This is to certify that the

thesis entitled

Rates of Removal of Dissolved Gasoline Components from Laboratory Soil Columns with and without Microbiological Activity

presented by

Mara E. Hollinbeck

has been accepted towards fulfillment of the requirements for

M.S. degree in Environmental Engineering

Major professor

Date April, 1995

RATES OF REMOVAL OF DISSOLVED GASOLINE COMPONENTS FROM LABORATORY SOIL COLUMNS WITH AND WITHOUT MICROBIOLOGICAL ACTIVITY

By

Mara E. Hollinbeck

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Civil and Environmental Engineering

1995

ABSTRACT

RATES OF REMOVAL OF DISSOLVED GASOLINE COMPONENTS FROM LABORATORY SOIL COLUMNS WITH AND WITHOUT MICROBIOLOGICAL ACTIVITY

By

Mara E. Hollinbeck

A preliminary study was performed to identify the factors affecting remediation of gasoline contaminated soil under saturated flow conditions. After contamination with groundwater containing benzene, toluene, ethylbenzene, *o*-xylene, and naphthalene, a series of "active" columns were seeded with an acclimated mixed culture of microorganisms and flushed with highly oxygenated nutrient-enhanced groundwater. "Inactive" columns were flushed using a deoxygenated non-nutrient-enhanced solution. Effluent contaminant concentrations, dissolved oxygen levels, microbiological activity levels, and solid phase contaminant concentrations from sacrificed columns were measured as flushing proceeded.

Microbiological conditions within each column system were significantly different based on dissolved oxygen data and microbial plating results. Flow interruption showed non-equilibrium partitioning was occurring. Both column systems approached similar liquid remediation levels rapidly indicating that microorganisms did not offer a significant advantage in the extent of contaminant depletion. Mass balance calculations indicate substantial error associated with solid phase analysis.

TABLE OF CONTENTS

LIST OF TABLES

TABLE

LIST OF FIGURES

FIGURE

CHAPTER 1

INTRODUCTION AND RESEARCH OBJECTIVES

1.1. Introduction.

Significant attention has been focused on the issue of groundwater remediation specifically related to petroleum hydrocarbons due to contamination from leaking underground storage tanks (USTs). From a regulatory standpoint, the alkylbenzene components (or light aromatic compounds), which include benzene, toluene, ethylbenzene, and xylene (BTEX), are of particular concern due to their potential for rapid migration relative to other contaminants (Voice, 1993). Drinking water contamination is the primary route of human exposure to these chronically toxic compounds (Voice, 1993). Heavier components of gasoline, including naphthalene (a polyaromatic hydrocarbon, PAH), are also of interest for comparative purposes due to their increased persistence.

Pumping contaminated groundwater to above ground treatment facilities (pump and treat) has been, and continues to be, an accepted and common method of treatment, however, sorption of contaminants to soil and organic matter tends to complicate removal rates. Pumping a system until the removed water meets a desired level of treatment does not ensure complete aquifer restoration (Piwoni and Keeley, 1990). Desorption, driven by the concentration gradient between the sorbed and liquid phases, slowly releases the

contaminants, resulting in long treatment times. In addition, after removal from the ground, the contaminated water still requires treatment using above ground bioreactors or activated carbon adsorption systems.

In comparison, in-situ biodegradation has the potential to convert the contamination to innocuous byproducts without removal from the ground. In addition to this advantage, in-situ bioremediation is receiving more attention as a result of the problems associated with pump and treat technologies. Sufficient information on successful remediations, however, has not been provided which prevents assessment of the degree to which each process and parameter influences the performance of bioremediation (Abriola and Chen, 1993). In either case, there remain unanswered questions regarding the relationship between sorption and biodegradation.

The purpose of this study was to make a direct comparison between two systems which loosely represent the two technologies commonly used to remediate contaminated groundwater, namely pump and treat and in-situ biodegradation. Of primary interest was the rate of remediation observed using either technology to decrease the level of soil contamination in two one-dimensional saturated soil column systems contaminated with dissolved gasoline components. The two systems differed only in that one was biologically active (to represent in-situ biodegradation) while the second was inactive (to represent removal by pumping alone). This direct, quantitative comparison was intended to allow conclusions to be drawn regarding the relative rate of clean-up allowed in either system

along with possible factors affecting this rate. The target compounds include benzene, toluene, ethylbenzene, and *o*-xylene (BTEX) along with naphthalene.

1.2. Research Objectives.

The hypothesis underlying this research is that the degree to which mass transfer limits solid phase removal rates of dissolved gasoline components from saturated soil columns will be decreased by the presence of degradative microorganisms. The underlying assumption is that solid phase removal by degradation is unlikely, therefore, under desorption limiting conditions (maintained by optimizing degradation conditions) the effect of microorganisms can be assessed by comparing the two systems under similar conditions. The microorganisms may increase solid phase removal rates by decreasing liquid phase concentrations which results in a higher driving force for desorption. Alternately, or in addition to this effect, the microorganisms may have surfactant-like properties which improve desorption.

Based on the above paragraph the three-fold objective can be explicitly stated as follows. (1) To compare two saturated soil systems one of which is microbiologically active while the second is inactive; (2) To quantify the solid phase removal rates of dissolved gasoline components sorbed to saturated soil through either flushing alone or in the presence of degradative microorganisms; and (3) To assess the effect microorganisms have on the desorption limitation. The results of this study were intended to provide a quantitative comparison between the two primary technologies used to remediate contaminated

groundwater.

1.3. Research Approach.

The first step in meeting the specified research objectives was to design a system with the features necessary to isolate the variables of interest. This process of broadly addressing each issue constitutes the research approach. The importance of this preliminary stage is justified when considering it the foundation for the entire project. In addition, the fundamental reasoning behind use of the experimental system is beneficial when attempting to apply conceptual ideas to further research or research in related areas.

There are essentially four factors upon which the core system features were based. The first factor was the interest in groundwater contamination which specifies that packed soil column systems be used as representative of that dynamic environment. The second factor is the measurement of solid phase contaminant levels which requires the use of multiple columns due to the destructive nature of this procedure. The third factor is related to the comparison aspect which requires two series of columns to be used which differ only in that one

contains microbiological activity while the other is sterile. This assists in isolating the effect microorganisms have on removal rates. Finally, the interest in desorption limitation effects dictates a system in which measurements are taken during column flushing after contaminants have been previously sorbed to the soil.

The result when combining the mentioned factors is a system which includes two series of multiple packed soil columns (active and inactive) flushed to remove previously sorbed contaminants. The remaining components which make up the system are important yet don't change the overall operating system.

CHAPTER 2

LITERATURE REVIEW

Significant attention has been focused on the issue of groundwater remediation specifically related to petroleum hydrocarbons due to contamination from leaking underground storage tanks (USTs). From a regulatory standpoint, the alkylbenzene components (or light aromatic compounds), which include benzene, toluene, ethylbenzene, and xylene (BTEX), are of particular concern due to their potential for rapid migration relative to other contaminants (Voice, 1993). Drinking water contamination is the primary route of human exposure to these chronically toxic compounds (Voice, 1993). Heavier components of gasoline, including naphthalene (a polyaromatic hydrocarbon, PAH), are also of interest for comparative purposes due to their increased persistence.

Pumping contaminated groundwater to above ground treatment facilities (pump and treat) has been and continues to be an accepted and common method of treatment, however, sorption of contaminants to soil and organic matter tends to complicate removal rates. Pumping a system until the removed water meets a desired level of treatment does not ensure complete aquifer restoration (Piwoni and Keeley, 1990). Desorption, driven by the concentration gradient between the sorbed and liquid phases, slowly releases the contaminants, resulting in long treatment times. In addition, after removal from the ground,

the contaminated water still requires treatment using above ground bioreactors or activated carbon adsorption systems.

In comparison, in-situ biodegradation has the potential to convert the contamination to innocuous byproducts without removal from the ground. In-situ bioremediation is also receiving more attention as a result of the problems associated with pump and treat technologies, however, there remains unanswered questions regarding the relationship between sorption and biodegradation. In addition, sufficient information on successful remediations has not been provided which prevents assessment of the degree to which each process and parameter influences the performance of bioremediation (Abriola and Chen, 1993).

Coupled-process (biodegradation and sorption) research was initiated by soil scientists studying the fate of pesticides in soils (Angley *et al.*, 1992). More current research has focused on the bioavailability of sorbed contaminants to microbial species. Sorption, theoretically can cause either an increase or a decrease in bioconversion rate (Rijnaarts *et al.*, 1990). An increase would result if the contaminant is toxic to the microbes since sorption will reduce the level of solution contaminant thereby lowering the impact on the microbes using only dissolved substrates. More often a decrease in rate is seen for one of two reasons. The first is due to a decrease in aqueous phase concentration below threshold levels while a second effect is realized when desorption controls bioconversion which limits high potential rates due to slow desorption velocities.

Numerous studies have been performed with the specific intent of assessing the relationship between sorption and biodegradation. Results vary depending on the contaminant and contaminated matrix (sediments or soil), organic matter content, moisture content, microorganism(s) involved, age of contamination, and type of system (batch or column). Generally the studies can be divided into three interrelated areas of interest: bioavailability, modeling issues, and rate limitations.

Modeling is of value not only as a method for quantifying the transport and degradation of contaminants but for use as a predictive tool and way to understand the mechanisms involved in both processes. Sorption was typically found to follow a two-phase process whereby a fraction sorbs instantaneously while the remainder sorbs in a time-dependent or rate-limited manner (van Genuchten *et al.*, 1989; Angley *et al.*, 1992). Rate limited sorption models were accordingly found to fit data better than models which assume local equilibrium exists (Angley *et al.*, 1992) except for compounds which sorb to a lesser degree (i.e. benzene). Models also exist which include the use of the local equilibrium assumption (LEA) (Scow and Hutson, 1992). The validity of this assumption can be tested using the flow interruption technique as described by Pennell *et al.* (1993).

Non-linear sorption has been attributed to structural heterogeneity at the intraparticle scale in terms of microporosity while hysteresis was found to contribute due to irreversible sorption to organic matter (Farrell and Reinhard, 1994). Linear sorption is also a common assumption (Scow and Hutson, 1992). Slow release (desorption) of compounds has been attributed to

restricted diffusion through both soil organic matter and intraaggregate pores (Farrell and Reinhard, 1994). Residual contaminants also resist mobilization and microbial degradation due to entrapment in intraparticle micropores (Steinberg *et al.*, 1987). Biodegradation is often assumed to follow first-order kinetics (Angley *et al.*, 1992) however, exceptions do exist. For example, Scow and Hutson (1992) found that biodegradation was initially fast due to solution phase degradation followed by a slower rate, limited by desorption/diffusion. It is also interesting to note that while some researchers have identified the possibility of enhanced desorption due to microorganisms (Wszolek and Alexander, 1979; Gordon and Millero, 1985; Rijnaarts *et al.*, 1990), no models can accommodate this effect.

Bioavailability of sorbed compounds to degradation has also been addressed in some detail. The most cited of these studies found that sorbed 2,4-dichlorophenoxyacetic acid (2,4-D) was completely protected from microbial degradation but solution 2,4-D was degraded with equal efficiency by both solution and sorbed microorganisms (Ogram *et al.*, 1985). On the other hand, adsorption of phenol, an antimicrobial agent at high concentrations, has been found to increase degradation rates (Scott *et al.*, 1983; van Loosdrecht *et al.*, 1990; Shimp and Young, 1988). Chlorophenols fall somewhere between the two extremes since a "surface" fraction of bound substance was found to be releasable while the remainder is bound to a "core" which is entirely inaccessible to microorganisms (Dec and Bollag, 1988).

Gordon and Millero (1985) tested various substrates that sorb to different degrees on hydroxyapatite and found decreased availability to be a function of the degree of adsorption.

Organism-specific properties were found to dictate whether and to what extent sorbed naphthalene is biologically available by Guerin and Boyd (1992). Finally, quaternary ammonium compounds (QACs - surfactants) were found to be available in the sorbed phase with the explanation being that they were utilized as sole carbon and energy source and have specific enzymes to bind and transport the QACs (Larson and Vashon, 1983).

Rate limitations are primarily thought to be due to desorption (van Loosdrecht *et al.*, 1990; Rijnaarts *et al.*, 1990), however, this may not initially be limiting (Robinson et. al., 1990). Alvarez-Cohen *et al.* (1993) found the transformation reaction to be rate limiting while Weissenfels *et al.* (1992) associated the type of binding with degradation limitations. Finally, diffusion/desorption was found to be faster in non-sterile systems due to a steeper concentration gradient resulting from microbial removal and decreased diffusion distances (Rijnaarts *et al.*, 1990; van Loosdrecht *et al.*, 1990).

Of the research related to this proposal, two studies are of particular interest. One of these performed a direct comparison experiment to test the effect sorption has on biodegradation with the use of three systems (Voice *et al.*, 1992). One system allowed contaminant removal using both sorption and biodegradation (biologically active carbon fluidized bed) while the other two considered removal by only one of the mechanisms, either sorption (granular activated carbon fluidized bed) or biodegradation alone (non-activated carbon with biofilm). This work concluded that substrate sorbed to the outer portions of the particles is readily available to microorganisms. More importantly, the study provided incentive regarding the

type of system necessary to isolate the information of interest to this work.

