**July 1, 1993**

**LCMR Final Status Report - Summary - Research**

**I. Program Title: Demonstration of Bioremediation Technology for Removing Organic Chemicals from Aquifers and Groundwaters**

**Issue Area and Program # :** WATER A20

**Program Manager:** Walter J. Maier, 625-3016
Civil and Mineral Engineering Dept.
University of Minnesota, Minneapolis 55455
Tel. # 625-3016, 625-5522

**A. Legal Citation:** ML. 91, Ch.254, Art. 1, Sec. 14, Subd. 4 (e)

BIOREMEDIAL TECHNOLOGY FOR GROUNDWATER This appropriation is to the University of Minnesota, Department of Civil and Mineral Engineering, for a pilot demonstration of technology for insitu biodegradation of organic pollutants in groundwater.

appropriation : $96,000.00 Balance: $0.00

**II. Narrative:**

Abstract:

Contaminated aquifer materials will be tested to demonstrate and define practical applications of insitu microbial degradation. Two organic chemicals representing major groundwater pollutants (pentachlorophenol and phenanthrene) will be tested in soil columns under groundwater flow conditions to determine rates of biodegradation, and the benefits of injecting additional sources of oxygen, nutrients, and/or acclimated microbial cells.

Statement of the Problem, Importance, and Extent of the Problem:

Contamination of soils and groundwaters poses significant risks to public water supplies and potential damage to the ecosystem of existing polluted sites is an enormous task for which more effective and lower cost solutions are sought. Minnesota Pollution Control Agency has identified 179 sites where the spread of contaminants is of direct concern to the communities. A Minnesota Health Department survey of public and private water supply wells shows that 50% of the wells surveyed have traces of commonly used agricultural pesticides. Clean up of these problems has been mandated by State and Federal laws.

Insitu bioremediation of contaminated aquifer soils and groundwater is environmentally sound and potentially much less costly than alternative methods for removing organic pollutants. Biodegradation has potential applications for cleaning up contamination problems resulting form fuel spills, industrial discharges such as volatile organics, and agricultural chemical residues. It builds on the same underlying principles that are used in sewage treatment, namely the ability of microbes to decompose organic chemicals and ultimately to mineralize them. However, additional factors such as adsorption of pollutants on aquifer-soil surfaces, availability of oxygen and nutrients, colonization/transport of microbes, accumulation of biomass and the effects of flow velocity have to be considered in designing and implementing insitu biodegradation in subsurface environments. Experimental testing and demonstration of the combined effects of these variables under realistic but controlled conditions that approximate subsurface conditions are needed.

## III. Objectives:

### Objective A:

### 1. Narrative:

Laboratory soil column studies will be continued on model organic chemicals to obtain quantitative descriptions of insitu biodegradation in representative aquifer materials. The broad objective is to measure the effects of controllable process variables and to correlate the data in a form that can be used to design bioremediation scenarios for removing organic chemicals from polluted soils.

As stated in the original proposal, phenanthrene and pentachlorophenol (PCP) will be studied. These chemicals were chosen in consultation with MPCA staff because (a) they have been identified at numerous Superfund sites, (b) they are representative of two important classes of pollutants, (c) pentachlorophenol is representative of pesticides that are toxic at elevated concentrations, and (d) phenanthrene is representative of slightly soluble fuel compounds for which biodegradation rates are limited by solubility (availability for biodegradation). In addition, studies are being carried out on octadecane as a substrate because this chemical is of interest in fuel spills as well as in connection with biodegradation of waxes and oils. The work on octadecane is funded from other sources but the results will enhance the applications from the LCMR funded program.

Specific objectives are:

a. To measure rates and extent of biodegradation and transport of phenanthrene and pentachlorophenol in soil columns that have been spiked with known concentrations of the respective chemicals. These initial column tests are designed to establish the potential for biodegradation to occur in soils that have not been previously exposed to such chemical pollutants.

   A crucial question regarding PCP, which is toxic, is to determine whether the naturally occurring microbial flora is capable of acclimating and initiating biodegradation in a continuous flow soil column environment. The crucial question regarding phenanthrene, which is very slightly soluble in water (0.8 mg/L), is to establish whether the naturally occurring flora is capable of initiating biodegradation or whether there is a threshold concentration effect as has been proposed by some investigators.

b. A related objective is to demonstrate the feasibility of accelerating bioremediation by inoculating the soil with suspensions of acclimated cells. Strains adapted to growth on very low concentrations of the test chemicals will be tested to demonstrate feasibility of complete removal of trace concentrations.

c. A series of column tests will be made to assess the need for adding nutrients and/or additional sources of oxygen to accelerate biodegradation rates.

d. An important question is whether insitu biodegradation is capable of completely removing the target chemicals down to concentrations that are acceptable for potable water use. If the initial column tests show that there is a threshold concentration below which there is leakage of chemicals, addition of secondary substrates or co-metabolites will be tested to determine whether threshold concentration can be reduced.

e. A major objective of the column studies is to demonstrate the feasibility of insitu biodegradation on site contaminated soils. To this end, a series of column tests will be made using selected soils from well characterized polluted sites.

## 2. Apparatus and Procedures:

Column Apparatus:

Custom-made glass columns 2 inches in diameter and 36 inches long with sampling ports located 6 inches apart will be used. The sampling ports are constructed such that either liquid samples or small amount of soil samples can be taken. Inflow medium will be fed into the column using peristaltic pumps with either a closed-loop or open-ended setup. All tubings and connecting hardware will be made of either teflon, glass, or stainless steel to prevent adsorption of or chemical reactions with the test chemicals. The columns are housed in a constant temperature room that allows operating in the 10-25°C range.

Short columns (20 cm long), 2.5 cm in diameter, will be used to study contaminated soils. These columns are designed to be loaded with soil materials obtained by soil borings as described below.

Soil Materials:

Two uncontaminated soils will be used in this study: Jordan sandstone and glacial outwash. Jordan Aquifer sand underlies Central Minnesota. It is a high silica, low organic content material that has been characterized in previous studies with fuel-related contamination problems. Glacial outwash deposits are particularly important in the context of subsoil contamination because they are widely distributed, highly permeable and span both the vadose and saturated zones. The St. Croix Valley deposit outside of Hudson, Minnesota is a good source of very slightly aggregated low organic content material. It is currently being mined and used as a source of sand and fine gravel; it is readily accessible at faces to a depth of 70 ft. Materials range from fine sand to gravel.

Initial plans for obtaining and testing soils from contaminated sites have been formulated by a planning committee consisting of M. Scott, Senior Engineer-Site Response Section (MPCA), J. Blum, Hydrogeologist, Minnesota Department of Health, and E. Tam and W. Maier from the University of Minnesota.

The first phase of the study of site contaminated soils will make use of small diameter, short columns that will be loaded with cores taken from soil borings. This requires relatively small amounts of sample. Procedures for obtaining samples, handling and storage are outlined below. The second phase will be carried out in the box-microcosm described in part B.

Contaminated Soils - Sampling Methods

Siting and collecting soil samples for characterizing the presence of pollutants has been the subject of numerous papers that address special problems such as minimizing cross-contamination, loss of chemicals, or modifying microbiological properties. Modifications of the traditional soil sampling procedures described in the following ASTM procedures for soil sampling will be used:

| | |
|---|---|
| D 1586 | Method of penetration test and split barrel sampling of soils |
| D 1587 | Practice for thin-walled tube sampling of soils |
| D 3350 | Practice for ring-lined barrel sampling of soils |
| D 4220 | Preserving and transporting soil samples |
| D 422 | Particle size analysis of soils |

Modifications of the ASTM procedures are in the nature of precautions to prevent cross contamination and loss of chemicals. The boring operations will be field supervised by staff from PCA and the University. It is not deemed essential to impose aseptic sampling techniques.

Column Tests:

1. The first series of column tests will look at the potential and extent of biodegradation of the test chemicals by natural microbial flora present in the soils. The initial procedure has been modified to simplify the testing protocol, namely, the columns will be dosed with solutions containing solubilized substrate. This allows for concurrent

development of procedures for impregnating soils. Columns will be loaded with uncontaminated soil in the bottom half. Then the top half will be filled with soil that is uniformly impregnated with respective chemicals. Soil will be sampled occasionally.

The column will be continuously dosed with a buffered synthetic groundwater. Liquid samples will be taken periodically along the length of the column and analyzed for the soluble concentration of test chemicals in the aqueous phase. A control column with autoclaved soil will be run concurrently. Rate of dissolution and transport of test chemical will be determined from the control column. Biodegradation of test chemicals will be reflected by a gradual decrease in the soluble concentration of test chemical. Samples will be extracted and analyzed by gas chromatography, HPLC, and UV absorption of hexane extracts of phenanthrene as described under Analytical Procedures below. We have used all three methods. HPLC analysis of PCP biodegradation products is particularly useful for measuring by-products or intermediates. UV absorbance of hexane extracts is a rapid method that is effective for monitoring that requires testing large numbers of samples.

2. The second series of column tests will look at the biodegradation of test chemicals with the addition of acclimated cells. The study will determine if externally added cells are capable of competing with the natural flora, can colonize the soil matrix, and accelerate the degradation of test chemicals. Cell culture will be prepared by enrichment technique as described below. Preliminary experiments to determine rates of substrate utilization, oxygen utilization, maximum growth rate coefficient, cell mass yield, and half saturation constant under controlled environmental conditions, will be performed using standard batch test apparatus. The apparatus and procedures have been used by our graduate students and will not be described here.

   Soil will be inoculated by mixing before loading to obtain uniform distribution. Alternatively, inoculum can be dosed into the column. The loaded column will be run as (1) above. The water phase will be sampled periodically and analyzed for target chemical. Concurrently, soil samples will also be taken along the column and analyzed for distribution of cell mass using the Lowry protein assay as described below.



3. In the third set of column tests, columns will be dosed with synthetic groundwater containing limited concentrations of dissolved oxygen to determine the effects on insitu biodegradation. If rates of biodegradation are significantly lower, addition of $NO_3^-$ or $H_2O_2$ in the feed water will be tested. Tests in which higher concentrations of nutrients are added will also be made to see whether there are any process advantages.

4. Column tests with site contaminated soils will be similar to the first series of column tests except that the soil will contain a variety of other potentially biodegradable organics. Details of this test and analytical procedures remain to be worked out.

Analytical Procedures:

Analyses of the test chemicals (PCP and phenanthrene) and their metabolic intermediates in column samples will be performed using the extraction, cleanup, and gas-chromatographic procedures as recommended by the Environmental Protection Agency (Test Methods for Evaluating Solid Waste, Vol 1B: Laboratory Manual Physical/Chemical Methods). Determination of cell mass in aqueous samples and in soil column will be performed by the Lowry protein assay method or the standard bacteriological colony plate count method.

1. Analysis of Pentachlorophenol:

   Aqueous samples containing pentachlorophenol will be serially extracted by acidifying to pH<2 with methylene chloride using a separatory funnel. The extract will be dried by passing through a drying column of anhydrous sodium sulfate, concentrated to appropriate volume in a K-D concentrator, and exchanged to cyclohexane. For soil samples containing PCP, the slurry will be

extracted by stirring in acetone acidified with $H_2SO_4$. Slurries will be stirred for 2 hr, and then partitioned between equal volumes of 0.1N HCl/0.1N KCl and cyclohexane. The cyclohexane extracts from the aqueous or soil samples will be cleaned up by transferring to activated sodium sulfate/silica gel column. The PCP/hexane extract will be derivatized with diazoethane in hexane and injected into a gas chromatograph equipped with a glass column of 1.95% OV-210 and 1.5% OV-17 of Chrom W-HP, 100-200 mesh. The column will be set at 160° C for 7 min and bakeout at 250°C for 5 min. Peaks will be detected using a $^{63}Ni$ Electron Capture Detector. When the GC temperature program is modified, the above procedure may also be used to quantify PCP metabolites, including the various tetrachlorophenols, tetrachlorocatechol, and tetrachlorohydroquinone.

When a quick method of determining the PCP concentration in an aqueous sample is required, the sample will be analyzed using UV spectrophotometry. At pH 7.3, PCP has a $Lambda_{max}$ at 320nm (e=4150).

2. Analysis of Phenanthrene:

Aqueous samples containing phenanthrene will be extracted, dried,exchanged, and cleaned up using the same method as for pentachlorophenol. Soil samples containing phenanthrene will be mixed with anhydrous sodium sulfate, placed in an extraction thimble or between two plugs of glass wool, and extracted with methylene chloride in a Soxhlet extractor. The extract will be exchanged into cyclohexane, and transferred to an activated sodium sulfate/silica gel column for cleanup. The extract will be eluted with methylene chloride/pentane (2:3)(v/v) into a K-D flask and concentrated to the appropriate amount for GC analysis.

The cleaned extract will be analyzed with a gas chromatograph equipped with a Flame Ionization Detector. The column will be a 30-m x 0.25-mm I.D. SE-54 fused silica capillary column. Column temperature will be set at 35°C for 2 min, and then programmed at 10°C/min to 265°C and hold for 12 min. Helium will be used as carrier gas.
Aqueous samples with pure phenanthrene can also be analyzed by extracted with hexane and measured the UV absorbance at a wavelength of 252 nm.

3. Analyses for Cell Mass:

The total cell concentration in various aqueous and soil samples will be analyzed with the following procedures:

a. To determine cell number from aqueous or soil samples with pure bacterial cultures, direct plate counts in various growth medium will be used. Growth mediums include agar plates with pentachlorophenol, phenanthrene, or a complex medium such as nutrient broth or peptone/yeast extract. Plate with pentachlorophenol can be easily prepared by dissolving pentachlorophenol/sodium salt in buffer at pH 7.2. Plates with phenanthrene can be prepared by first spreading the bacteria onto a buffered agar plate, and an ethereal solution of phenanthrene sprayed onto the surface with a mist sprayer. after the ether is evaporated, a thin film of phenanthrene will remain.

b. Total cell mass protein will be assayed using the Lowry protein assay method. For soil samples with adsorbed bacterial cells, the cells will be solubilized in 1N NaOH at 90°C for 20 min before the Lowry assay is performed.

Preparation of Enrichment Cultures:

The substrates to be used in this study have been shown to be biodegradable by microorganisms present in sewage and soils. Development of acclimated enrichment cultures will therefore follow a classical sequence of acclimation in shake flasks followed by expansion in chemostats. The procedures are well known and will not be described here.

Chemostat enrichment results in growth of stable consortia but usually does not produce pure cultures. It may be desirable to carry out studies with pure cultures if preliminary screening studies show that there are significant differences in surface properties and physiology of the members of the enrichment cultures. A decision will have to be made regarding the use of pure cultures in transport studies.

It has been noted that the metabolic capacity for biodegradation of PCP as sole carbon source has been reported for pure cultures of Flavobacterium, Arthrobacter, Pseudomonas, and consortia that contain Pseudomonas. One consortium exhibited very high sensitivity to substrate, a second consortium was less susceptible to substrate inhibition. All of these species are considered to be soil related microorganisms and could serve as cultures for insitu biodegradation. However, there is no information regarding the relative susceptibility of these species to transport through soils and/or colonization on surfaces. It is therefore proposed to grow at least three of the above cultures for use in screening studies. If transport and colonization properties are significantly different and warrant further study, the experimental program will be modified to include these cultures for more complete evaluation.

Analysis of Data and Development of Models and Correlations:

Transport, adsorption, and biodegradation of organic chemicals will be analyzed using available computer models. Program U3 utilizes the numerical solution algorithm for one dimensional flow presented by Van Genuchten. This model describes time and space distributions based on the mass balance relationship described below.

Concentration data obtained from the column tests will be analyzed using a mass balance. A separate mass balance is formulated for substrate and cell mass respectively. However the two equations are coupled.

ACCUMULATION = DISPERSION - ADVECTION - RETARDATION + GROWTH - DECAY



in which each of the above terms has the units mass concentration of chemical or bacterial cells in the liquid phase per unit time ($M_xL^{-3}T^{-1}$). RETARDATION can include both adsorption of substrate or cell mass onto soil particles. Retention of cell mass by filtration (straining and enmeshment) is incorporated as a first order rate process. DECAY includes maintenance requirements and predation. While the GROWTH and DECAY terms may not be important in the short column studies, their effect on the movement of bacterial mass through long soil columns and in field studies can not be ignored.

In situations where transport occurs primarily in one-direction, such as in laboratory soil columns, the above mass balance equation can be written as

$$\frac{dX}{dt} = D_z \frac{d^2X}{dz^2} - U \frac{dX}{dz} - \text{RETARDATION} \pm \mu X - K_d X$$

in which X is the concentration of chemical or bacteria in the liquid phase ($M_xL^{-3}$), t is time (T), z is distance in the z-direction (L), $D_z$ is the dispersion coefficient in the z-direction ($L^2T^{-1}$), U is the average water velocity in the z-direction ($LT^{-1}$), RETARDATION has the units of $M_xL^{-3}T^{-1}$, $\mu$ is the specific decay rate ($T^{-1}$). The RETARDATION term in the above mass balance equation can be described either by an adsorption model or by filtration model.

U3 has a parameter estimation capability that allows calculating the best fit value of one or more parameters from measured values of concentration as a function of depth. The parameter estimating option will be used to calculate the best fit value of dispersion and adsorption coefficients from the test run with heat-sterilized soil in which there are no biological reactions. Test results from the column with biodegradation will be fitted using U3 in order to determine the effective rates of biodegradation as opposed to the rates of biodegradation under ideal conditions. Quantitative descriptions of the kinetics that can be obtained in the soil environment are essential for design studies. Cell mass accumulation in

the column are calculated from a mass balance on substrate removal throughout the column.

Modifications of this model that can be used to correlate the concurrent effects of oxygen depletion and its effect on kinetics of aerobic biodegradation is needed and has been developed during the first six months period. Further modification to correlate the effects of cell mass accumulation are planned. It is also planned to incorporate a function that allows correlating the rate of solubilization of substrate for slightly soluble chemicals such as phenanthrene and octadecane.

## 3. Budget:

Amount budgeted: $ 49,000.00 Balance: $ 0.00

## 4. Timeline:

| | Jan 92 | Jun 92 | Jan 93 | Jun 93 |
|---|---|---|---|---|
| Column Tests A1 | ——— | | | |
| Column Tests A2 | ——— | | | |
| Column Test A3 | | | ——— | |
| Column Tests A4-A5 | | | ——— | |
| Analysis Models Correlation | ——— | ——— | ——— | ——— |

## 5. Status

A series of laboratory tests to demonstrate the feasibility of biodegradation of selected model chemical pollutants have been completed. The results are documented in two Ph.D. theses and one M.S. thesis. The data have been analyzed and described in a series of papers published in technical journals covering work funded by this LCMR grant as well as related studies that were carried out earlier and provided the basis for this more comprehensive study of bioremediation of aquifers and groundwaters.



Rates of biodegradation of the three model pollutant chemicals, pentachlorophenol (PCP), phenanthrene (Ph), and octadecane (C18), have been determined in batch reactor and column tests using acclimated enrichment cultures obtained from soils and sewage as well as indigenous soil microorganisms that are present in contaminated soils. The data have been analyzed in terms of a modified Monod growth rate equation to determine kinetic coefficient.

PCP was found to be toxic at elevated concentrations, however, it was completely and effectively biodegraded by controlling PCP concentrations. This information was used to guide the subsequent column test work on PCP and facilitate analysis of test results from column studies. Soil column tests with PCP have shown that it is completely removed (biodegraded) provided adequate concentrations of oxygen are available. Inoculation is not necessary but can be used to accelerate initiation of biodegradation processes. PCP is quite soluble in water (14 mg/L) if pH is above the pKa of 4.74. PCP-impregnated soils therefore release PCP into solution readily. The kinetics of biodegradation of PCP have therefore been tested by dosing columns of soil with known solutions of PCP ranging up to 100 mg/L. Results from this work have been published and a complete report is presented in the Ph.D. thesis of C. Kim.

Phenanthrene and octadecane are only slightly water soluble and techniques for impregnating soils have been developed. Studies to measure rates of solubilization and biodegradation in batch tests and soil column tests are continuing.

Results from a series of batch tests to measure and characterize adsorption, desorption, and biodegradation of phenanthrene and octadecane in the absence and presence of soil have been published in the conference proceedings of the Purdue Industrial Waste Conference (May 11-13, 1992). The paper entitled "Sorption, Desorption, and Biodegradation of C-labeled Phenanthrene" summarizes results on a series of different organic content soils. This information is crucial in assessing the fate of phenanthrene in soils that contain significant concentrations of naturally occurring organic matter (humics). The papers entitled

"Surfactant Enhancement of Octadecane Biodegradation" and "Biodegradation of Phenanthrene in the Presence of Nonionic Surfactants" summarize a series of test results that were carried out to assess the feasibility of accelerating the rates of removal of these target chemicals. The results show that: (a) the naturally occurring microbial flora in contaminated soils is capable of acclimating to and biodegrading these chemicals; (b) enrichment from sewage derived cells is not necessary; (c) essentially complete oxidation of both substrates is observed. Batch reactor tests have been analyzed in terms of a modified Monod growth rate equation to determine kinetic coefficients for the biodegradation of phenanthrene and octadecane. The studies of phenanthrene biodegradation in the presence and absence of soils are documented in Kauser Jahan's Ph.D. thesis dated 1993 and entitled "Biodegradation of Phenanthrene in Soils in the Presence of Surfactants". The studies on octadecane biodegradation in the presence and absence of soils are documented in Le Thai's M.S. thesis dated 1993 and entitled " ".

Short column tests have been carried out using phenanthrene coated soils and dosing the columns with water to simulate groundwater flow. Data from studies in the absence of microbial activity (removal by washing alone) show rapid initial removal of a small fraction of the total phenanthrene followed by a very slow removal of the bulk of the chemical. By contrast the combined processes of washing and biodegradation result in significantly more rapid removal. These data are fully documented in Jahan's thesis. Papers that describe the rates of removal by washing and the combined effects of washing and in situ biodegradation have been submitted for publication.

A comprehensive analysis of the data using computerized models (described below) is still ongoing. As indicated below, model development and data analysis has been funded from this project.

A new type of column for testing contaminated soil samples has been designed, constructed, and is currently being used. It consists of a 2.5-inch stainless steel tube that is inserted into a splitspoon samples which allows taking minimally disturbed subsurface soil samples. After filling, the steel tube is fitted with sampling ports and subjected to controlled flow rates of water. A series of these columns have been used to sample a Superfund site. Three of the filled columns are currently being tested in the laboratory.

The soils were taken from a creosote contaminated site. Analysis of the soils and leachates shows that the soils contain a large suite of polynuclear aromatic hydrocarbons as well as related phenolics and heterocyclics. The columns were initially dosed with deoxygenated water to measure washing. The results of this phase of the study have been presented to MPCA. More recently the columns are being dosed with aerated water to simulate microbial degradation. Analysis of the effluents show that there is complete utilization of oxygen. The studies are continuing and will be documented in Mark Nelson's M.S. thesis which is scheduled for completion in Fall quarter of 1993.

In a related study, it has been demonstrated that all the organic chemicals in the effluent are biodegradable provided that sufficient oxygen is made available. Nutrients do not appear to be a limiting factor. Based on the observations that nutrients are not a limiting factor for the contaminated soils used in these studies, the nutrient enrichment studies were curtailed and experimental work was focused on defining oxygen limitations. The results of this phase of the column studies are being documented in Jian Shin Chen's Ph.D. thesis which is scheduled for completion end of 1993.

Modeling Studies

A simplified mathematical model (U3) that describes the salient features of insitu biodegradation of organic pollutants in contaminate soils/aquifers has been programmed. The model described the concurrent effects of transport, adsorption, solubilization, and biodegradation, in subsurface environments in terms of a first order rate coefficient. U3 has been used extensively for preliminary interpretation of column test data. It has also been incorporated into the teaching programs where it is used as an engineering tool for simulating the fate of organic chemicals in soil environments under different flow conditions, different rates of diffusion, and different rates of biodegradation.

Analysis of the column data using U3 also showed that the need for a more comprehensive model to fully describe the time space distributions of chemicals, bacteria, and oxygen in soil columns. A more comprehensive program, TPMBX has been developed that allows concurrent description of oxygen, substrate concentration, cell mass accumulation, and dissolution of separate phase excess chemical pollutants as a function of column depth and time.

A PC version of TPMBX was presented at the 60th annual meeting of the Minnesota Academy of Science on April 24, 1992. Graphical simulation of time and spatial variations in concentration of pollutant, oxygen, and cell mass were presented. The graphical output gives a dynamic display of the coupled effects of the major variables on rates and effectiveness of insitu biodegradation for cleaning up contaminated aquifers. A report, entitled "Computer Modelling of Solute Transport in Porous Media with Biodegradation and Solubilization of Excess Substrate" by Andrea Stalf (Plan B MSCE paper), describing the computer program has been prepared including a sensitivity analysis of the major controllable variables.

Analysis and modeling of the column test data is continuing. Because this effort requires highly specialized skills in numerical analysis, modeling, and programming, a subcontract has been arranged with University of Minnesota staff at the Army High Performance Computer Center to allow utilizing the Supercomputer facilities for this phase of the work. This work is expected to be completed by end of Summer 1993. No additional funding is requested from LCMR.

## 6. Benefits:

The results will provide quantitative description of insitu biodegradation under saturated groundwater flow conditions. Discussions with staff from MPCA and consulting firms (STS and Barr) confirm that such information is needed for assessing the feasibility of field applications of bioremediation principles.

## Objective B:

## 1. Narrative:

A better understanding of bioremediation in unsaturated soils (vadose zone) and the underlying saturated water zone is essential for effective field applications because:

a. The vadose zone is the passage-way and most common point of entry of pollutants.
b. The vadose zone has been shown to be a significant source of contamination of underlying groundwater due to leaching.
c. The vadose zone has the potential for supporting an active flora of microorganisms that can serve as a protection for underlying aquifers.

The objectives of this part of the study are to measure the effects of controllable process variables and to correlate the data obtained under unsaturated flow conditions that simulate the vadose zone in contact with a water table.

Transport and biodegradation of PCP and phenanthrene will be measured in a box-shaped microcosm that is designed to simulate the combined effects of vertical infiltration into the vadose zone and horizontal flow in the underlying saturated zone. Flow to the microcosm should be a combination of surface infiltration and horizontal flow. The objective is to define environmental conditions to maximize biologically mediated interception of chemicals in the vadose zone and minimize offsite transport of chemicals in the associated saturated groundwater. This phase of the study is a small scale demonstration that builds on the principles established in the previous column tests.

Specific objectives are:

1. To measure the effects of adding inoculum with infiltration water in order to minimize transport of chemicals to the water table.

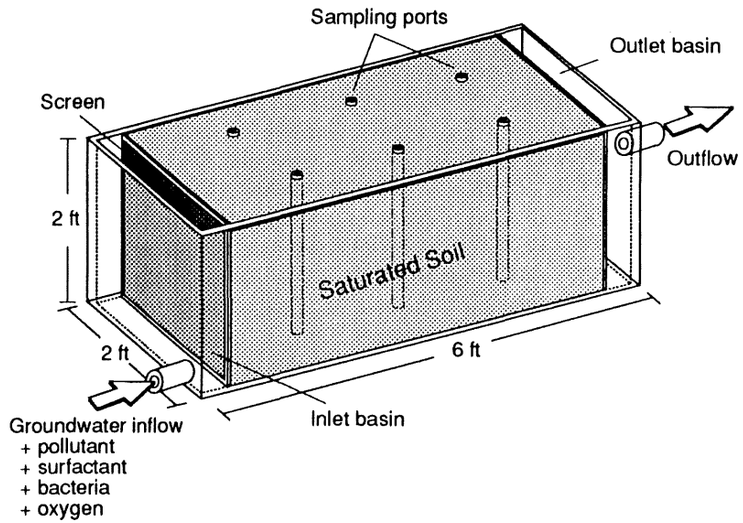
2. To determine whether addition of excess nutrients is beneficial.
3. To determine whether the application of frequent but small doses of "irrigation" water have beneficial effects in accelerating biodegradation thereby minimizing the subsequent transport of pollutants to the groundwater table.
4. The microcosm will also be used to measure the effects of periodic raising and lowering of the water table on rates of insitu biodegradation. Manipulation of the water table is seen as having potential for accelerated aerobic biodegradation in the vadose zone and the near surface saturated zone.

## 2. Procedures:

Microcosm Studies:

Construction, instrumentation, and operation of the box-microcosm is essentially the same as that used at EPA's Ada-Oklahoma laboratory. It is recognized as an essential laboratory testing method for obtaining reliable data on unsaturated groundwater flow conditions and its interface with the saturated zone. The approximate dimensions and configuration of the inlet, outlet, and sampling ports of the microcosm are illustrated below:

Microcosm for demonstration of
in-situ biodegradation in aquifer materials




The microcosm will be initially loaded with Jordan aquifer sand that has been sieved to remove small quantities of less than 200 mesh material. Capillary sampling tubes will be inserted at several locations and depths.

Aquifer Material for Microcosm Study:

The objective of the microcosm study is to test soils under saturated and vadose flow conditions that simulate subsurface environments. The box microcosm is designed to be large enough to allow simulating groundwater flow conditions that involve the combined or separate effects of saturated gradient flow and surface seepage. It is small enough to be practical for laboratory manipulation and allows precise control of flows, gradients, and air exposure which can not be

achieved in field tests. The laboratory scale microcosm also allows carrying out studies in which there is need for controlled addition of alternative sources of oxygen or nutrients, or the injection of acclimated microorganisms.

The techniques for loading the microorganism have not been finalized. Initial calibration studies will be carried out with Jordan Aquifer material. It consists of well rounded, uniform grain size, high silica, low organic content sand. This material has been studied extensively in continuous flow columns. The columns were subjected to saturated flow and/or unsaturated flow conditions to simulated conditions below and above a hypothetical groundwater table. The techniques used for obtaining uniform loading of the columns will be adapted to loading the microcosm (Ph.D. Thesis, C. Kim, 1986).

For studying contaminated aquifer materials, the goal is to load the microcosm with minimally disturbed material in order to retain its physical and hydraulic characteristics. We are looking at several possibilities.

One approach is to excavate blocks of aquifer material. This involves digging and trenching around the block to be removed. The block would then be encased to maintain its structure prior to moving it.. The possibility of splicing small blocks to facilitate handling will be considered.

The use of large diameter "coring tubes" has been developed for sampling unconsolidated soil vertically. We are looking at possible modification of this approach for obtaining a horizontal block sample starting at the vertically exposed face of an excavation.

The staff at U.S.E.P.A.'s Ada, Oklahoma, Kerr Laboratory will be contacted to find out whether they have developed procedures that could be used for loading the microcosm with minimally disturbed field samples. We are particularly interested in studying the potential for insitu biodegradation in a Drift Aquifer which contains a variety of geological materials. Available data from earlier borings show that there are lenses of outwash ranging from coarse sand-gravel to silt, as well as peat. These materials are known to have significantly different hydraulic characteristics. At this time, we do not know the size distribution or the homogeneity of the lenses. If the lenses are composed of mixtures rather than homogeneous materials, it may be desirable to load the microcosm with a randomized distribution of aquifer material. Data from the phase one vertical sample profiles and available data from previous borings will be used to characterize the size distribution and homogeneity of the lenses. The size and homogeneity of the lenses will influence the decision of whether to test undisturbed blocks or carry out tests on mixtures of materials in which the distribution is randomized and therefore does not require retaining the original physical structure.

Base case experiments using salt solutions will be carried out to calibrate flow and dispersion characteristics. Chloride concentrations will be measured using a chloride electrode. A parallel set of experiments will be carried out with solutions of phenanthrene and pentachlorophenol to measure transport and adsorption at selected feed concentrations. The analytical procedures are the same as described above.

Cultures of microorganisms capable of utilizing the respective chemicals as sole source of carbon for growth will be prepared as for the column test and injected into the microcosm. Inoculum will be added for a period of several hours followed by flushing with water to distribute the cells. Previous tests have shown that most of the cells pass through the soil and only a small fraction are retained. Adsorbed cell mass concentrations will be measured by taking soil samples.

<u>Insitu Biodegradation Studies</u>:

Two scenarios will be tested to measure rates of biodegradation:

1. Jordan sandstone that has been impregnated with both organic chemicals (as described in objective A) will be subjected to periodic infiltration.
2. Low concentration solutions of carbon-14 labeled chemicals will be spiked into the infiltration water to measure the fate of mobilized chemicals.

Water phase concentrations of the organic chemical will be measured as a function of time and location. Initial studies will use high oxygen content water feed and excess nutrients. Mass balance calculations will be used to describe total accumulation of mass in the soil. Cell mass distribution and residual chemical in the soil will be measured by sampling the soil periodically.

