve more comprehensive experiments with model systems (Phase II). The efficiency of immobilized enzymes (HRP and tyrosinase) and immobilized transition metal oxides in promoting oxidative polymerization will be investigated.



Finally, some additional work will be done with engineered systems (Phase III). The multi-port column will be used to simulate a reactive wall technology for remediation of contaminated subsurface environments.