A second study used computer modeling to compare pump and treat, biorestoration, or a combination of the two technologies under varying conditions (Marquis and Dineen, 1994). Limited published documentation involving direct comparisons between the systems was the impetus for the study. Either in-situ biorestoration alone or in combination with pump and treat were found to be remedial options for sand and gravel aquifers contaminated with biodegradable organic compounds.

The overwhelming interest in comparing remediation alternatives based on efficiency and expediency of site clean-up combined with the scarcity of direct quantitative comparisons validates the need for this work. In particular, the relative merits of pump and treat versus in-situ bioremediation technologies are debated with each side convinced their choice is the "best" often times based only on qualitative comparisons. This study intends to quantify this on-going debate and provide additional "food for thought".

CHAPTER 3

MATERIALS AND METHODS

The two series of packed soil columns which were operated simultaneously are referred to as active and inactive systems based on the presence or absence of microbiological activity, respectively.

3.1. Soil Characteristics.

The soil used for this work was a sandy soil collected from Clare County, Michigan in the spring of 1989. The organic matter content of the soil was 0.5% as determined using a soil adapted CHN analyzer. Since a direct comparison was made between the two sets of columns, it was not necessary to quantify soil parameters such as porosity and bulk density except to verify consistency from column to column. The porosity was 0.35 ± 0.01 while the bulk density was found to be 1.73 ± 0.04 g/cm^3. The pore volume was thus calculated to be 4.22 and used as a basis for graphical comparisons. Equilibrium sorption/desorption partitioning coefficients for this soil were determined to assist in estimation of solid phase contaminant levels using effluent concentrations. The results of this sorption study are presented in Appendix C.

3.2. Contamination.

Simulated gasoline-saturated water reservoirs were generated and used as a uniform contaminant source during contaminant saturation. The target compounds, benzene, toluene, ethylbenzene, *o*-xylene, and naphthalene (BTEXN), were dissolved in each other in the ratios found in gasoline and spiked into water to create levels similar to those found in gasoline-saturated water. The concentrated BTEXN stock solution was prepared by dissolving 0.9 g naphthalene in 40 mL toluene and adding 7.4 mL of this solution to 5.6 mL benzene, 2 mL ethylbenzene, and 1.1 mL *o*-xylene. Multiple reservoirs were then generated by spiking the concentrated BTEXN stock solution into 160 mL serum vials containing deionized water passed through a 0.22 micron filter, two drops of 6 M HCl, two (2) 4 mm glass beads, and approximately 10 mL of headspace. Each reservoir was then sealed, tumbled for two (2) days to promote dissolution and uniform distribution, and refrigerated until use. The initial concentrations of each component were determined and verified periodically throughout the experiment prior to filling the feed syringes. Using the five (5) components mentioned represents the various fractions of contaminants present in gasoline.

The hydrochloric acid was used to prevent microbiological degradation of the substrates prior to or during contaminant saturation. A result of its use was a decrease in column pH which may have created conditions unfavorable for microbiological growth. For this reason, sodium hydroxide (NaOH) was added to the final contaminant solution to produce neutral pH conditions within the columns prior to inoculation.

3.3. Apparatus/Operating Conditions.

3.3.1. Packed Soil Columns.

Two series of 10 borosilicate glass columns (Kontes, 1.0 cm in diameter x 15 cm long) were used with identical fittings and physical constraints. All soil was sterilized initially by gamma-irradiation (2 Mrad). This was necessary to prevent microbiological activity in the inactive columns and was subsequently necessary for the soil in the active columns and for that used in standard curve generation. This precaution addresses the possible effect gamma-irradiation has on the structure of the soil organic matter which could change the sorption characteristics of the soil.

Aseptic column packing included use of sterile gloves and autoclaved equipment in a laminar flow hood. Soil was packed into the columns incrementally using a glass rod to mix after each addition to create uniform packing and prevent layering effects. The bulk density was calculated using the soil mass (found by weighing the columns before and after soil packing) in conjunction with the column volume occupied by soil. The column pore volume and porosity were also determined using these parameters to verify consistency among columns.

3.3.2. Carbon Dioxide Flush and Water Saturation.

The dry soil columns were initially flushed with 240 mL of carbon dioxide (CO_2), at a rate of 10 mL/hr, to replace the air in the pore space. This prevents entrapment of gas during water saturation since the CO_2 will subsequently dissolve. A feed rate of 10

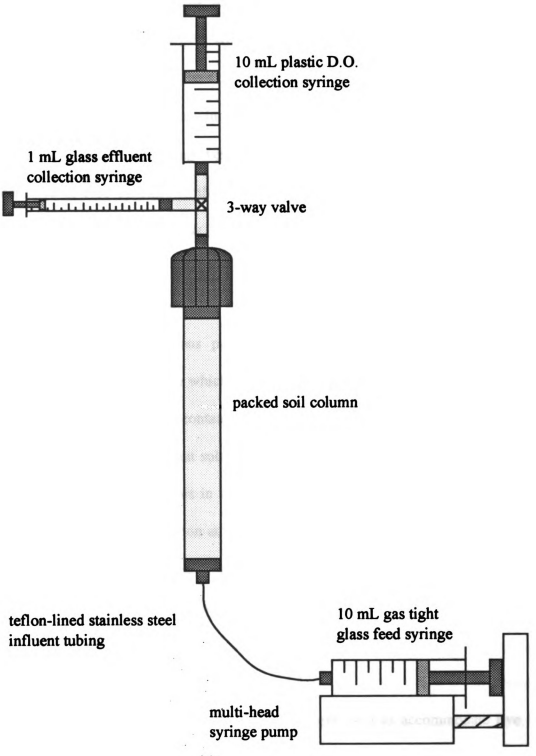

Figure 3.1. Schematic of Operating System

mL/day was used to water saturate the columns in an upflow mode. The water was obtained from the aged (to ensure volatilization of any chlorine residual) tap water reservoir maintained throughout the experiment for consistency. Prior to use, this water was autoclaved to assist in maintaining sterile conditions during contaminant saturation. Plastic syringes and teflon tubing were used during this portion of the experiment to connect influent feed syringes to the base of the columns. Feeding was consistently performed using Harvard Apparatus (Model 22) syringe pumps with multi-head adapters.

3.3.3. Contaminant Saturation.

Maintenance of sterile conditions prevented the presence of microorganisms from impeding contaminant saturation which could cause a concentration gradient to develop instead of the desired uniform contamination. This was accomplished by filtering the water used to prepare contaminant solutions, autoclaving the feed syringes, and soaking the valves, tubing, and connectors in a 70% ethyl alcohol solution. The columns were saturated with contaminant solution at an increased rate until the effluent concentrations stabilized. After this point, the rate was decreased to 10 mL/day to verify that saturation levels were reached. Contaminant saturation continued for a total of approximately 3.5 months.

Four (4) syringe pumps with multi-head adapters were used to accommodate five (5) syringes apiece to ensure the columns received identical feed rates. It was inevitable that losses still occurred due to the nature of the columns however keeping uniform influent

concentrations and monitoring effluent concentrations allowed the degree of consistency among columns to be assessed. Use of gas-tight glass syringes (10.0 mL) during contaminant saturation helped to ensure uniform influent conditions and prevented significant losses due to sorption or volatilization. Gas-tight glass syringes (sterilized by autoclaving for 20 minutes at 121°C) were also used during flushing to prevent oxygen loss or gain (depending on the system and corresponding concentration gradients) due to diffusion. Teflon lined stainless steel tubing (1/8") was used to prevent changes in influent oxygen levels during flushing due to diffusion through teflon or other types of tubing.

3.3.4. Seed Solution and Seeding Procedure.

A mixed culture obtained from an acclimated fluidized bed reactor used to treat BTX contaminated groundwater for three (3) years was used to seed the active columns. Growth of this biomass seed solution included addition of 250 mL of reactor seed to tap water to create a total volume of 1000 mL. Trace nutrients were present in the tap water and bulk nutrients were provided by addition of 3 mL of concentrated solution containing 33 g/L NH_4Cl and 7.8 g/L KH_2PO_4. Substrate consisted of the concentrated BTEXN stock solution prepared in conjunction with the contaminant solutions described previously. The stock substrate solution was added to the seed solution to create a toluene concentration of approximately 100 ppm (45 µL BTEXN stock). The seed solution was then sealed with a rubber stopper and mixed continuously using a stir bar and plate. Periodic addition of additional substrate and nutrient solutions ensured

continued bioactivity prior to column seeding. The approximate organism density was determined by plate counts.

The seeding procedure consisted of adding 15 mL of seed solution at the high rate of 100 mL/day using plastic syringes, teflon tubing, and syringe pumps. The inactive columns were also flushed at the same rate but using 15 mL of the appropriate flushing solution to maintain comparable conditions. Influent and effluent samples were plated using a series dilution procedure to estimate the percentage of biomass retained in the columns.

3.3.5. Flushing Solutions.

3.3.5.1. Inactive Columns.

The use of thimerosal, a mercury derived biocide, to maintain sterile conditions within the inactive columns was tested and found to affect sorption of the compounds significantly. For this reason, a nitrogen flushed reservoir was used to displace the oxygen in the water and maintain low oxygen levels in the influent flushing solution. The reservoir consisted of a water filled 1.0 L graduated cylinder flushed with nitrogen gas by use of a flow meter, tubing, and a diffuser. Maintaining low influent oxygen levels was intended to prevent significant microbiological activity within the columns. Addition of microorganisms from the flushing solution was prevented by filtering the water through a 0.22 μm filtering unit (Millipore Sterivex-GS) when filling the feed syringes. Sodium sulfite was also tested in conjunction with nitrogen flushing to serve as an oxygen scavenger and remove the remaining trace levels of oxygen, however, it was also found

to have an effect on sorption therefore could not be used.

3.3.5.2. Active Columns.

The seeded organisms were enhanced using an oxygenated nutrient flushing solution generated by addition of nitrogen and phosphorus to aged tap water (to ensure volatilization of any chlorine residual). Sufficient nutrient levels were determined based on the maximum decrease in chemical oxygen demand under maximum oxygen provision to prevent nutrient limitations. A flowmeter was used to mix gaseous nitrogen and pure oxygen (≈50% of each) prior to addition to a 1.0 L graduated cylinder using a diffuser to create a consistent reservoir containing between 13 and 28 mg O_2/L as needed. The effluent oxygen levels were ideally maintained above 4 mg O_2/L by adjusting the influent oxygen levels to ensure an oxygen limitation wasn't encountered.

3.4. Operating Procedure.

Operation of the aseptically packed soil column systems consisted essentially of five stages. Each is described in detail elsewhere however the following is a summary to integrate each procedure in terms of stages.

Stage 1: Carbon dioxide flush. The intention here was to prevent air entrapment by replacing the gas in the pore space with carbon dioxide which subsequently dissolves upon water saturation.

Stage 2: Water saturation. Performed to create the saturated soil conditions of interest to this study.

Stage 3: Contaminant saturation. This stage continued for some time after effluent levels stabilized to allow time for intraparticle sorption to occur. These conditions more closely resemble the contamination encountered in the field although it is acknowledged that the time frame involved does not adequately address the issue of long term contamination.

Stage 4: Inoculation of active columns. Performed to provide an acclimated microbiological culture to "activate" the microbiologically active system. The inactive system was also flushed during this stage to keep the systems similar for comparison purposes.

Stage 5: Flushing. The previous stages were in preparation for this final stage which was intended to address the objectives of this research by looking at removal of sorbed contaminants from soil by flushing with non-contaminated water. The active columns were flushed with oxygenated nutrient solution while the inactive columns were flushed with deoxygenated nutrient-free water. Sampling was performed during this desorption mode of operation to allow the effect of desorption on removal rates in both cases to be investigated and offer insight into the effect that microorganisms have on desorption. Partitioning coefficients (determined in the batch sorption study) were used to

back-calculate the solid phase contaminant levels in each column based on effluent concentrations. This would ideally allow the columns to be sacrificed at intervals which appropriately generate the removal curves in their entirety.

3.5. Sampling Procedures.

3.5.1. Liquid Samples.

Both influent and effluent contaminant concentrations were determined during initial column contamination to determine the time it takes to reach saturation ("complete" adsorption). During flushing, 0.2 mL effluent samples were collected in triplicate using 1.0 mL glass syringes connected to valves on the effluent end of each column. Influent and effluent dissolved oxygen (D.O.) levels were monitored (using an oxygen probe) in the active columns to determine the oxygen uptake. These measurements were intended to provide an indication of conditions in the columns and assist in attributing losses to the appropriate cause while also verifying that the mixed culture seed had acclimated. Dissolved oxygen levels in the inactive columns were also monitored to verify that oxygen in the system remained low as intended.

All liquid samples were collected by increasing the feed rate to 5 mL/day to allow collection in a short, discrete time period. The importance of this method of sample collection is two-fold: (1) to avoid loss of contaminants due to volatilization during collection and (2) to prevent biased oxygen results from changes in effluent oxygen levels with time due to diffusion through the plastic collection syringes. Additionally, in

both systems, 10 μL effluent samples were periodically plated on agar pour plates to qualitatively assess that bioactivity was present (in the active system) or absent (in the inactive system) as desired.

3.5.2. Solid Samples

Initial solid phase contaminant levels were assessed by sacrificing and analyzing the soil in the first column from each series prior to flushing but after the microbiological inoculation procedure. The consistency of contaminant conditions among columns was verified by monitoring effluent concentrations which stabilized to similar levels after sufficient contamination occurred.