One important unanswered question regarding the effectiveness of insitu biodegradation is whether there is any release of organic byproducts. This question will be examined by analyzing samples to test for intermediates and byproducts. Metabolic pathways for the biodegradation of both test chemicals have been published, and analytical methods for the intermediates are also well-documented.

Test runs on the microcosm are expected to last for periods of several months. During this time, the effects of short periods of operation at different rates of percolation and nutrient concentrations will be carried out.

If time permits, the effects of limited concentrations of dissolved oxygen will be tested by controlling the feed water D.O. Initially, short periods of low oxygen will be tested to measure its effect on removal of the target substrate. Subsequently, various schemes for adding alternative sources of oxygen will be tested. Use of hydrogen peroxide as supplemental oxygen source and nitrate as alternative electron acceptor will be tested in short batch type experiments before injection into the microcosm. The concept of raising/lowering the water table will be examined. Analytical procedures and data correlation are the same as described under objective A.

## 3. Budget:

Amount budgeted: $ 16,000.00 Balance: $ 0.00

## 4. Timeline:

| | Jun 92 | Jan 93 | Jun 93 |
|---|---|---|---|
| Microcosm construction and calibration | | | |
| Insitu biodegradation | | _____ | |
| Analysis/correlation of data | | _____ | _____ |

## 5. Status

As indicated in the July 1, 1992 status report, construction of the microcosm was deferred in order to concentrate efforts on column studies. As indicated in A3, the newly designed steel columns with six sampling ports have been very effective for studying the removal of a specific model organic pollutant from well defined soils that had been impregnated with phenanthrene as well as for studying removal of creosote type mixtures from a Superfund site (Reilly site). The columns have been used to study rates of removal under anoxic (low dissolved oxygen concentrations) as well as aerobic conditions. Operation of the column under anoxic and aerobic conditions combined with the capability of sampling at intermediate sampling ports has facilitated obtaining additional data that serve the same purpose as some of the proposed microcosm studies. Furthermore, the column studies (by running three columns at the same time) allow measuring the effects of additional variables such as different soil samples, flow rate, and availability of oxygen.

Construction of the microcosm is now completed. It consists of a stainless steel box 3 feet long, 2 feet deep, and 1 foot wide. Sampling ports are spaced vertically and longitudinally to allow measuring soil water and soil air samples. A detailed description of the apparatus will be presented in the detailed version of this final work program status report. The critical design features focus on simulating the combined effects of

vertical infiltration into the vadose zone and horizontal flow in the underlying saturated zone. Flow through the microcosm is a combination of surface infiltration and horizontal flow. The objective is to define environmental conditions to maximize biologically mediated interception of chemicals in the vadose zone and minimize offsite transport of chemicals in the associated saturated groundwater. It is recognized that deferred construction of the microcosm has extended the research beyond the LCMR funding period. However funding from other sources are available and the studies will continue beyond the June 1993 period. Negotiations are underway to obtain representative contaminated soils from the Reilly Superfund Site for use in the initial studies.

### 6. Benefits:

The results of the microcosm studies will provide quantitative description of insitu biodegradation in the combined region of unsaturated and saturated flow. The interaction of infiltration flows from the unsaturated zone into the horizontal flowing groundwater are poorly understood. Discussions with staff from MPCA and consulting firms (STS and Barr) confirm that such information is needed for assessing the feasibility of field applications of bioremediation principles.

## IV. Evaluation:

The status of the program will be evaluated in terms of the following milestones:

1. Completion of a report describing the column studies with pentachlorophenol.
2. Completion of a report describing the column studies with phenanthrene.



3. Presentation of generalized correlation of insitu biodegradation in saturated soil/groundwaters.
4. Report on calibration of microcosm apparatus.
5. Analysis of insitu biodegradation rates in box microcosm.

## V. Context:

Insitu biorestoration of aquifer soils and groundwaters is a developing technology that is environmentally sound and potentially much less costly than alternative methods for removing organic pollutants. It has potential applications in cleaning up contamination problems resulting from fuel spills, industrial discharges, and agricultural chemical residues.

We have done extensive basic research on the kinetics of biodegradation of organic chemicals and on the transport properties of microbial cells. The results of this prior research, which was funded by federal agencies, provides a solid foundation for the proposed development of engineering design tools.

## VI. Qualifications:

### 1. Program Manager:

| | |
|---|---|
| Walter J. Maier, | Professor, Dept. of Civil and Mineral Engineering,University Minnesota |
| Academic training: | B.S. Chemical Engineering, Rensselaer Polytechnic Institute |
| | Ph.D. Environmental /Civil Engineering, Cornell University |
| Professional Experience: | Research Engineer , Exxon Research & Engineering Company |
| | Visiting Professor , Dow Chemical Company |
| | Visiting Professor Princeton University |
| | Professional Engineer, Minnesota Registration |

Major Research Interests :
Kinetics and dynamics of biological wastewater treatment processes
Biochemical treatment of xenobiotics and plastics
Bioremediation of soils and groundwaters

Publications:

54 publications and presentations in the areas of microbiological processing, environmental water quality , and water supply technology. Related recent publications:

Kinetics of biodegradation of chlorinated organics in activated sludge type systems, Journ. WPCF, 52:8, 2158-2166, 1980.

Kinetics of biodegradation of 2,4-dichlorophenoxy acetic acid in the presence of glucose, Biotech. and Bioeng., XXIV, 2001-2011, 1982.

Dynamics of biodegradation of 2,4-dichlorophenoxyacetic acid in the presence of glucose, Biotech. and Bioeng., XXIV, 2337-2346, 1983.

A new modeling technique and computer simulation of bacterial growth, Biotech. and Bioeng., XXVI, 275-284, 1984.

Kinetics of microbial growth on pentachlorophenol, Applied and Environ. Microbiol., 49:1, 46-53, 1985.

Acclimation and biodegradation of chlorinated organic compounds in the presence of alternate substrates, Journ. WPCF, Feb. 1986.

Biodegradation kinetics of mixtures of substrates that exhibit both inhibition and enhancement, International Conf. on Innovative Biological Treatment of Toxic Wastewaters, Arlington, VA, June 1986.

Effects of soil surfaces on biokinetics of PCP biodegradation, International Conf. on Innovative Biological Treatment of Toxic Wastewaters, Arlington, VA, June 1986.

Biodegradation of pentachlorophenol in soil environments, Proceedings, Purdue Industrial Waste Conference, 1986.

On-site biodegradation of organic pollutants in contaminated soils and groundwater, Bulletin #123, Water Resources Research Center, University of Minnesota, 1987.

Process considrations in biological treatment of low concentration wastewaters:I. Steady State, Z. Wasser-Abwasser Forsch., 20, 85-90, 1987.

Process considrations in biological treatment of low concentration wastewaters: II. Dynamics, Z. Wasser-Abwasser Forsch., 20, 108-112, 1987.

Kinetics of microbial growth on mixtures of pentachlorophenol and chlorinated aromatic compounds, Biotech. and Bioeng., XXXI, March 1988.

Transport characteristics of a pentachlorophenol acclimated bacterium through aquifer sand columns, IAWPRC Meeting, Stanford University,1989

## 2. Cooperators:

Eric C. Tam
Assistant Scientist
Department of Civil and Mineral Engineering, University of Minnesota

Education and Experience:

B.S., University of Wisconsin-Whitewater, 1976
M.S., University of Wisconsin-Madison, 1984 (Microbiology)
Instructor, Bacteriology, University of Wisconsin-Madison, 1983-84
Junior Scientist, Gray Freshwater Biological Institute, 1984-87

Publications:

Tam, E., and J.L. Pate. Amino Acid Transport by Prosthecae of Asticcacaulis biprosthecum: Evidence for a Broad Range Transport System. J. Gen. Microbiol., 131: 2687-2699.

Newman, R., J.A. Perry, E. Tam, and R.L. Crawford. Effects of Chronic Exposure of Chlorine on Litter Processing in Outdoor Experimental Channels. Manuscript in Preparation.

**3. Other Investigators**:

The following poeple have been involved in formulating the research plan and have agreed to serve as unpaid consultants on this project:

Michael Scott, Senior Engineer, MPCA, Tel: (612) 296-7297.

Justin Blum, Hydrogeologist, Minnesota State Health Department, Tel: (612) 627-5165.

Dr. Lawrence Wackett, Microbiologist, Gray Freshwater Biological Institute, UM-Minneapolis, Tel: (612) 471-9493.

Dr. Michael Sadowsky,Soil Microbiologist, Dept. of Soils, UM-Minneapolis, Tel: (612) 624-2706.

Dr. Raina M. Miller, Microbial Ecologist,Dept. of Soils, U of Arizona, Tel: (515) 294-0527.

**1991 RESEARCH PROJECT ABSTRACT**
FOR THE PERIOD ENDING JUNE 30, 1993
This project was supported by LCMR Funds

| | |
|---|---|
| **TITLE:** | Demonstration of Bioremediation Technology for Removing Organic Chemicals from Aquifers and Groundwaters |
| **PROGRAM MANAGER:** | Walter J. Maier |
| **ORGANIZATION:** | Civil and Mineral Engineering Department<br>University of Minnesota, Minneapolis 55455 |
| **LEGAL CITATION:** | ML. 91 Ch_Sec_Subd: 4 (e) $ |
| **APPROP. AMOUNT:** | $96,000 |

## STATEMENT OF OBJECTIVES

Contamination of soils and groundwaters is a significant problem that has impacted numerous sites throughout the State. Environmentally sound and cost effective cleanup methods are sought. To this end, studies were carried out to develop/demonstrate in situ bioremediation technology. The objective is development of engineering criteria for the design of bioremediation scenarios for removing slightly soluble and potentially toxic organic chemicals. This includes assessment of needs for addition of oxygen, nutrients and adapted microorganisms to ensure complete removal and accelerate cleanup. Because of the importance of in situ soil conditions on the effectiveness of biodegradation, an important objective was to develop protocols for testing and characterizing minimally disturbed soils cores under field conditions.

## RESULTS

Kinetic coefficients that describe rates of biodegradation of selected model compounds (pentachlorophenol, phenanthrene, and octadecane) in the absence and presence of soils have been determined. It was shown that they were completely oxidized. Tests using higher molecular weight polynuclear aromatic hydrocarbons also showed complete removal by soil resident organisms provided that there was adequate supply of oxygen. However, rates of biodegradation of octadecane and phenanthrene were shown to be relatively slow due to their low solubility in water. One outgrowth of these findings is that funding from another agency was obtained to carry out a parallel study of the use of surfactants to facilitate solubilization and accelerate biodegradation of slightly soluble chemicals.

Protocol for testing minimally disturbed soil cores using a 2.5 inch diameter stainless steel insert in a split spoon sampler have been developed. The columns are fitted with intermediate sampling ports that allows testing the spatial distribution of pollutants as well as oxygen. A series of such soil cores, taken from the Reilly Superfund Site in St. Louis Park, have been studied. Reilly is one of many sites contaminated with creosote in which the polynuclear aromatic hydrocarbon (PAH's) are the chemicals of concern, The results show that rates of biodegradation were limited by oxygen availability. Oxygen enrichment was shown to result in complete biodegradation of all PAH's to below detection limits. There was no need to add either nutrients or specially adapted microorganisms. These studies are ongoing using funds from other agencies.

The column studies have generated a large database of information on rates of transport as well as in situ biodegradation. This has given us much better insight on the dynamics of transport and biodegradation of chemicals in soils. Data analysis has been pursued at

several levels. A simplified mathematical program (U3) has been used to interpret changes in soluble and adsorbed PAH concentrations versus time and serves as a guide for the experimental program. A more comprehensive mathematical model (TPMBX2) has been developed for analyzing concurrent changes in oxygen concentration, biomass accumulation, and solubilization of separate phase organic chemicals. This model has been most useful for simulating the dynamics of oxygen deficiencies. It is therefore a useful engineering tool for evaluating alternative scenarios of oxygen addition as a means of achieving complete biodegradation without offsite migration of chemicals. However, it became clear that this model was not adequate for fitting the column test results because it was not designed for parameter estimation. This has been resolved by working with the staff at the Army High Performance Computer Center to develop a computer model that is capable of fitting effluent concentration data to find best fit values of selected coefficients that cannot be measured directly. The results of this modeling effort will be published in the near future.

## DISSEMINATION OF RESULTS

Results have been presented at two conferences and respective conference proceedings. In addition, five papers have been submitted for publication as listed below.

Results from a series of batch tests to measure and characterize adsorption, desorption, and biodegradation of phenanthrene and octadecane in the absence and presence of soil have recently been published in the conference proceedings of the Purdue Industrial Waste Conference (May 11-13, 1992). The paper entitled "Sorption, Desorption, and Biodegradation of C-labeled Phenanthrene" summarizes results on a series of different organic content soils. This information is crucial in assessing the fate of phenanthrene in soils that contain significant concentrations of naturally occurring organic matter (humics). The papers entitled "Surfactant Enhancement of Octadecane Biodegradation" and "Biodegradation of Phenanthrene in the Presence of Nonionic Surfactants" summarize a series of test results that were carried out to assess the feasibility of accelerating the rates of removal of these target chemicals.

A PC version of TPMBX was presented at the 60$^{th}$ annual meeting of the Minnesota Academy of Science on April 24, 1992. Graphical simulation of time and spatial variations in concentration of pollutant, oxygen, and cell mass were presented. The graphical output gives a dynamic display of the coupled effects of the major variables on rates and effectiveness of in situ biodegradation for cleaning up contaminated aquifers. A report, entitled "Computer Modeling of Solute Transport in Porous Media with Biodegradation and Solubilization of Excess Substrate" by Andrea Stalf (Plan B MSCE paper), describing the computer program has been prepared including a sensitivity analysis of the major controllable variables.

The following graduate students have received partial funding from this project in conjunction with their thesis research:

Dr. Kauser Jahan, Ph.D. Thesis, January 1993. Biodegradation of Phenanthrene in Soils in the Presence of Surfactants. Three papers have recently been submitted for publication.

Le Thai, M.S. Thesis, March 1993. Solubilization and Biodegradation of Octadecane in the Presence of Nonionic Surfactants. Two papers have recently been submitted for publication.

Andrea Stalf, M.S. Plan B Paper. Computer Modeling of Solute Transport in Porous Media with Biodegradation and Solubilization of Excess Substrate.

## APPLICATION OF RESULTS

The protocol for taking and testing minimally disturbed soil cores have been used several times at the Reilly Site and are being considered at another Superfund Site (Kummer Landfill Site, Bemidji, MN).

Model TPMBX2 has been used for evaluation of laboratory column test data as well as for the analysis of field test data from the Kummer Landfill Site (Plan B Project Paper, M.S. degree, Steven Barrett, June 1993).

September 21, 1993

# LCMR FINAL REPORT

## I. PROGRAM TITLE

Demonstration of Bioremediation Technology for Removing Organic Chemicals from Aquifers and Groundwaters

Issue Area and Program # : WATER A20

Program Manager: Walter J. Maier, 625-3016
Civil and Mineral Engineering Dept.
University of Minnesota, Minneapolis 55455
Tel. # 625-3016, 625-5522

A. Legal Citation: ML. 91, Ch.254, Art. 1, Sec. 14, Subd. 4 (e)

BIOREMEDIAL TECHNOLOGY FOR GROUNDWATER This appropriation is to the University of Minnesota, Department of Civil and Mineral Engineering, for a pilot demonstration of technology for insitu biodegradation of organic pollutants in groundwater.

appropriation : $96,000.00 Balance: $0.00

## II. NARRATIVE

### Abstract:

Contaminated aquifer materials will be tested to demonstrate and define practical applications of insitu microbial degradation. Two organic chemicals representing major groundwater pollutants (pentachlorophenol and phenanthrene) will be tested in soil columns under groundwater flow conditions to determine rates of biodegradation, and the benefits of injecting additional sources of oxygen, nutrients, and/or acclimated microbial cells.



### Statement of the Problem, Importance, and Extent of the Problem:

Contamination of soils and groundwaters poses significant risks to public water supplies and potential damage to the ecosystem of existing polluted sites is an enormous task for which more effective and lower cost solutions are sought. Minnesota Pollution Control Agency has identified 179 sites where the spread of contaminants is of direct concern to the communities. A Minnesota Health Department survey of public and private water supply wells shows that 50% of the wells surveyed have traces of commonly used agricultural pesticides. Clean up of these problems has been mandated by State and Federal laws.

Insitu bioremediation of contaminated aquifer soils and groundwater is environmentally sound and potentially much less costly than alternative methods for removing organic pollutants. Biodegradation has potential applications for cleaning up contamination problems resulting form fuel spills, industrial discharges such as volatile organics, and agricultural chemical residues. It builds on the same underlying principles that are used in sewage treatment, namely the ability of microbes to decompose organic chemicals and ultimately to mineralize them. However, additional factors such as adsorption of pollutants on aquifer-soil surfaces, availability of oxygen and nutrients, colonization/transport of microbes, accumulation of biomass and the effects of flow velocity have to be considered in designing and implementing insitu biodegradation in subsurface environments. Experimental testing and demonstration of the combined effects of these variables under realistic but controlled conditions that approximate subsurface conditions are needed.

## III. RESULTS

### Part A Results:

This final status report for Part A is presented in 3 parts that address the research objectives as outlined in the Work Plan.

As indicated in the original work plan, the major objective is to demonstrate the feasibility of in situ biodegradation of contaminated soils. The subobjectives include:

(a) measuring rates and extent of biodegradation and transport of selected organic chemicals in soil columns

(b) demonstrating the need for and the feasibility of accelerating bioremediation by inoculation with acclimated organisms

(c) assessing the need for adding nutrients and/or oxygen to accelerate biodegradation

(d) demonstrating complete removal of target pollutants and/or whether there is a threshold concentration.

Part 1 describes the results of column studies of enhanced washing of phenanthrene contaminated Jordan aquifer sand.

Part 2 describes the results of column studies to characterize in situ biodegradation of Jordan sand.

Part 3 describes studies using site contaminated soils from a Superfund site.

It should be noted that an extensive literature review was carried out before each phase of the experimental work. The literature references are not included in this status report to keep the report to a reasonable size. However, any reader interested in seeing the literature review and list of references can consult the following public documents in the University of Minnesota Library and/or Archives.

Kauser Jahan, Ph.D. thesis, 1993.
Le Thai, M.S. thesis, 1993.
J. Chen, Ph.D. thesis in preparation.

---

Part 1:

**Column Studies Of Enhanced Washing Of Phenanthrene From Jordan Sand**



1. ABSTRACT:

Continuous flow column studies were conducted to measure rates of washing of phenanthrene from contaminated Jordan aquifer sands and to assess the suitability of four commercial nonionic surfactants to accelerate washing phenanthrene from coated soil. The nonionic surfactants selected were : Triron X114, Corexit 0600, Brij 35 and Tween 40. Phenanthrene was precoated on sand as a methylene chloride solution and allowing the solvent to evaporate, leaving the desired phenanthrene concentration as a surface coating. Phenanthrene coated sand was designed to simulate soil contaminated with excess phenanthrene which remained after evaporation of lighter hydrocarbon solvents. This coated sand was washed with surfactant solutions at a low concentration of 25 mg/L. This selected concentration was below the critical micelle concentration of all four surfactants. The columns were washed at a low and high flow rate. Results indicated that the selected surfactant concentration marginally enhanced the washing of phenanthrene from the Jordan sand. The performance of Tween 40 was better as compared to the other surfactants. Results indicated that there is a rapid release of the phenanthrene initially and then a continuous slow release with time. Highest effluent concentrations were observed during the first few hours of flushing, corresponding to saturated solutions. After approximately 5 hours (20 pore volume displacements) effluent concentrations decreased significantly. The slower release with time is ascribed due to the decrease in readily available surface area of coated phenanthrene to the flowing water phase. Sorption of phenanthrene with the organic fraction of the sand also plays a role. There was an enhanced rate of washing of phenanthrene initially at the higher flow rate. Analysis of the phenanthrene distribution remaining at the end of the tests showed that most of the phenanthrene was washed from the upper parts of the columns, with lesser removal from the lower parts of the column.

2. INTRODUCTION:

Many environmental pollutants, such as polynuclear aromatic hydrocarbons (PAHs), polychlorinated biphenyls and dioxins are characterized by their low aqueous solubility and hydrophobicity. These properties contribute to the strong sorption of these compounds to the

soil porous matrix and hence slow migration through the hydrogeologic system. Thus these compounds are found in the soil phase at concentrations much higher than in the aqueous phase. Sorption of these compounds is highly dependent on the soil organic fraction. Results from several studies have indicated that the removal of such compounds from soil and aquifer systems is resistant to water washing. Remediation techniques based on pump and treat are lengthy and expensive. Clean up of such contaminated soil sites are costly and include excavation and disposal in a hazardous waste facility or excavation and incineration. Thus more cost effective treatment methods are required for the cleanup of soil sites contaminated with low aqueous solubility and hydrophobic compounds. This phase of the laboratory study was designed to measure rates of phenanthrene removal by flushing.

In recent years, there has been growing interest in use of surfactants for enhancing removal of hydrophobic contaminants in aquifer restoration. The use of surfactants to increase hydrocarbon mobility is well established and has been well documented. The mechanism of mobility enhancement may involve increased solubility at the molecular level, dispersion of colloid particles, as well as partitioning into surfactant micelles. The presence of surfactants may also affect the availability of insoluble hydrocarbons for microbial degradation. Several researchers have investigated the effect of surfactants on the solubilization of hydrocarbons and have assessed the potential of surfactants for treating contaminated soils. The flushing studies were therefore expanded to include tests with four nonionic surfactants.

Phenanthrene coated sand was prepared for testing, in order to simulate the dispersion of excess phase phenanthrene (above solubility) in contaminated soil. It is known that phenanthrene is commonly introduced into soils as a solute in a acarrier solvent such as creosote. Examination of creosote contaminted soils suggest that the lighter hydrocarbons evaporate leaving residual PAHs including phenanthrene as a quasi solid/high viscosity liquid phase matrix that covers the sand surfaces, fills pore spaces and acts as a binder for sand particles.

Nonionic surfactants were selected as they are known to have greater hydrocarbon solubilizing power, less toxicity to microbial populations, minimal adsorption to charged surfaces and low foaming property.

Phenanthrene was used as a model compound because it has a low aqueous solubility and high sorptive properties. Its physical characteristics are typical of PAHs and it has been identified as a major chemical pollutant at numerous sites. Column studies were conducted to simulate soil water contacting conditions analogous to soil/groundwater situations that would be encountered in insitu aquifer restoration.

## 3. MATERIALS AND METHODS:

The aquifer material used in this study was obtained from a subsidiary of J.L. Shiely Company, Minnesota Frac Sand Company, which mines Jordan sandstone near Jordan, Minnesota. This sand is almost pure quartz characterized by a low foc (0.0001). The sand was washed , oven-dried and sieved . The sand fraction used for all experiments was that passing through sieve # 30 and that retained on sieve # 100, in order to collect the fraction representing the materials in sandy aquifer. The sand was sterilized by autoclaving prior to all experiments. Autoclaving was carried out at a temperature of 121$^{o}$C and 15 psi pressure.

Tests were conducted in short glass columns constructed by fusing two 25 mm all glass Millipore filter assemblies with stainless steel meshes to hold the sand. The Millipore filters were purchased from Fisher Scientific Co. (Pittsburg, PA.). An all glass assembly was preferred over the commercial column apparatus as there have been several reports on the sorption effect of hydrophobic column components like Teflon. The apparatus is illustrated in Figure 1. All connecting tubes and fittings were either Viton or stainless steel. A Gilson peristaltic pump (Model Minipuls 2) was used to pump the aqueous surfactant solutions or deionized water through the columns. Distilled-deionized water from a Corning megapure water system was used for all experiments.

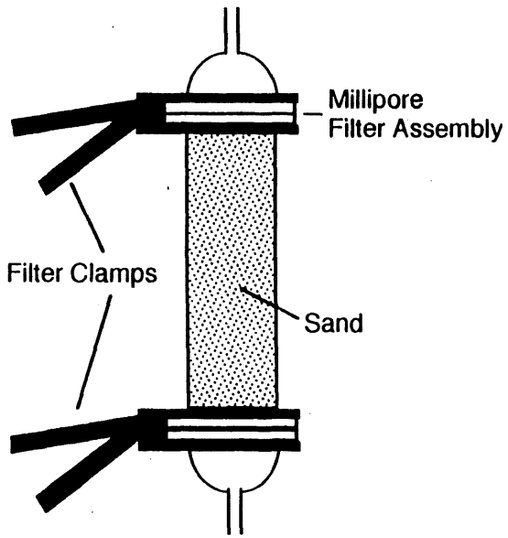



**Figure 1**

Reagent grade radiolabeled phenanthrene -9-$^{14}$C with a specific activity of 13.1 mCi/mmol (purity>98%) was obtained from Sigma Chemical Co., St. Louis, MO. This was supplied as a solid and was extricated from its package with 1 mL of HPLC grade methanol to prepare a $^{14}$C-labeled solution. The radiolabled solution was kept refrigerated in a foil-wrapped glass vial equipped with a Teflon-lined septum to minimize PAH losses. Nonradiolabeled zone-refined phenanthrene was also obtained from the same distributor. A phenanthrene stock solution consisting of a predetermined mass ratio of radiolabled to nonlabeled phenanthrene was prepared in HPLC grade methylene chloride (Fisher Scientific Co., Pittsburg, PA). Radiolabeled phenanthrene measurements were made on a Beckman LS 1801 liquid scintillation counter (Beckman Instruments Inc., Irvine CA), using the H# quench monitoring technique with automatic quench compensation.

Four commercial nonionic surfactants were used in this study. The Triton X 114, Brij 35 and Tween 40 surfactants were purchased from Sigma Chemical Co., St. Louis, MO. The Corexit 0600 surfactant was obtained from Exxon Chemical Co., Houston, TX. The surfactants were selected on the basis of their favourable solubility at groundwater temperatures, lack of toxicity and range of structures. They were used without further purification.

## 4. EXPERIMENTAL PROCEDURE:

The glass columns were packed by pouring a continuous thin stream of phenanthrene precoated sand while tapping the side of the column to achieve a uniform porous medium. The sand was precoated with phenanthrene using a mixture of prepared labeled and nonlabeled phenanthrene solution in methylene chloride. It was added to Jordan sand in a beaker to saturate the sand and pond on the surface. The sand was then continuously stirred with a glass rod to uniformly distribute the phenanthrene within the sand. The methylene chloride was then slowly evaporated in a vented fume hood. Ten samples of 1gm of dried coated sand were extracted back into 30 ml of methylene chloride and shaken for 24 hours. 0.5 ml of each sample was added to 5.0 mL scintillation cocktail (Ecoscint A, National Diagnostic Laboratory, Mannville, NJ) and counted in the scintillation counter. Phenanthrene recovery from coated sand averaged 96%. Some loss was due to phenanthrene coating of the glass beaker. The influent solutions consisted of deionized water and the four selected surfactants at a concentration of 25 mg/L.

All solutions were pumped at a rate of 0.243 ml/min through the column. Samples were collected in glass vials with crimp top caps having Teflon-lined septa. The septa were covered with aluminium foil to prevent losses to the hydrophobic septa. 0.5 mL of each sample was added to 5 mL of scintillation cocktail and counted on the liquid scintillation counter. Similar experiments were also conducted at a flowrate of 1.5 mL/min for water and Tween 40 as the wash solutions.

At the end of the experiments the sand was removed from the columns in three equal portions, representing the top, mid and bottom third of the column. This sand was then weighed and dried. 30 mL of methylene chloride was added to this sand in a crimp top vial and put on a shaker for 36 hours. 0.5 mL of the supernatant from the vial was added to 10 mL scintiallation cocktail for counting. The sand was subjected to sequential

extractions with methylene chloride until the counts fell to background levels. Counts were converted to $\mu g$ of phenanthrene.

A chloride tracer (NaCl) study was also carried out to investigate channeling and determine the dispersion and retardation coefficients. Chloride concentrations were analyzed by ion chromatography with a Dionex Ion Chromatograph (Model DX 100, Dionex Corporation, Sunnyvale CA).

## 5. RESULTS AND DISCUSSION:

Results for the $Cl^-$ elution from the column are presented in Figure (2). The results were analyzed using a nonlinear least-squares inversion method to determine the retardation coefficient R and the dispersion coefficient. R was estimated at 1.0 indicating that the chloride behaved as a nonsorbing tracer. The dispersion coefficient was estimated to be 0.0001 $cm^2/hr$.

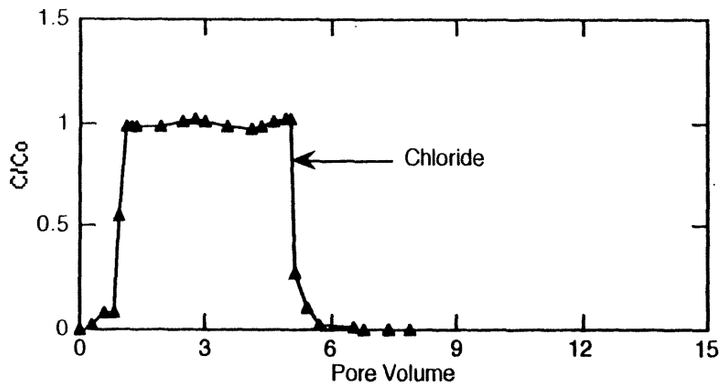



**Figure 2**

The results of experiments conducted at a low flow rate of 0.243 mL/min are presented in Figures (3) through (6). Figures (3a) and (3b) present the percent mass of phenanthrene remaining versus the number of washings or pore volumes. It decreases initially rapidly during the first 20 pore volume displacements. The removal rate then decreased significantly in all cases. All the surfactants at the selected concentration enhanced the washing rate slightly as compared to the control column which was washed with water alone. Of the four surfactants studied the Tween 40 was the most effective in removing the coated phenanthrene from the sand.

Comparison of the results in terms of phenanthrene remaining versus time (Figures (4a) and (4b)) also show the same trend. The phenanthrene concentration in the column effluent versus time is presented in Figures (5a) and (5b). Highest phenanthrene concentrations were observed during the first five hours and decreased thereafter. With water alone the maximum concentration (0.8-0.85 mg/L) corresponds to saturation solubility. This indicates that initially there is enough contact and adequate contact time with the flowing water to reach saturation. As the washing progresses, rates of dissolution become limiting because readily available phenanthrene surfaces are depleted and dissolution and mass transport from smaller diameter pores occur at a slower rate. The presence of dead end or isolated pores ultimately results in very slow rates of washing. This explains the very slow removal of phenanthrene at the later stages of washing. During the first 10 hours of washing, approximately 40% of the coated phenanthrene has been washed out. Another 25% is removed in the next 40 hours.

As indicated in Figures (5a) and (5b), maximum effluent concentrations with surfactant solutions were significantly higher than with water during the first four hours of washing. Maximum concentrations were1.08 mg/L with Tween 40, 0.96 mg/L with Brij 35 and Corexit 0600 and 0.93 mg/L with Triton X 114 versus 0.8 to 0.85 mg/L for water alone. This can be observed clearly in Figure (6) where the effluent concentration for the first 10 hours of washing is presented. For the period 5-16 hours effluent concentrations were actually higher with water than with the surfactant solutions. However, as the washing period extended beyond 27 hours phenanthrene concentrations in surfactant solutions were consistently higher than with water.

The overall higher washing rate with surfactants can be ascribed to more rapid rates of dissolution in the presence of surfactant molecules and/or the effects of lower surface tension. Earlier experiments (Chapter 2) indicated that the surface tension for the selected surfactants at 25 mg/L was as follows: 35.0 dynes/cm for Corexit 0600, 35.4 dynes/cm for Triton X 114, 37.95 dynes/cm for Tween 40 and 41.1 dynes/cm for Brij 35. The higher concentrations with water that was observed after four hours of washing is ascribed to the fact that solubilization rates in the larger diameter pores were still contributing a significant part of the phenanthrene being flushed from the column with water. However after 16 hours the water washed column caught up with the surfactant washed columns in that the contribution from large diameter pores became insignificant. The underlying assumption of this explanation is that surfactants intrinsically increase rates of solubilization. This explanation will be tested by using modified transport models in which dissolution rates of chemicals are described in terms of pore flow velocity distribution that reflect pore size distribution as defined by soil porosity and uniformity index considerations.