After a column was sacrificed (removed from operation), it was sealed and frozen (to prevent volatilization losses) and removed as a slug which was physically partitioned into uniformly sized pieces (≈1 g/sample) to allow a comparison of conditions at multiple locations within each column in addition to the total contaminant mass. Headspace vials were weighed prior to use to allow soil mass per sample to be determined after analysis and drying to remove moisture.

3.6. Analysis Procedures.

3.6.1. Gas Chromatography.

Liquid samples were analyzed using a combination Perkin-Elmer headspace sampler (model HS-40) and gas chromatograph (GC Autosystem) with a megabore column (PE

624: 30 m x 0.53 mm) fitted with a flame ionization detector (FID) using helium as the carrier gas. Solid samples were analyzed using the static headspace analysis method described by Voice and Kolb (1994). Briefly, this entails adding 5 mL chilled deionized water to each frozen soil sample contained in a headspace vial. The samples were sealed using teflon-coated septa and aluminum crimp tops. Soil calibration standards were chilled prior to contaminant spiking to minimize volatilization losses followed by rapid shaking for 1 hour and equilibration overnight prior to preparation and analysis. Sonication was tested and not found to improve recovery therefore was not used as part of the analysis procedure.

The operating conditions of the GC were as follows. Each liquid sample was equilibrated at 90°C for 1 hour while the solid samples had a thermostating time and temperature of 1 hour at 95°C. The GC oven temperature started at 60°C and ramped at 15°C/min to a final temperature of 200°C which was held constant for 1 minute. Both high and low sensitivity methods were used to span the full range of each contaminant encountered. A minimum of three check standards and blanks were analyzed in conjunction with each sample analysis period. The detection limits for all compounds was 1 μg/L or less.

3.6.2. Soil Biomass Plating.

To obtain a quantitative estimate of the microbial numbers on the column soil, the biomass was removed from one column from each system, plated and incubated in growth chambers. In this manner, a comparison between the levels of biological activity

specifically due to growth on the substrates of interest in either the active or inactive columns was afforded. This comparison is important to attribute any difference in solid phase contaminant concentration to the presence of microbial activity. The specific procedure and equipment used are described in the following text.

Agar pour plates were prepared in advance by adding the following to each liter of deionized water: 5 grams of bacto-agar (no carbon source), 1.0 mL trace element solution, and 100 mL major ion solution. The trace element and major ion solutions were prepared as described by Ridgway *et al.* (1990). The entire solution was autoclaved at 121°C for 20 minutes, tempered, and poured into sterile plates (60x15 mm Corning polystyrene tissue culture dishes). The pH 7 phosphate buffer solution contained per liter of deionized water 2 g monobasic phosphate (KH_2PO_4) and 3.5 g dibasic phosphate ($K_2H_2PO_4$). Disposable culture tubes (Baxter 13x100 mm, T1290-4) were filled with 1.8 mL of this buffer solution, capped (Kimble glass natural kim-kaps, 13 mm, T1291-13A), and autoclaved prior to use.

Triplicate 1 g soil samples were aseptically removed from each sacrificed column, weighed, and added to the first dilution tubes (containing phosphate buffer) in the triplicate dilution series. Each was mixed for 3 minutes (using a touch mode Thermolyne mixer) followed by a 2 minute wait in a 3-cycle period for a total of 15 minutes. This first stage was intended to remove the biomass from the soil. A dilution series was then performed by adding 200 µL from the first tube to the second and 200 µL from the second to the third and so on to give successive 1:10 dilutions. Ten (10) µL from each

tube was then plated to give 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} , and 10^{-8} dilutions. The dilutions and plating were all done using new sterile automatic pipette tips prior to each step to prevent cross contamination. Plates were spread using a sterilized glass rod and turntable.

After plating, seven (7) bacto-agar plates were placed in a growth chamber containing a headspace vial saturated with concentrated BTEXN substrate solution and cotton to prevent leakage. The concentrated BTEXN solution was prepared as described for contaminant solution preparation. Vapors then serve as the carbon source for growth of organisms. Growth will not occur if the concentration is too high (inhibitory to the microbes) or too low (carbon limiting) however, it was found previously that adding 0.5 mL of BTEXN solution was appropriate to allow for growth if organisms with the ability to degrade these compounds are present.

Fourteen (14) additional plates were prepared to serve as controls. Seven (7) of these consisted of 10 µL from each dilution tube spread on bacto-agar plates incubated outside of the growth chambers. This provided an indication of the number of organisms which may grow on trace substrates found in the agar due to impurities. The remaining 7 plates contained 10 µL from each dilution tube on nutrient agar (23 g/L H_2O) also grown outside of the growth chambers. These plates allow the total number of organisms present to be quantified and provide a comparison to the number which grow on BTEXN.

The appropriate incubation period was determined by plating 10 µL of the seed solution

on replicate bacto-agar plates, placing them in a growth chamber with the appropriate vapor carbon source, and monitoring growth. When no new colonies appeared from one day to the next, this time period was selected as the appropriate incubation period and all plates were counted using this guideline of 5 days.

CHAPTER 4

RESULTS

4.1. Microbiological Data.

The density of the microbiological seed used to inoculate the active columns was determined by plating on nutrient agar pour plates and on bacto-agar pour plates incubated for five (5) days in growth chambers which contained contaminant vapors. In addition, the effluent from three columns (to generate triplicate samples) was collected during inoculation and plated on nutrient agar pour plates to estimate the percent of organisms which were retained in the columns.

It was found that $2.66 \pm 0.33 \times 10^4$ colony forming units per milliliter (CFU/mL) of acclimated target substrate degraders were present in the seed solution based on the growth chamber incubated bacto-agar plates. Growth of this culture on nutrient plates resulted in $1.24 \pm 3.6 \times 10^4$ CFU/mL which is statistically similar to the growth chamber results indicating that the vast majority of organisms had the ability initially to degrade benzene, toluene, ethylbenzene, *o*-xylene, and/or naphthalene. Effluent plating produced variable results of $2.10 \pm 1.7 \times 10^2$ CFU/mL which indicates a maximum retainage of approximately 98%.

Triplicate soil biomass plating was performed on one column from each system after flushing for 17.5 days (41.5 pore volumes). Samples were collected from three (3) locations within the columns to try to assess the distribution of organisms. These locations included the column inlet and two points in the middle of the soil columns. Triplicate plates consisting of bacto-agar in growth chambers and controls of bacto-agar with no substrate plus nutrient agar plates were counted.

The non-incubated bacto-agar controls contained more colonies after 5 days than the incubated bacto-agar plates. This indicates that significant growth occurred on trace substrates present in the agar with possible inhibition occurring in the presence of the target substrates. Nutrient agar plates were therefore used as an indication of the soil microbial numbers assuming the majority of growth is comprised of BTEXN degraders. No trend was seen in terms of location within the columns, however, the active column contained an average of 4.28×10^8 CFU/g soil compared to 3.20×10^6 CFU/g soil in the inactive column.

4.2. Oxygen Data.

Influent oxygen levels were measured daily and maintained consistently. Effluent oxygen samples were collected periodically by increasing the flushing rate to 5 mL/hr to allow collection in a shortened period of time in an attempt to minimize bias due to atmospheric diffusion. Figure 4.2.1. provides a summary of influent and effluent dissolved oxygen concentrations. Effluent levels were averaged over the columns in each

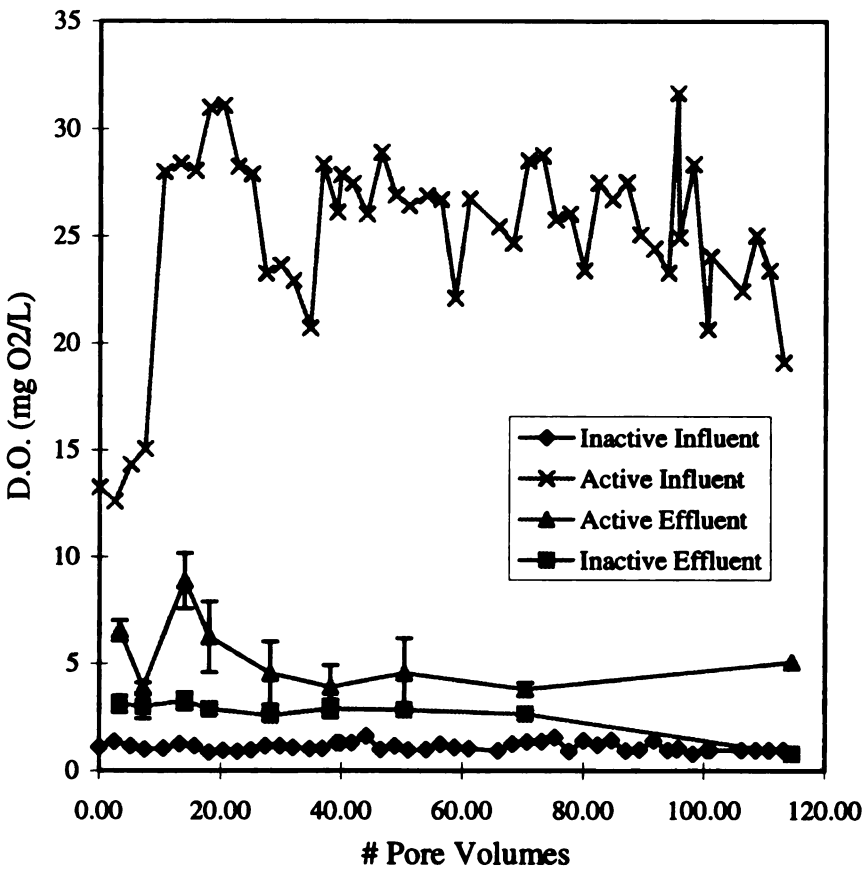

Figure 4.2.1. Influent and Effluent Dissolved Oxygen.

series with typical deviations of less than 15% except for the later active column values which increased in variability to a maximum of 35%. An increase in oxygen concentration by approximately 2 mg/L was associated with the influent fed to the inactive columns while approximately 20 mg/L of oxygen was consistently consumed in the active columns.

4.3. Liquid Phase Data.

Liquid phase data consists primarily of effluent contaminant concentrations analyzed using headspace gas chromatography. Between one and three 0.2 mL samples were analyzed from each column at regular intervals and average values for each series of columns were used for interpretation. Deviations from column to column within the column series were typically less than 10% however this variability increased to a maximum average of 25% as the sample size decreased due to column sacrifice and as concentrations dropped into the lower ranges. Figures 4.3.1 - 6 provide a comparison between active and inactive concentrations for each target compound in addition to a combined removal represented by chemical oxygen demand.

To test whether equilbrium partitioning was occurring in the column systems, flow was interrupted for a period of four days starting on day 40. Effluent samples were collected prior to interuption and after flow was resumed. Comparison of the contaminant concentrations in the samples showed a two-fold increase in the effluent levels after resumption (see Figures 4.3.1 - 6). This indicates non-equilibrium partitioning was

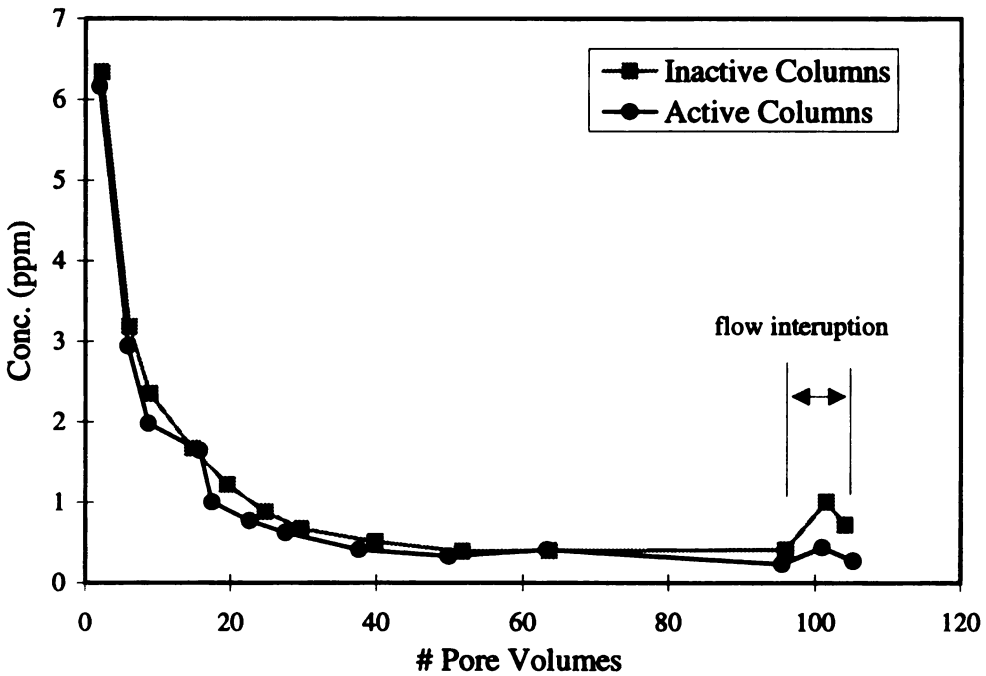

Figure 4.3.1. Effluent Benzene Concentrations

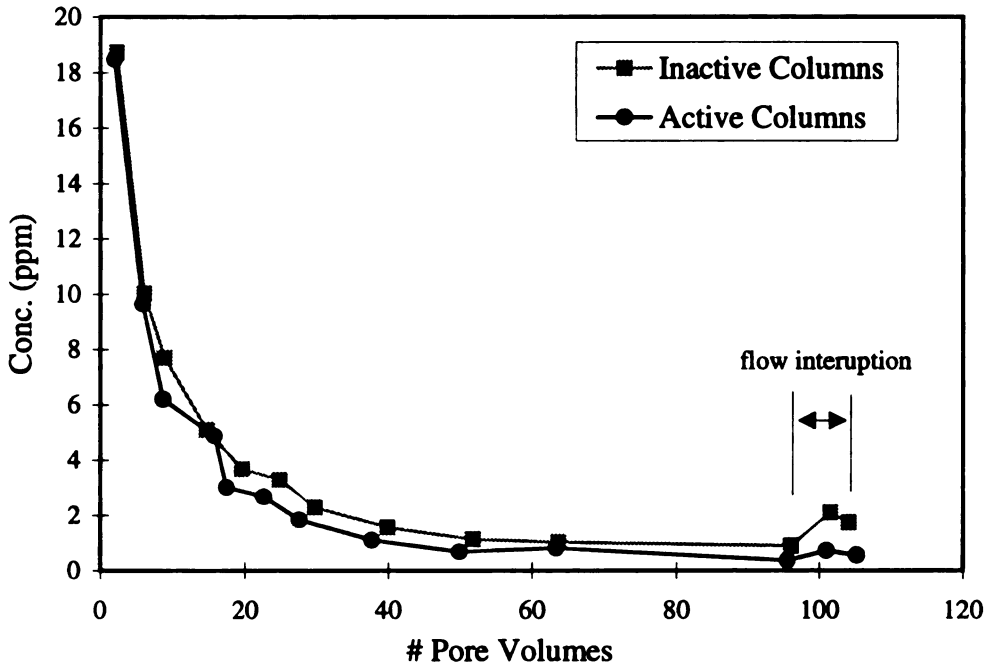

Figure 4.3.2. Effluent Toluene Concentrations

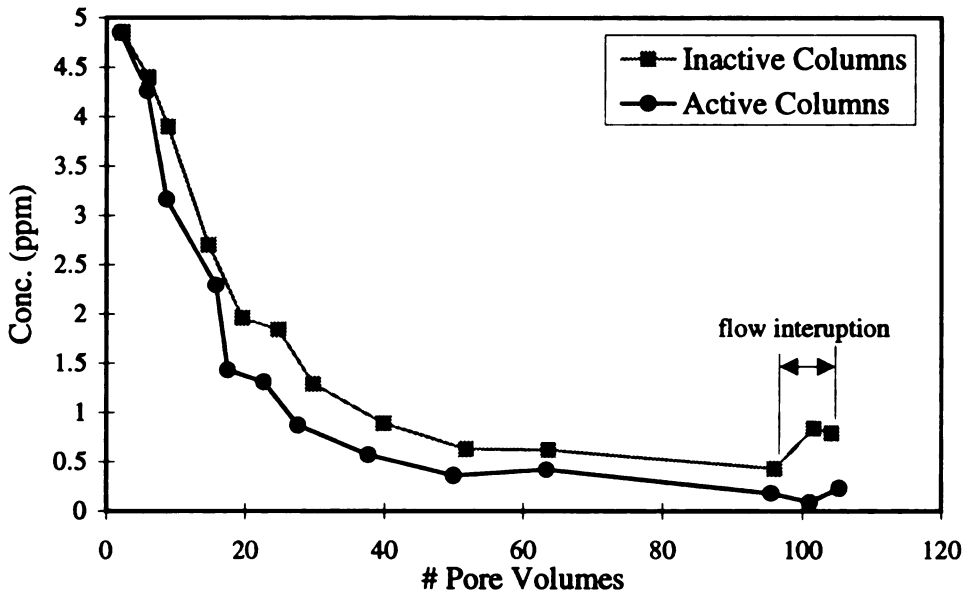

Figure 4.3.3. Effluent Ethylbenzene Concentrations

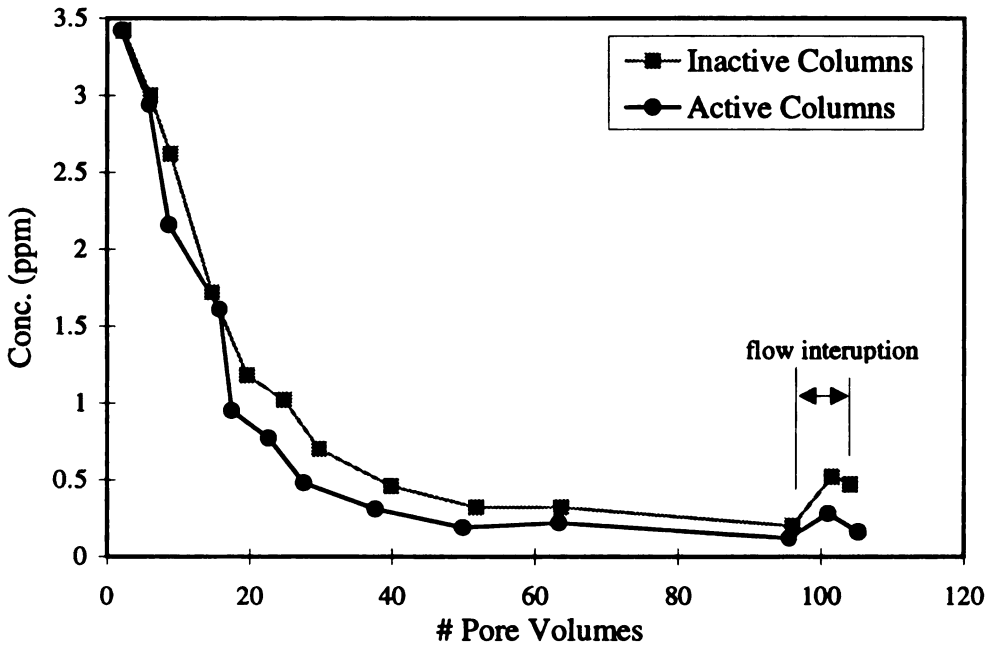

Figure 4.3.4. Effluent *o* -Xylene Concentrations

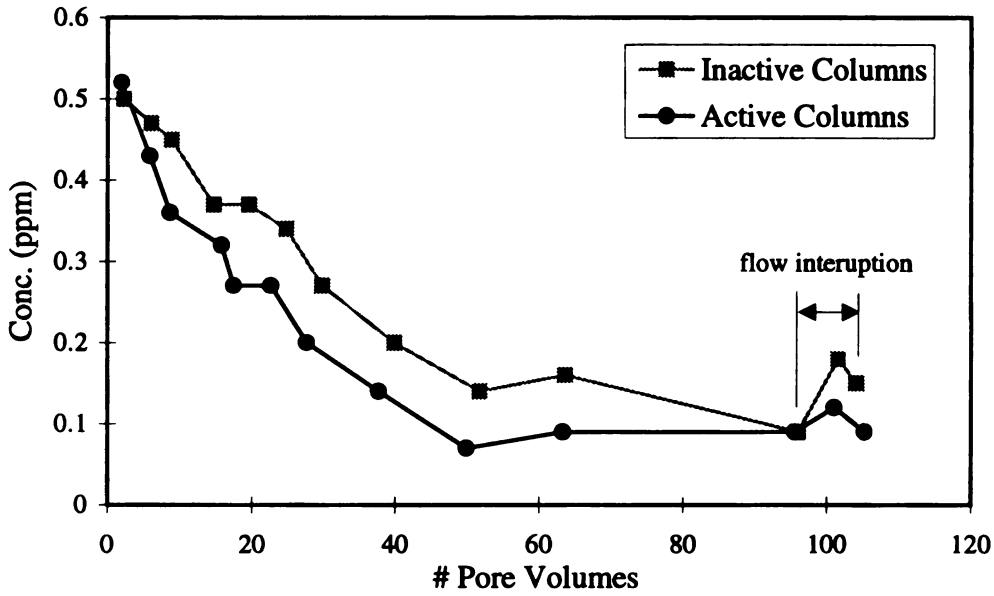

Figure 4.3.5. Effluent Naphthalene Concentrations

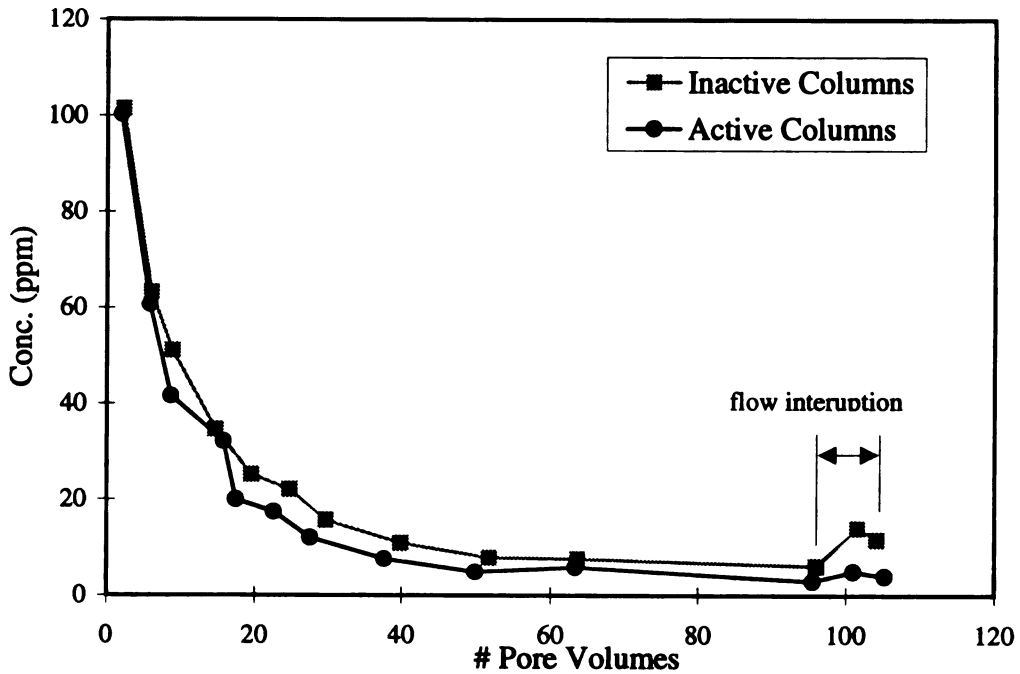

Figure 4.3.6. Effluent COD

occurring.

4.4. Solid Phase Data.

Sacrificed soil columns provided the primary data of interest to this study. The "true" starting contaminant levels were those determined after inoculation and initial flushing of the inactive system. Sacrifice of one column from each system followed by solid phase analysis provided the initial concentrations. In the active system, these levels were 1.44 mg/kg benzene, 10.77 mg/kg toluene, 10.88 mg/kg ethylbenzene, 6.83 mg/kg *o*-xylene, and 3.25 mg/kg naphthalene. Conversion to chemical oxgyen demand (COD) resulted in an initial concentration of 84.85 mg/kg. The inactive system levels were 0.95 mg/kg for benzene, 9.08 mg/kg toluene, 11.87 mg/kg ethylbenzene, 7.26 mg/kg *o*-xylene, 3.2 mg/kg naphthalene, and 97.11 mg/kg COD.

No trend was seen when incremental concentrations were plotted versus depth up through the columns, therefore, interpretation of changes due to location could not be assessed. For this reason, the total contaminant mass on each column served as single data points during plotting of solid phase removal rates. Figures 4.4.1 - 6 are presented to compare solid phase removal in either system and for each contaminant. Figures 4.4.7-12 represent a mass balance by comparing the liquid to solid removal levels.