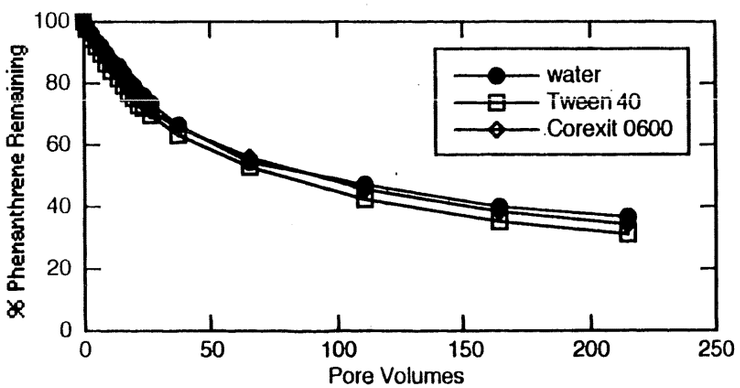



**Figure 3a**

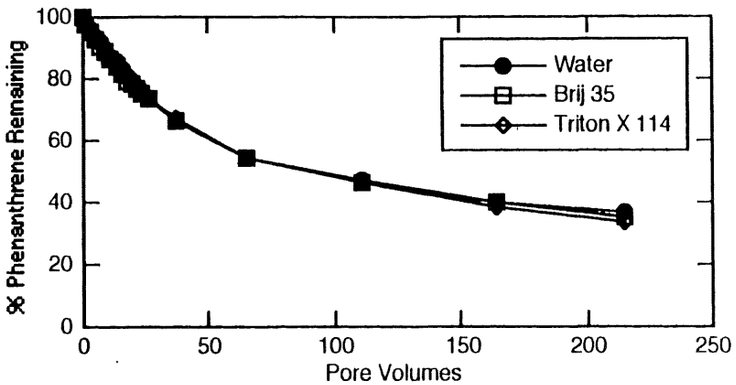



**Figure 3b**

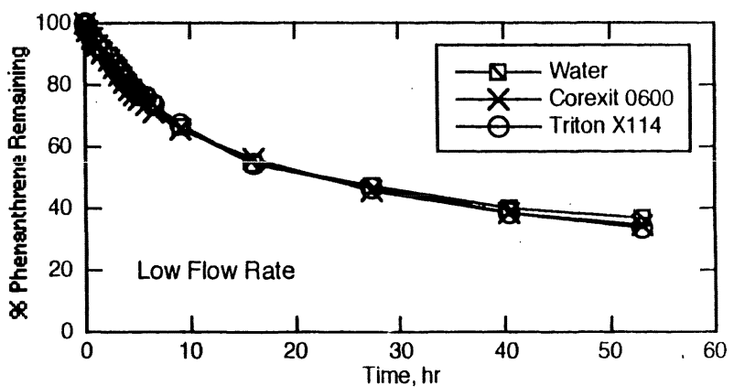



**Figure 4a**

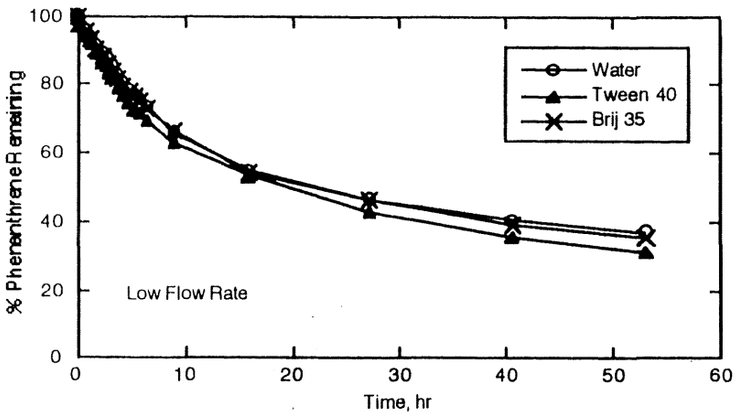



**Figure 4b**

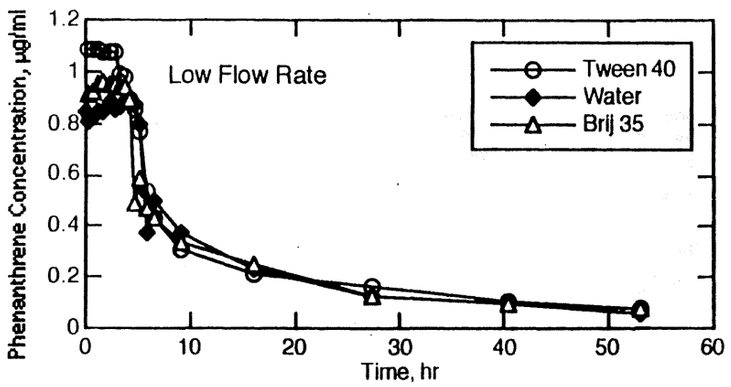



**Figure 5a**

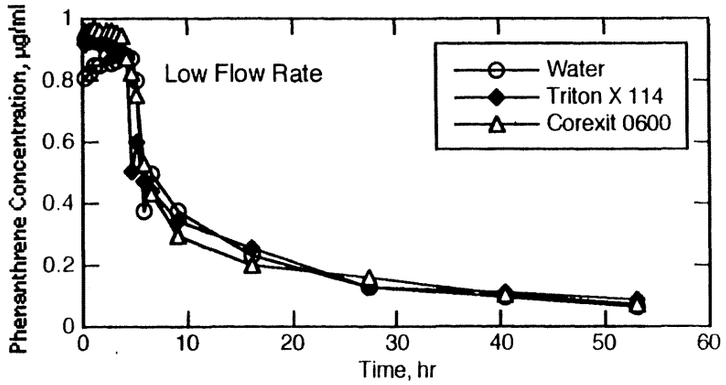



**Figure 5b**

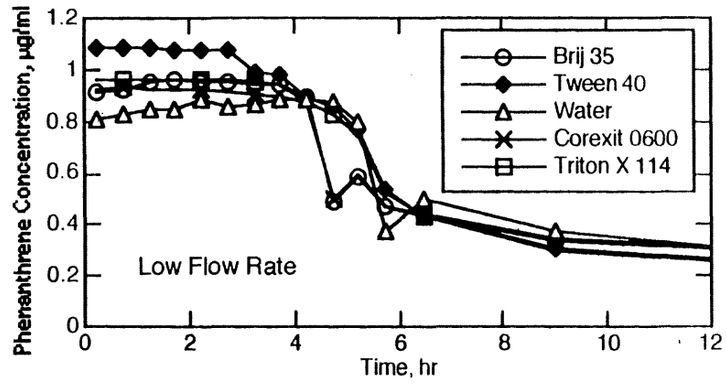



**Figure 6**

Figure (7) shows the distribution of phenanthrene remaining on the sand at the end of the experiment. The plots show that most of the phenanthrene has been removed from the upper third of the column. The same trend is seen in all the columns regardless of the surfactant used. Phenanthrene recovery from the columns at the end of the

experiments was sufficient to close the mass balance. In all cases the total phenanthrene recovered after a mass balance was 93.2% on an average for all the columns.

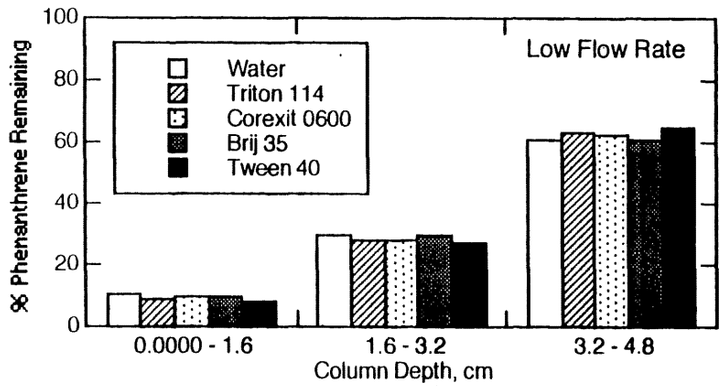



**Figure 7**

The effects of using higher dosing rates were tested with water and with Tween 40 at 25 mg/L. Results for the first ten hours of dosing with 1.5 mL/min versus 0.243 mL/min is shown in Figure(8a) as percent phenanthrene remaining versus dosing time; the corresponding data for the whole duration of the test is shown in (8b). It can be seen that more phenanthrene was removed at higher flow rates during the first ten hours whereas removal at high flow rate was less during the latter period (10-30 hours).

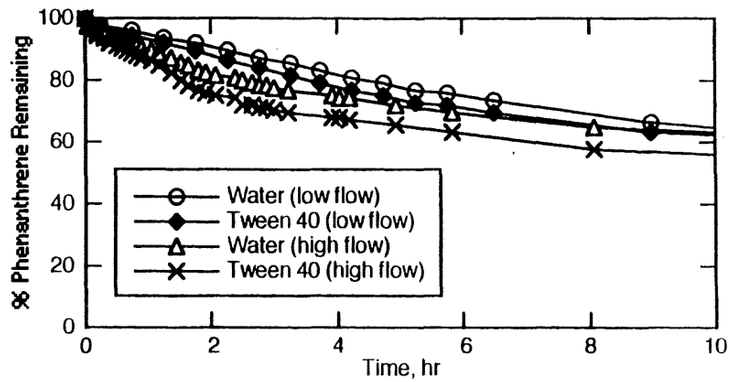



**Figure 8a**

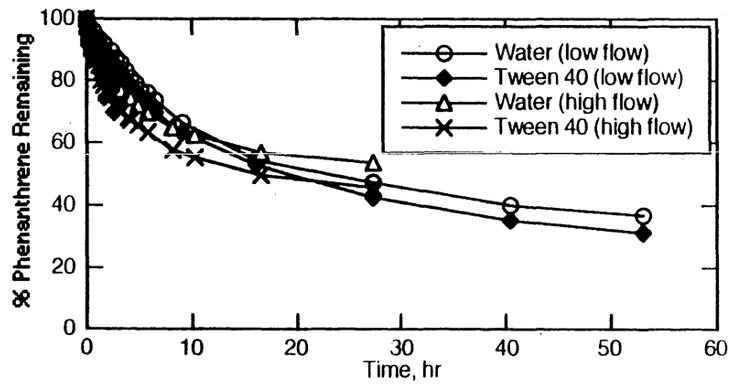



**Figure 8b**

Effluent concentrations of phenanthrene were significantly lower at the higher flow rate indicating that the residence time was too short for the effluent to reach saturation. This is indicated in Figures (9a) and (9b). The effluent concentrations are significantly lower than the saturation concentration of phenanthrene. Comparison of the effluent

concentrations at the higher and lower flow rates is shown in Figure (10). It is evident that the contact time at the lower flow rate was adequate for the wash solution to be saturated with phenanthrene. The plots further indicate that there is a slight enhancement in the solubility of phenanthrene in the presence of the surfactants both at the higher and lower flow rates.

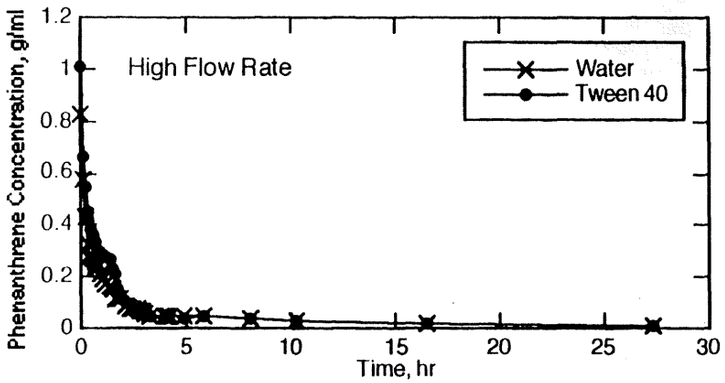



**Figure 9a**

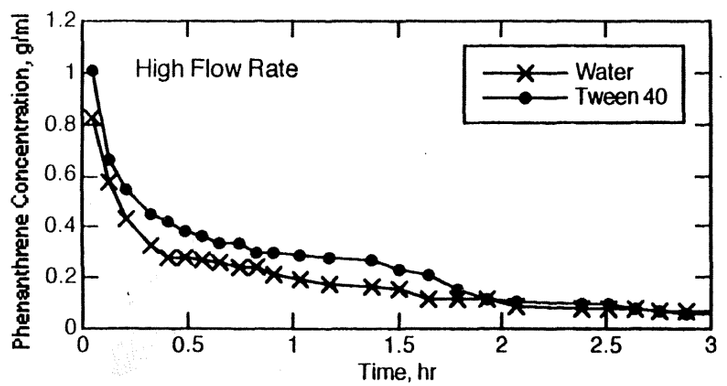



**Figure 9b**

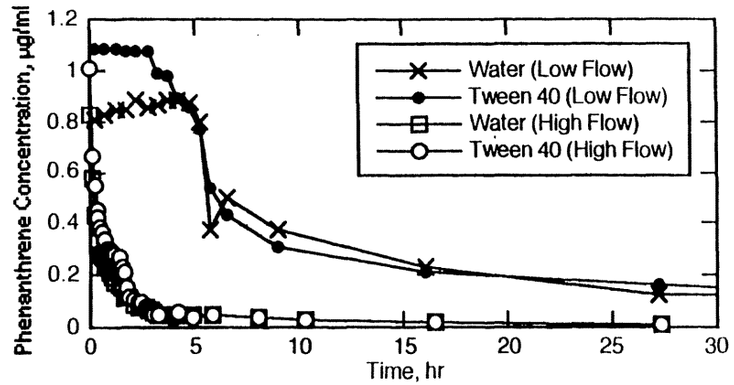



**Figure 10**

As shown in Figure (11), overall removal at low flowrates was significantly better than at high flow rates. The high flow columns retained more than 45-53% of the phenanthrene after 684 pore volume displacements whereas the low flow columns retained only 31-37% after 215 pore volume displacements. This effect of flow rate was not predicted by intuition. It had been anticipated that using higher flow rates would result in lower effluent concentrations due to shorter residence times but would nevertheless remove more phenanthrene because concentration gradients would be greater. However, the results at low flow rate are clearly better, leading to greater removal albeit over a longer period of time (53 versus 28 hours).

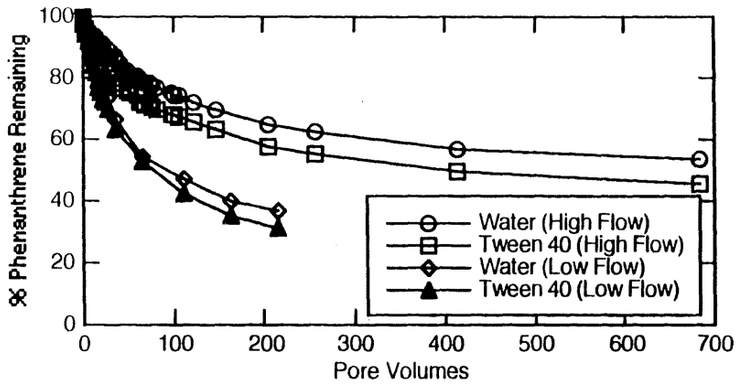



**Figure 11**

The distribution of phenanthrene remaining in the column at the end of the experiment at the higher flow rate follows the same general trend, with more phenanthrene remaining at the bottom third of the column.The distribution of phenanthrene remaining in the column is presented in Figure (12). Phenanthrene distribution in the upper two-thirds of the column at the high flow rate were greater than those found at the low flow rate.

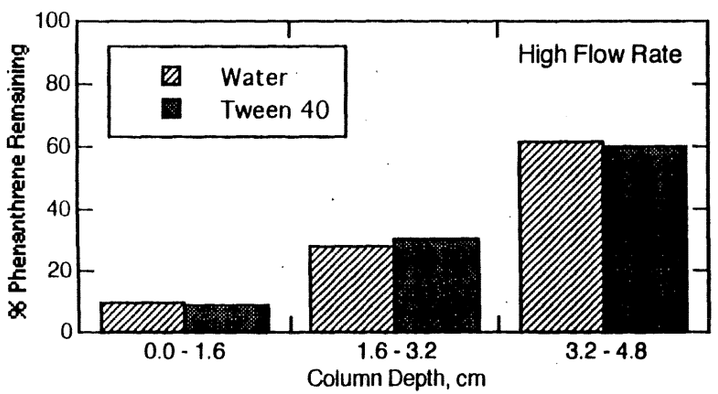



**Figure 12**

The mass balance on the phenanthrene lost in the effluent and retained on the sand are presented in Table ( ). Satisfactory recovery efficiencies ranging from 89.9% to 96.1%, with an average of 93.2 % were obtained. The distribution of phenanthrene inside the columns at the end of the experiments are also indicated in the same table.

The performance of the surfactants at enhancing the washing rates can be explained in terms of their nonpolar contents. It has been reported that the enhanced solvency of hydrocarbons could be correlated to the inner nonpolar core of the surfactants. Tween 40 has the highest nonpolar content among the selected surfactants. This could explain its better performance. The nonpolar content in increasing order for the selected surfactants is as follows: Tween 40> Triton X 114> Brij 35. Although Triton X114 has a close nonpolar content (35%) to the Tween 40, earlier sorption experiments indicated that it has a higher partition coefficient for the Jordan sand. The Triton X 114 was also seen to disperse soil colloids in earlier batch tests. This could lead to soil clogging and formation of dead end pores. Partition coefficients for the other three surfactants to Jordan sand were comparable. The sorption of phenanthrene in presence of these surfactants had also been determined. Partition coefficients determined in batch tests indicated that the Tween 40 had the lowest value followed by Triton X 114, Corexit 0600 and Brij 35.

Other investigators have reported that the dispersion of octadecane by a rhamnolipid biosurfactant was greatest at surfactant concentrations below the CMC where decrease in surface tension was minimum. From the surface tension data reported before, Corexiit 0600, Triton X 114 and the Tween 40 surfactants should have given comparable results. The Tween 40 gave the best results, an indication that more than one physical parameter is responsible for solubility enhancement. This was also concluded by Vigon and Rubin (1989) where they evaluated performance parameters that included surface tension minimization, CMC, HLB number and the partition coefficient. The ability of a surfactant to solubilize a PAH from soil is dependent on various factors of which interaction of the PAH with surfactant monomers and the sorption of the PAH and surfactant to the soil are the most important.

With regards to field applications, higher surfactant concentrations will induce greater losses to the subsurface material. Surfactant losses due to adsorption and its recovery may increase remediation costs. On the other hand lower concentrations will require larger number of washings. This increase in the number of washing could also increase the costs. The present study indicates that low concentrations of surfactants below their critical micelle concentration may enhance the washing of contaminated soil. This slight enhancement may lead to greater bioavailability thereby increasing mineralization rates.

## 6. CONCLUSIONS:

Continuous flow column tests show that flushing with water results in a short period of saturated effluent concentration followed by a period of rapidly decreasing effluent concentrations. Effluent concentrations at low flow conditions level out at 0.05 - 0.1 mg/L whereas high flow rate concentrations are significantly lower. Residual soil concentrations after some 55 hours of flushing show that 90% of the phenanthrene has been removed from the inlet but less than 40% removal in the bottom third of the column.

Increasing flow rate was effective for removing phenanthrene for the first few hours but did not change the distribution of residual phenanthrene. Furthermore, when compared on a pore volumne basis, the low flow rate flushing resulted in greater overall removal.

the observation that low flow rates are more effective indicates that dissolution is rate limiting. The initial flush (first few hours) is the only period when saturation of the water phase was observed. Most of the time, effluent concentrations are significantly below saturation. The addition of surfactants was only marginally helpful in removing phenanthrene. It is postulated that saturation concentrations are obtained as long as there is phenanthrene on the walls of the large pores. Transport of solubilized phenanthrene from the small pores or dead end spaces into the water flowing through the large pores is by molecular diffusion and therefore very slow.

The concentration versus time data are being analyzed using computerized solution of the classical transport models with a modified term to incorporate the effects of diffusion. This analysis is in progress but is expected to be completed by end of summer.

This study indicates that the addition of surfactants at a low concentration may be slightly beneficial in cleanup of contamianted soils. The presence of surfactants does enhance the dispersion of phenanthrene/surfactant complexes even at a surfactant concentration below the critical micelle concentration. However actual success in the field will depend on the site conditions, geology and material composition. In practical applications the surfactant adsorptive losses would represent a significant material cost. Significant organic carbon content of the soil will also increase the loss of surfactants due to sorption. Higher flushing rates maybe beneficial to some extent.

---

# Part 2:

## Column Studies Of Biodegradation Of Phenanthrene In Precontaminated Jordan Soil

## 1. ABSTRACT:

Biodegradation of phenanthrene coated on sand was studied in continuous flow columns dosed with nutrient water solution and in the presence of four commercial nonionic surfactants. Phenanthrene (carbon-14 labeled) coated sand was designed to simulate soil contaminated with excess phenanthrene which remained after evaporation of lighter hydrocarbon solvents. The coated sand was loaded in a all glass column and nutrient buffer with 25 mg/l of the selected surfactants was pumped by a peristaltic pump at a flow rate of 0.125 ml/min. A mixed enriched culture aoclimated to phenanthrene was used to inoculate the columns. Columns were operated in the downflow mode. Parameters monitored during the course of this study were: $^{14}CO_2$ production, total counts remaining in the sample after $CO_2$ trapping and counts remaining in the same sample after centrifugation. The difference between the latter two counts was the pellet count which represents $^{14}C$ label incorporated into cell mass. The count after centrifugation represents label concentration remaining in solution. It is

combination of unmetabolized phenanthrene plus byproducts of metabolism. Dissolved oxygen and pH were also monitored with time. Intermediate concentrations measured as resorcinol equivalents were monitored. At the end of the experiment the column was taken apart and analysed for residual protein and $^{14}C$ counts remaining along the depth of each column. All experiments were performed in duplicates.

Additional experiments involving cell transport in the columns were also carried out. The cells were radiolabeled and their transport in the columns was observed. Results indicated that 40% of the labeled cells were retained in the sand column. The duplicate test results show that approximately 80% of the retained cells are trapped in the top one-third of the column. The other 20% are essentially uniformly distributed. However after flushing with water containing nutrients (no phenanthrene) there was considerable redistribution of cells. Redistribution is the result of the combined effects of new cell growth as well as downstream transport by flushing. The final distribution shows 50-60% in the top third, 30+% in the mid third section, and 20-% in the bottom section.

Comparisons of phenanthrene washed out of the columns shows that the inoculated columns had relatively small effluent concentrations compared to the columns that had received no inoculum clearly indicating the potential for in situ biodegradation.

Biodegradation experiments indicated that the presence of the surfactants enhanced the biodegradation of phenanthrene significantly.

## 2. INTRODUCTION:

The introduction to this section is the same as presented above for the soil washing experiments except as noted below.

Many researchers have indicated that bacteria are capable of producing their own emulsifying or solubilizing agents to enhance degradation of insoluble hydrocarbons. Chemical dispersants and surfactants have also been used for this purpose. A stimulation of microbial degradation of lubricating oil in soil was observed in the presence of a dispersant, and other investigators have reported that low concentrations of surfactants may promote the mineralization of sorbed aromatic compounds. A



number of researchers have demonstrated that this increase in solubility leads to greater substrate availability thereby enhancing mineralization. On the other hand complete inhibition of phenanthrene mineralization by selected nonionic surfactants in soil-water systems has also been reported. Inhibition was noted at supra-cmc concentrations of the surfactants. However inhibition was reversible after diluting the surfactant to a concentration below CMC values. The diversity of results that have been presented in the literature on the effects of surfactants on biodegradation of slightly soluble hydrocarbons leads to the conclusion that there is a need for further research in this area.

This research first addresses the effect of inoculation with acclimated microorganisms and characterizes the ensuing in situ biodegradation. The second part addresses effects of nonionic surfactants at a low concentration on biodegradation of phenanthrene from a contaminated soil. Phenanthrene coated sand was prepared for testing, in order to simulate the dispersion of excess phase phenanthrene (above solubility) in contaminated soil. It is known that phenanthrene is commonly introduced into soils as a solute in a carrier solvent such as creosote. Examination of creosote-contaminated soils suggest that the lighter hydrocarbons evaporate leaving residual PAHs including phenanthrene as a quasi solid/high viscosity liquid phase matrix that covers the sand surfaces, fills pore spaces and acts as a binder for sand particles. Nonionic surfactants were selected as they are known to have greater hydrocarbon solubilizing power, less toxicity to microbial populations, no adsorption to charged surfaces and low foaming property. The model compound of choice is phenanthrene, which has a relatively low aqueous solubility and high sorptive properties. Its physical characteristics are representative of PAHs and it has been identified as a major chemical pollutant at numerous sites. This chapter describes column studies that were conducted to simulate process conditions analogous to in situ aquifer restoration. Low concentrations of surfactants were used to investigate whether their presence would promote mineralization of the hydrocarbon without inhibiting bacterial activity.

3. MATERIALS AND METHODS:

The materials and methods used in this phase of the study are the same as described in the section on "Column Studies of Enhanced Washing of Contaminated Jordan Sand" except as noted below.

Cell Transport Tests: Acclimated enrichment cultures capable of degrading phenanthrene were developed from contaminated soil. Samples of petroleum contaminated soil were collected from the Bemidji (Minnesota) Oil Spill Site. Enrichment cultures were developed in batch reactors incubated at room temperature (22±1°) with phenanthrene as the sole carbon source. Phenanthrene was added as particles. The culture was acclimated to phenanthrene through repeated transfers into fresh autoclaved nutrient-buffer media. Growth media and nutrient buffer were prepared in distilled water similar in composition to the Watanabe media. The media consisted of (per liter of distilled water) 0.5 g $NaNO_3$, 0.65 g $K_2HPO_4$, 0.17 $KH_2PO_4$, 0.1 g $MgSO_4.7H_2O$, 0.03g $CaCl_2$ and 0.00375 g $FeSO_4.7H_2O$. pH was maintained at 7.2.

A Roche OXI/FERM TUBE (Fisher Scientific, Pittsburg PA) was used for identification of the organisms which had been previously plated out on 2% Difco Agar. Six different organisms were isolated. Gram staining, motility studies along with identification using the OXY/FERM TUBE indicated that five of the isolated strains belonged to the Pseudomonas species while one belonged to the Flavobacterium species.

In order to investigate the transport of cells in the sand columns, the above culture was radiolabeled. Twenty mL of the acclimated enrichment culture was centrifuged at 10,000 rpm on a Eppendorf microfuge (Model 5415) and the resulting cell pellet was resuspended in 5 mL of nutrient buffer. This was inoculated into a flask containing 0.1g phenanthrene (labeled and nonlabeled) in 300 mL nutrient buffer and incubated to produce active cell mass. 0.1 mL of the medium was immediately counted for initial activity and 1mL of the sample was analyzed for initial protein concentration. Protein concentrations throughout the experiments were measured by the method of Lowry on a spectrophotometer (Bausch and Lomb, Spectronic 1001) . Bovine serum albumin (Sigma Chemicals, St. Louis, MO.) was used as the protein standard. Similar samples were analyzed for $^{14}C$ incorporation into the cell mass and the accumulation of protein at various times during the incubation. The tests were conducted in triplicate. These radiolabeled cells were used to inoculate four sand columns.

Two columns each were prepared with sand coated with phenanthrene while the other two received clean sand. One hundred mL of the labeled cell suspension was centrifuged and the pellet was washed with 50 mL of nutrient buffer. The final pellet was resuspended in 50 mL of nutrient buffer. Each column received this 50 ml labeled cell suspension. Cells were loaded into each column by pumping the cell suspensions through aTeflon tube at a flow rate of 0.233 mL/min. The columns had been presaturated with pH buffered nutrient medium. The cells were pumped for 3.58 hours followed by flushing with pH buffered nutrient medium. Column effluent was collected in glass scintillation vials as a function of time. Each sample was counted before and after centrifugation to measure total and cell free $^{14}C$ activity. At the end of the experiment the columns were taken apart and the sand was analyzed for residual activity and protein.

Mineralization Tests: Columns were operated in the downflow mode under saturated flow conditions. Phenanthrene was coated on the Jordan sand as described above. This coated sand was packed into the columns by pouring a continouos thin stream with gentle tapping to achieve a uniform packing. A peristaltic pump (Gilson Minipuls 2) at a flow rate of 0.125 mL/min was used to pump nutrient buffer with or without surfactants. The buffer feed bottle was continously sparged with air to obtain saturation dissolved oxygen concentrations approximately of 8.2 mg/L. Each column recieved a total of 997 $\mu$g of phenanthrene (labled and nonlabeled). The activity of $^{14}C$ phenanthrene in these tests were 0.5 $\mu$Ci. As indiated above duplicate mineralization experiments were carried out for each test condition. Average values are presented for test results.

Samples were collected in glass vials with crimp top caps having Teflon-lined septa. The septa were covered with aluminium foil to prevent losses to the hydrophobic septa. Sample bottles were acidified with 1 mL concentrated sulfuric acid to lower the pH to about 1.09. $^{14}CO_2$ released from the mineralization of phenanthrene was then trapped as decribed below.

Mineralization of $^{14}C$-labeled phenanthrene was detected by trapping and analyzing liberated $^{14}CO_2$. This type of system was devised by Marinucci and Bartha (1979) for monitoring the mineralization of volatile $^{14}C$-labeled compounds. The apparatus consists of three 10 mL glass scintillation vials. All vials contained 10 mL Permaflour (Packard Instrument Co., Downer Grove, IL) while the second and third vials also contained 1 mL of Carbo-Sorb E (Packard Instrument Co., Downer Grove, IL). The first vial was designed to trap any volatilized phenanthrene and the second and third vials were designed for $^{14}CO_2$ collection. Each vial has an inflow 22 gauge X 7.5 cm stainless steel needle and an outflow 22 gauge X 1.27 cm stainless steel needle. All vials were interconnected with 0.25 inch (o.d.) stainless steel tubing. In this apparatus air is sucked through the sequence of the three vials to purge $CO_2$. This type of trapping system eliminated the error of measuring volatile parent compound as $^{14}CO_2$. The trapping efficiency of the system was checked by evolving $^{14}CO_2$ from acidified radiolabeled $NaHCO_3$. An overall 99% recovery of $^{14}CO_2$ was achieved in eight minutes of flushing. The scintillation vials were stored overnight in the dark to minimize chemiluminescence. After the $^{14}CO_2$ had been purged from the serum bottles, 0.5 mL of each sample was added to 10 mL of scintillation cocktail and and counted on the liquid scintillation counter to measure total counts. One mL of the same sample was centrifuged at 10,000 rpm on a Eppendorf Centrifuge (Model 5415) for 8 minutes. 0. 5 mL supernatant of the centrifuged sample was counted to measure the soluble C-14. The difference in the total and supernatant counts was ascribed to cell mass associated C-14. The pellet was analyzed for protein concentrations.

The acclimated enrichment culture as described above was used to inoculate the columns. 50 ml of harvested cell suspension was pumped into each of the columns. The protein concentration of this cell suspension was also determined at the start of the experiment.

At the end of the experiments the sand was removed from the columns in three equal portions, representing the top, mid and bottom third of the column. Each portion was analyzed for residual protein and C-14 remaining. For the sand, 0.2 g sand portions in triplicates were heated with 0.5 mL NaOH at 90oC for 15 minutes in a water bath. 1.0 mL of water was added to the samples and centrifuged at 14,000 rpm in an Eppendorf Microcentrifuge (Model 5415) for 8 minutes. The supernatant was then anayzed for protein by the method of Lowry.

Duplicate 1.0 g air dried sand samples from the above mentioned three sand fractions were added to 30 mL of methylene chloride in a crimp top vial and put on a shaker for 24 hours. 0.5 mL of the supernatant from the vial was added to 10 mL scintillation cocktail for counting. The sand was subjected to sequential extractions with methylene chloride until the counts fell to background levels. Column parameters for the mineralization tests are presented in Table 1.