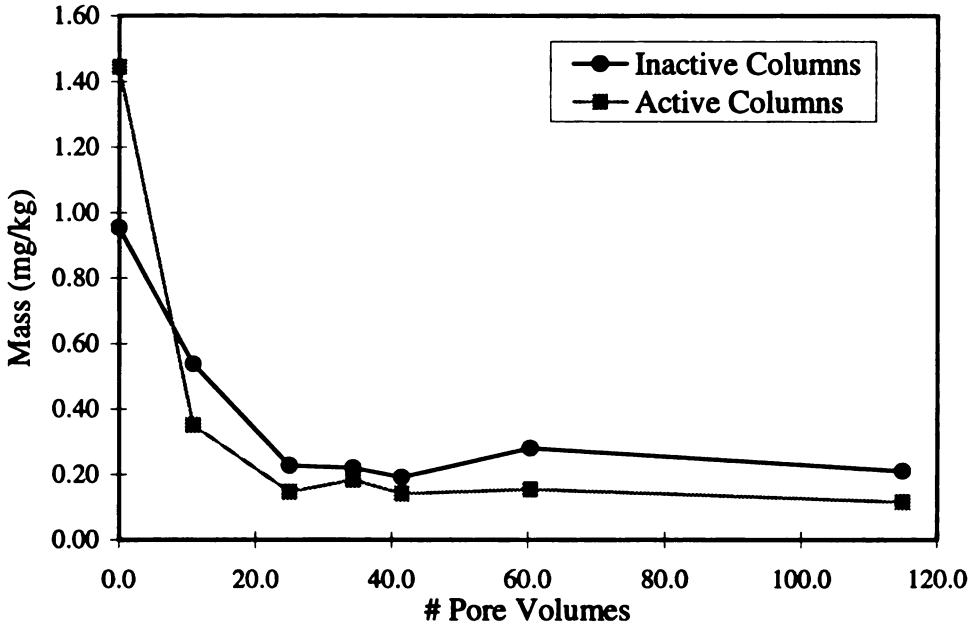

Figure 4.4.1. Solid Phase Benzene Concentrations

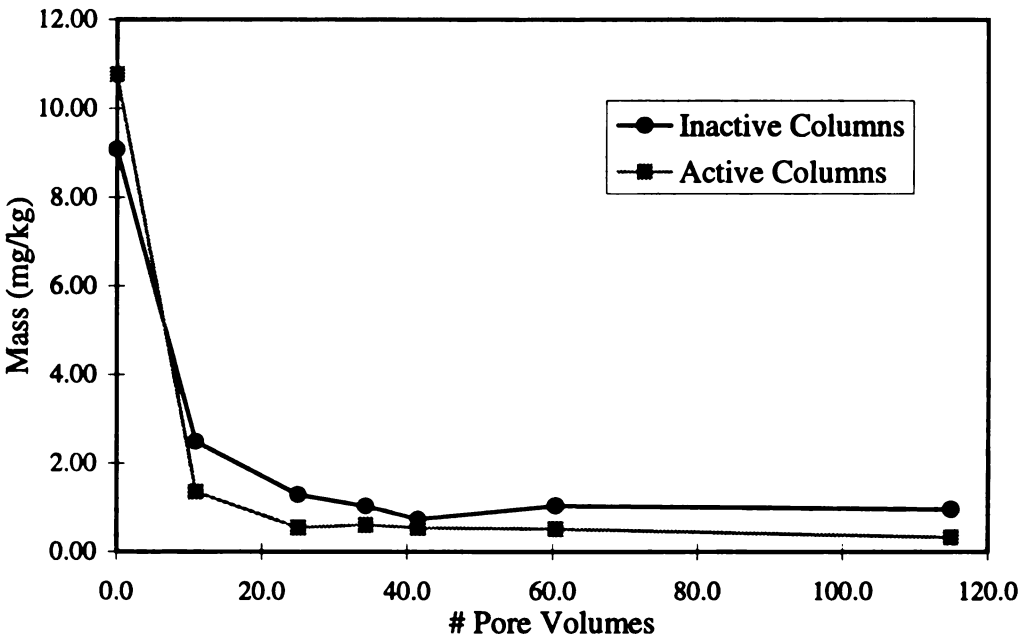

Figure 4.4.2. Solid Phase Toluene Concentrations

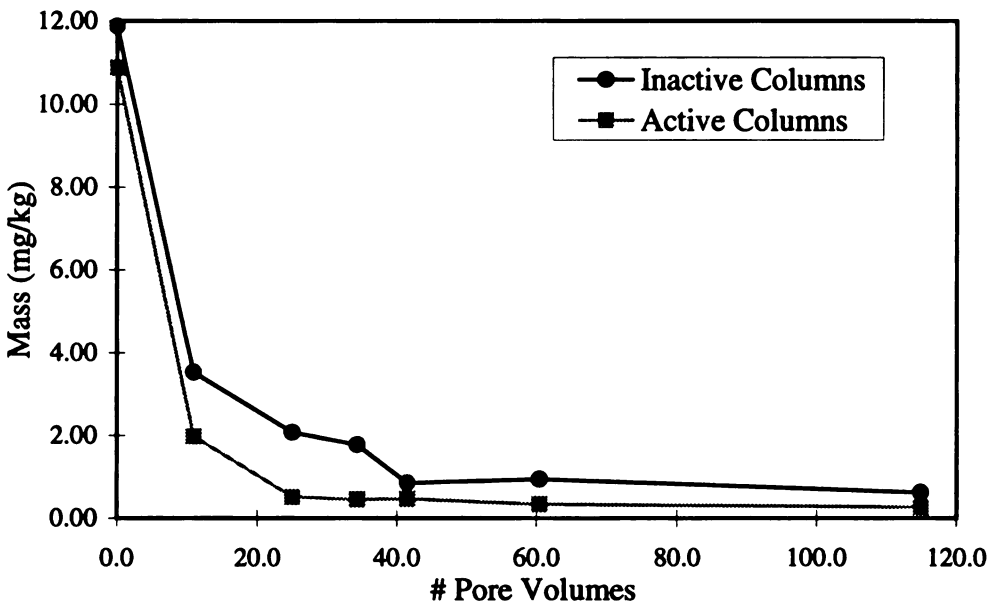

Figure 4.4.3. Solid Phase Ethylbenzene Concentrations

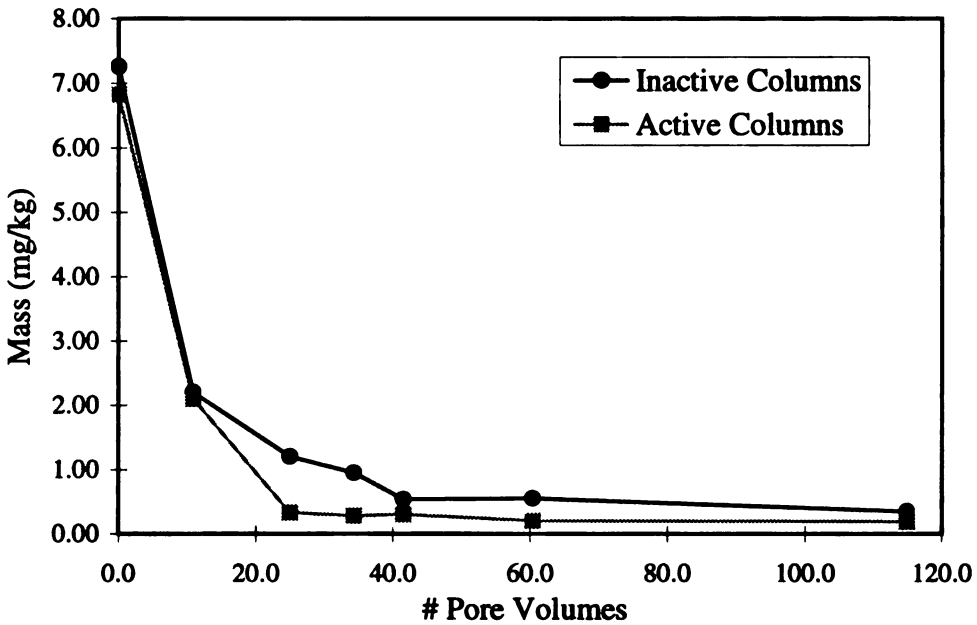

Figure 4.4.4. Solid Phase *o* -Xylene Concentrations

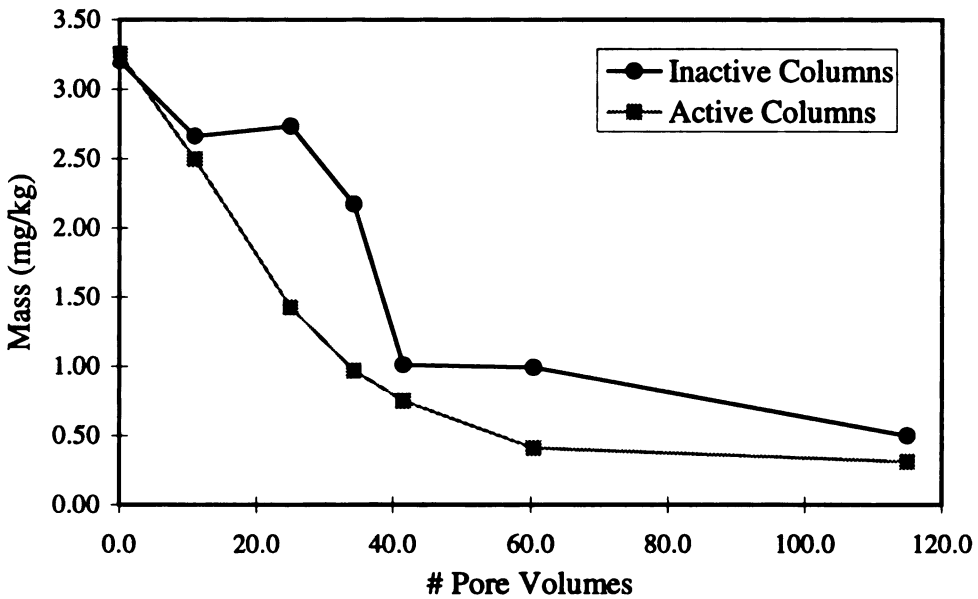

Figure 4.4.5. Solid Phase Naphthalene Concentrations

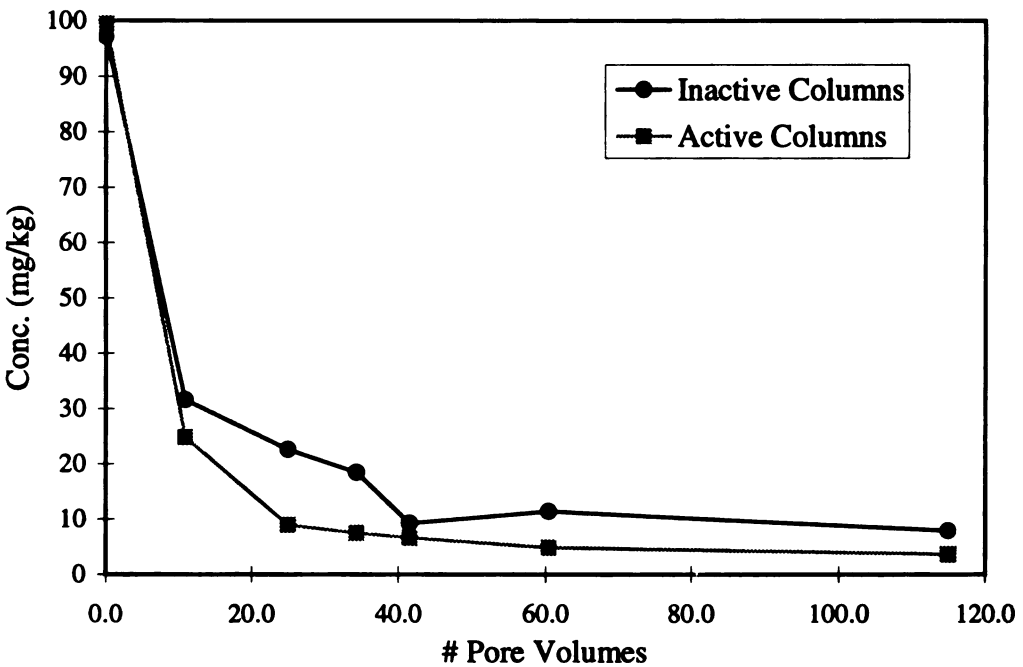

Figure 4.4.6. Solid Phase COD

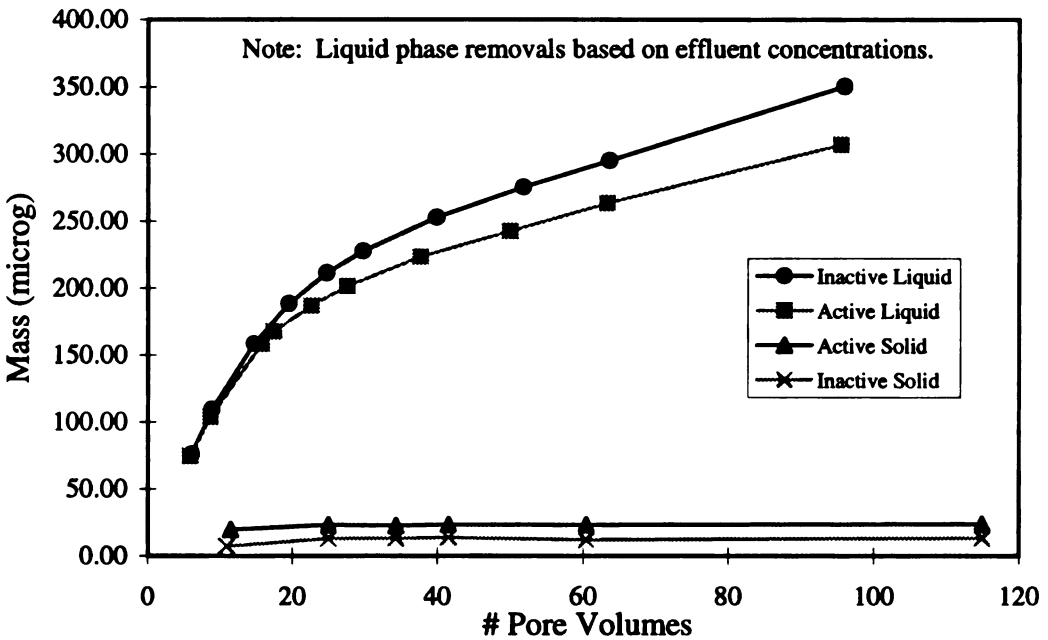

Figure 4.4.7. Cumulative Mass of Benzene Removed

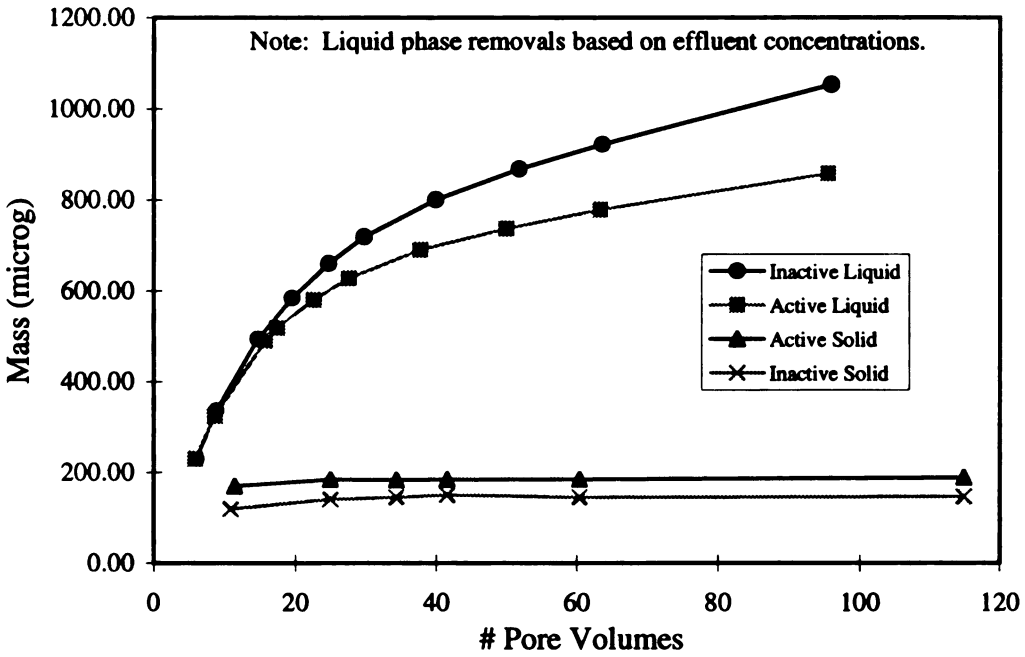

Figure 4.4.8. Cumulative Mass of Toluene Removed

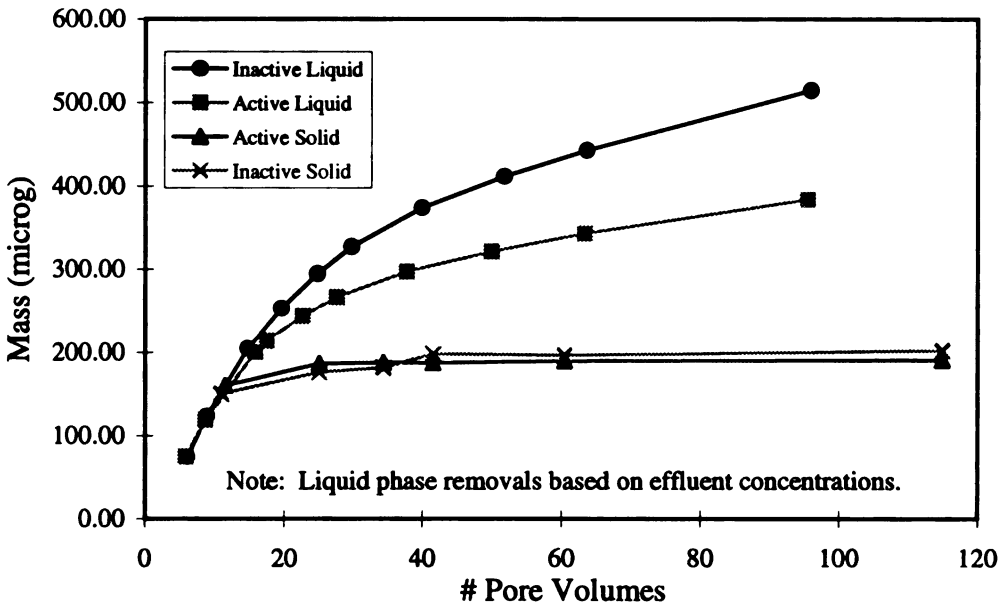

Figure 4.4.9. Cumulative Mass of Ethylbenzene Removed

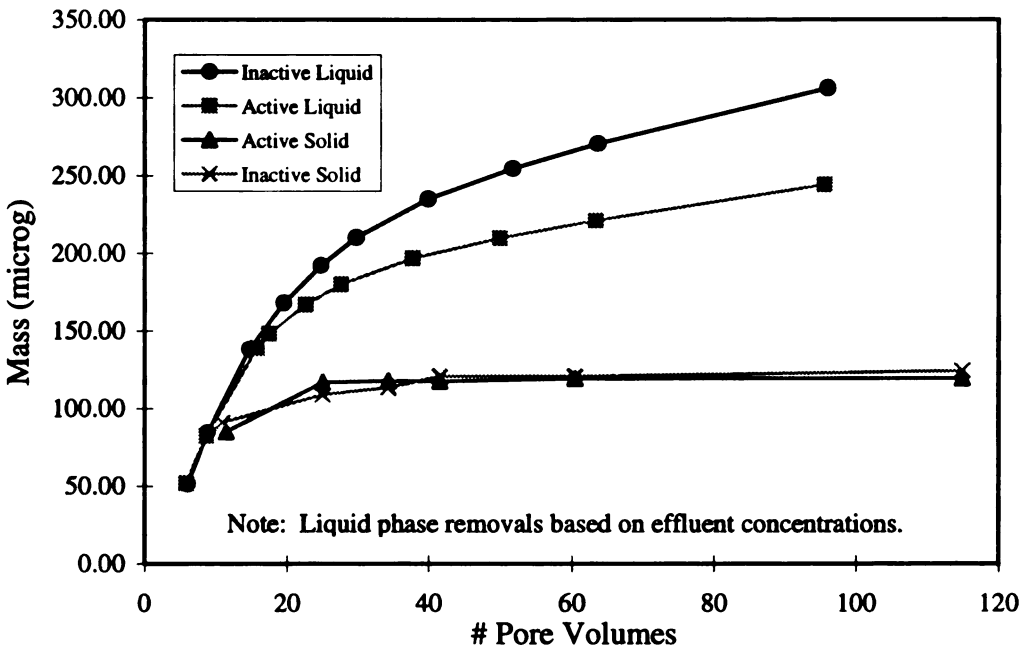

Figure 4.4.10. Cumulative Mass of *o* -Xylene Removed

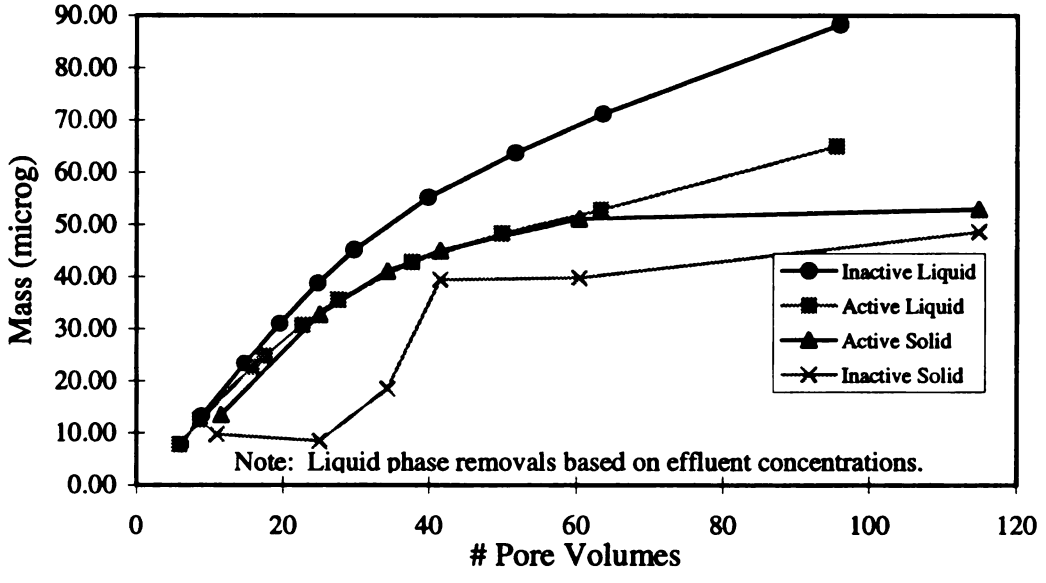

Figure 4.4.11. Cumulative Mass of Naphthalene Removed

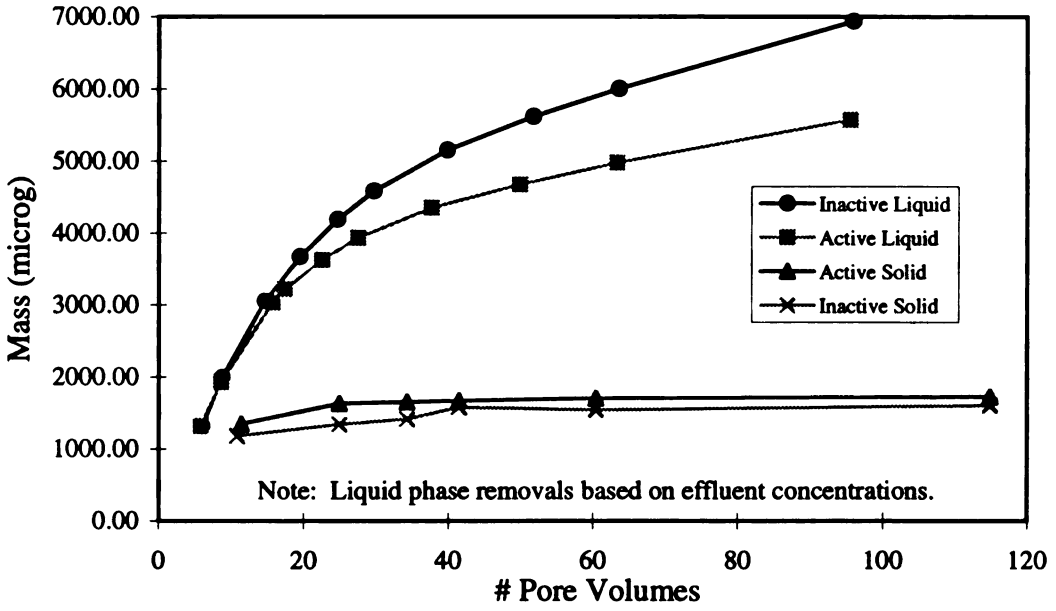

Figure 4.4.12. Cumulative Mass of COD Removed

Table 4.4.1 contains data generated to assess the validity of sample data. It essentially is a comparison of estimated data and measured data to determine whether the results are similar to those expected. This is useful to locate potential errors while also identifying unexpected results which may be of particular interest. The table contains the same information for each target compound including converted COD data.

The contaminant saturation levels are the concentrations fed during contaminant saturation. These numbers represent the maximum possible concentration of each compound loaded prior to flushing. The distribution coefficients (K_d) were estimated during the batch sorption experiment as discussed in Appendix C. The maximum possible contaminant mass on the soil was then found by multiplying the liquid levels by the distribution coefficients and is represented as estimated mass on soil. Cumulative mass removals for both active and inactive columns were calculated based on the effluent concentrations measured during flushing. The measured mass on soil were determined using headspace gas chromatography to analyze the column soil.