**Table 1**. Initial parameters of soil column experiments

Run #1: Flow rate 0.125 ml/min

| Column # | Phenanthrene (ug) | Mass of Sand (g) | Bulk Density (g/mL) | Pore Volume (mL) | Wash Solution |
|---|---|---|---|---|---|
| 1 | 997 | 18.5 | 1.76 | 3.59 | Buffer |
| 2 | 997 | 18.6 | 1.77 | 3.59 | Buffer(Cells) |
| 3 | 997 | 18.5 | 1.76 | 3.59 | Corexit 0600 |
| 4 | 997 | 18.6 | 1.77 | 3.59 | Tween 40 |
| 5 | 997 | 18.6 | 1.77 | 3.59 | Triton X114 |
| 6 | 997 | 18.5 | 1.76 | 3.59 | Brij 35 |

Run #2: Flow rate 0.125 ml/min

| Column # | Phenanthrene (ug) | Mass of Sand (g) | Bulk Density (g/mL) | Pore Volume (mL) | Wash Solution |
|---|---|---|---|---|---|
| 1 | 994 | 18.5 | 1.76 | 3.59 | Buffer |
| 2 | 994 | 18.6 | 1.76 | 3.59 | Buffer,Cells |
| 3 | 994 | 18.5 | 1.76 | 3.59 | Corexit 0600 |
| 4 | 994 | 18.6 | 1.76 | 3.59 | Tween 40 |
| 5 | 994 | 18.6 | 1.76 | 3.59 | Triton X114 |
| 6 | 994 | 18.5 | 1.76 | 3.59 | Brij 35 |

| | |
|---|---|
| Column Length: | 4.8 cm |
| Column Diameter: | 1.668 cm |
| Temperature: | 22±0.5°C |

4. RESULTS AND DISCUSSION:

a. Radiolabel Cell Transport in the Sand Columns:

Cell retention and transport in the saturated sand columns were monitored by measuring outflow concentration of radiolabeled cells. Columns with clean sand and sand coated with 997 $\mu$g phenanthrene were set up in duplicate. Each column recieved 50 mL of labeled cell inoculum. The cell suspension was pumped at a rate of 0.233 mL/min for 3.58 hours and followed by nutrient buffer for another 4.42 hours. Samples were collected every half an hour in glass vials and analyzed for total counts and counts remaining in the supernatant after centrifugation. The difference between the total and supernatant counts was ascribed to cell mass incorporated C-14. Results from the tests are reported as the C-14 incorporated with the cell pellet as a % of the total C-14 added to the column.

Figures 13a and 13b show the cumulative removal of the cells in the effluent of the columns with the uncoated and the coated sand. The duplicate column tests gave very similar results indicating that the experiments and test procedures are very reproducable including the packing of the columns. During the initial 3.58 hours when the cell suspension was pumped into the columns, an average of 35.48% of the added activity was removed in the effluent of the uncoated columns. An additional 3.75% was removed when the feed was switched to nutrient buffer. The same trend was observed for the column effluent in the columns loaded with coated sand; 36.965% was removed for the initial 3.58 hours and 4.47% removed while flushing the column with nutrient buffer. The incremental 14C associated with the cell pellet in the column effluent versus time is indicated in Figures 14a and 14b. These figures indicate that the major washout of cells (35.5%) from the columns occured during the initial 3.58 hours. This time corresponds to the time when the cell suspension was pumped into each column. An additional 4.11% was removed during flushing with cell free medium. Flushing with cell free medium was terminated when there was no significant change in $^{14}C$ measurements in the column effluents. Figures 15a and 15b shows the comparison of the effluent in the clean and contaminated soil columns. Figures 15a and 15b indicate that there was slightly more washout of cells from the precoated soil; however the differences are not considered to be significant in terms of practical implications for innoculating soils.

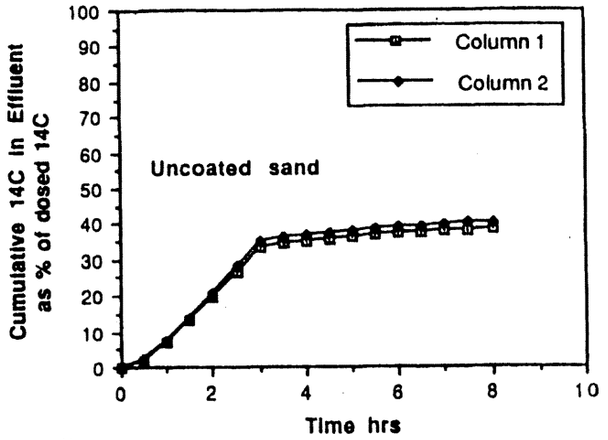



**Figure 13a**. Cumulative pellet associated %14C in effluent as % of dosed 14C

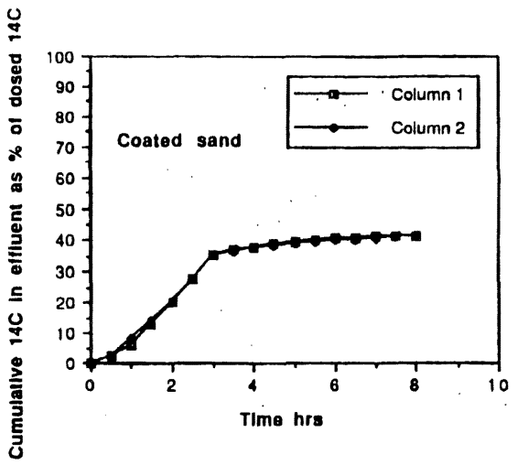



**Figure 13b**. Cumulative pellet associated %14C in effluent as % of dosed 14C

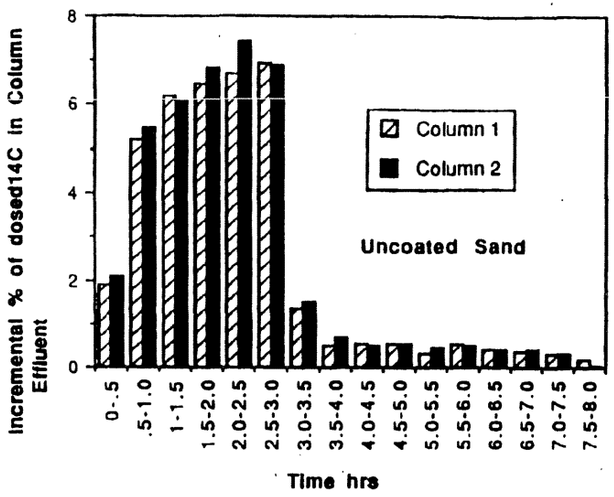



**Figure 14a**. Incremental % of dosed 14C in column effluent

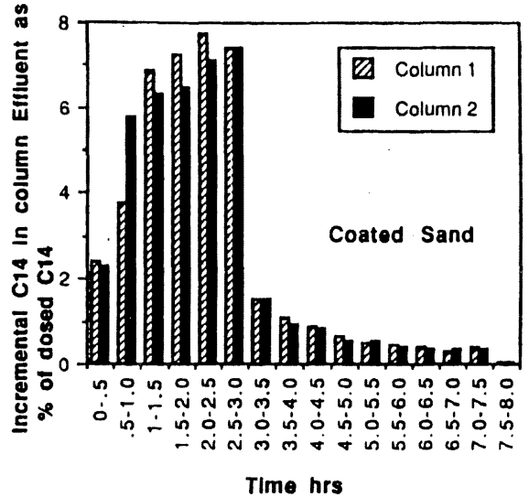



**Figure 14b**. Incremental % of dosed 14C in column effluent

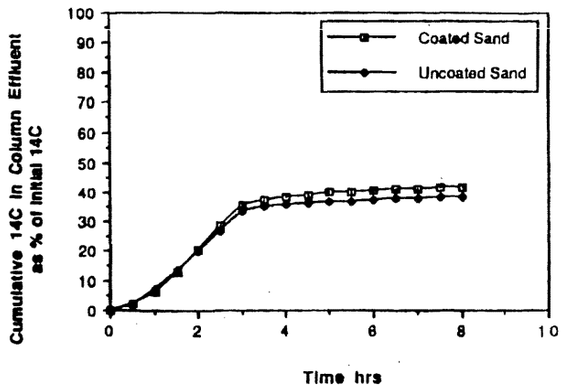



**Figure 15a**. Comparison of coated and uncoated column effluent

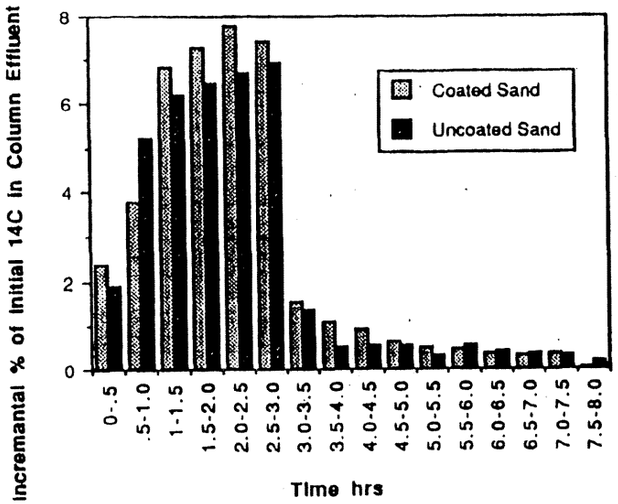



**Figure 15b**. Comparison of coated and uncoated data

The distribution of retained labeled cells along the column depth is shown in Figure 16a and 16b. The most significant feature is that higher concentrations of cells were retained in the upper 1.3 cm of the column while the other two sections had much lower but similar distributions. An average of 31.46% was retained in the upper third of the uncoated columns with 4.36% and 3 .64% retention in the middle and bottom thirds of the column. Similar distributions were observed for all four columns. Mass balance on the C-14 are presented in Table 2. Recoveries based on $^{14}C$ measurements range from 91.35 to 96.59% with an average recovery of 93.1% which is considered good. Recoveries of less than 100% are not unexpected because there may be some loss of C14 activity due to release of soluble materials from the pellets. Some variability in soil associated C14 activity is also to be expected due to extraction efficiencies. A third source of variation may be due to the difficulty of obtaining truly representative samples from each of the top, mid and bottom thirds of the column. Average recovery efficiency was 93.185%. Protein measurements are also reported in Table 2 as mg protein retained per gram of sand. The latter were used as estimates of the cell mass retained in the columns and represents the initial values of cell mass expected in subsequent biodegradation studies.

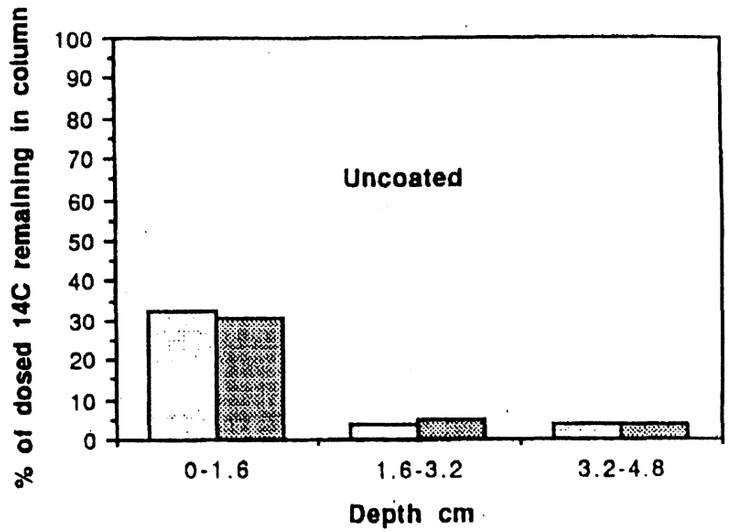



**Figure 16a**. Distribution of C14 inside column

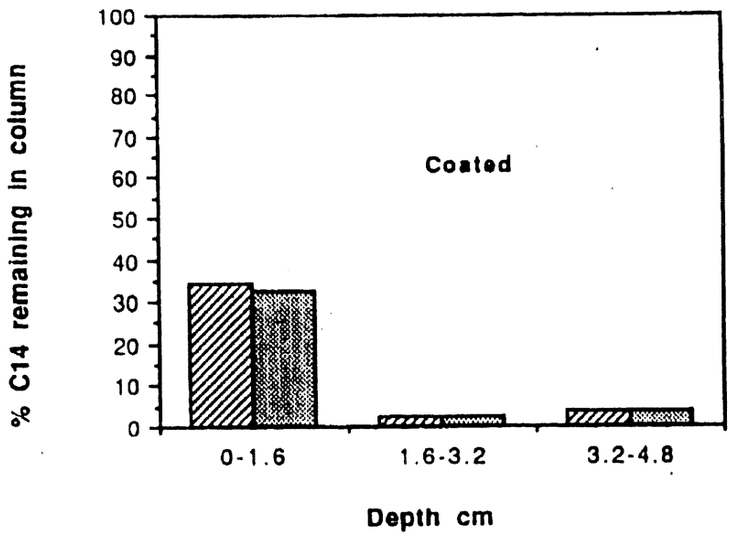



**Figure 16b**. Distribution of C14 remaining in column

**Table 2**. Mass balance on C14 counts for cell transport experiments.

| Column # | Column Type | % 14C Effluent | % 14C Sand | Protein mg | Protein mg/g sand | % 14C Recovered |
|---|---|---|---|---|---|---|
| 1 | Coated | 56.36 | 40.23 | 5.43 | 0.294 | 96.59 |
| 2 | Coated | 54.86 | 38.15 | 5.15 | 0.278 | 93.01 |
| 3 | Uncoated | 52.75 | 39.04 | 5.27 | 0.285 | 91.79 |
| 4 | Uncoated | 51.44 | 39.91 | 5.38 | 0.291 | 91.35 |

Column Length: 4.8 cm
Column Diameter: 1.668 cm
Temperature: 22±0.5°C

The results clearly show that the mixed culture was capable of attaching to the sand particles and thus would be suitable for use as innoculum in further mineralization studies in the sand columns. As mentioned earlier, the culture consisted of species belonging to Pseudomonads and Flavobacterium. Previous studies in our laboratory have reported that there was 10% retention of a pure Pseudomonas culture on the same sand used in this study in similar column experiments. The low retention of bacteria in these experiments was attributed to the high motility of the culture. On the other hand a 90% retention of cells belonging to a Flavobacterium sp. was found on the same sand in column experiments. This species is characterized by non-motile features. The findings in this study are therefore consistent with previous test results.

The literature on factors affecting adsorption include species type, soil type, surface charge, pH, and the time of contact, has been reviewed and has been presented in the University of Minnesota thesis of Kauser Jahan, Ph.D., 1993, and Le Thai, M.S., 1993. the references will not be presented in this report in order to limit the pages; only the salient features are summarized. Surface structure of microbial cells that could participate in the attachment process include cell walls, capsules and extracellular slime. The latter has physical characteristics such as high viscosity and gelling properties. Adsorption of bacteria can also be enhanced by the presence of flagella. Most bacteria as well as most solid surfaces carry a net negative surface charge so that electrostatic repulsion is to be expected to minimize bacterial attachment. However, when a charged surface is immersed in an aqueous solution, counter-ions are concentrated in the diffuse electrical double layer. The thickness of this double layer is decreased through compression at high electrolyte concentrations or if the valency of ions is increased thereby reducing repulsion energies. The relative importance of each of these effects on the retention of a particular culture are not known. For this reason, it was deemed essential to measure the cell mass retention as described above in order to define the initial cell mass concentrations to be expected in the following biodegradation tests.

b. Washing of Phenanthrene Coated Sand:

Biological mineralization of phenanthrene was monitored by measuring $^{14}CO_2$ concentrations in column effluent samples as a function of time.

Six columns with sand coated with a mixture of labeled and nonlabeled phenanthrene (995 $\mu$g) were tested. The operating conditions are listed in Table 1. The first column was dosed with nutrient buffer without any cells and was designed to measure how much and how fast phenanthrene was washed from the sand by dissolution and flushing. The other columns were pretreated with 50 mL of acclimated inoculum as described above. Initial protein concentrations of the inoculum were measured and found to be 275 mg/L (13.75 mg in 50mL).

Figure 17(a,b) and 18(a,b) summarize the data on phenanthrene removal from the column which received no cells. Effluent phenanthrene concentrations were measured by collecting samples every 10 hours and measuring C14 concentrations. Sodium azide was added to the samples to prevent any microbial activity. The data are presented in four different ways, namely % cumulative phenanthrene and as concentration in mg/L washed out of the column versus time and versus number of pore volume displacements repectively. Figure 17a and 17b shows the cumulative carbon 14 in the effluent versus time and number ofpore volume displacements expressed as % of initial dpm. 59% of the total counts were recovered from the column effluent in 220 hours which corresponds to 460 pore volumes. The cumulative % mass curve indicates that there is a fairly rapid removal of phenanthrene (50%) in first 130 hours. After 200 hours the rate of removal is less than 0.5% per ten hours whereas the initial rates were approximately 6% per ten hours. There is only an additional 9% removal in the following 90 hours. This slower removal towards the later stage indicates that the residual phenanthrene is not as available for dissolution from the soil. This maybe due to the presence of pore spaces in which there is restricted contact with the flowing water. Another possible explanation is that phenanthrene is initially present as a separate phase on the sand as a coating and is thus readily available for dissolution. During the later stages, phenanthrene may become sorbed to the sand and thus not as readily available for dissolution. As the coated phenanthrene is washed off, the sorption sites may become available.

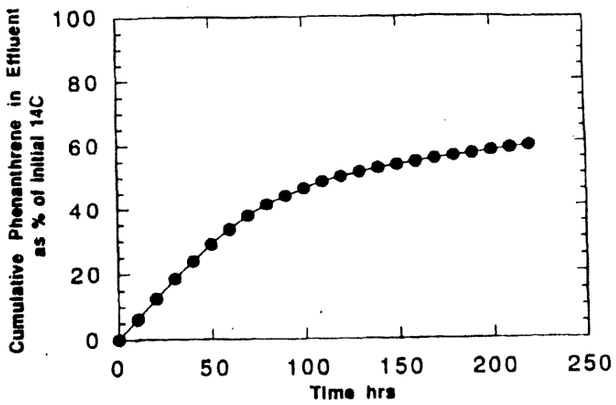



**Figure 17a**. %14C in column effluent

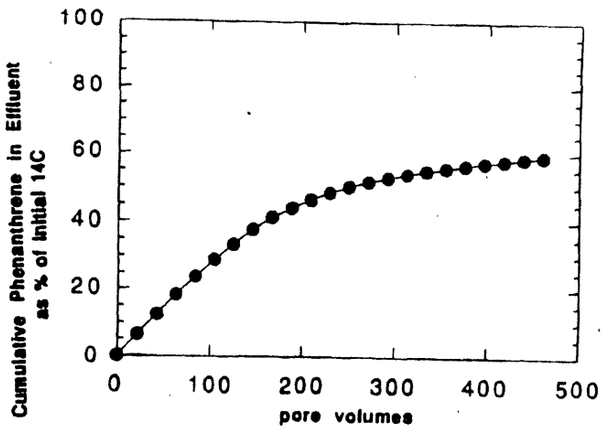



**Figure 17b**. %14C in column effluent

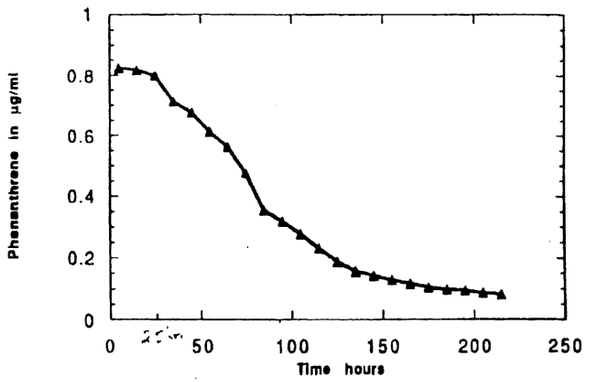



**Figure 18a**. Phenanthrene concentration in column effluent

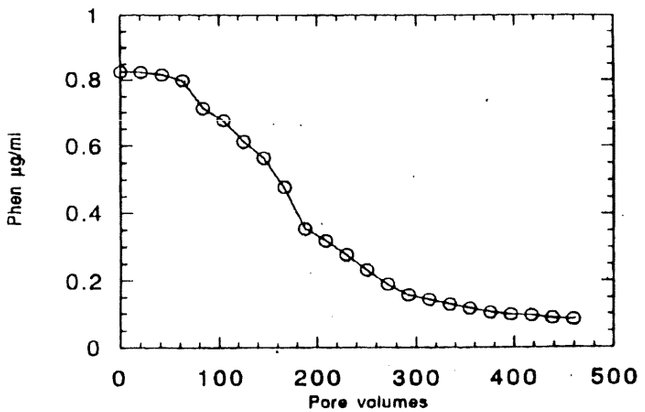



**Figure 18b**. Phenanthrene concentration in column effluent

Calculated phenanthrene concentrations in the effluent versus time and number of washings are shown in Figure 18a and 18b. These figures indicate that for the first initial 40 hours the column effluent is saturated with phenanthrene. The effluent concentration then decreases almost linearly with time in the following 70-80 hours. After 130 hours effluent concentration decreases more slowly. These figures also indicate that initially there is enough contact and adequate contact time with the flowing water to reach saturation. As the washing progresses, the rate of dissolution is decreased because readily available phenanthrene coated surfaces are depleted. Mass transport from smaller diameter pores will also occur at a much slower rate. The presence of dead end pores will finally result in very low rates of washing. Approximately 50% of the coated phenanthrene is removed after 270 pore volume displacements and an additional 9% is removed in another 188 pore volume displacements.

The finding that washing to remove slightly soluble organic chemicals from soils is a very slow process is consistent with laboratory and field studies. It is recognized that desorption maybe a much more slower process than adsorption thus contributing to the slow rate of removal. In this work, it can be seen that the rate of dissolution of phenanthrene that was originally placed as a separate phase (coating) is also very slow as washing proceeds.

Additional analysis of these data is in progress. Conventional and modified transport models are being used to fit the time-space distribution of phenanthrene in the effluent and remaining in the column at the end of the experiment. the results of the modeling studies will be published within the coming year.

c. Phenanthrene Mineralization Tests:

Effluent from the columns were collected every ten hours and analyzed for C14 distribution. Each sample was purged for trapping 14CO2 and then measured for total counts and counts remaining after centrifugation. CO2 production is a direct indicator of the extent of mineralization and hence a quantitative descriptor of the rate of biodegradation. The total counts in the column effluents comprised of undegraded soluble phenanthrene, soluble by products and cell mass. The effluent from the

column was analyzed to measure soluble phenanthrene and by products and cell mass by measuring the C14 counts before (Total) and after centrifugation. The difference between the total and the supernatant counts is the C14 associated with cell mass (termed as pellet counts). The total counts in the supernatant are a measure of soluble phenanthrene and by products.

Data on $14CO_2$ production in columns 2 to 6 after inoculation are shown in Figure 19a and 19b as the cumulative mass of C14 leaving the column versus time. The control column (#2) received nutrient buffer only whereas columns 3 to 6 were dosed with nutrient buffer media containing 25 mg/L of the surfactant indicated. Biodegradation is enhanced significantly in the presence of the Tween 40 and Corexit 0600 surfactants. The enhancement in the presence of the other two surfactants is not significantly different from the control column. The rapid initial release of CO2 indicates that the culture was well acclimated to phenanthrene. The rate decreases after the first hundred hours. Approximately 55% of the initial 14C is converted to 14CO2 for the columns which recieved the Tween 40 surfactant in the first 130 hours. This corresponds to 272 pore volume displacements. Cumulative 14CO2 production for Corexit 0600, Triton X114 and Brij 35 for the same time interval are respectively 50.5%, 41.7% and 41.6% compared with a 41.3% conversion in the control column. During the period beyond the 130 hours an additional 2 to5% of 14C has been converted to 14CO2 . This decrease in rate of mineralization is attributed to a decrease in soluble substrate availability as described in the washing experiment.

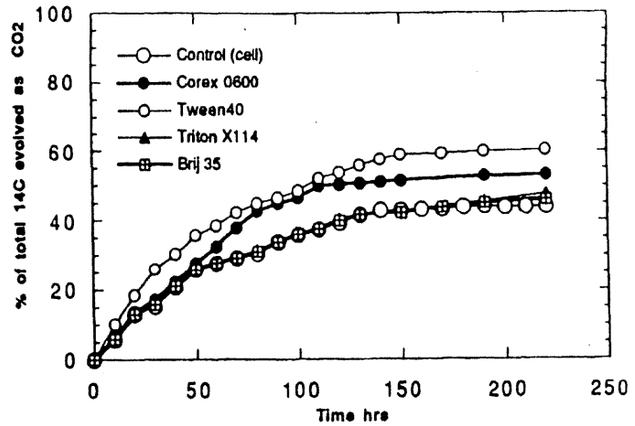



**Figure 19a**. %14C evolved as $CO_2$ versus time

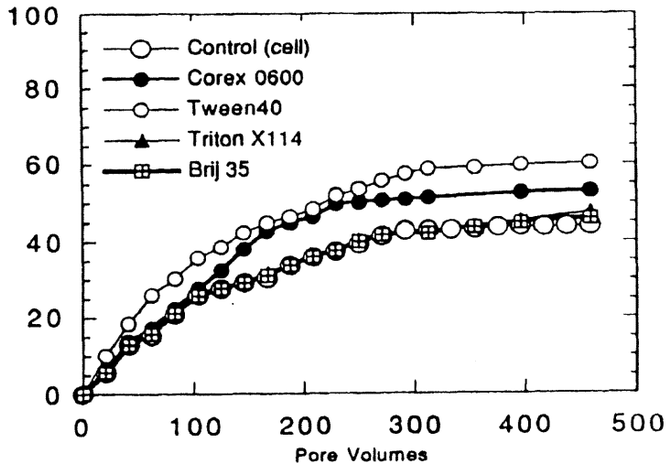



**Figure 19b**. %14C evolved as $CO_2$ versus time

The comparison of the effluent supernatant in the biodegradation columns is compared to the effluent of the uninoculated column in Figure 20a. The curve indicates that elution of phenanthrene and soluble intemediates is significantly lower than the uninoculated column. The supernatant counts in the biodegradation columns were further converted to equivalent phenanthrene concentrations and compared to the effluent concentration in the uninoculated column. This is shown in Figure 20b. It can be clearly seen that the concentration in the effluent for the biodegradation columns is significantly below saturation whereas the uninoculated effluent was saturated for the first few hours. The effluent concentration in presence of the surfactants is slightly higher for Tween 40 and Corexit 0600 as expected to the control. This indicates that more phenanthrene was flushed from the columns by these surfactants.

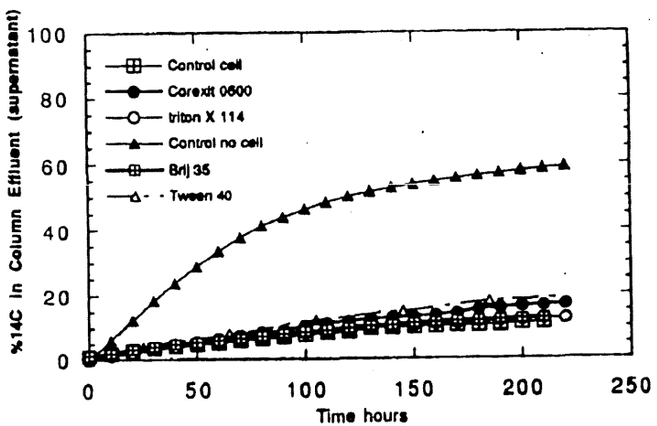



**Figure 20a**. Comparison of effluent supernatant in inoculated and uninoculated column

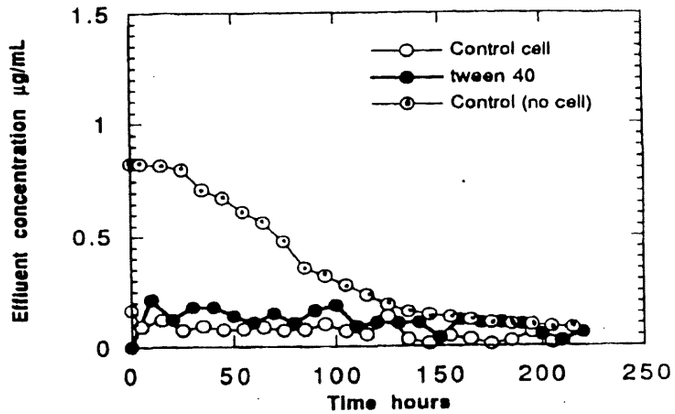



**Figure 20b**. Comparison of effluent concentration in inoculated and uninoculated column

The total counts in the supernatant and in the cell pellet recovered in the column effluent are presented in Figures 21a through 21e. Higher counts were eluted in the presence of the Tween 40 and Corexit 0600 surfactants as compared to the control and the other two surfactants. An average of 22% of the total counts were recovered as soluble dpm in the column effluent, indicating that removal by biodegradation is significantly higher than loss of solubilized phenanthrene and cell mass. In all the five columns, pellet associated counts were significant during the first 20 hours. After 20 hours, pellet associated dpm were relatively small for a total of 7-9% at the end of 230 hours. The pellets obtained after centrifugation were also analyzed for protein concentrations.

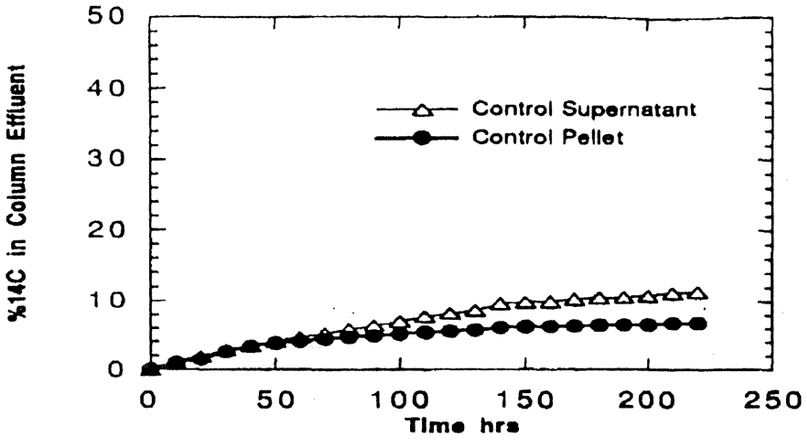



Figure 21a

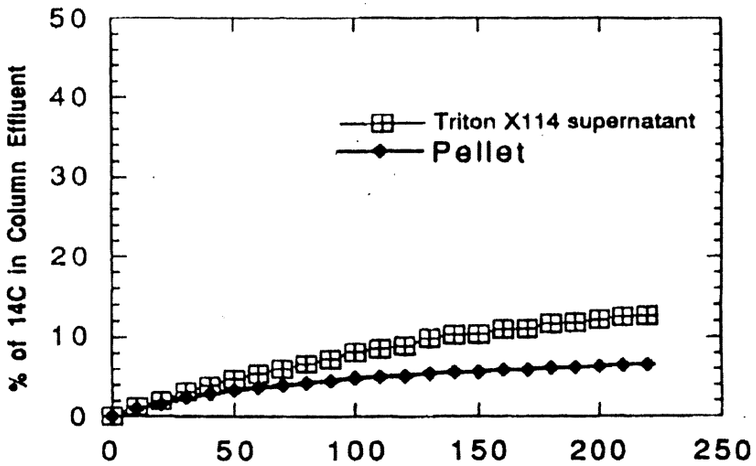



Figure 21b

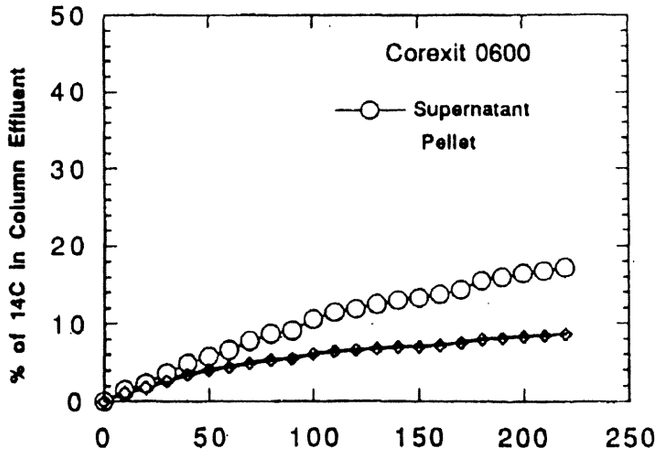



Figure 21c

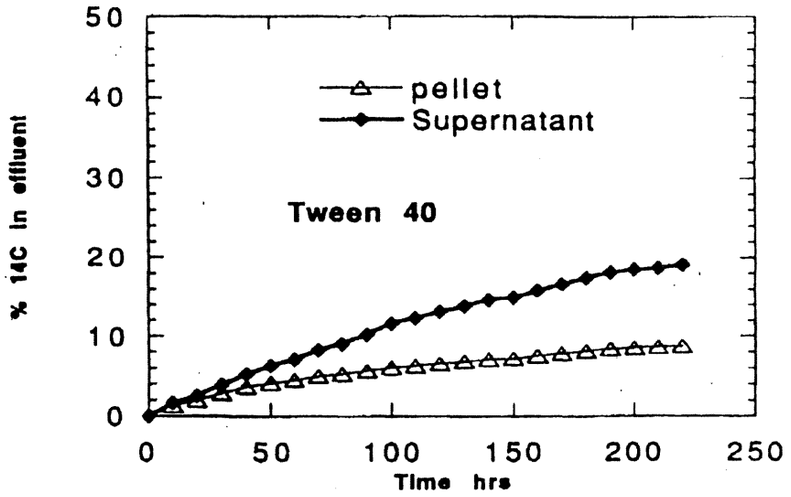
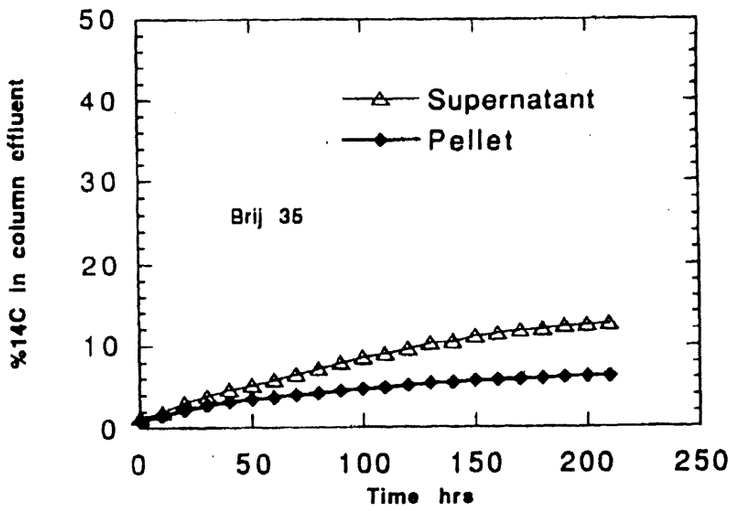



Figure 21d




**Figures 21e**. %14C in column effluent as supernatant and pellet

d. Analysis of Protein:

Accumulated cell mass concentrations on the soils at the end of the column tests were measured as residual protein. Protein measurements give a measure of total cell mass which includes that retained in the column from the initial inoculum (not C14 labeled) as well as new labeled cell mass produced by biodegradation of coated labeled phenanthrene. Total protein distributions in the top, mid and bottom third of the columns is shown in Table 3.

**Table 3**. Protein distribution inside columns at end of experiment

| | Total Recovered, mg | Column % 0.0 - 1.6 cm | Depth % 1.6 - 3.2 cm | % 3.2 - 4.8 cm |
|---|---|---|---|---|
| Buffer ( Cells ) | 4.4 | 54.2 | 30.6 | 15.2 |
| Triton X 114 | 4.5 | 56.1 | 31.8 | 12.1 |
| Corexit 0600 | 5.2 | 60.2 | 24.3 | 15.5 |
| Brij 35 | 4.6 | 55.9 | 31.5 | 12.6 |
| Tween 40 | 5.8 | 65.3 | 26.2 | 8.5 |

The table indicates that an average of 58% of the residual protein in the columns was present in the top 1.6 cm; 29% in the middle and 12.78% in the lower third portions. Although the data is the average of three samples analysed for protein at each depth, the results may not indicate the true protein value if the cells were not uniformly distributed on the sand. Some error could be introduced due to sampling of each section of the column. The protein distribution along the column depth at the end of the experiment is shown in Figures 22a and 22b. All columns showed similar trends in cell retention, with more cells being retained in the upper 1.6 cm. Protein concentrations were significantly higher in the presence of the Tween 40 and Corexit 0600 surfactants. These surfactants also had the highest mineralization rates. This table indicates that 67% of the inoculated cells were lost in the column effluent. This includes cell loss in effluent during inoculation and loss during subsequent flushing with the wash solutions.

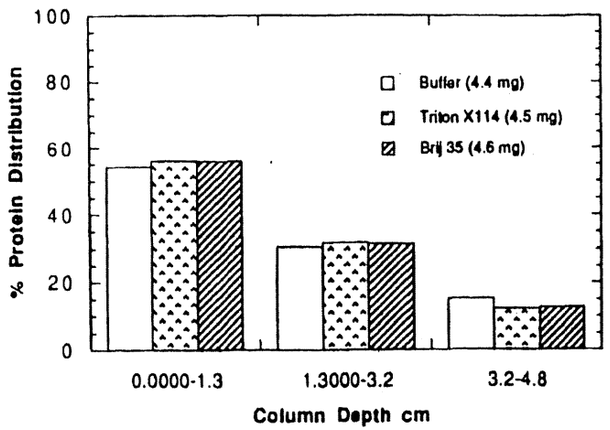



**Figure 22a**

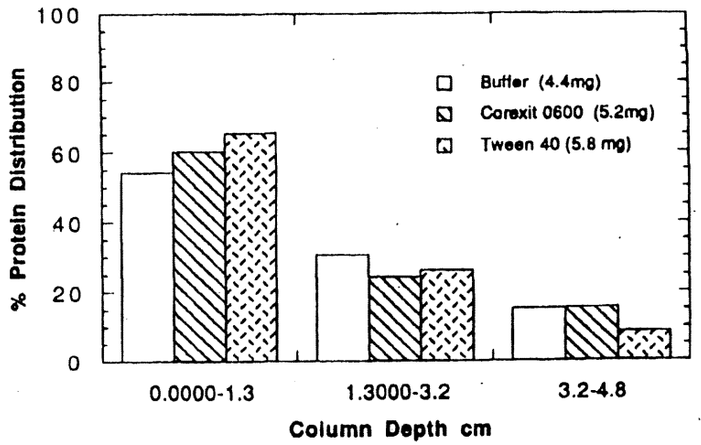



**Figure 22 b**. % Protein distribution along column depth

On an average 51% of the total counts was converted to 14CO2. This indicates that 508 $\mu$g of phenanthrene has been degraded. Earlier experiments indicated a yield value of 0.58 g/g. This would give a dry cell mass of 295 $\mu$g. Protein concentrations have been reported to be 52% of the dry cell weight. This indicates 153 $\mu$g of protein or 0.153 mg. Thus microbial growth appears to be negligible compared to initial inoculum. Thus results on cell retention with coated sand with and without biodegradation should be similar. Comparison of Figure 16b and Figures 22(a,b) show that there was redistribution of cells in the mineralization experiments. This is attributed to the longer periods of test runs 220 hours as opposed to 8 hours in the cell transport experiments. The flowrate in these tests were 53% lower than the flowrate used in the cell transpor tests. Longer residence times would provide more contact time for the cells to adsorb to the sand. Redistribution of the cells is also evidenced as there is a significant increase in cell retention in the middle and outlet portions in the mineralization experiments. The cell distribution in the columns in the mineralization experiments are also more uniformly distributed.

e. C14 Mass Balance in Column Studies:

Table 4 indicates the mass balance on the total counts in each column. The total counts in each column can be divided as follows:

14C (Total) = 14CO2 + 14C (Effluent) + 14C (sand)

14C material balances indicated recovery efficiencies ranging from 91 to 95% with an average of approximately 93%. As indicated previously, the measurements of C14 in soil phase is subject to sampling errors; small losses may also occur due to volitilization. Comparison of these recoveries as 14CO2, elution and residual on sand indicate that mineralization rates were significant in all columns as compared to the column which did not receive any inoculum. Elution of phenanthrene in the water phase accounted for 59% in the control compared to 18-27% in the inoculated columns.

**Table 4**. Mass balance on total C14 counts

| | % 14C as $CO_2$ | % 14C Eluted in Water | % 14C Recovered from Sand | Total % Recovered |
|---|---|---|---|---|
| Buffer Only | -------- | 59.134 | 36.166 | 95.3 |
| Buffer (Cells) | 43.896 | 18.030 | 33.074 | 95.0 |
| Triton X 114 | 47.455 | 19.150 | 25.095 | 91.7 |
| Corexit 0600 | 52.987 | 24.790 | 14.023 | 91.8 |
| Brij 35 | 45.899 | 18.980 | 27.421 | 92.3 |
| Tween 40 | 60.213 | 27.860 | 5.727 | 93.8 |

f. Overall Discussion of Column Tests:

Comparisons of the inoculated columns with and without surfactants show:

(a) more mineralization was obtained with surfactants with the performance in the order of Tween 40>Corexit 0600>Triton X 114> Brij 35>Control

(b) Although the % C14 eluted with the water phase represents the sum of the concentration of phenanthrene, intermediates and cell mass being flushed out, it will be shown in subsequent discussions that the cell mass contribution is very small. In any case, it is seen that inoculation reduced this category significantly; 59% in uninoculated column versus 18-27% in inoculated columns. However it is interesting to note that there are significant differences between surfactants. Tween 40 and the Corexit 0600 showed higher mineralization and elution losses as compared to the other surfactants and control. It is clear that higher mineralization goes hand in hand with higher elution losses of phenanthrene.

(c) Residual C14 on the sand at the end of the experiments were significantly different, varying from 5.7 to 36.2 %. Carbon 14 residuals on the sand were measured by extraction of the soil and therefore include residual phenanthrene and cell mass. It can be seen that the Tween is more effective followed by Corexit 0600, Triton X 114 and Brij 35. The absence of surfactant resulted in much higher residual C14 (33%) which is marginally better than flushing without biodegradation (36%). Assessments of the possible sources of lower mass balances indicates that the soil measurement are the least reliable primarily due to sampling problems. If the deviations from 100% recovery are assigned to %14C recovered from sand, the relative efficiencies of removal remain unchanged.

During the time course of these experiments there was no dissolved oxygen limitations and the system was well buffered. The average pH value was at approximately 7.1. The acclimated culture used in these experiments had been earlier used in batch biodegradation tests. In these tests none of the selected surfactants were biodegraded during the time course (10 days) of the batch experiments. The surfactants selected in this study are not readily biodegradable although there is is literature available on such surfactant biodegradation at much lower concentrations than that employed in this study. The fact that the mixed culture was preacclimated to phenanthrene and earlier tests havenot demonstrated the biodegradation of the surfactants in preference to phenanthrene, support that the surfactant s were not biodegraded in these experiments. The effluent samples were analyzed for intermediate concentrations expressed as resorcinol equivalents. The production of intermediates is significant when the initial phenanthrene concentraion is high. Intermediate concentrations as resorcinol equivalents could not be detected in the column effluent samples. The surfactants never exhibited inhibitory effects on mineralization or toxic effects on the inoculum during earlier batch tests and the current experiments described in this paper.

Earlier batch studies on biodegradation of phenanthrene indicated that the Tween 40 and Corexit 0600 surfactants enhanced the biodegradation rates significantly. This was also observed in these column tests. The better performance of these two surfactants in comparison to the other two can be explained by looking at some important physical parameters. Enhanced mineralization is attributed to the enhanced solubilization of phenanthrene in presence of the surfactants. The performance of the surfactants at enhancing the solubilization rates can be explained in terms of their nonpolar contents. Tween 40 has the highest nonpolar content among the selected surfactants. This could explain its better performance. The nonpolar content in increasing order for the selected surfactants is as follows:

Tween 40> Triton X 114> Brij 35. Although Triton X114 has a close nonpolar content (35%) to the Tween 40, earlier sorption experiments indicated that it has a higher partition coefficient for the Jordan sand. The Triton X 114 was also seen to disperse soil colloids in earlier batch tests. This could lead to soil clogging and formation of dead end pores. Partition coefficients for the other three surfactants to Jordan sand were comparable. The sorption of phenanthrene in presence of these surfactants had also been determined. Partition coefficients determined in Chapter 6 indicated that the Tween 40 had the lowest value followed by Triton X 114, Corexit 0600 and Brij 35.

Other investigators have reported that the dispersion of octadecane by a rhamnolipid biosurfactant was greatest at surfactant concentrations below the CMC where decrease in surface tension was minimum. From the surface tension data reported before, Corexiit 0600, Triton X 114 and the Tween 40 surfactants should have given comparable results. The Tween 40 gave the best results, an indication that more than one physical parameter is responsible for solubility enhancement. Other parameters that included surface tension minimization, CMC, HLB number and the partition coefficient have been reported to affect solubilization. However, it appears that the interaction of the PAH with surfactant monomers and the sorption of the PAH and surfactant to the soil are the most important factors governing decontamination and bioavailability of this slightly soluble hydrocarbon in soils.

## 5. SUMMARY OF PART 1 AND PART 2

A series of column studies using phenanthrene precontaminated Jordan auqifer sand were carried out to measure rates of flushing under abiotic conditions and under biotic conditions that approximate in situ biodegradation

A large body of data describing time and spatial distribution of phenanthrene and cell mass concentrations in columns as well as the time variable effluent concentrations of phenanthrene has been generated.



Phenanthrene was used as a model compound because it has low aqueous solubility and high sorptive properties. Phenanthrene has been identified as a major chemical pollutant at numerous sites where wood treating operations using creosote have been carried out in the past. As such it is a model compound for a broad spectrum of organic pollutants. Prior to these column studies, biodegradation studies were also carried out with phenanthrene available in the particulate form in batch reactors (data reported elsewhere.) Phenanthrene coated sand was designed to simulate soil contaminated with excess phenanthrene which remained after evaporation of lighter hydrocarbon solvents. The sand was characterized by a low fractional organic content (0.0001).

Because the previous batch reactor tests and review of the literature indicated that the use of surfactants can enhance biodegradation of slightly soluble organic compounds by increasing solubilization, a series of column tests were carried out. Nonionic surfactants were selected as they are known to have greater hydrocarbon solubilizing power, less toxicity to microbial populations, and minimal adsorption to charged surfaces. Surfactants were used at a concentration of 25 mg/L in all experiments as this was below their critical micelle concentration. The study focused on low surfactant concentrations because it seems unlikely that high concentrations of surfactants would be practical for in situ decontamination of soils by biodegradation both from the standpoint of toxicity to bacteria and potential oxygen demand created by ultimate biodegradation of the surfactant.

Batch reactors solubilization studies of phenanthrene by water alone and with nonionic surfactants indicated that the process was mainly a micellar phenomenon. Batch biodegradation studies showed that the rates of biodegradation were enhanced by the presence of surfactants.

The continuous flow column described in this report were conducted to assess the solubilization, flushing, and in situ biodegradation of phenanthrene under conditions that approximate contacting of soil and groundwater flow in subsurface environments. Suitability of the surfactants for washing phenanthrene coated soil were also evaluated. Column studies were conducted to simulate soil water contacting conditions analogous to soil/groundwater situations that would be encountered in in situ aquifer restoration. The abiotic column flushing studies indicated that the effect of surfactants at low concentration was

marginally beneficially. However, under biotic conditions the presence of surfactants enhanced the biodegradation of phenanthrene significantly.

Cell transport tests indicated that the acclimated culture could associate with sand. This may have favorable implications for enhancing in situ biodegradation of soils contaminated by excess phase hydrocarbons. Sorption of phenanthrene on the sand could be represented by the linear isotherm model. The sorption of phenanthrene was significantly enhanced in the presence of the surfactants indicating that surfactants can sorb and increase the sorptive properties of soil, thereby minimizing downstream transport and allowing greater opportunity for in situ biodegradation. Surfactant sorption could also be represented by the linear model. The sorption studies imply that surfactants, when used on low-carbon aquifer materials with fractional organic carbon contents less than 0.001, can dominate the partitioning of phenanthrene and impact its fate and transport. Selection of surfactants for bioremediation of contaminated coils depends on a large number of factors, and site specific studies may be desirable to ensure maximum benefits.

The performance of the Tween 40 surfactant was superior as compared to the other surfactants. It enhanced mineralization rates of phenanthrene and exhibited low sorptive properties. Solubilization was also enhanced by this surfactant. Surfactant characterized by greater hydrocarbon solubilizing power, low toxicity to bacteria, and poor sorptive properties would be the best choice. Ultimate biodegradation of the surfactants would also be desirable for in situ bioremediation scenarios.

6. ONGOING AND FUTURE WORK:

Analysis and modeling of the data are continuing. The objectives are:

a. To describe the rates of solubilization and transport of phenantrene using conventional and modified transport equations. Initial results using conventional models have shown that rates of dissolution are not properly described. A model using a time variable mass transfer coefficient to describe the slow rates of flushing that have been observed shows promise. The time variable mass transfer coefficient is a surrogate correlation tool for incorperating the slow rates of flushing that occur from pore spaces that are not subjected to advective flow and hence mass transport is by diffusional processes. This work is being prepared for publication.

b. A related objective is to model the time space distribution of cell mass as defined by cell mass accumulation at the end of the experiments as well as by the effluent concentrations of cells that were measured during the column studies. A first order removal (capture of cell mass) rate has been tested and shows promise for characterizing the distribution of cells over short periods of time (hours) as for example during dosing and in the absence of significant frowth metabolism. However, the fate of cells under growth conditions is clearly different. There is much less mobility and an appropriate modification of the model is needed to incorporate the effects of permanent attachments of cells to surfaces which occurs when there is metabolism and holdfasts are generated.

c. The most important objective of the ongoing modeling studies is to calibrate a comprehensive model of the concurrent effects of chemical transport, cell mass accumulation, availability of oxygen, and rate of solubilization of excess phase chemical. A first cut model (TPMBX2) is available and being used to examine column test results.

---

## Part 3:

### Test Program on Contaminated Soils from Superfund Site

A series of column tests using minimally disturbed soil cores from a creosote contaminated superfund site have been carried out. the Reilly site was chosen because it is representative of a large number of sites that have been contaminated as a result of wood treating operations in which creosote and related solvents were accidentally discharged to soils and groundwaters. Creosote is a petroleum distillate that contains large concentrations of Polynuclear Aromatic Hydrocarbons (PAH's), heterocyclics and Phenolics. The PAH's are of particular concern because of their toxicity.

The Reilly site, St. Louis Park, MN, is of particular concern because it has impacted groundwaters in the Metropolitan area leading to potential public health concerns. Because the work is site specific, supplemental funding covering some of the laboratory studies and the site sampling were obtained from MPCA.

## 1. OBJECTIVES

The purpose of this study of the Reilly Superfund site is to investigate insitu remediation strategies for removing polynuclear aromatic hydrocarbons (PAH) which are major constituents of creosote impacted subsurface environments. Because this is a very common problem, the study has stressed development of inovative protocols that may have applications at other sites.

The rationale of the program reflects the findings that creosote associated organic chemicals are ultimately biodegradable, although rates of solubilization and biodegradation are known to depend on molecular weight-structure of the individual compounds. The program was designed to answer questions regarding:

- spatial distribution and composition of organic chemicals in the subsurface.
- water mobility of the major chemicals
- limitations on insitu biodegradationas due to oxygen and nutrients
- permeability of the soils and its relation to insitu biodegradation
- development of mathematical models for design of bioremediation strategies.

This status report describes some of the new protocols that have been developed to obtain data to answer these questions. It also presents illustrative test data that have been obtained on soils and column tests. The first section describes procedures for sampling and analysis of subsurface soils. Procurement and setting up of minimally disturbed vertical profile soil cores for continuous flow testing are described in some detail because this protocol is capable of measuring fates of organics under conditions that approximate the field site. The third section presents data on rates of elution of PAH'S and related organics under no-growth conditions and with concurrent insitu biodegradation.



Discussions of the mathematical modeling effort will be published separately because it is still in progress.

## 2. SAMPLING

The study has focused on the area referred to as Mount Reilly which was created as a temporary storage site for contaminated soils and was covered with top soil pending decisions regarding ultimate disposal. Because of the nonhomogenous nature of the site a series of borings were carried out to obtain soil samples for testing.

Figure 23 illustrates the sample gathering protocol for Drilling Site #1 which is one of three borings carried out December 11, 1991. A seven inch flight augur was used to initiate drilling. Minimally disturbed soil cores were taken in the form of two feet long, 2.5 inch diameter, stainless steel, split spoon inserts at the depths indicated. In addition, grab samples of soil were taken at the locations indicated when the bore hole was widened between split spoon sampling takes.

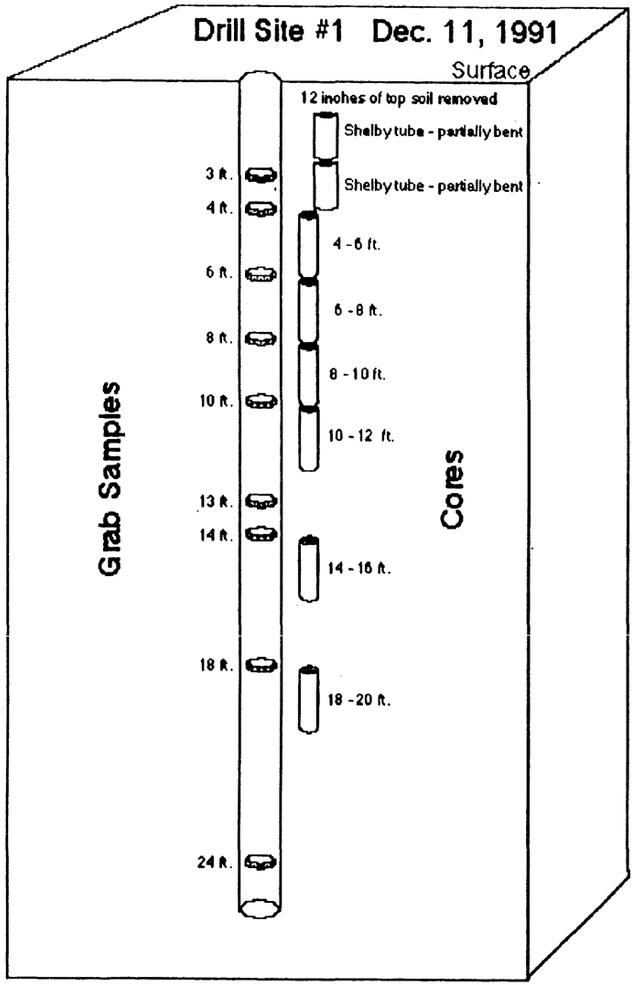



**Figure 23**. Sampling protocol on 12/11/91 for site #1 of the St. Louis Park Reilly Site

Two shelby tube samples were taken near the surface. But use of shelby tubes was discontinued because the tubes were too severely damaged. Depth locations of the split spoon core samples and the grab samples are identified in Figure 23. It can be seen that the split spoon sampling cores can be used to obtain an essentially complete vertical soil profile for laboratory testing. Test results on the grab samples and cores are presented in subsequent sections.

3. ANALYSIS OF POLYNUCLEAR AROMATIC HYDROCARBONS (PAH'S) IN SOIL SAMPLES.

a. Chemicals of Concern (COC)

PAH's listed below have been identified as the chemicals of concern based on previous surveys of the Reilly Site reported by MPCA , and the analytical protocols are designed to measure these PAH's:

| | | |
|---|---|---|
| Naphthalene | Phenanthrene | Benzo(a)anthracene |
| Benzo(a)pyrene | Acenaphthylene | Anthracene |
| Chrysene | Indeno(1,2,3-cd)pyrene | Acenaphthene |
| Fluoranthene | Benzo(b)fluoranthene | Dibenzo(a,h)anthracene |
| Fluorene | Pyrene | Benzo(k)fluoranthene |
| Benzo(g,h,i)perylene | | |

In creosote, the predominant PAH's include some 17 compounds ranging from naphthalene with a solubility of 31.7 mg/L to Benzo(a)pyrene with a solubility of 0.003 mg/L. Phenolic and heterocyclic compounds are also present in creosote. However, the predominant phenols are very soluble as are most of the predominant heterocyclics. It is therefore likely that many of these chemicals will have been solubilized and transported off site. Nevertheless, a study is underway to estimate the total mass of organic chemicals that are present. More specifically, the total organic content as measured by combustion is being compared with the identified PAH concentrations to estimate unidentified mass of organic chemicals. The results will be published as separate reports.

b. Soil Samples

The following soil samples were collected from the Reilly site in 250-ml glass bottles with gas-tight aluminum-lined lids, and were transported to the laboratory in coolers and stored in a 4°C cold room immediately upon arrival.

| Sampling Date | Site# | Sampling Depths, ft. |
|---|---|---|
| 10/9/91 | 1 | 4, 8, 13, 18, 24, 29 |
| | 2 | 2, 9, 14, 18 |
| 12/11/91 | 1 | 3, 4, 6, 8, 10, 13, 14, 18, 24 |

c. Soxhlet Extraction Procedures

The Soxhlet Extraction procedures described under EPA method 3540 was followed. The method is for extraction of nonvolatile and semivolatile organic compounds from solids, sludges, and wastes, and is applicable to the isolation and concentration of water-insoluble and slightly water-soluble organics in preparation for a variety of chromatographic procedures.

1. Apparatus and Materials

   a. Soxhlet extractor:
   40-mm I.D., with 500-ml round-bottom flask.

   b. Kuderna-Danish (K-D) apparatus:
   Concentrator tube, 10-ml, graduated, Kontes.
   Evaporation flask, 500-ml, Kontes.
   Snyder column, 3-ball macro, Ace Glass,Inc.

   c. Boiling chips:
   PTFE boiling stones, solvent extracted, Norton co.

   d. Extraction thimbles:
   Cellulose extraction thimbles, single thickness, Whatman.

   e. Heating mantle:
   3-sample model, individually rheostat controlled, Fisher Scientific Co.

   f. Reagent water:
   Corning Mega-Pure water purification system.

   g. Sodium sulfate:
   Granular anhydrous, purified by washing with methylene chloride
   followed by heating at 400°C for 4 hours.

   h. Methylene chloride:
   OPTIMA grade, Fisher Scientific Co.

   i. 2-Fluorobiphenyl:
   Surrogate, 1000 mg/ml in methanol.

   j. Glassware:
   All laboratory glassware was cleaned according to the following procedures:
      a. Laboratory grade detergent wash and rinse.
      b. Multiple deionized water rinses.
      c. Acetone rinse.
      d. Oven dried (105°C) overnight.

2. Sample Preparation

   a. Foreign objects such as sticks, leaves, and gravels were discarded from the soil sample.

   b. Ten (10) grams of soil sample were blended with 20 grams of anhydrous sodium sulfate until a dry homogeneous mixture was obtained.

   c. The mixture was transferred to an extraction thimble, and 100 $\mu$g of 2-Fluorobiphenyl was added to the soil by transferring 100 $\mu$L of the surrogate stock solution using a syringe.

   d. When moisture content of the soil sample was required, 10 grams of soil sample was weighed into a tared crucible and

dried overnight at 105°C. The dried sample was weighed again after being cooled in a dessicator:

$$\% \text{ moisture} = \frac{\text{g of sample - g of dry sample}}{\text{g of sample}} \times 100$$

3. Extraction

   a. Five hundred (500) ml of methylene chloride was added to the 500-ml round bottom flask containing one or two boiling chips.

   b. The flask was attached to the extractor and extracted for 16-24 hours.

   c. After the extraction was cooled and the extract cooled, the extract was concentrated to 10-ml using the K-D concentrator and the 3-ball Snyder column. The water temperature for concentration was adjusted (about 70°C) such that the concentration procedure was completed in approximately 30 minutes.

   d. The concentrated extract was then analyzed for PAH's in the gas chromatograph.

4. Gas Chromatograph Analysis

   EPA method 8000 (General Gas Chromatography) and EPA method 8100 (GC Analysis of Polynuclear Aromatic Hydrogen) were followed.

   a. External Standard Calibration

      For each PAH of interest, calibration standards at a minimum of five concentration levels were prepared. One of the external standards was at a concentration near, but above, the method detection limit. The other concentrations corresponded to the expected range of concentrations found in real samples. Retention times were recorded for the identificatin of the analytes. Peak area responses were



tabulated against the mass injected, and a calibration curve for each analyte was prepared. A second-order curve fitting equation was obatined for each analyte.

   b. GC Analysis

      Conditions for GC analysis were as follows:

| | |
|---|---|
| GC: | Hewlett Packard 5890 Gas Chromatograph with auto-sampler |
| Integrator: | Hewlett Packard 3396A Integrator |
| Column: | Hewlett Packard HP-5, 25m x 0.2mm x 0.33$\mu$m |
| Detector: | FID |
| Carrier gas: | hydrogen, 2ml/min |
| Initial T°: | 40°C |
| Initial time: | 1 min |
| Rate: | 5°C/min |
| Final T°: | 300°C |
| Final time: | 10 min |
| Injection vol: | 2 $\mu$L |

   d. Results

      Results of PAH analysis of soil samples obtained from the St. Louis Park Reilly site on 10/9/91 and 12/11/91 are shown in Table 5. Sixteen PAH's have been identified in measurable quantities in most of the samples. Total concentrations are listed in the last column. All the profiles show vertical variations in concentrations. The following discussion of these variations focuses on the Site #1 samples of 12/11/91.