Table 4.4.1. Comparison of Expected and Actual Results

Compound	Contaminant Saturation Level (mg/L)	K_d [(mg/kg)/ppm]	Estimated Mass on Soil (ug*)	Cumulative Mass Removed in Effluent		Measured Mass on Soil (ug*)
				Inactive	Active	
Benzene	32	1.11	639.36	376.39	320.96	17.10
Toluene	41	1.21	892.98	1108.94	882.08	163.44
Ethylbenzene	10	2.06	370.80	538.41	389.94	213.66
o-Xylene	6	1.90	205.20	319.86	252.93	130.68
Naphthalene	1	3.53	63.54	93.22	69.31	57.60
COD	270	----	6515.64	7309.82	5744.15	1747.98

* ug = micrograms

CHAPTER 5

DISCUSSION, CONCLUSIONS, AND RECOMMENDATIONS

5.1. Discussion.

A number of interrelated issues arose during experimentation which merit discussion in relation to the conclusions and understanding which can be gained from this research effort. There are essentially six (6) areas into which these "issues" can be grouped. The first is related to the solid phase data, while the others include generation and use of oxygen data, microbial quantification, similarity of conditions among columns, soil organic matter content, and the possible effect of oxygen on the sorption characteristics of the soil.

The large difference between cumulative mass removal of contaminants when comparing solid phase and liquid phase data indicates a loss in contaminant mass which has not been accounted for (refer to Figures 4.4.7 - 12). One possibility could be low recovery of contaminants from soil due to the type and extent of sorption. The effect of contaminant age on recovery was, however, investigated as described in Appendix B. The results did not indicate a strong contaminant age correlation with decreased recovery for the length of this study. For this reason, loss during column sacrifice, physical partitioning of the soil columns, and/or analysis must be considered.