**Table 5**. PAH Analysis of Soil Samples Obtained from the St. Louis Park Reilly Site on 10/9/91 and 12/11/91

| Sampling Date | Site No. | Depth (ft) | PAH Concentration, mg per Kg Soil | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | NAPH | ACNY | ACNE | FLUO | PHEN | ANTH | FLAN | PYRN | BAAN | CHRY | BBFN | BKFN | BAPY | INPY | DBAN | BPER | Total |
| 10/9/91 | 1 | 4 | 40.12 | 7.17 | 22.23 | 32.57 | 75.54 | 59.81 | 51.06 | 37.89 | 19.24 | 22.78 | 15.94 | 8.95 | 16.37 | 24.38 | 19.86 | 19.60 | 473.51 |
| | | 8 | 92.04 | 6.16 | 75.77 | 69.74 | 163.97 | 54.09 | 126.16 | 89.23 | 29.65 | 33.97 | 19.93 | 15.49 | 18.23 | 20.78 | 16.76 | 17.03 | 849.00 |
| | | 13 | 42.21 | 10.32 | 31.58 | 33.18 | 91.08 | 31.88 | 107.88 | 86.66 | 33.41 | 45.52 | 30.87 | 21.94 | 28.36 | 29.05 | 20.30 | 25.39 | 669.63 |
| | | 18 | 36.88 | 6.64 | 33.15 | 33.34 | 78.04 | 33.11 | 75.50 | 58.37 | 24.21 | 30.01 | 20.15 | 17.89 | 19.42 | 23.94 | 19.69 | 20.06 | 530.40 |
| | | 24 | 6.33 | 1.29 | 8.64 | 8.80 | 25.25 | 9.89 | 22.23 | 16.72 | 5.94 | 7.88 | 5.05 | 2.80 | 4.59 | 4.58 | 3.09 | 4.16 | 137.24 |
| | | 29 | 10.09 | 1.42 | 11.00 | 10.95 | 31.21 | 12.19 | 26.29 | 19.52 | 6.54 | 8.50 | 5.51 | 2.86 | 5.00 | 4.88 | 3.12 | 4.22 | 163.30 |
| | 2 | 2 | 1.47 | 1.38 | 0.87 | 1.89 | 4.78 | 3.70 | 6.34 | 5.80 | 3.65 | 5.59 | 5.24 | 2.57 | 4.05 | 6.23 | 3.34 | 7.04 | 63.94 |
| | | 9 | 0.24 | 0.13 | 0.28 | 0.48 | 1.59 | 1.29 | 1.45 | 1.55 | 0.58 | 1.06 | 0.62 | 0.52 | 0.43 | 0.57 | 0.35 | 0.51 | 11.65 |
| | | 14 | 0.35 | 0.22 | 0.79 | 0.75 | 2.59 | 2.65 | 4.88 | 4.00 | 1.57 | 2.43 | 1.59 | 1.21 | 1.29 | 1.12 | 0.51 | 1.01 | 26.96 |
| | | 18 | 0.24 | 0.15 | 0.29 | 0.27 | 0.61 | 0.48 | 1.29 | 1.09 | 0.54 | 0.98 | 0.71 | 0.39 | 0.52 | 0.65 | 0.39 | 0.67 | 9.27 |
| 12/11/91 | 1 | 3 | 6.43 | 3.98 | 9.38 | 19.42 | 58.28 | 32.10 | 49.25 | 39.61 | 19.23 | 25.42 | 19.92 | 13.41 | 17.55 | 13.61 | 0.52 | 13.21 | 341.32 |
| | | 4 | 16.99 | 5.02 | 40.59 | 47.91 | 195.11 | 71.86 | 203.99 | 155.76 | 72.15 | 89.31 | 69.68 | 64.66 | 61.02 | 36.51 | 10.32 | 36.07 | 1176.95 |
| | | 6 | 80.63 | 2.99 | 75.15 | 74.83 | 195.49 | 116.51 | 131.24 | 94.97 | 38.96 | 51.49 | 34.82 | ND | 27.02 | 19.01 | ND | 18.80 | 961.91 |
| | | 8 | 61.14 | 2.64 | 62.92 | 54.84 | 135.16 | 60.49 | 133.82 | 99.83 | 44.59 | 63.19 | 45.26 | 21.69 | 34.46 | 22.19 | ND | 21.72 | 863.94 |
| | | 10 | 25.11 | 1.59 | 33.07 | 31.84 | 86.31 | 70.80 | 81.52 | 67.18 | 27.89 | 43.79 | 28.44 | ND | 21.83 | 13.42 | 1.45 | 15.10 | 549.34 |
| | | 13 | 62.51 | 10.10 | 49.17 | 47.83 | 148.94 | 60.89 | 161.77 | 136.26 | 69.34 | 112.49 | 74.96 | 64.08 | 63.78 | 37.69 | 13.61 | 36.77 | 1150.19 |
| | | 14 | 70.08 | 14.46 | 68.98 | 70.33 | 241.15 | 86.64 | 244.97 | 207.87 | 97.90 | 169.26 | 100.37 | 75.80 | 85.43 | 51.27 | 21.22 | 46.94 | 1652.67 |
| | | 18 | 58.08 | 6.61 | 50.19 | 46.02 | 153.06 | 72.60 | 158.47 | 132.49 | 59.80 | 88.91 | 57.52 | 51.91 | 48.94 | 30.48 | 9.20 | 28.58 | 1052.86 |
| | | 24 | 29.17 | 4.41 | 35.63 | 35.53 | 115.07 | 51.44 | 116.93 | 93.95 | 44.82 | 66.50 | 39.21 | 36.88 | 33.39 | 21.01 | 5.03 | 18.50 | 747.47 |

**Legends**

| | | | | | |
|---|---|---|---|---|---|
| NAPH | Naphthylene | BAAN | Benzo(a)anthracene | ND | Non-detectable |
| ACNY | Acenathylene | CHRY | Chrysene | | |
| ACNE | Acenaphthene | BBFN | Benzo(b)fluoranthene | | |
| FLUO | Fluorene | BKFN | Benzo(k)fluoranthene | | |
| PHEN | Phenanthrene | BAPY | Benzo(a)pyrene | | |
| ANTH | Anthracene | INPY | Indeno(1,2,3-cd)pyrene | | |
| FLAN | Fluoranthene | DBAN | Dibenzo(a,h)anthracene | | |
| PYRN | Pyrene | BPER | Benzo(g,h,i)perylene | | |

**Table 6**. Percentage Distribution of Total PAH's in Soil Samples from Site 1 of the St. Louis Park Reilly Site

| Sampling Date | Site No. | Depth (ft) | % of Total PAH's | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | NAPH | ACNY | ACNE | FLUO | PHEN | ANTH | FLAN | PYRN | BAAN | CHRY | BBFN | BKFN | BAPY | INPY | DBAN | BPER |
| 12/11/91 | 1 | 3 | 1.88 | 1.17 | 2.75 | 5.69 | 17.07 | 9.40 | 14.43 | 11.60 | 5.63 | 7.45 | 5.84 | 3.93 | 5.14 | 3.99 | 0.15 | 3.87 |
| | | 4 | 1.44 | 0.43 | 3.45 | 4.07 | 16.58 | 6.11 | 17.33 | 13.23 | 6.13 | 7.59 | 5.92 | 5.49 | 5.18 | 3.10 | 0.88 | 3.06 |
| | | 6 | 8.38 | 0.31 | 7.81 | 7.78 | 20.32 | 12.11 | 13.64 | 9.87 | 4.05 | 5.35 | 3.62 | 0.00 | 2.81 | 1.98 | 0.00 | 1.95 |
| | | 8 | 7.08 | 0.31 | 7.28 | 6.35 | 15.64 | 7.00 | 15.49 | 11.56 | 5.16 | 7.31 | 5.24 | 2.51 | 3.99 | 2.57 | 0.00 | 2.51 |
| | | 10 | 4.57 | 0.29 | 6.02 | 5.80 | 15.71 | 12.89 | 14.84 | 12.23 | 5.08 | 7.97 | 5.18 | 0.00 | 3.97 | 2.44 | 0.26 | 2.75 |
| | | 13 | 5.43 | 0.88 | 4.27 | 4.16 | 12.95 | 5.29 | 14.06 | 11.85 | 6.03 | 9.78 | 6.52 | 5.57 | 5.55 | 3.28 | 1.18 | 3.20 |
| | | 14 | 4.24 | 0.87 | 4.17 | 4.26 | 14.59 | 5.24 | 14.82 | 12.58 | 5.92 | 10.24 | 6.07 | 4.59 | 5.17 | 3.10 | 1.28 | 2.84 |
| | | 18 | 5.52 | 0.63 | 4.77 | 4.37 | 14.54 | 6.90 | 15.05 | 12.58 | 5.68 | 8.44 | 5.46 | 4.93 | 4.65 | 2.89 | 0.87 | 2.71 |
| | | 24 | 3.90 | 0.59 | 4.77 | 4.75 | 15.39 | 6.88 | 15.64 | 12.57 | 6.00 | 8.90 | 5.25 | 4.93 | 4.47 | 2.81 | 0.67 | 2.48 |

**Legends**

NAPH Naphthylene
ACNY Acenathylene
ACNE Acenaphthene
FLUO Fluorene
PHEN Phenanthrene
ANTH Anthracene
FLAN Fluoranthene
PYRN Pyrene
BAAN Benzo(a)anthracene
CHRY Chrysene
BBFN Benzo(b)fluoranthene
BKFN Benzo(k)fluoranthene
BAPY Benzo(a)pyrene
INPY Indeno(1,2,3-cd)pyrene
DBAN Dibenzo(a,h)anthracene
BPER Benzo(g,h,i)perylene

Figure 25 presents a histogram of the concentration profile. There are considerable variation in concentrations as a function of depth. The pattern is almost bimodal with high levels at depths 4-6 feet and in the vicinity of 14 feet. It has been suggested that the variations are due to concentration differences of the original depositied materials that were obtained from different parts of the site.

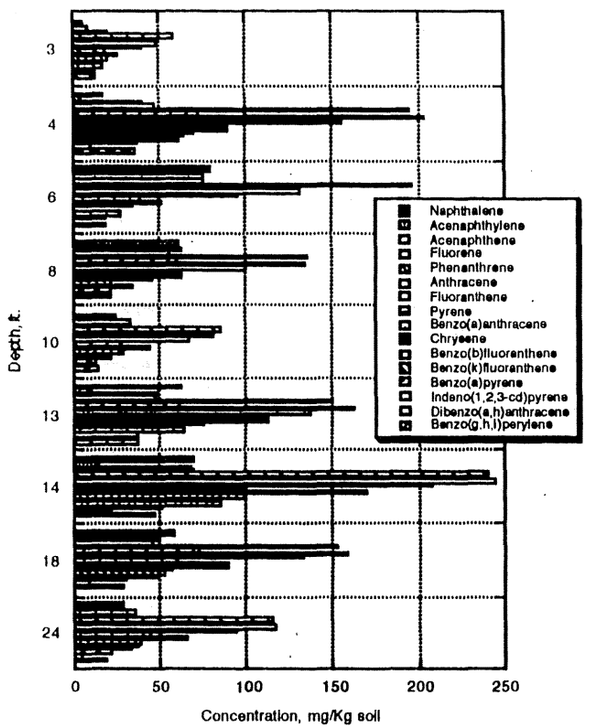



**Figure 25**. PAH concentrations in soil samples from site #1 of the St. Louis Park Reilly Site (sampled on 12/11/91)

It is interesting to note that the "% of Total" for each compound is relatively constant as a function of depth as shown in Table 6. This suggests that the original chemical mixtures are from similar sources. Naphthalene shows the largest variations in the upper soil horizons. This may be the result of vapor losses and/or the more rapid rate of biodegradation of this compound. The bar graph in Figure 26 shows the same data to highlight the variations in naphthalene as opposed to the almost constant "% of Total" values for phenanthrene and Benzo(a)pyrene as a function of soil depth.

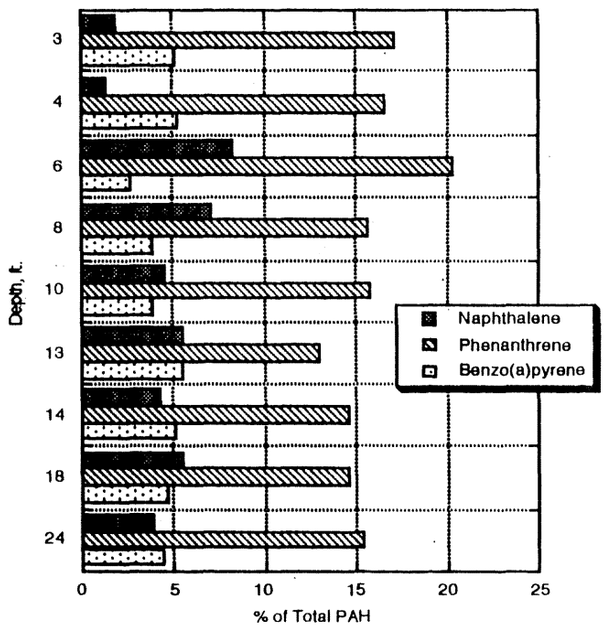



**Figure 26**. Distribution of total PAH's for Naphthalene, Phenanthrene, and Benzo(a)pyrene in soil samples from site #1 of the St. Louis Park Reilly site (sampled on 12/11/91.

Vertical variations in total PAH concentrations ranged from 341 to 1,652 mg/Kg, roughly a 5-fold variation. By contrast some of the specific PAH's show nearly 15-fold variations. However these larger variations occur in the more volatile constituents in which losses due to volatilization probably played a role.

Information on relative rates of biodegradation suggests that the more volatile PAH's are biodegraded more rapidly than the higher molecular weight chemicals, probably because of the previously mentioned differences in solubility. It is therefore reasonable to assume that the larger vertical variations shown by the more volatile PAH's are not rate limiting as regards cleanup. It would however be prudent to base cleanup calculations on a weight averaged value that reflects relative solubilities and hence potential for biodegradation.

Solubilization is recognized as the most likely rate limiting factor in both flushing and in situ biodegradation. Rate data on solubilization of each of the major PAH's is therefore essential. Such data has been developed as part of this study. In addition, rates of biodegradation of 7 individual PAH's have been measured in batch tests in a related study (not included in this report.) These data will be used to model relative rates of biodegradation in order to provide a weight averaging basis for calculating the cleanup time required for soils that exhibit vertical variations in PAH distribution. This phase of the data analysis/modeling is still in progress and will be published separately.

e. Conclusions

The following conclusions can be drawn:

1. The sampling and analytical protocols give quantitative descriptions of the concentration distributions of the major PAH compounds. This information is essential for guiding bioremediation tests and for assessing oxygen and nutrient requirements.

2. The similarity in the vertical percentage distribution of all compounds indicates that the source materials were similar. This information is useful because it gives support to the idea that the kinetics of dissolution and biodegradation will be similar throughout the site.

f. Ongoing and Future Work

Correlations for analyzing and interpreting the effects of spatial variations in pollutant concentrations on the time needed for cleanup by flushing and in situ biodegradation are needed. Data on relative rates of solubilization and biodegradation of mixtures that has been generated in this phase of the study need to be analyzed to develop such correlation. Funding for such efforts will be sought.

4. SPLIT SPOON INSERT COLUMN STUDIES COLUMN SETUP AND ANALYTICAL PROTOCOL

a. Apparatus for testing Minimally Disturbed Aquifer Materials
Split Spoon Insert Columns (SSIC)

Development of protocols for obtaining minimally disturbed samples of contaminated soils for testing in the laboratory was a critical initial objective of this research program. Testing of minimally disturbed soils is deemed essential for defining rate data needed to assess the feasibility of insitu cleanup of contaminated aquifers. For this reason, a program for taking cores of aquifer materials by drilling with split spoon samplers with stainless steel inserts and adapting the filled tubes for column studies was undertaken. Needless to say, the procedures could have application at other sites. Construction and operating procedures of Split Spoon Insert Columns (SSIC) are described below.

1. Split Spoon Insert Column Description

The column is the insert from a split spoon sampler containing an undisturbed soil core. The column is 24 inches long with an id of 2 3/8 inches and an O.D. of 2 1/2 inches. The ends of the column are sealed at either end with an aluminum cap. The caps

are square blocks of aluminum, 4.5 inches per side with a thickness of 1/2 inch. A circular groove the same diameter as the column and 0.125 inches wide was cut into the center of aluminum block. A rubber o-ring was inserted in this groove to seal the column to the cap. Three bolts running from the bottom base plate to the top plate secure the base plates to the column. The center of the cap was drilled and tapped to fit an 1/8 inch stainless steel swagelock fitting.

The column is equipped with 7 sampling ports located at 3 inch intervals on alternating sides of the column. The ports are constructed from 1/2 inch diameter stainless steel sleeves inserted into holes drilled into the side of the column. The sleeves are sealed to the column with a silicon epoxy. A mini-inert valve is inserted into each sleeve. The valve, as shown in Figure ( ), is of all Teflon construction and is equipped with a rubber septa which allows insertion of a needle to withdraw water samples from the soil core while maintaining an airtight seal. The mininert valves can be removed to allow removal of soil samples.

The water is fed to the column through 1/8 inch stainless steel tubing. The tubing is connected to the column with 1/8 inch swagelock stainless steel fittings. The water is pumped through the column using a Gilson Minipuls 2, 4-channel pump. Each column is assigned to one of the four channels The pump is set up with a 1.52 mm I. D. pump tube with a flow rate of 0.3 ml/min. Both higher and lower flow rates have been tested.

2. Core Descriptions

At the present time three soil cores are being tested, and are numbered sequentially one to three from the date testing started. The cores were obtained from different depths at the same site located on the ridge running along the western edge of the Reilly site:



| Number | Date Started* | Depth |
|---|---|---|
| 1 | 3/1/92 | 6'-8' |
| 2 | 6/3/92 | 10'-12' |
| 3 | 6/5/92 | 4'-6' |

*Columns are still operating; tentative shut down scheduled for Fall 1993.

The soil in the columns contains a mix of layers of different soil types including silty sand, medium sand, peat and clay. The water content of the soil was approximately 10% by weight. The porosity of the soils was 30 % and the average solids density was 2.64 g/cm$^3$. The chemical properties of the soil taken from the inlet and outlet of each column have been tested. When the columns are finally shut down and dismantled, soil tests will be made to define the vertical concentration profiles.

b. Column Preparation

The drilling to obtain the split spoon insert cores was done on December 11, 1991. The full split spoon inserts were weighed and the ends sealed with plastic caps in the field. The inserts were stored in a temperature controlled room at 10°C until needed. Before testing the split spoon insert was opened and approximately 50 grams of soil was remove from both the top and bottom of the core. This soil was tested to find the physical and chemical characteristics of the soil. A Soxhlet extraction was performed on a 10 g sample of the removed soil to find the concentration of the targeted contaminants in the soil.

After the soil samples are removed the top and bottom of the insert is sealed with a Styrofoam pug and resealed with the plastic cap. The insert was taken to the Civil and Mineral Engineering machine shop. The personnel at the machine shop installed the seven sampling ports and returned the insert to the lab. In the lab the Styrofoam was removed from both ends of the insert. The space between the soil core and the end of the column was filled with washed and sieved Jordan aquifer sand. Two screens were placed on the top of the sand. The first screen is a fine mesh designed to allow water to enter and exit but retain the soil particles. The second screen is a course

mesh with drainage channels cut into the center. It is designed to direct the water flow to the outflow fitting of the column. The insert is capped on both ends and installed onto a stand.

The water initially supplied to the column was deoxygenated to an oxygen content of 0.1-0.5 mg/l by sparging nitrogen into a 5 gallon carboy of de ionized water. To begin the flow of water through the column the tubes were connected to the column to provide up flow. The reverse flow was used to ensure that the soil core was completely saturated. The pump flow rate was set to 0.3 ml/min. and the column was filled until water began to flow from the outlet. Once the flow from the top of the column was established the inlet and outlet tubes were reversed so that the remainder of the experiment was performed with down flow.

c. Sampling Procedures-Anoxic Conditions

1. Glassware

All of the glassware was cleaned using the following procedure:
a. Soap wash
b. Rinsed and soaked in de-ionized water
c. Air dried
d. Rinsed in acetone
e. Oven dried at 105°C for 24 hours

2. Effluent Sampling

Initial operations were designed to have "no growth" conditions in order to measure rates of removal of chemicals by flushing. Anoxic water was run through the column to prevent biological activity in the column. To inhibit bacterial growth in the collection vessel the effluent was collected in 500 ml Erlenmeyer flasks which contained approximately 0.75g of Sodium Azide as a bactericide. Also the flask was purged with nitrogen prior to being used.



Effluent sample was collected over a 24 hour period yielding a total sample volume of 400 to 435 milliliters depending on the average flow rate of the sampling period.

d. Effluent Analysis

1. Dissolved Oxygen and pH Measurements

The effluent was tested for dissolve oxygen concentration immediately after the flask was removed from the column. The oxygen level was determined with an Orion oxygen electrode connected to an Orion SA 520 pH meter. The pH of the sample was taken with the same meter.

2. Water Sample analysis Using Solid Phase Extraction

A 10 ml aliquot of the sample was removed from the flask with a 10 ml gas tight syringe and injected into a 10 ml screw cap test tube. Five µl of a surrogate spike, 2-Fluorobiphenyl 5000 mg/l in methanol, was injected into the 10 ml aliquot. The spike was added to track the efficiency of the solid phase extraction of the samples. The non-polar organic in the aliquot are extracted and concentrated using a solid phase extraction technique available from Varian.

a. Equipment:

Varian 500 mg Bond Elut Octadecyl (C-18) cartridges
Varian Vac Elut vacuum source
2 ml class A volumetric test tubes.
Fisher Optima grade hexane
Fisher Optima grade methanol

b. Procedure:

The procedure used is prescribed by Varian for general non-polar isolates. It involves filtering the water sample through a solid packing coated with C-18 adsorbent. The cartridge is inserted into the vacuum source. One to two ml of methanol

is passed through the cartridge to activate the sorbent. The cartridge is immediately rinsed with 1 to 2 ml of de-ionized water. Immediately after the rinse water has passed, the sample is applied to the cartridge. After the sample has passed through the cartridge the cartridge is removed from the vacuum source and allowed to dry over night.

The non-polar isolates are extracted from the C-18 sorbent by passing a 2-ml aliquot of hexane through the cartridge and collecting it in a 2-ml volumetric test tube. The hexane which is lost due to volatilization during the extraction process is replaced so that the test tube is filled to the 2-ml mark. It follows that when 10 ml water samples are filtered the final extract is a five-fold concentration of the original sample aliquot. Larger volumes of water can be filtered to increase the concentration factor accordingly. In this work 200 ml water samples have been filtered successfully.

3. Gas Chromatographic Analysis

EPA method 8000 (General Gas Chromatography) and EPA method 8100 (GC Analysis of Polynuclear Aromatic Hydrocarbons) were followed.

a. External Standard Calibration:

For each PAH of interest, calibration standards were made using 99.9% pure compounds dissolved in Optima grade hexane. A minimum of five concentration levels were prepared. The concentration levels began near but not below the detection limit of the instrument, the rest of the standards at levels expected of the real samples. The peak areas were compared against the mass injected and a calibration curve was developed for each analyte. A second order polynomial fit was prepared for each analyte.

b. GC Analysis Parameters:

Two injections from each sample extract was performed on the GC under the following conditions:

| | |
|---|---|
| GC | Hewlett Packard 5890 with auto sampler |
| Integrator | HP 3396 |
| Column | HP-5, 25m x 0.2 mm x .33$\mu$m |
| Detector | FID |
| CarrierGas | Hydrogen, 2ml/min |
| Initial Temp | 40°C |
| Initial Time | 1 min. |
| Rate | 6°C/min |
| Final Temp | 260°C |
| Final Time | 10 min |
| Injection vol | 2 $\mu$l |

E. Intermediate Column Samples

Intermediate samples were taken along the length of the column through the mininert valve sampling ports. These sample were taken about every few weeks.

1. Port Sampling Method

To sample the ports a 10-ml syringe, with the plunger removed and a 4-inch, 22 gauge needle was used to penetrate the septum of the mininert valve in the sampling port. Once the septum was penetrated and a flow into the syringes was established the syringe was clamped to a stand and allowed to fill. The syringe filled by the flow from the port alone the plunger was not used to draw out a sample. This was done to prevent desaturating the column and to provide a more representative sample of the column water at the position of the port.

The ports were sampled sequentially from the top of the column to the bottom. Each sample takes about 20-30 minutes to collect.

2. Analysis

The samples from the ports were extracted and analyzed using the same methods as the effluent samples,except that pH and dissolved oxygen content were not measured.

5. RESULTS:

A. General Operations of the Split Spoon Insert Columns (SSIC)

Three split spoon insert column soil cores from the Reilly site are currently being tested to measure rates of removal of creosote related organic chemicals. PAH Analysis of Column Soils

Prior to initiating the tests, composite samples of soil taken from the inlet and outlet of each column were analyzed. The chemical composition of the composite is consistent with the composition of the grab samples for the same depth of soil shown in Table 7. Concentrations are expressed in terms of mg-compound /Kg soil. Fifteen compounds were identified, ranging from naphthalene to Benzo(g,h,i)perylene which represent progressively higher molecular weights and lower volatility's. Only four of the 15 found were monitored throughout the experiments on the columns, It is noteworthy that the intermediate compounds show the highest concentration in line with the measurements of the grab samples described above. It is not known to what extent this reflects the effects of weathering and insitu biodegradation of the more volatile species as opposed to differences in initial distribution and composition of the creosote source materials.

**Table 7.**

| Compound | mg/Kg Soil | Total Weight (mg) | Leached Amt (mg) | Percent Leached |
|---|---|---|---|---|
| **Initial Soil Analysis Column One** | | | | |
| Naphthalene | 43.6 | 104.6 | 30.5 | 29.1 |
| Acenaphthene | 80.6 | 193.4 | 18.3 | 9.5 |
| Fluorene | 75.8 | 181.9 | 16.6 | 9.1 |
| Phenanthrene | 213.7 | 512.9 | 11.3 | 2.2 |
| **Initial Soil Analysis Column Two** | | | | |
| Naphthalene | 6.0 | 14.4 | 6.5 | 45.1 |
| Acenaphthene | 30.5 | 73.2 | 32.7 | 44.7 |
| Fluorene | 37.1 | 89.0 | 24.6 | 27.6 |
| Phenanthrene | 110.5 | 265.2 | 21.7 | 8.2 |
| **Initial Soil Analysis Column Three** | | | | |
| Naphthalene | 7.2 | 20.2 | 71.4 | 354.2 |
| Acenaphthene | 61.6 | 172.5 | 15.4 | 8.9 |
| Fluorene | 54.6 | 152.9 | 12.3 | 8.1 |
| Phenanthrene | 201.8 | 565.0 | 10.5 | 1.9 |

Conclusions:

1. Soxhlet analyses of the soil are useful as indicators of the chemicals that are expected to be measured in the effluent water samples, keeping in mind that water solubility's are progressively lower with increasing molecular weight.

2. For future reference, consideration should be given to taking samples of soil at intermediate points to define initial

concentration distribution more precisely albeit this will result in some disturbance of the hydraulic characteristics of the soil core.

B. Column Operation

1. Initial Operations

The columns were dosed continuously with pure deionized water. The flow rate and oxygen content of the water was varied to determine the effect those parameters had on the dissolution and aqueous concentrations of the PAH's. A description of the various conditions of flow and the results of each condition of flow will be detailed in the following sections.

Column #1 has been in operation for over 12000 hours with continuous dosing. Columns #2 and #3 have been in operation for over 10000 hours. During this period, samples of water from the outlet and at intermediate sampling port have been taken periodically and analyzed using an adaptation of EPA prescribed analytical procedures for concentrating and measuring PAH's by GC analysis as described in the previous section.

Effluent concentrations of four PAH's, naphthalene, acenaphthene, fluorene and phenanthrene were monitored during the first 700 hours of dosing are shown in Table 4.2. The same data are shown graphically in Figures 4.1 - 4.8. The day to day variations in concentration were larger than expected and it was found that most of these variations were due to experimental problems that have since been eliminated. The causes of these problems and their solution are briefly summarized to show that the SSIC testing protocol is reliable but requires attention to operating procedures, particularly with regard to access to molecular oxygen and handling of samples prior to analysis.

One problem was caused by diffusion of oxygen from the air through the teflon tubing that was used to pump deoxygenated water to the column. It had been decided to initiate the test by using anoxic conditions to test removal of organics by flushing without biodegradation. As a result availability of oxygen and



hence degree of removal of organics varied. Teflon tubing was replaced with stainless steel. The second problem resulted from biodegradation of organics in the collection vessel caused by the presence of microbial cells and oxygen. This problem was solved by adding sodium azide to the collected effluent to stop microbial activity. It should be noted that the observed biological removal of organics is actually desirable from a practical viewpoint because it is evidence that microbial activity is capable of removing the target chemicals. However, for this first phase of the study, the objective was to measure the rates of elution with minimal biological activity. This type of information is needed to quantify the physical rates of removal, namely desorption and dissolution and subsequent transport of the solubilized species through the rest of the column.

2. Stabilized Operations

a. Anoxic Conditions

The purpose of this portion of the experiment was to determine the long term dissolution behavior of the PAH's with no biotransformation. After solving the problems described above the columns were dosed with deionized water which was deoxygenated at a flow rate of 0.3 ml/ min. This flow rate corresponds to a Darcy velocity of 1.75 x 10-4 cm/s and a pore velocity of 5.83 x 10-4 cm/s. These conditions were maintained from day 35 to day 184 in column one, day 1 to day 144 in column two and day 1 to day 142 in column Three.

The leaching of the four monitored PAH's follows a pattern which can be generalized to all four PAH's and all three columns. The effluent concentrations peak at or near the start of column operation and gradually decrease to a stable value. These results are summarized in Figures 27-38.

Naphthalene had the highest effluent concentration in columns one and three. The naphthalene concentration in these columns peaked at about 1.0 mg/l and then dropped

to a steady state concentration of approximately .05 in column one and 0.3 mg/l in column three. The naphthalene concentration in column two was very low from the very beginning and never reached a well defined peak. The effluent concentration from column two was fairly constant ranging in value from .01 to .015 mg/l during the time of this portion of the experiment. None of the concentrations reached a value close to the saturation concentration of 31.7 mg/l.

Acenaphthene leaching was similar in all three columns. In each column the acenaphthene reached a peak value of between 0.15 and 0.20 mg/l. The concentrations then dropped to values between 0.06 to 0.09 mg/l and remained fairly steady with very little variation in concentrations. The peak concentrations did not approach the acenaphthene saturation concentration of 3.93 mg/l.

The fluorene leaching was more variable between the three columns. In column one the effluent concentration reached a peak of 0.2 mg/l after approximately 80 days of operation. The concentration then fell to a steady concentration of 0.06 mg/l after approximately 150 days. In column two a peak concentration of 0.078 mg/l was reached after approximately 70 days of operation. The effluent in column two did not drop significantly, dropping only 0.02 to 0.04 mg/l after 130 days. In column three the fluorene concentration peaked at 0.1 mg/l after 35 days of operation then gradually decreased to a low of 0.04 mg/l after 130 days of operation. As with naphthalene and acenaphthene the fluorene concentration was not ever near the saturation concentration of 1.98 mg/l.

The phenanthrene effluent concentration also showed some variation between the three columns. In column one the phenanthrene concentrations vary from .03 to 0.10 mg/l. The initial leaching shows relatively steady concentrations of approximately 0.035 mg/l for the first 110 days. The concentration then forms a hump where the effluent slow builds to 0.1 mg/l from 110 to 140 days and then drops of to 0.05 mg/l at 160 days. The phenanthrene in the effluent of column two over the first 50 days shows a large amount of variation, from 0 to 0.07 back down to 0.02 mg/l in the first 20 days of operation. The concentration then rises to and remains fairly constant at 0.03 to 0.04 mg/l. In column three the effluent concentration varied from a high of 0.1 to 0.025 mg/l over the first 60 days. The concentration became fairly constant after 110 days at 0.03 to 0.04 mg/l.

The effluent concentrations of the monitored PAH's never approached the aqueous saturation values of these compounds. This indicates that the effluent concentration is controlled by other factors. The other factors which influence the effluent concentration include, dissolution rates between an immobile boundary layer between the moving water and soil particles, desorption from the organic carbon component of the soil and the Raoult's law. It seems, given the variety of concentrations in the effluent of the three columns that the concentration is controlled by a complex interplay of the above factors.

b. Ambient Oxygen Concentration

The purpose of this portion of the experiment is to determine if ambient oxygen concentrations are sufficient to oxygenate the entire length of the column and to observe the effects of biodegradation on effluent concentrations of the PAH's. The water fed into the columns was allowed to equilibrate with the atmospheric oxygen resulting in an influent oxygen concentration of approximately 8 mg/l. All other operating parameters remained the same. These conditions were maintained from day 185 to day 282 in column one, day 145 to day 198 in column two and day 143 to 196 in column three. The results of this portion of the experiment are shown in Figures 27-38.

The effluent concentrations of the monitored PAH's were not significantly affected by the addition of low amount of oxygen. The effluent concentrations dropped slightly in some instances over the first ten days of oxygen addition but then most recovered to the concentrations recorded prior to

oxygen addition. These concentrations remained fairly constant through the remainder of the time the columns were run under these conditions. These results suggest that limited biodegradation has little or no effect on the effluent concentrations of the PAH's.

c. Ambient Oxygen with Varying Flow Rates

The purpose of this portion of the experiment was to investigate the effect of flow rate on the dissolution of PAH's into aqueous solution. This was done by running the columns at three different flow rates. The first flow rate was the lowest, with an average of 0.130 ml/min. This flow rate corresponds to a darcy velocity of 7.6 x 10-5 cm/ s and an average pore velocity of 2.5 x 10-4 cm/s. The second flow rate was identical to the one used in the anoxic and ambient oxygen conditions. The final flow rate was highest with an average of .510 ml/min, corresponding to a darcy velocity of 2.98 x 10-4 cm/s and an average pore velocity of 9.91 x 10-4 cm/s. The timing and duration of the three flow rates is summarized in table 8.

**Table 8**

Varying Flow Rate Conditions

| Flow Rate (ml/min) | Time of Conditions (day started-day ended) Column One | Column Two | Column Three |
|---|---|---|---|
| 0.13 | 305-333 | 222-262 | 220-260 |
| 0.3 | 335-368 | 264-285 | 262-283 |
| 0.51 | 400-427 | 317-344 | 316-342 |

The effect of varying the flow rate is summarized in Figures 39-50 and in Table 9

**Table 9**

Column One
Average Effluent Concentration (mg/L) at:

| PAH | Low Flow | Medium Flow | High Flow |
|---|---|---|---|
| Naphthalene | 0.1073 | 0.0768 | 0.0422 |
| Acenaphthene | 0.1440 | 0.1185 | 0.0830 |
| Fluorene | 0.0787 | 0.0600 | 0.0434 |
| Phenanthrene | 0.0797 | 0.0542 | 0.0465 |

Column Two
Average Effluent Concentration (mg/L) at:

| PAH | Low Flow | Medium Flow | High Flow |
|---|---|---|---|
| Naphthalene | 0.0186 | 0.0127 | 0.0103 |
| Acenaphthene | 0.0709 | 0.0521 | 0.0447 |
| Fluorene | 0.0598 | 0.0403 | 0.0317 |
| Phenanthrene | 0.0514 | 0.0353 | 0.0258 |

Column Two
Average Effluent Concentration (mg/L) at:

| PAH | Low Flow | Medium Flow | High Flow |
|---|---|---|---|
| Naphthalene | 0.4165 | 0.3606 | 0.2750 |
| Acenaphthene | 0.1118 | 0.1061 | 0.0788 |
| Fluorene | 0.0815 | 0.0731 | 0.0565 |
| Phenanthrene | 0.0617 | 0.0655 | 0.0530 |

The results show that the effluent concentrations decrease with increasing flow rates. The only exception is the phenanthrene concentration increases slightly when the flow rate is increased from low to medium. This inverse correlation between flow rate is misleading, it suggests that the rate of dissolution decreases with increasing flow rate. If the concentration is adjusted by the flow rate the amount of PAH removed in a set period of time is increased with

increasing flow rate. For example, in column three at .13 ml/min 0.078 mg of naphthalene is removed in 24 hours, at .3 ml/min .155 mg is removed and at .51 ml/ min .202 mg is removed. This suggests that the effluent concentrations are boundary layer diffusion controlled. Unfortunately this does not hold true for all of the compounds in all of the columns. In column one the amount removed in 24 hours decreases from .033 to .031 when the flow is increased from .3 to .51 ml/min. This is a slight decrease yet it suggests that other factors may play a role and must be investigated further.

d. Saturated Oxygen Concentration and Varying Flow Rate

The purpose of this portion of the experiment is to determine the flow rate that would allow oxygen breakthrough and to determine the effects of sparging with pure oxygen on the PAH dissolution and effluent concentrations. The influent deionized water was sparged with pure oxygen to achieve dissolved oxygen concentrations of 35 to 40 mg/l. The initial average inflow rate was .280 ml/min. This rate was maintained for 26 days without any breakthrough of oxygen in the effluent. The flow rate was increased to an average of .570 ml/min. At this flow rate there was oxygen breakthrough in columns one and two but not in column three. The operating parameters are summarized in Table 10

**Table 10**

Varying Flow Rate Conditions

| Flow Rate (ml/min) | Time of Conditions (day started - day ended) Column One | Column Two | Column Three |
|---|---|---|---|
| 0.28 | 450-476 | 367-393 | 365-391 |
| 0.58 | 477-513 | 394-430 | 392-428 |

The effect of the pure oxygen on the effluent concentrations is shown graphically in Figures 51-62. The graphs show that the initial effect of the increased oxygen is minimal at the lower flow rates. In all three columns and all four monitored PAH's showed very little change in effluent concentration. This changes, however with the increased flow rate. At the increased flow rate the effluent concentrations of all of the PAH's drops rapidly in all three columns. Simultaneously, with the exception of column three, the effluent oxygen concentration begins to increase. In columns one and two, after significant oxygen breakthrough, the concentration of the monitored PAH's dropped to non detectable levels. There was no significant oxygen breakthrough in column three and there was always detectable levels of PAH's thought they did drop off rapidly with the increased flow rate.

e. Conclusions:

1. Column tests generate a large data base which gives insight on relative rates of leaching of specific compounds.

2. Rates of leaching are slow but significant. The low concentration of PAH's in solution is well below saturation concentrations suggesting that the leaching influenced by boundary layer diffusion rates and Raoult's law of dissolution of mixed compounds.

3. Overall mass balances on each chemical give qualitative insight on rates of leaching but will have to be checked by taking soil samples in the near future.

4. The effect increasing flow rate decreases average effluent concentrations suggests that the rate of leaching is diffusion controlled but the immobile boundary layer does not affect the diffusion rate as expected.

5. The biodegradation of the dissolved PAH's is limited by available oxygen. If the columns are fully oxygenated the effluent concentration of PAH's declines to below detectable amounts.

6. Data must be analyzed using transport models to quantify the partitioning relationships of each compound between the water phase and soil phases. This is essential for developing the necessary correlation's as tools for engineering application studies.

3. Concentration Profiles at Intermediate Points

As indicated in the procedures section, water samples were taken from each of the intermediate sampling ports in order to measure concentration distributions at selected time intervals. The depth measurement is given with reference to the top of the column. .

The results of the intermediate sampling ports are summarized in Figures 63-65. The results show some interesting trends. In all three columns the concentrations increase in the downstream direction and then decrease in the lower half of the column. Increases in concentration in the downstream direction were not unexpected because of the cumulative contact time between water and soil phases. However, the downward concentration trends in the lower half of the column are due to other causes. One possibility is that there was non uniform distribution of chemicals in the bed initially. Another possibility is that continuous leaching of the column has resulted in a substantial concentration gradient in soil organic content (foc). This could result in readsorption of chemicals in those parts of the bed with the highest foc.

The intermediate sampling during the saturated oxygen conditions also shows some interesting results. In column one, for example, the intermediate ports show some significant concentrations of PAH's where the effluent concentrations have dropped to below detectable levels. Column two is similar, for the sample taken on day 413 there is detectable levels of PAH's at depths of 18 and 21 inches but the effluent concentration on that day was non detectable. These results suggest that the dissolution rate is faster than biodegradation rates in certain portions of the column. The biodegradation is sufficient, however, remove the PAH's before they reach the collection vessel as they pass through the packed sand at the end of the column and the plumbing of the column.

Conclusion:

1. Concentration profiles (snapshots in time) show some expected as well as unexpected results.

2. Explanations will be sought by modeling/parameter estimation.

C. Sampling and Analysis of Soils from Intermediate Points in the Columns

In order to gain further insight about the leaching behaviour of the PAH's shown by the water samples taken from the intermediate ports soils samples were take from the sampling ports. Three ports were sampled located 6, 12 and 18 inches from the top of the column. The PAH's were extracted and analyzed by soxhlet extraction and GC analysis procedures described previously. The results of the analysis are given in Table 11.

**Table 11**

Intermediate Port Soil Analysis
Column One

| Depth (in from top) | Compound (mg/Kg) Naphthalene | Acenaphthene | Fluorene | Phenanthrene | foc |
|---|---|---|---|---|---|
| 6 | 30.2 | 232.6 | 206.5 | 622.7 | 1.74 |
| 12 | 23.6 | 160.4 | 161.5 | 458.3 | 8.56 |
| 18 | 0 | 0 | 0 | 3.1 | 0.364 |

Intermediate Port Soil Analysis
Column Two

| Depth (in from top) | Compound (mg/Kg) Naphthalene | Acenaphthene | Fluorene | Phenanthrene | foc |
|---|---|---|---|---|---|
| 6 | 1 | 47.97 | 46.16 | 133.13 | 1.56 |
| 12 | 0 | 2.3 | 0.9 | 10.8 | 1.91 |
| 18 | 17.1 | 164.7 | 128.6 | 402.42 | 3.24 |

Intermediate Port Soil Analysis
Column Three

| Depth (in from top) | Compound (mg/Kg) Naphthalene | Acenaphthene | Fluorene | Phenanthrene | foc |
|---|---|---|---|---|---|
| 6 | 0.5 | 3.6 | 7.4 | 42.5 | 1.58 |
| 12 | 161.6 | 358.4 | 371.1 | 1097.1 | 1.39 |
| 18 | 18.4 | 23.9 | 28.8 | 97.8 | 1.15 |

The results of the analysis are mixed. They show that the PAH contamination in the cores is very spatially variable. In column three the phenanthrene contamination varies from 42.5 to 1097.1 mg/kg in a space of 6 inches. When compared to the aqueous samples taken from the ports it shows that where the soil content of PAH's is low the water content is also low. The aqueous concentration in column two is consistently higher in the samples taken from a depth of 18 inches, the soil analysis shows that is also the position of the highest soil concentrations. This also hold true at the 12 inch depth in column two. This suggests that there may be some dynamic equilibrium where the dissolution of the PAH's occurs in the highly contaminated soils with some readsorption in the less contaminated soils.

The soil samples taken from the ports were also analyzed to determine the fraction of organic carbon (foc). The organic carbon content plays an important role in the adsorption of organics in soil systems. In general the higher the foc would indicate higher contamination levels due to the adsorption of the PAH's to the organic carbon fraction of the soil. These results show that there is little correlation between foc and the level of PAH contamination.