One method used to identify the source for the error detected during performance of a mass balance was described in the results section with the accompanying data shown in Table 4.4.1. Comparing the mass removed in either the active or inactive columns (based on effluent data) to the total contaminant mass on the soil (estimated from liquid data and distribution coefficients) provides an indication of order of magnitude similarities. Since there is some error associated both with the estimated distribution coefficients and effluent concentrations, in addition to the conservative estimate of saturation levels (probably lower due to losses), the values are fairly similar. On the other hand, looking at the initial soil levels determined from analysis, the numbers are much less than the same predicted values. Coupling this with the fact that the effluent data (Figures 4.3.1 - 6) is similar for both systems but somewhat lower for the active columns as expected indicates that the error is likely due to analysis of soil samples.

A comparison of liquid and solid removals for the increasingly volatile compounds (benzene > toluene > *o*-xylene > ethylbenzene > naphthalene) show larger errors in mass balance terms indicating possible loss due to volatilization. In addition, prior to analysis of the final pair of columns, half of the samples in sealed headspace vials from each column were sonicated for 1 hour at 50°C in an attempt to improve contaminant recovery during analysis. The use of sonication had been previously tested and found to insignificantly effect the results however, the possible effect may have become more significant during analysis of lower concentrations which was the reason for a second assessment. The results were somewhat varied but there was no trend indicating

improved recovery associated with sonication.

The oxygen issue can be sub-divided into (1) diffusion effects in the column system and during sampling and (2) abiotic oxygen uptake. Figure 4.2.1 shows the influent and effluent oxygen data for both series of columns. The effluent oxygen from the inactive columns was somewhat higher than the influent due to the first issue of diffusion and sampling effects. The effluent oxygen from the active columns was maintained at very low levels which indicates a constant oxygen uptake. This could be due to abiotic effects or microbiological activity however both would decrease over time. The average oxygen uptake of 20 mg/L corresponds to a COD of approximately 4,400 mg/L when 2 moles of oxygen is consumed per mole of COD degraded. This level of removal should be represented by the difference in initial contaminant mass on the soil (6,515.64 mg/L COD from Table 4.4.1) and cumulative mass removed from the active columns (5,744.15 mg/L COD) however, it's significantly higher. These comparisons indicate an error is associated with using the collected oxygen data to estimate removal due to degradation as intended.

The change in dissolved oxygen levels during column flushing due to atmospheric diffusion was minimized through the use of gas-tight glass syringes and teflon-lined stainless steel tubing. Some diffusion remained unavoidable due to the columns and the connections, however, this level was estimated by flushing a sterilized ottawa sand packed column with high levels of oxygen. The resulting effluent dissolved oxygen

levels were approximately 2 - 3 mg/L lower than the average influent levels of 15 mg O_2/L.

Biased results during sampling and analysis were addressed by using an increased sampling rate to minimize the time for diffusion into the effluent collection syringes. After collection, a needle was used to rapidly introduce the 2 mL sample into the bottom of a customized oxygen vial designed to allow for reduced volume analysis. This diffusion effect was also better understood by operating the last three inactive columns in an anaerobic glove box. Influent and effluent oxygen levels were the same and constant at approximately 0.8 mg O_2/L. It is believed that there was in fact "no" oxygen in the nitrogen flushed solution therefore the 0.8 was introduced during transfer from the columns to the vial for measurement. The effluent levels of 2 - 3 mg O_2/L minus 0.8 mg O_2/L would therefore be due to diffusion into the columns during operation.

Abiotic oxygen uptake was quantified by flushing a non-contaminated sterilized packed soil column with highly oxygenated water and measuring the effluent dissolved oxygen levels. Significant oxygen uptake was observed, however, the study did not continue long enough to understand the maximum abiotic uptake levels. It is understood that these losses will reduce the consumption by microorganisms, however, it would be suprising if this abiotic consumption is a continuous oxygen sink therefore it does not serve as an explanation for the large and continuous oxygen uptake.

Quantification of microbial numbers was problematic in some respects. Initially a protein assay was performed and intended to allow for conversion to microbial concentration at different locations within the columns. Humic acids present in the soil clouded the results of this procedure. The soil biomass plating procedure was then considered, however, due to freezing of sacrificed columns (to minimize volatilization losses of contaminants) prior to headspace analysis, the organisms would suffer. Effluent plating was not found to be representative of soil microbial levels, therefore, was also ruled out.

Quantification of soil microorganism numbers occurred at a single point in time through the sacrifice of two (2) columns solely for the purpose of microbial analysis. While this provided some indication of the comparative concentrations, the point of measurement may not correspond to the steady-state microbial levels due to decreasing substrate availability. In addition, use of bacto-agar plates in growth chambers did not identify the number of organisms present which were capable of degrading the target compounds. For this reason, a most probable number (MPN) procedure is recommended using column effluent to quantify the microbial numbers capable of degrading BTEXN. Alternately, soil biomass plating using high purity agarose media in growth chambers could have provided improved results.

Similarity in conditions from column to column is an important factor since each column represents a point in the treatment series. Numerous sources of variability were introduced due to non-uniformity of column packing, contamination, microbial

distribution, and flow. Considering these effects, the deviation in results is not suprising or extensive.

Use of a higher organic matter content soil may have been useful to increase the sorption/desorption effect. Desorption from the soil used in this study appeared to be rapid resulting in a relatively short experimental run time. While being representative of groundwater environments, the time frame may have affected the results.

The finding that sodium sulfite effects soil sorption characteristics was suprising and may indicate that the change in oxygen level actually produced the effect. If this is true, the results would be highly dependent on the vast difference in influent oxygen levels in either column system which warrants further investigation.

5.2. Conclusions.

Using the results summarized in the previous chapter, the following conclusions can be drawn.

1. The microbial conditions within each series of columns were significantly different based on the dissolved oxygen data and microbial plating results.

2. Non-equilibrium partitioning occurred and indicates the desorption limitation was achieved.

3. Both column systems approached similar liquid remediation levels rapidly, indicating that microorganisms did not offer a significant advantage. The somewhat higher removal in the active columns is potentially due to an increased concentration gradient.

4. Mass balance calculations indicate substantial loss of contaminants prior to or during solid phase analysis.

5. There was a poor correlation between oxygen data and substrate removal due to biodegradation.

6. Methods used to quantify solid phase microbiological activity levels were problematic.

5.3. Recommendations.

Due to the preliminary nature of this study, there are a broad range of areas into which further effort could be invested. The comparative nature offers decided advantages when attempting to isolate system differences. For this reason, addressing some of the focus issues introduced here could maximize the effectiveness of this type of experiment in future study.

The recommendations can be divided into four (4) areas. The first is further study to isolate the potential reasons for soil analysis error. This can be subdivided into three studies to understand whether contaminant age effects, contaminant recovery, or losses during column sacrifice and preparation were responsible for low headspace responses.

The second recommendation is related to the type of column system used. A series of small packed soil columns connected by valves to allow influent and effluent sampling to be directly associated with the solid slug is recommended. In addition, glass columns with stainless steel fittings would minimize losses due to diffusion and sorption.

The "inactive" system could also be operated and sampled in a nitrogen atmosphere to minimize atmospheric oxygen bias. Gamma-irradiation after the columns have been packed could also decrease the possibility of microbial contamination. Oxygen levels in the inactive column flushing solution could also be decreased closer to zero through the use of zero-grade nitrogen.

The third recommendation is to develop a more appropriate soil biomass quantification procedure or method. This is necessary to allow a better correlation between removal rate and relationship to microbiological activity level to be developed. The final recommendation is for additional study to address the effect of oxygen level on sorption, abiotic soil uptake, and contaminant loss during soil analysis.

APPENDICES

APPENDIX A

CONTAMINANT RECOVERY FROM SOIL

In order to determine what percentage of each contaminant was recoverable from soil samples during analysis by headspace gas chromatography a recovery study was performed. This study consisted of analyzing replicate samples with and without soil spiked with varying levels of low concentration standard solution. This standard solution contained 226.24 mg/L benzene, 248.48 mg/L toluene, 138.4 mg/L ethylbenzene, 93.04 mg/L *o*-xylene, and 24.088 mg/L naphthalene. Additionally, the effect of variation in soil mass on analysis results was investigated.

A.1. Contaminant Recovery.

Soil samples were prepared using the standard procedure (as described elsewhere) to contaminate five (5) replicate 1.0 g soil samples to the same level using the BTEXN standard solution. Samples containing this same level of contamination spiked directly into empty headspace vials were also prepared. Both soil containing and soil free samples were then shaken, equilibrated, and chilled prior to addition of 5 mL chilled deionized water. Preparation of these samples in a similar manner was necessary to maintain similar loss conditions and prevent biased results. Check standards were also prepared by spiking contaminant solution directly into 5 mL deionized water. All of the

above samples were then analyzed by headspace gas chromatography.

The difference between the samples with and without soil allowed contaminant recovery to be determined. Naphthalene recovery was based on a comparison between the check standard response and soil response due to loss of naphthalene in the soil free systems due to solidification onto the headspace vials. Table A.1.1. provides a summary of these results which indicate that a significant portion of the contamination present is available for analysis. Recovery based on varying levels of contamination was also tested and found to have an insignificant effect.

Table A.1.1. Contaminant Recovery from Spiked Soil.

Contaminant Recovery (%)				
Benzene	Toluene	Ethylbenzene	*o*-Xylene	Naphthalene
96.0	96.7	96.3	95.3	68.4

A.2. Effect of Soil Mass.

The effect of soil mass on analysis results was investigated by spiking similar levels of contaminant solution onto soil samples which varied in mass by 0.2 g in the range from 0.4 g to 1.6 g. The results of this study are presented in Table A.2.1. The deviations in GC response over this entire range (60% variation in mass) for each component were not significant, therefore, soil mass was not incorporated into the analysis procedure except for use during calculation of concentration per mass of soil analyzed.

Table A.2.1. Effect of Soil Mass on GC Response.

	GC Response				
Soil Mass, g	Benzene	Toluene	Ethylbenzene	*o*-Xylene	Naphthalene
0.4	398128.64	469834.54	252575.16	172305.93	27055.44
0.6	433103.40	516573.74	286552.79	195810.98	27285.79
0.8	434138.04	520757.97	279001.10	193205.33	27419.72
1.0	450783.56	554753.85	311977.41	213143.30	25725.27
1.2	443538.94	546382.41	307157.29	210835.05	25025.01
1.4	447359.65	556838.77	315531.35	213661.53	23682.55
1.6	412723.38	521173.29	304083.42	208636.37	25122.53
Average	431397	526616	293840	201085	25902
Deviation	19339	30235	22575	15132	1407
% Deviation	4.5	5.7	7.7	7.5	5.4

APPENDIX B

EFFECT OF CONTAMINANT AGE ON RECOVERY

Research has shown that the recovery of contaminants from soil decreases with time due to interparticle and irreversible sorption (Farrell and Reinhard, 1994; Steinberg *et al.*, 1987). To quantify this effect for the contaminants and soil used in this research a recovery study was designed and implemented.

Neutral, clear-glass ampoules (OIC No. 130021) were used to create a sealed environment to allow the effect of decreased recovery to be assessed. Six (6) series of six (6) ampoules were used including triplicate samples and triplicate controls for each series. All were prepared at the same time and analyzed to coordinate with column sacrifice times to allow a direct comparison to be made.

The samples consisted of 1 g of gamma-irradiated soil with the remaining volume containing contaminant reservoir solution. The approximate concentrations of each target compound were 28 mg/L (benzene), 34 mg/L (toluene), 9 mg/L (ethylbenzene), 5.5 mg/L (*o*-xylene), and 0.9 mg/L (naphthalene). The controls contained this same solution without soil. All ampoules were sealed using a purging and sealing unit (OI Corporation Model 524PS) with minimal headspace followed by periodic shaking to ensure

equilibrium sorption occurred. At the time of sacrifice the soil containing samples were frozen to allow the soil to be removed with minimal volatilization losses prior to preparation and analysis. The controls were sampled using a glass syringe to remove triplicate 1.0 mL samples after the glass top was cut away from the ampoule body.

The data generated was used to calculate the solid phase contaminant mass of each component and the accompanying change over time. The change with time will presumably be due to decreased recovery from the soil which allows a recovery with time curve to be generated. This information is useful in and of itself to quantify an effect which previously hasn't been quantified. In addition, associating the ampoule recovery study with the analysis of the packed soil columns may allow the effect of decreased recovery to be taken into consideration upon data reduction and use.

Table B.1. Estimated K_d Values Based on Contaminant Age.

	K_d [(mg/kg)/ppm]				
# Days	Benzene	Toluene	Ethylbenzene	*o*-Xylene	Naphthalene
5	.46	.6	.86	.85	1.81
10	1.06	1.32	1.8	1.73	2.93
12	1.29	1.52	1.97	1.85	2.79
18	.78	.99	1.47	1.39	2.41
27	1.47	1.76	2.40	2.28	3.55
27	1.21	1.48	1.99	1.91	3.25

The results presented in Table B.1. contain significant variation, however, the values estimated from the last two days were significantly similar and high indicating no decreased recovery.

APPENDIX C

BATCH SORPTION EXPERIMENT

Estimation of solid phase contaminant levels based on effluent analysis is important to determine column sacrifice intervals. To obtain a reasonable estimate, it is necessary to quantify the partitioning coefficient (K_d) of each contaminant. For this reason, a batch sorption experiment was performed to obtain these values while also assessing whether different concentrations affect the results. In addition, thimerosal and sodium sulfite use was investigated to observe whether changes in equilibrium sorption characteristics of the soil occurred.