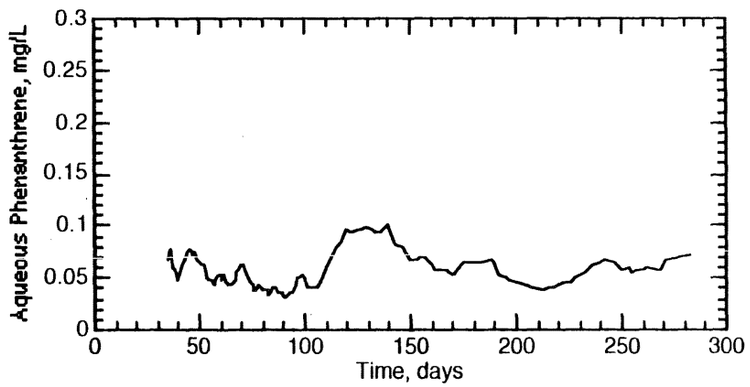



**Figure 27**. Column 1: Phenanthrene effluent concentration under anoxic and ambient oxygen conditions.

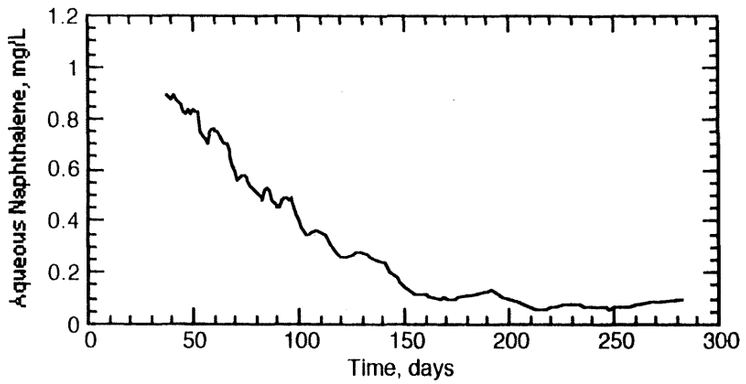



**Figure 28**. Column 1: Naphthalene effluent concentration under anoxic and ambient oxygen conditions.

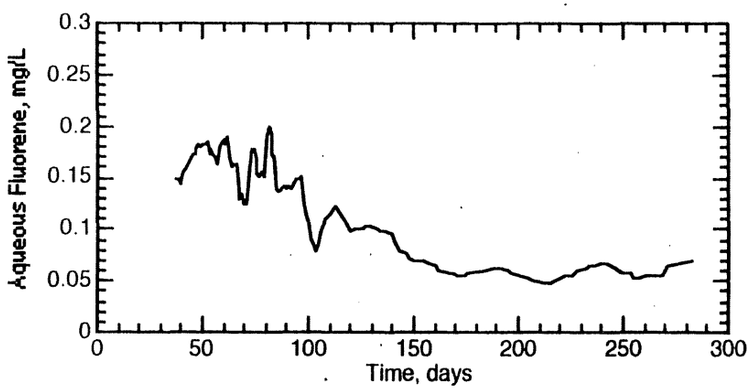



**Figure 29**. Column 1: Fluorene effluent concentration under anoxic and ambient oxygen conditions.

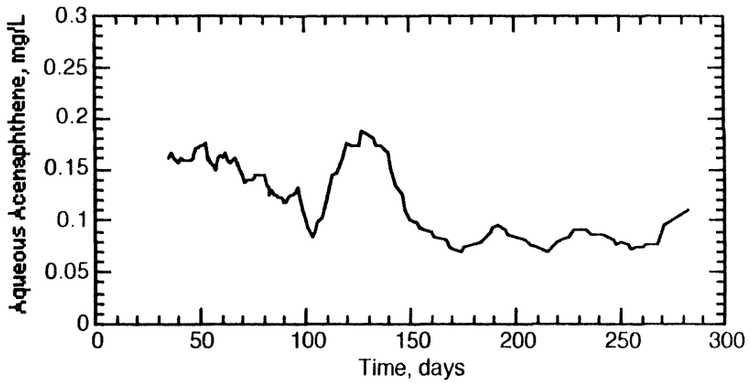



**Figure 30**. Column 1: Acenaphthene effluent concentration under anoxic and ambient oxygen conditions.

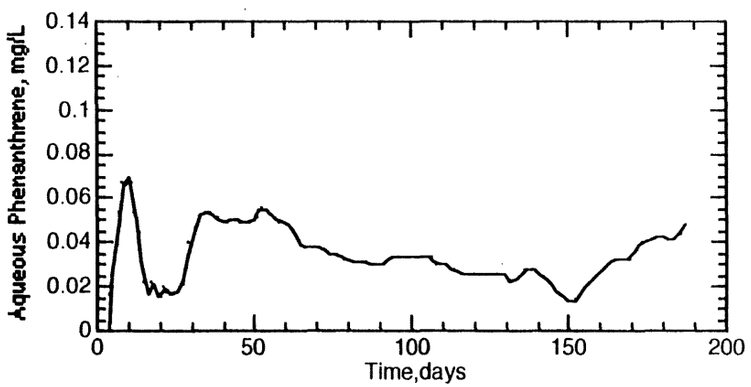



**Figure 31**. Column 2: Phenanthrene effluent concentration under anoxic and ambient oxygen conditions.

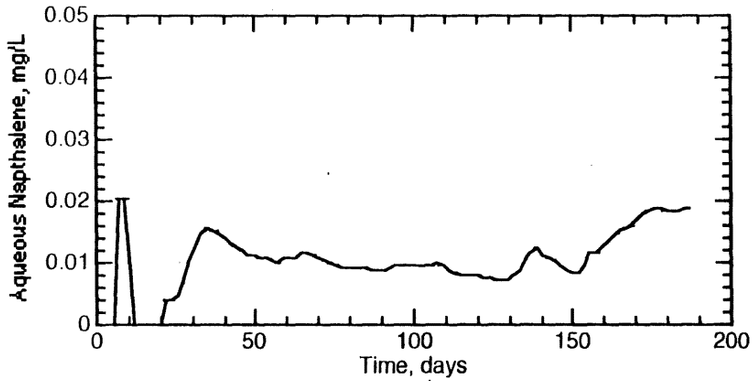



**Figure 32**. Column 2: Naphthalene effluent concentration under anoxic and ambient oxygen conditions.

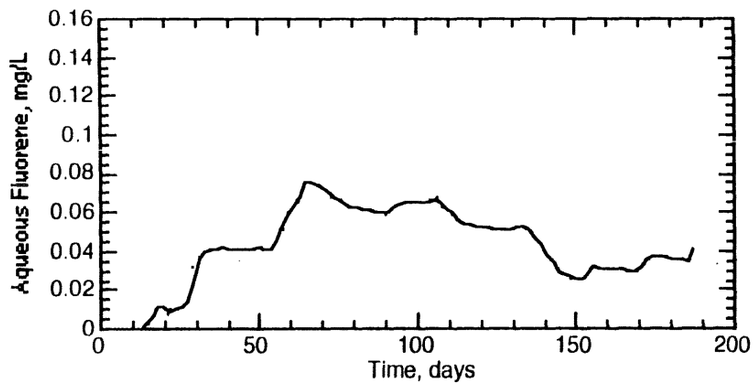



**Figure 33**. Column 2: Fluorene effluent concentration under anoxic and ambient oxygen conditions.

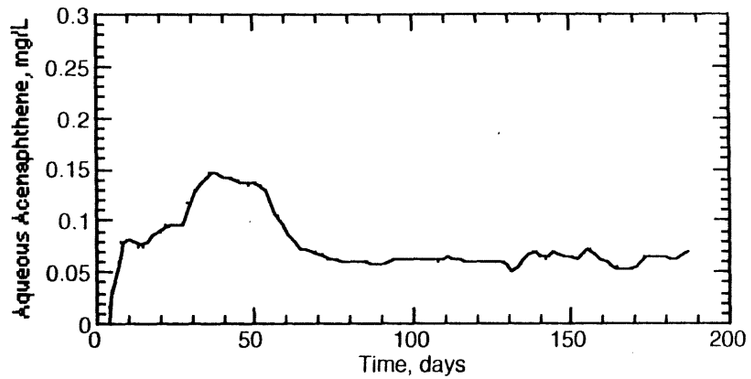



**Figure 34**. Column 2: Acenaphthene effluent concentration under anoxic and ambient oxygen conditions.

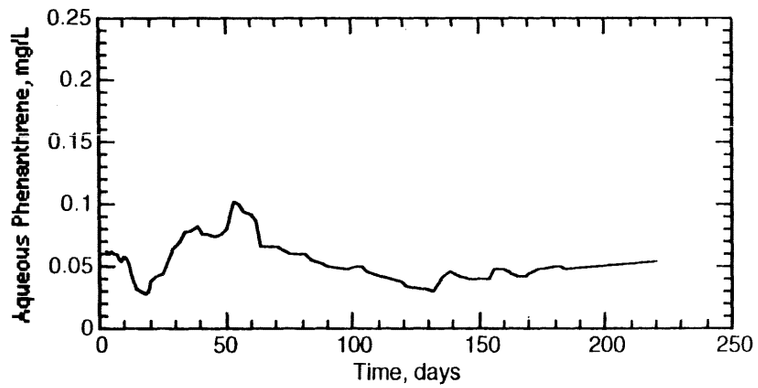



**Figure 35**. Column 3: Phenanthrene effluent concentration under anoxic and ambient oxygen conditions.

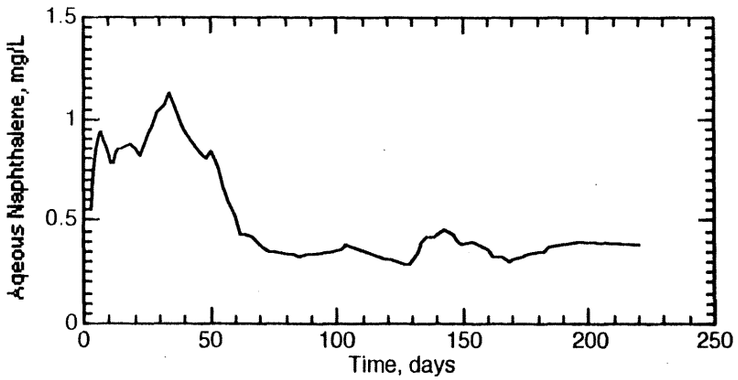



**Figure 36**. Column 3: Naphthalene effluent concentration under anoxic and ambient oxygen conditions.

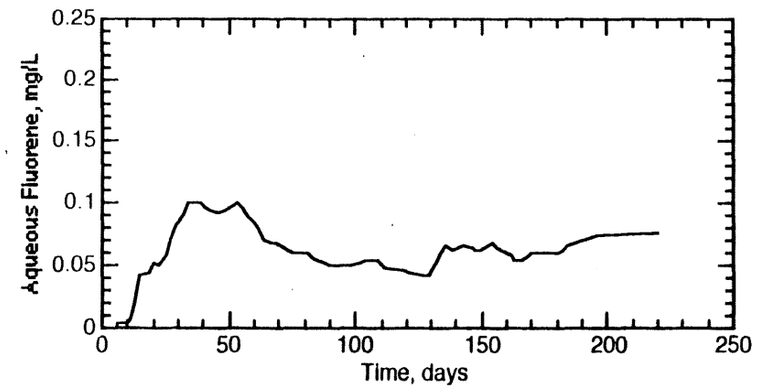



**Figure 37**. Column 3: Fluorene effluent concentration under anoxic and ambient oxygen conditions.

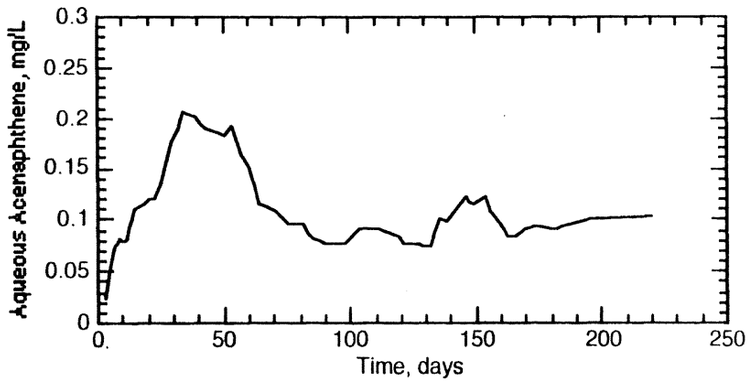



**Figure 38**. Column 3: Acenaphthene effluent concentration under anoxic and ambient oxygen conditions.

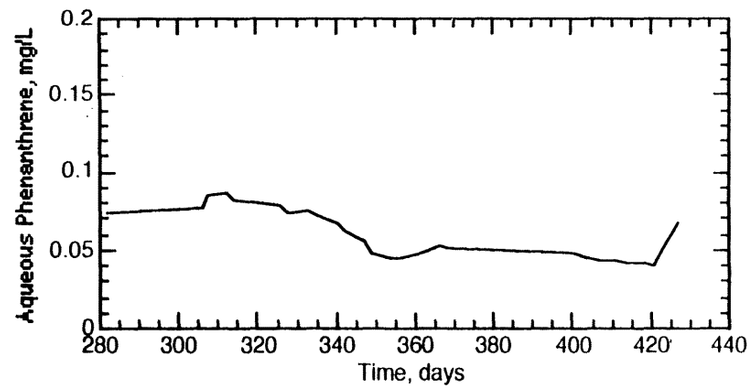



**Figure 39**. Column 1: Phenanthrene effluent concentration under low and high flow conditions.

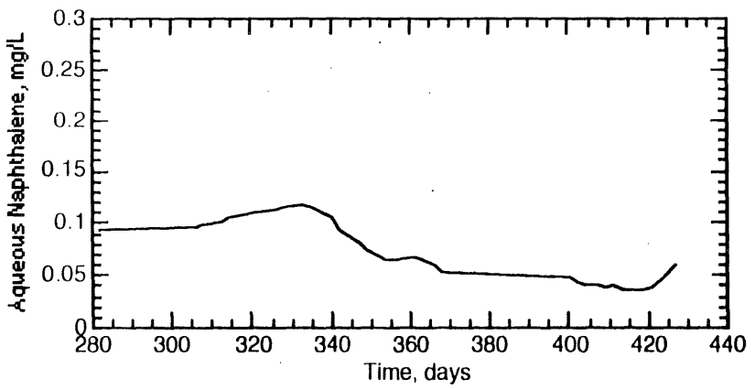



**Figure 40**. Column 1: Naphthalene effluent concentration under low and high flow conditions.

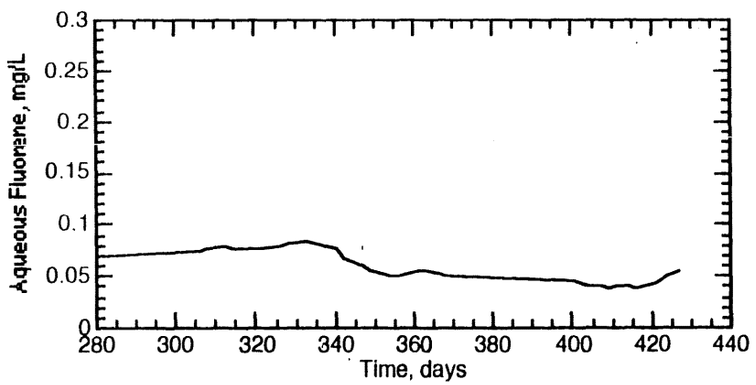



**Figure 41**. Column 1: Fluorene effluent concentration under low and high flow conditions.

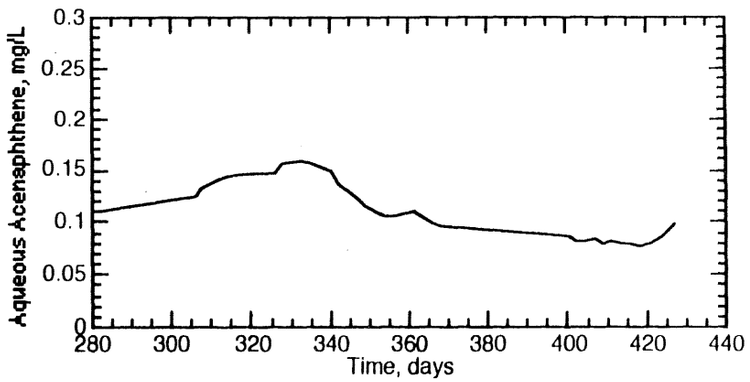



**Figure 42**. Column 1: Acenaphthene effluent concentration under low and high flow conditions.

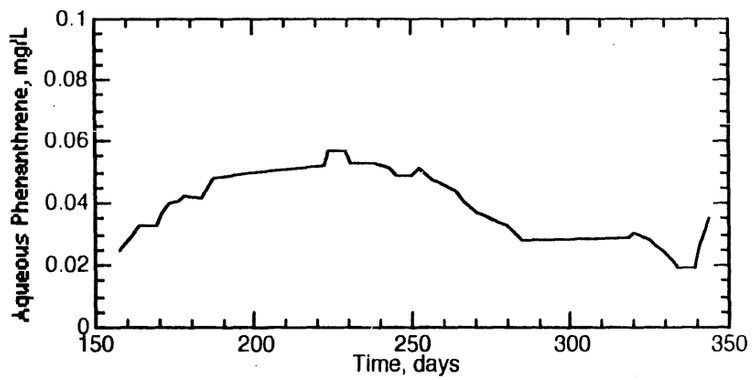



**Figure 43**. Column 2: Phenanthrene effluent concentration under low and high flow conditions.

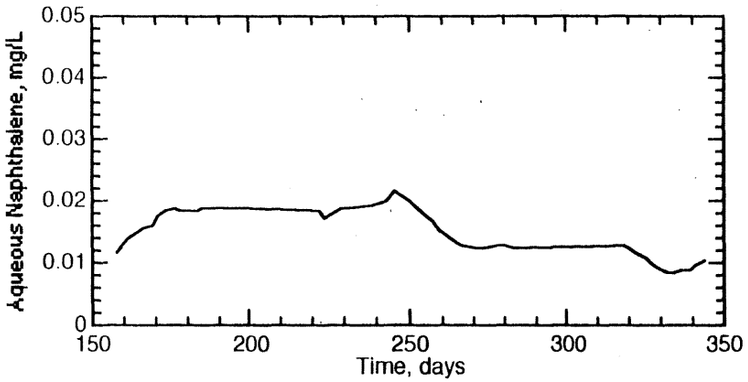



**Figure 44**. Column 2: Naphthalene effluent concentration under low and high flow conditions.

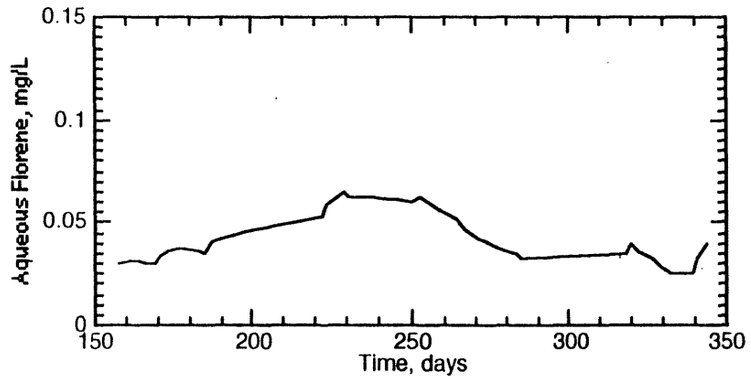



**Figure 45**. Column 2: Fluorene effluent concentration under low and high flow conditions.

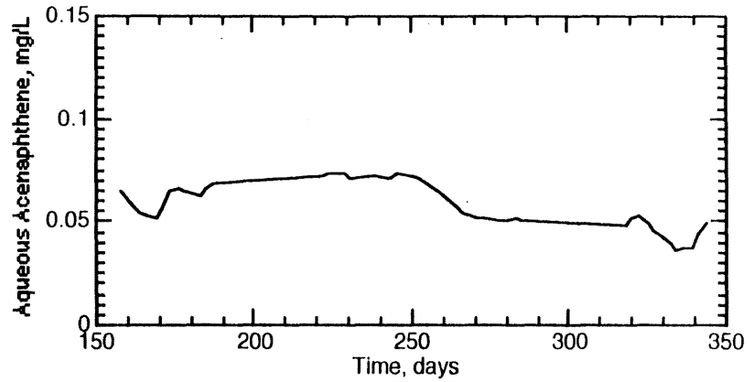



**Figure 46**. Column 2: Acenaphthene effluent concentration under low and high flow conditions.

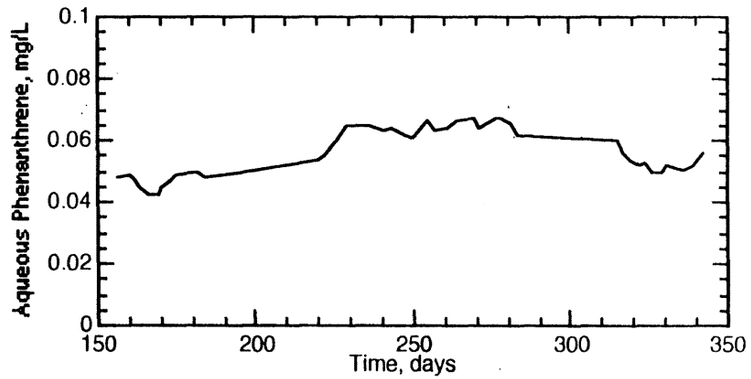



**Figure 47**. Column 3: Phenanthrene effluent concentration under low and high flow conditions.

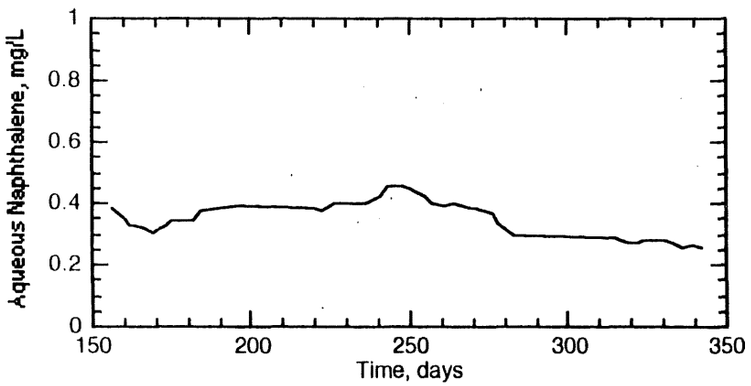



**Figure 48**. Column 3: Naphthalene effluent concentration under low and high flow conditions.

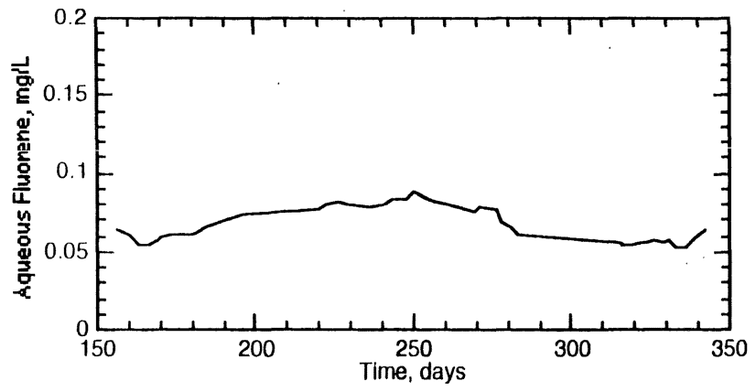



**Figure 49**. Column 3: Fluroene effluent concentration under low and high flow conditions.

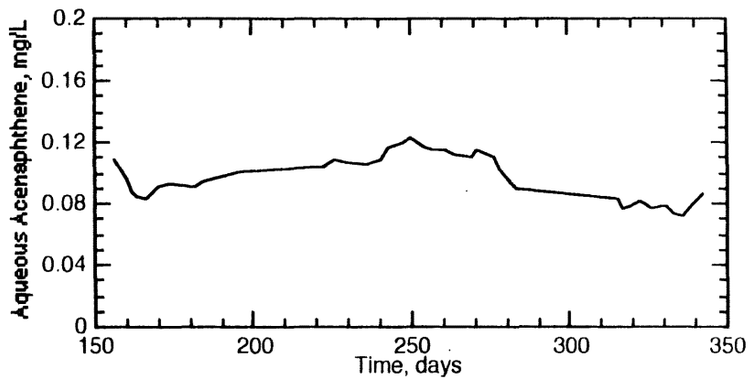



**Figure 50**. Column 3: Acenaphthene effluent concentration under low and high flow conditions.

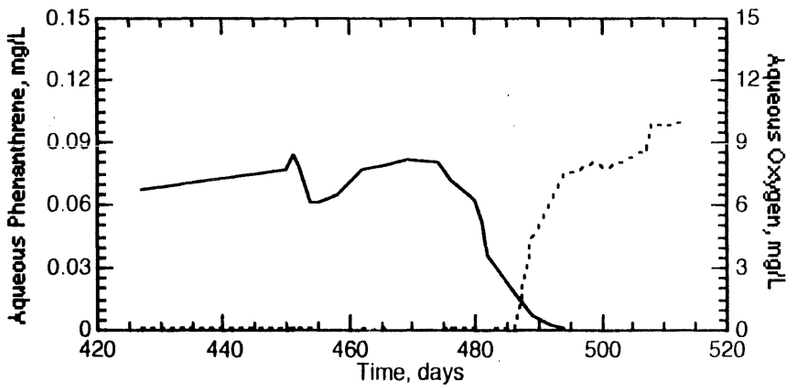



**Figure 51**. Column 1: Phenanthrene and oxygen effluent concentrations under saturated oxygen conditions.

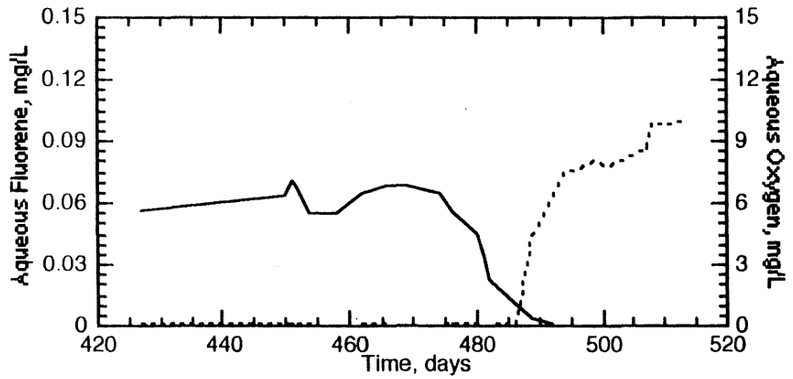



**Figure 52**. Column 1: Fluorene and oxygen effluent concentrations under saturated oxygen conditions.

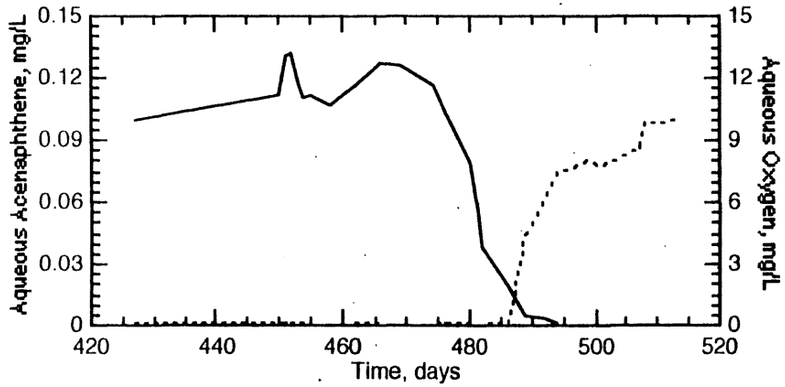



**Figure 53**. Column 1: Acenaphthene and oxygen effluent concentrations under saturated oxygen conditions.

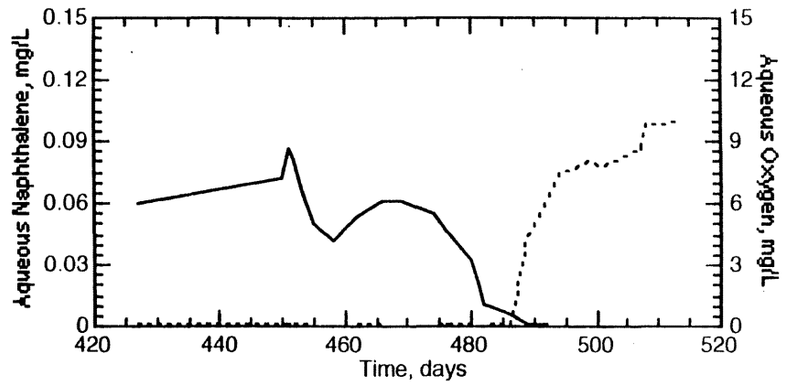



**Figure 54**. Column 1: Naphthalene and oxygen effluent concentrations under saturated oxygen conditions.

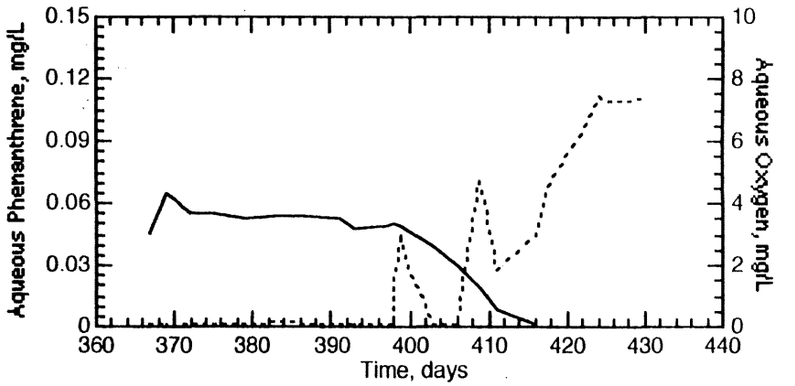



**Figure 55**. Column 2: Phenanthrene and oxygen effluent concentrations under saturated oxygen conditions.

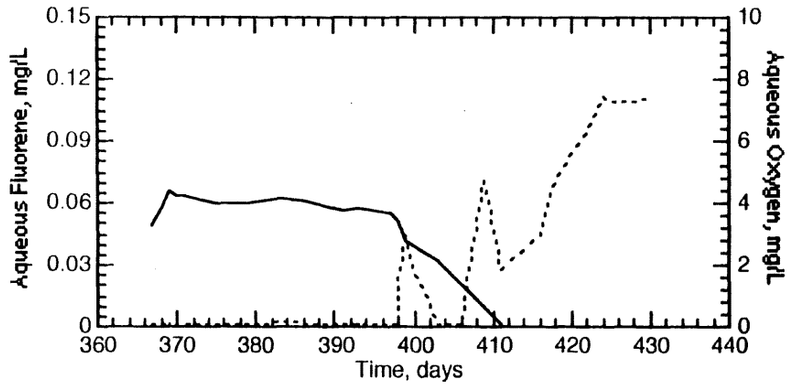



**Figure 56**. Column 2: Fluorene and oxygen effluent concentrations under saturated oxygen conditions.

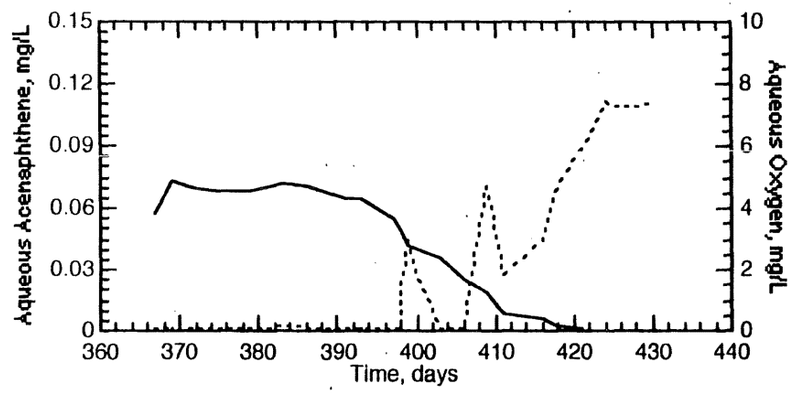



**Figure 57**. Column 2: Acenaphthene and oxygen effluent concentrations under saturated oxygen conditions.

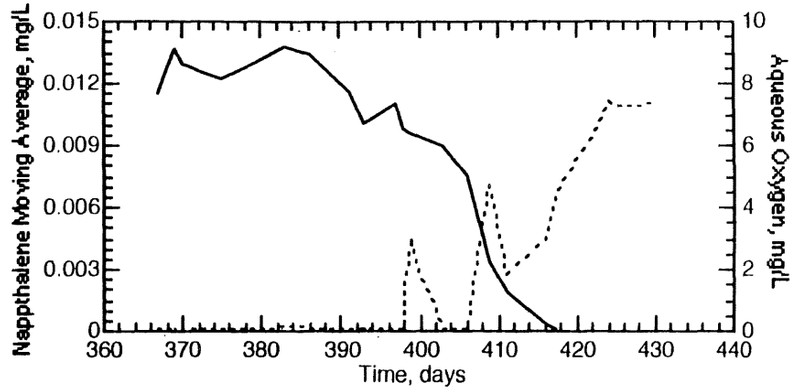



**Figure 58**. Column 2: Naphthalene and oxygen effluent concentrations under saturated oxygen conditions.

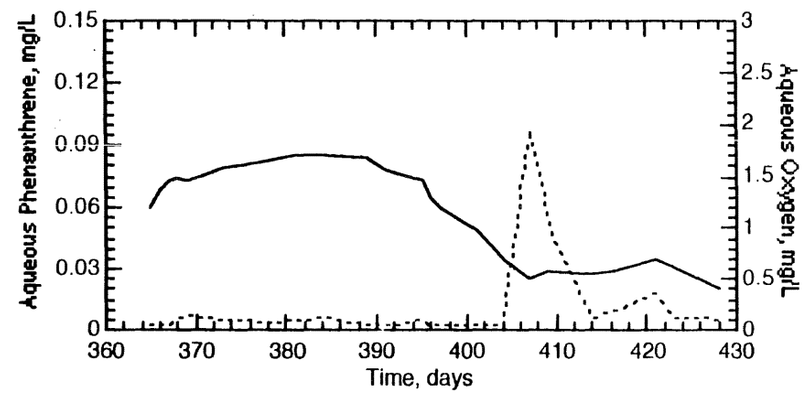



**Figure 59**. Column 3: Phenanthrene and oxygen effluent concentrations under saturated oxygen conditions.

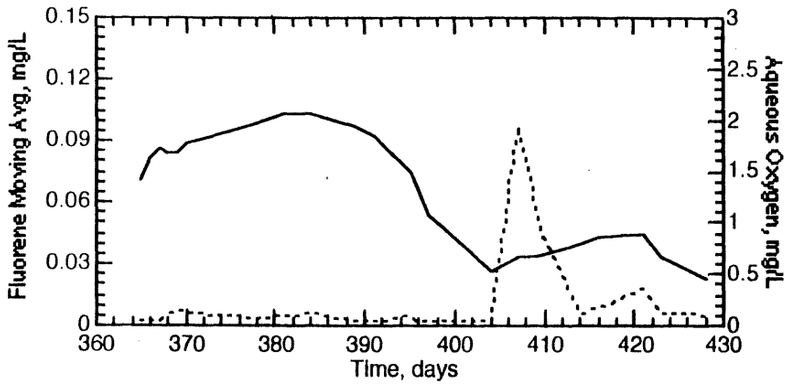



**Figure 60**. Column 3: Fluorene and oxygen effluent concentrations under saturated oxygen conditions.

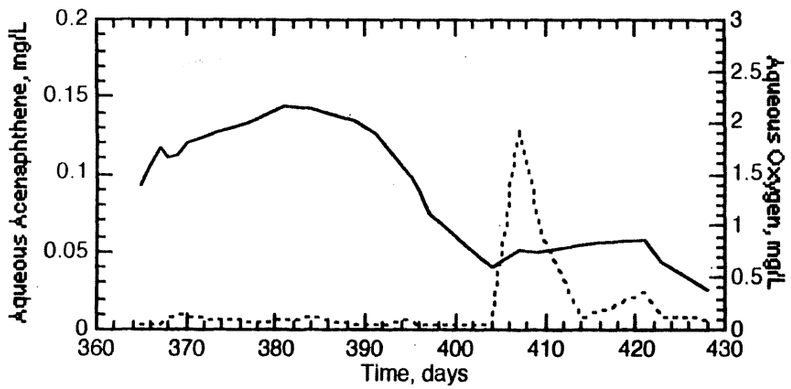



**Figure 61**. Column 3: Acenaphthene and oxygen effluent concentrations under saturated oxygen conditions.

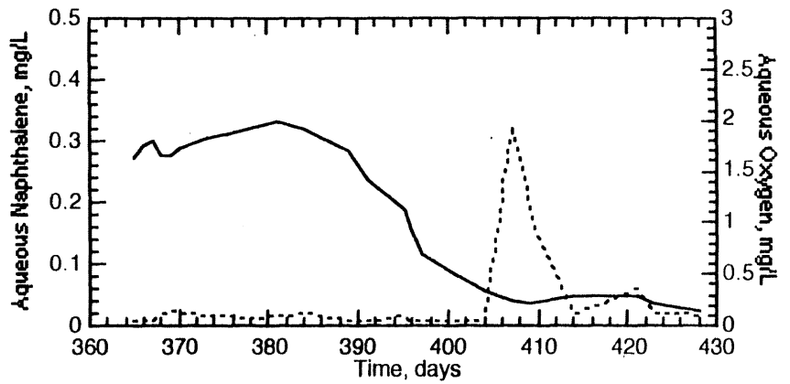



**Figure 62**. Column 3: Naphthalene and oxygen effluent concentrations under saturated oxygen conditions.

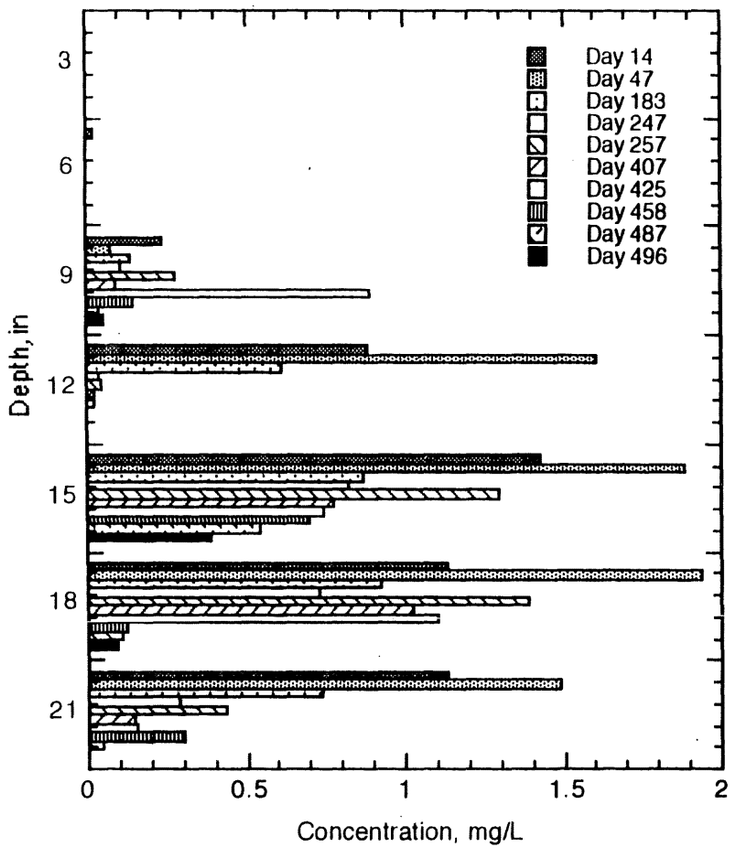



**Figure 63**. Total PAH Profile from column 1 sampling ports.

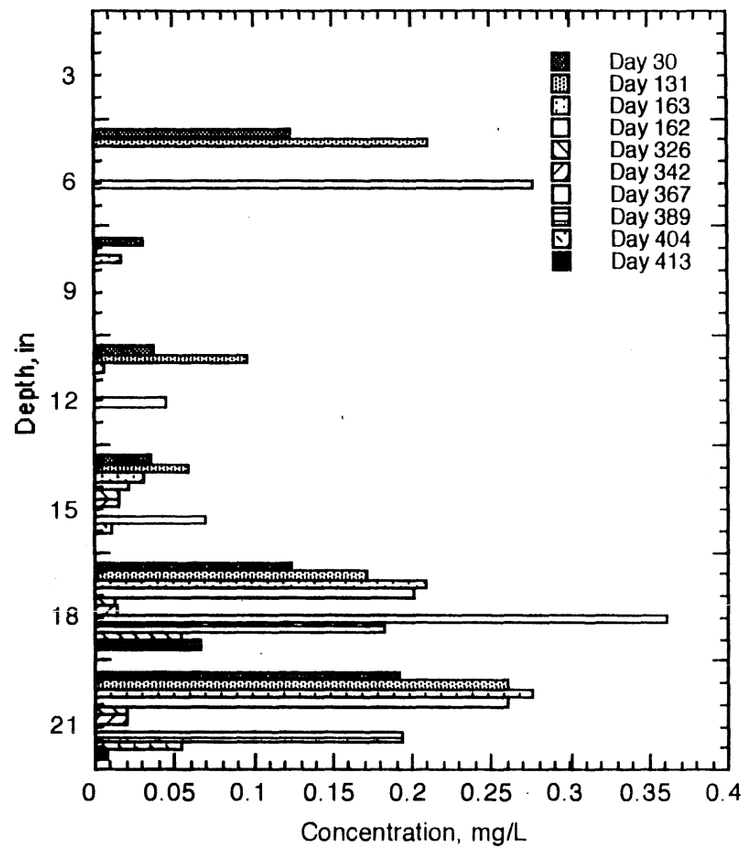



**Figure 64**. Total PAH Profile from column 2 sampling ports.

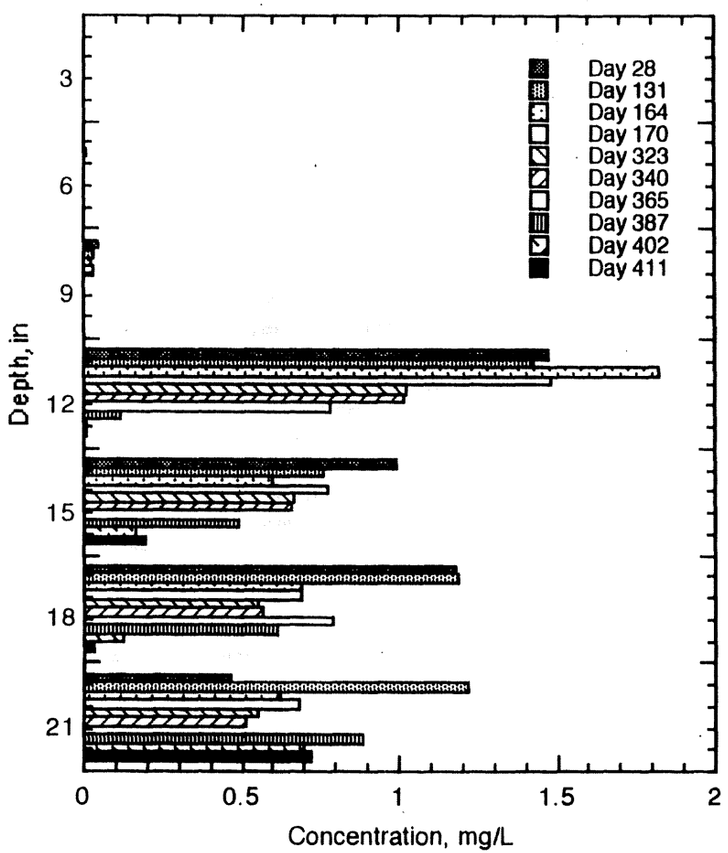



**Figure 65**. Total PAH Profile from column 3 sampling ports.

**Part A Budget:**

Amount budgeted: $ 49,000.00 Balance: $ 0.00

**Part A Timeline**

| | Jan 92 | Jun 92 | Jan 93 | Jun 93 |
|---|---|---|---|---|
| Column Tests A1 | ---------- | | | |
| Column Tests A2 | ---------- | | | |
| Column Test A3 | | | ------ | |
| Column Tests A4-A5 | | | ---------- | |
| Analysis Models Correlation | ---------- | ---------- | ---------- | ---------- |

**Part B Results:**

1. NARRATIVE:

A better understanding of bioremediation in unsaturated soils (vadose zone) and the underlying saturated water zone is essential for effective field applications because:

a. The vadose zone is the passage-way and most common point of entry of pollutants.
b. The vadose zone has been shown to be a significant source of contamination of underlying groundwater due to leaching.
c. The vadose zone has the potential for supporting an active flora of microorganisms that can serve as a protection for underlying aquifers.

The objectives of this part of the study are to measure the effects of controllable process variables and to correlate the data obtained under unsaturated flow conditions that simulate the vadose zone in contact with a water table.

Transport and biodegradation of PAH's will be measured in a box-shaped microcosm that is designed to simulate the combined effects of vertical infiltration into the vadose zone and horizontal flow in the underlying saturated zone. Flow to the microcosm should be a combination of surface infiltration and horizontal flow. The objective is to define environmental conditions to maximize biologically mediated interception of chemicals in the vadose zone and minimize offsite transport of chemicals in the associated saturated groundwater. This phase of the study is a small scale demonstration that builds on the principles established in the previous column tests.

Specific objectives are:

1. To measure the effects of adding inoculum with infiltration water in order to minimize transport of chemicals to the water table.

2. To determine whether addition of excess nutrients is beneficial.

3. To determine whether the application of frequent but small doses of "irrigation" water have beneficial effects in accelerating biodegradation thereby minimizing the subsequent transport of pollutants to the groundwater table.

4. The microcosm will also be used to measure the effects of periodic raising and lowering of the water table on rates of insitu biodegradation. Manipulation of the water table is seen as having potential for accelerated aerobic biodegradation in the vadose zone and the near surface saturated zone.

2. Procedures:

   a. Study of Pollutant Transport and Fate in Microcosm:

      A box shaped microcosm has been constructed for studying the fate and transport of groundwater and soil contaminants. This shape microcosm is needed to study the behavior of contaminated soil in the vicinity of the water table. This region is critical because it involves two dimensional flow and transport of chemicals. Infiltration flow in the vadose zone is vertical whereas groundwater flow below the water table is predominantly horizontal. The box type microcosm allows simulating the two dimensional flow regimes by introducing a saturated flow in the lower layer and vertical infiltration flow through the surface. Construction and operation protocols are described below.

      The microcosm was constructed with 0.125" stainless steel walls to minimize adsorption of chemicals on the walls. Its dimensions are 3' (L) X 1' (W) X 2' (H). Inlet and outlet chambers at each end of the microcosm are 3" (L) X 1' (W) X 2' (H). They serve as stilling basins that are designed to minimize flow disturbances in the bed. Figure 66 shows the side-view cross-section of the microcosm with the configuration of sampling points.

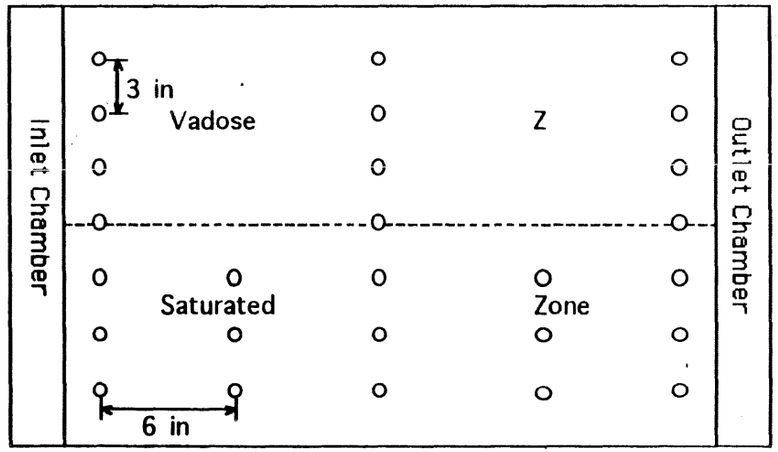



**Figure 66.** Location of sampling ports in the microcosm

1. Inlet and Outlet Chambers:

   The inlet and outlet chambers are separated from the soil bed by a 100 mesh stainless steel screen as shown in Picture 5. The upper half of the wall is solid steel sheeting. This arrangement is used to hold the soil in place. It allows creating a water saturated zone in the lower part of microcosm and a vadose zone condition in the upper half. The top half of both the inlet and outlet chambers was constructed with a stainless steel plate so that only vertical infiltration of water is allowed to enter the soil in the upper part of the microcosm.

   A magnetic stirrer is attached to the side wall of both chambers to facilitate mixing. A FlexMaster pump is used to pump and meter the flow of water to the inlet chamber through a 0.25" swagelock fitting. Effluent is allowed to overflowed from outlet chamber through a 0.25" teflon fitting for collection.

2. Sampling Wells:

   A map locating the sampling ports is attached as Figure 1. All sampling wells are installed horizontally in both unsaturated and saturated zone rather than vertically to minimize flow disturbances. Wells were constructed with 316 stainless steel tube with a 0.25" OD. In the saturated zone, each tube well is perforated, approximately 4 holes per well. The perforated area was then wrapped with 200 mesh stainless steel screen to prevent soil particle from plugging the holes. Sampling well in the unsaturated zone are constructed with high flow rate ceramic cups. Water samples are obtained by applying suction using a syringe; specific flow rate can be as high as 50 mL/hr/cm$^2$. All wells are connected to the wall of microcosm through a female connector and a luer lock connector. For sampling, a luer lock syringe is connected to the luer lock fitting to suck water out of the soil.

3. Piezometer:

   Four piezometer are placed on the back of the microcosm to monitor the water table level in the soil. The piezometer is constructed with 0.25" clear teflon tubing attached to a copper fitting. The level of water in the tube are measured to determine changes corresponding to the water table in the microcosm. The depth of the capillary fringe will be determined with reference to the water depth in the inlet compartment.

4. Procurement and Placement of Soil:

   The microcosm is being loaded with well mixed soil for the initial studies. Ultimately, minimally disturbed soil reactions will be tested. Clean Jordan aquifer sand is used to pack the bottom one foot of depth to simulate a low organic content aquifer that will serve as the saturated zone for groundwater flow. Placement of soil involves addition of approximately one inch layers to obtain uniform distribution. Enough sand to form about a 1" layer is dropped into the box. The sand is then compacted by moving back and forth across each lift with a piece of stainless steel plate. Tapping on the sides of the microcosm with a light rubber hammer cause the sand to settle and eliminate large channels. When the sand layer reaches a height at which sampling wells are installed, the wells are locked to the connectors and additional layers of sand added as above. Thus the presence of sampling wells does not interfere with packing of sand above or below the wells. It is however necessary to carefully pack sand around each well.

   Contaminated soil was obtained from the Reilly Site in sufficient quantity to fill the top half of the microcosm. It was obtained from approximately the same location that was sampled previously for use in the minimally disturbed soil columns described in part A. Soil was obtained using a flight auger over a depth range starting at 4 feet to a depth of 18 feet. The soil has been mixed to generate a uniform soil for loading. Composite samples have been analyzed to measure PAH's and total organic carbon. Water is not used before packing the soil in order to create an

unsaturated zone. The packing procedure is otherwise the same as for the Jordan sand.

5. Ongoing Experimental Program:

The objective of the microcosm study is to test the soil environment that exits in the vicinity of the water table and includes both saturated and vadose flow conditions. More specifically the microcosm study will provide additional direct evidence of the biodegradation of wood preserving chemicals by indigenous microorganisms during simulated aquifer treatment. This type of study is am important intermediate experiment prior to full-scale field application.

Specific objectives include:

1. Characterizing the hydrodynamic features of the system using a mixture of conservative tracers (chloride and bromide). Tracers test will be run at the beginning and again at the end of the study to see if any changes have occurred. The data will be analyzed to determine dispersion coefficients and to assess dispersion/mixing of the percolation water into the saturated groundwater flow zone.

2. Testing the effects of a range of infiltration rates on transport of chemicals into the saturated groundwater phase.

3. Evaluation of process benefits for increasing availability of oxygen.

4. Alternatives strategies for adding oxygen will be tested to determine relative effectiveness for increasing oxygen concentration.

As indicated above, this phase of the experimental program is in progress. It is am integral part of Jian-Shin Chen's Ph. D. thesis. Expected date of completion is early 1994. The results will be published in his thesis as well as in professional journals.

Financing for this extension of the program has been arranged from other sources

**Part B Budget**

Amount budgeted: $ 16,000.00 Balance: $ 0.00

**PartB Timeline**

| | Jun 92 | Jan 93 | Jun 93 |
|---|---|---|---|
| Microcosm construction and calibration | | | |
| Insitu biodegradation | ---------- | | |
| Analysis/correlation of data | | ---------- | |

## IV. EVALUATION:

The status of the program will be evaluated in terms of the following milestones:

1. Completion of a report describing the column studies with pentachlorophenol.
2. Completion of a report describing the column studies with phenanthrene.
3. Presentation of generalized correlation of insitu biodegradation in saturated soil/groundwaters.
4. Report on calibration of microcosm apparatus.
5. Analysis of insitu biodegradation rates in box microcosm.

## V. CONTEXT:

Insitu biorestoration of aquifer soils and groundwaters is a developing technology that is environmentally sound and potentially much less costly than alternative methods for removing organic pollutants. It has potential applications

in cleaning up contamination problems resulting from fuel spills, industrial discharges, and agricultural chemical residues.

We have done extensive basic research on the kinetics of biodegradation of organic chemicals and on the transport properties of microbial cells. The results of this prior research, which was funded by federal agencies, provides a solid foundation for the proposed development of engineering design tools.

## VI. QUALIFICATIONS:

1. Program Manager:

**Walter J. Maier**
Professor, Dept. of Civil and Mineral Engineering,University of Minnesota

| | |
|---|---|
| Academic training: | B.S. Chemical Engineering, Rensselaer Polytechnic Institute<br>Ph.D. Environmental /Civil Engineering, Cornell University |
| Professional Experience: | Research Engineer , Exxon Research & Engineering Company<br>Visiting Professor , Dow Chemical Company<br>Visiting Professor Princeton University<br>Professional Engineer, Minnesota Registration |
| Major Research Interests : | Kinetics and dynamics of biological wastewater treatment processes<br>Biochemical treatment of xenobiotics and plastics<br>Bioremediation of soils and groundwaters |

Publications:

54 publications and presentations in the areas of microbiological processing, environmental water quality , and water supply technology. Related recent publications:



Kinetics of biodegradation of chlorinated organics in activated sludge type systems, Journ. WPCF, 52:8, 2158-2166, 1980.

Kinetics of biodegradation of 2,4-dichlorophenoxy acetic acid in the presence of glucose, Biotech. and Bioeng., XXIV, 2001-2011, 1982.

Dynamics of biodegradation of 2,4-dichlorophenoxyacetic acid in the presence of glucose, Biotech. and Bioeng., XXIV, 2337-2346, 1983.

A new modeling technique and computer simulation of bacterial growth, Biotech. and Bioeng., XXVI, 275-284, 1984.

Kinetics of microbial growth on pentachlorophenol, Applied and Environ. Microbiol., 49:1, 46-53, 1985.

Acclimation and biodegradation of chlorinated organic compounds in the presence of alternate substrates, Journ. WPCF, Feb. 1986.

Biodegradation kinetics of mixtures of substrates that exhibit both inhibition and enhancement, International Conf. on Innovative Biological Treatment of Toxic Wastewaters, Arlington, VA, June 1986.

Effects of soil surfaces on biokinetics of PCP biodegradation, International Conf. on Innovative Biological Treatment of Toxic Wastewaters, Arlington, VA, June 1986.

Biodegradation of pentachlorophenol in soil environments, Proceedings, Purdue Industrial Waste Conference, 1986.

On-site biodegradation of organic pollutants in contaminated soils and groundwater, Bulletin #123, Water Resources Research Center, University of Minnesota, 1987.

Process considrations in biological treatment of low concentration wastewaters:I. Steady State, Z. Wasser-Abwasser Forsch., 20, 85-90, 1987.

Process considrations in biological treatment of low concentration wastewaters: II. Dynamics, Z. Wasser-Abwasser Forsch., 20, 108-112, 1987.

Kinetics of microbial growth on mixtures of pentachlorophenol and chlorinated aromatic compounds, Biotech. and Bioeng., XXXI, March 1988.

Transport characteristics of a pentachlorophenol acclimated bacterium through aquifer sand columns, IAWPRC Meeting, Stanford University,1989

2. Cooperators:

**Eric C. Tam**
Assistant Scientist
Department of Civil and Mineral Engineering, University of Minnesota

Education and Experience:

B.S., University of Wisconsin-Whitewater, 1976
M.S., University of Wisconsin-Madison, 1984 (Microbiology)
Instructor, Bacteriology, University of Wisconsin-Madison, 1983-84
Junior Scientist, Gray Freshwater Biological Institute, 1984-87

Publications:

Tam, E., and J.L. Pate. Amino Acid Transport by Prosthecae of Asticcacaulis biprosthecum: Evidence for a Broad Range Transport System. J. Gen. Microbiol., 131: 2687-2699.

Newman, R., J.A. Perry, E. Tam, and R.L. Crawford. Effects of Chronic Exposure of Chlorine on Litter Processing in Outdoor Experimental Channels. Manuscript in Preparation.

3. Other Investigators:

The following poeple have been involved in formulating the research plan and have agreed to serve as unpaid consultants on this project:

**Michael Scott**, Senior Engineer, MPCA, Tel: (612) 296-7297.

**Justin Blum**, Hydrogeologist, Minnesota State Health Department, Tel: (612) 627-5165.

**Dr. Lawrence Wackett**, Microbiologist, Gray Freshwater Biological Institute, UM-Minneapolis, Tel: (612) 471-9493.

**Dr. Michael Sadowsky**,Soil Microbiologist, Dept. of Soils, UM-Minneapolis, Tel: (612) 624-2706.

**Dr. Raina M. Miller**, Microbial Ecologist,Dept. of Soils, U of Arizona, Tel: (515) 294-0527.