Gamma-irradiated soil was used due to the potential change this procedure may have on the structure of the soil organic matter which in turn effects the sorption characteristics. The small quantity of this soil available dictated the use of a small system. Disposable 5 mL (volume is actually greater than this) glass centrifuge tubes with screw cap finish (Baxter C3795-5) in conjunction with screw thread vial caps (Baxter C4810-51) and silicone rubber septa (Baxter C4810-58) constituted the batch system. Approximately five (5) mL of liquid and 2 g of soil were used with thirty, 2 mm glass beads used to take up the excess volume to prevent the presence of headspace thereby avoiding gas-phase partitioning. These values (volume = V, mass of soil = M_s) were used to estimate the initial contaminant levels based on chosen equilibrium concentrations (C_e) and

partitioning coefficients (K_d) roughly estimated previously.

Three initial levels of contaminant (C_o) were used to assess whether sorption is linear. A mid-range concentration was representative of most values while a value 20% lower provided an idea of K_d changes at lower levels. High concentrations representative of influent values allowed K_d in this range to be found and compared. The following table (C.1) identifies the specific concentrations that were used.

Table C.1. Parameters used to Design Batch Sorption System.

		Benzene	Toluene	Ethylbenzene	*o*-Xylene	Naphthalene
	K_d [(mg/kg)/ppm]	0.996	1.129	1.531	1.4065	1.726
Low Concentration	C_o (ppm)	0.257	0.794	0.431	0.28	0.149
	C_e (ppm)	0.2	0.6	0.3	0.2	0.1
Mid-Concentration	C_o (ppm)	1.285	3.968	2.156	1.402	0.747
	C_e (ppm)	1	3	1.5	1	0.5
High Concentration	C_o (ppm)	41.106	55.548	15.812	8.411	1.493
	C_e (ppm)	32	42	11	6	1

The initial concentration levels (C_o) were prepared in 160 mL serum vials by spiking concentrated BTEXN solution (0.3, 1.5, and 20.5 µL) into 150 mL filtered D.I. water plus approximately 580 mg/L HCl to prevent microbial degradation of the constituents. Glass beads were added and the solutions mixed overnight on a rotary shaker, to insure dissolution and uniform distribution, prior to transferring 5 mL into the respective vials.

Three time periods (7, 14, and 21 days) were used to ensure equilibrium was reached. A

total of 33 vials were used as follows: three sets of three controls containing no soil tumbled for 7, 14, and 21 days, six high and mid-range vials in each period, three low concentration vials in 7-day period, and three vials containing sodium sulfite (Na_2SO_3) in the 7-day period. The Na_2SO_3 was added to the reservoir in sufficient quantity (0.03 g) to consume the oxygen present in the water and leave a residual level of approximately 100 mg/L. Thimerosal was also added to three vials to create a concentration of 200 mg/L.

The initial concentrations were measured in triplicate by sampling the serum vial reservoirs using glass syringes (triplicate 1 mL samples). Equilibrium concentrations were measured after centrifuging the batch vials and sampling the supernatant (triplicate 0.2 mL samples). Centrifugation occurred for 10 minutes at 3200 rpm using a six-place angle rotor Adams Compact II Centrifuge (Fisher, 05-100-90). Soil samples were also analyzed by freezing and removing the soil at the end of the experiment followed by preparation and analysis as described elsewhere.

Thimerosal Effects

The effect of thimerosal on sorption characteristics was significant for each compound excluding naphthalene. Table C.2. provides a comparison of K_d values for each contaminant in systems which either contain or don't contain thimerosal.

Table C.2. Thimerosal Effect on K_d.

	K_d [(mg/kg)/ppm]				
	Benzene	Toluene	Ethylbenzene	*o*-Xylene	Naphthalene
With Thimerosal	0.6335	0.7760	1.1660	1.0815	1.6865
Without Thimerosal	0.9960	1.1290	1.5310	1.4065	1.7260
% Difference	36.4	31.3	23.8	23.1	2.3

Sodium Sulfite Effects

Sodium sulfite was also found to have an effect on sorption of the target compounds therefore was not used. The following table (C.3.) summarizes the data to support this conclusion.

Table C.3. Sodium Sulfite Effect on K_d.

	Contaminant Concentration on Soil (mg/kg)				
	Benzene	Toluene	Ethylbenzene	*o*-Xylene	Naphthalene
With Na_2SO_3	1.84	1.81	0.71	0.31	0.16
Without Na_2SO_3	2.16	2.83	1.14	0.66	0.16
% Difference	14.8	36.0	37.7	53.0	0.0

K_d Estimation

Low, mid and high concentration vials produced similar results such that a single value of K_d for each contaminant was determined and used regardless of contaminant concentration. Due to the quantity of mid-concentration sorption vials these were then used to calculate K_d values. These values were estimated in two ways; using only liquid phase data to compare the difference between soil containing and soil free systems or using a combination of liquid phase and solid phase data collected from the soil

containing systems. The variability in results using only liquid phase data was significant and may be due to losses within the system. For this reason, the partition coefficients were determined based on the combination of liquid and solid phase data. Table A.3.4. below summarizes these values and provides a comparison for each equilibration period.

Table C.4. Partition Coefficients Based on Batch Sorption Experiment.

Equilibration Period	K_d [(mg/kg)/ppm]				
	Benzene	Toluene	Ethylbenzene	*o*-Xylene	Naphthalene
7-Day	1.11	1.09	2.07	1.89	4.00
14-Day	1.09	1.19	2.02	1.91	4.00
21-Day	1.14	1.34	2.09	1.90	2.60
Average	1.11 ± 0.03	1.21 ± 0.13	2.06 ± 0.04	1.90 ± 0.01	3.53 ± 0.81
% Deviation	2.26	10.43	1.75	0.53	22.88

LIST OF REFERENCES

LIST OF REFERENCES

Abriola, L.M. and Chen, Y.M. (1993) A Mathematical Model of BTX Biodegradation and Transport: Comparisons with Experimental Measurements. CoBioRem Conference. March 1-2. Lansing, MI.

Alvarez-Cohen, L., McCarty, P.L., and Roberts, P.V. (1993) Sorption of Trichloroethylene onto a Zeolite Accompanied by Methanotrophic Biotransformation. *Environ. Sci. Technol.* **27(10)**, 2141-2148.

Angley, J.T., Brusseau, M.L., Miller, W.L., and Delfino, J.J. (1992) Nonequilibrium Sorption and Aerobic Biodegradation of Dissolved Alkylbenzenes During Transport in Aquifer Material: Column Experiments and Evaluation of a Coupled-Process Model. *Environ. Sci. Technol.* **26(7)**, 1404-1410.

Boyd, S.A. and Guerin, W.F. (1992) Differential Bioavailability of Soil-Sorbed Naphthalene to Two Different Bacterial Species. *Appl. Environ. Microbiol.* **58(4)**, 1142-1152.

Criddle, C.S., Alvarez, L.M., and McCarty, P.L. (1991) Microbial Processes in Porous Media, In Transport Processes in Porous Media, Ed. J. Bear and Y. Corapcioglu, NATO Advanced Studies Institute Series E: Applied Sciences Vol. 202, Kluwer Academic Publishing Co., Dordrecht, The Netherlands, pp. 641-691.

Dec, J. and Bollag, J-M. (1988) Microbial Release and Degradation of Catechol and Chlorophenols Bound to Synthetic Humic Acid. *Soil Sci. Soc. Am. J.* **52**, 1366-1371.

Farrell, J. and Reinhard, M. (1994) Desorption of Halogenated Organics from Model Solids, Sediments, and Soil Under Unsaturated Conditions. 1. Isotherms. *Environ. Sci. Technol.* **28(1)**, 53-62.

Farrell, J. and Reinhard, M. (1994) Desorption of Halogenated Organics from Model Solids, Sediments, and Soil under Unsaturated Conditions. 2. Kinetics. *Environ. Sci. Technol.* **28(1)**, 63-72.

Gordon, A.S. and Millero, F.J. (1985) Adsorption Mediated Decrease in the Biodegradation Rate of Organic Compounds. *Microbial Ecology*. **11**, 289-298.

Hayden, N.J. (1992) Retention, Enhanced Volatilization, and Leaching of Gasoline in Unsaturated Soils. PhD Dissertation. Michigan State University.

Hu, C-Z, Korus, R.A., Levinson, W.E., and Crawford, R.L. (1994) Adsorption and Biodegradation of Pentachlorophenol by Polyurethane-Immobilized Flavobacterium. *Environ. Sci. Technol.* **28(3)**, 491-496.

Larson, R.J. and Vashon, R.D. (1983) Adsorption and Biodegradation of Cationic Surfactants in Laboratory and Environmental Systems. *Dev. Ind. Microbiol.* **24**, 425-434.

Marquis, S.A. Jr. and Dineen, D. (1994) Comparison Between Pump and Treat, Biorestoration, and Biorestoration/Pump and Treat Combined: Lessons from Computer Modeling. *Ground Water Monitoring Review.* 105-119.

Ogram, A.V., Jessup, R.E., Ou, L.T., and Rao, P.S.C. (1985) Effects of Sorption on Biological Degradation Rates of 2,4-Dichlorophenoxyacetic Acid in Soils. *Appl. Environ. Microbiol.* **49(3)**, 582-587.

Ridgway, H.F., Safarik, J., Phipps, D., Carl, P. and Clark, D. (1990) Identification and Catabolic Activity of Well-Derived Gasoline-Degrading Bacteria From a Contaminated Aquifer. *Appl. Environ. Microbiol.* **56(11)**, 3565-3575.

Rijnaarts, H.H.M., Bachman, A., Jumelet, J.C., and Zehnder, A.J.B. (1990) Effect of Desorption and Interparticle Mass Transfer on the Aerobic Biomineralization of α-Hexachlorocyclohexane in a Contaminated Calcareous Soil. *Environ. Sci. Technol.* **24(9)**, 1349-1354.

Robinson, K.G., Farmer, W.S., and Novak, J.T. (1990) Availability of Sorbed Toluene in Soils for Biodegradation by Acclimated Bacteria. *Wat. Res.* **24(3)**, 345-350.

Scott, H.D., Wolf, D.C., and Lavy, T.L. (1983) Adsorption and Degradation of Phenol at Low Concentrations in Soil. *J. Environ. Qual.* **12(1)**, 91-95.

Scow, K.M. and Hutson, J. (1992) Effect of Diffusion and Sorption on the Kinetics of Biodegradation: Theoretical Considerations. *Soil Sci. Soc. Am. J.* **56(1)**, 119-127.

Scow, K.M. (1993) Effect of Sorption-Desorption and Diffusion Processes on the Kinetics of Biodegradation of Organics Chemicals in Soil. Soil Science Society of America Special Publication No. 32. Madison, WI.

Shimp, R.J. and Young, R.L. (1988) Availability of Organic Chemicals for Biodegradation in Settled Bottom Sediments. *Ecotoxicol. Environ. Safety.* **15**, 31-45.

Smith, S.C., Ainsworth, C.C., Traina, S.J., and Hicks, R.J. (1992) Effect of Sorption on the Biodegradation of Quinoline. *Soil Sci. Soc. Am. J.* **56(3)**, 737-746.

Steinberg, S.M., Pignatello, J.J., and Sawhney, B.L. (1987) Persistence of 1,2-Dibromoethane in Soils: Entrapment in Intraparticle Micropores. *Environ. Sci. Technol.* **21(12)**, 1201-1207.

van Genuchten, M. Th. and Wagenet, R.J. (1989) Two-Site/Two-Region Models for Pesticide Transport and Degradation: Theoretical Development and Analytical Solutions. *Soil Sci. Soc. Am. J.* **53(5)**, 1303-1310.

van Loosdrecht, M.C.M., Lyklema, J., Norde, W., and Zehnder, A.J.B. (1990) Influences of Interfaces on Microbial Activity. *Microbiol. Rev.* **54(1)**, 75-87.

Voice, T.C. and Kolb, B. (1994) Comparison of European and American Techniques for the Analysis of Volatile Aromatic Compounds in Environmental Matrices. 1994 Pittsburgh Conference, February 27 - March 4, Chicago, IL.

Voice, T.C. and Wallace, R.B. (1994) Biodegradation Kinetics, Oxygen Transfer, and Bioavailability in Bioventing and Related Technologies (portion of proposal: Objective 3).

Voice, T.C. (1993) Fundamentals of Petroleum Spill Chemistry. CoBioRem Conference. March 1-2. Lansing, MI.

Voice, T.C., Pak, D., Zhao, X., Shi, J., and Hickey, R.F. (1992) Biological Activated Carbon in Fluidized bed Reactors for the Treatment of Groundwater Contaminated with Volatile Aromatic Hydrocarbons. *Wat. Res.* **26(10)**, 1389-1401.

Weissenfels, W.D., Klewer, H-J, and Langhoff, J. (1992) Adsorption of Polycyclic Aromatic Hydrocarbons (PAHs) by Soil Particles: Influence on Biodegradability and Biotoxicity. *Appl. Microbiol. Biotechnol.* **36**, 689-696.

Wszolek, P.C. and Alexander, M. (1979) Effect of Desorption Rate on the Biodegradation of n-Alkylamines Bound to Clay. *J. Agric. Food Chem.* **27(2)**, 410-414.