AN ASSESSMENT OF FACTORS CONTROLLING THE BIODEGRADATION OF BENZENE IN THE SUBSURFACE ENVIRONMENT

By

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(ABSTRACT)

The objectives of this research were to correlate benzene biodegradation with soil physical, chemical and biological properties, to determine if biodegradation could be predicted based on measured or observed soil properties, and to investigate the role of nutrients on benzene biodegradation in soil. Benzene disappearance over time was measured in aerobic active and control (autoclaved) microcosms prepared with previously-uncontaminated subsurface soils. Soil microcosm experiments were prepared with initial benzene concentrations of 1, 10 and 50 mg/L. For each soil, logistic, zero-, first-, and 3/2-order kinetic models were fit to benzene disappearance versus time data by regression analysis. The logistic and 3/2-order models fit the data better than zero- and first-order models for experiments prepared with initial benzene concentrations of 1 and 10 mg/L. For an initial benzene concentration of 50 mg/L, experimental data were often better fit by zero- or first-order kinetic models. To obtain predictive equations, logistic kinetic model rate constants were related to soil properties using multiple linear regression (MLR). The "best" MLR models and their regression coefficient estimates were statistically significant at $p \leq 0.05$. For experiments prepared with an initial benzene concentration of 1 mg/L, the resulting predictive equation contained soil phosphorus concentration and cation exchange capacity (CEC). For experiments prepared with an initial benzene concentration of 10 mg/L, the predictive equation contained soil copper, nitrate-N and phosphorus concentrations, CEC, and % sand. A comparison was made between benzene biodegradation in unamended soil microcosms and soil microcosms amended with ammonium and potassium phosphates (11 mM nitrogen, 6 mM phosphorus and 0.6 mM potassium). Benzene disappearance over time in soil microcosms was stimulated by nutrient addition in one (11%), 6 (50%), and 5 (45%) soils at initial benzene concentrations of 1, 10 and 50 mg/L, respectively. In general, nutrient addition had the greatest affect on benzene biodegradation in low pH soils.

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I. Introduction

The objectives of this research were to correlate benzene biodegradation with physical, chemical and biological properties of soil, to determine if biodegradation can be predicted based on measured or observed soil properties, and to investigate the role of nutrients on benzene biodegradation in soil. Benzene is a colorless, volatile, flammable and chemically unreactive hydrocarbon component of gasoline fuels. Benzene is a minor fuel constituent by volume, for example, 0.8% for gasoline fuels (AWWA, 1990). The global storage, distribution and consumption of gasoline has made benzene a common pollutant (Miller, *et al.*,1990; Salanitro, 1993). Partially soluble in water, benzene can move from the site of a spill through the soil with percolating water to contaminate groundwater. Typical co-contaminants are toluene, ethyl benzene, and the xylenes, known collectively as the BTEX fraction. Once in the subsurface environment benzene can volatilize and diffuse through the soil atmosphere, partition into soil organic matter, leach with moving groundwater or remain trapped in soil pore spaces. Benzene does not undergo significant adsorption reactions with soil minerals (Perlinger and Eisenreich, 1991) and is not hydrolyzed (Sittig, 1991). Benzene concentrations in the vicinity of a contaminant plume typically range up to 10 mg/L, with higher concentrations found in unweathered spills and nearer to spill sites (Aelion and Bradley, 1991; Borden, *et al.*, 1994; Hallbourg, *et al.*, 1992; Salanitro, 1993). Benzene is a known carcinogen based on both animal studies and studies of occupationally-exposed workers (Sittig, 1991). The United States Environmental Protection Agency (USEPA) drinking water maximum contaminant level (MCL) for benzene is 0.005 mg/L with a goal of zero. The low MCL for benzene can dictate subsurface remediation goals.

The biodegradation of benzene and its co-contaminants in the subsurface by indigeneous microorganisms is dependent upon not only the contaminant properties and concentrations, but also on local geology, weather, and soil physical, chemical and biological properties. Data from laboratory studies with soil microcosms and soil columns, field plot studies and pilot scale experiments, and *in situ* monitoring at contaminant spill sites have been used to define a general range of conditions for which *in*

situ biodegradation may be considered a viable remediation strategy (Allen-King, *et al.*, 1994; Borden, 1994; Buscheck, *et al.*, 1993; USEPA, 1994). Batch microcosm studies can reveal critical information on (1) the biodegradability of contaminant(s), (2) dependence on biodegradation of contaminant concentration, (3) sequence of biodegradation in contaminant mixtures, (4) lag or acclimation periods, and (5) biodegradation rate and reaction order (Alvarez and Vogel, 1991; Burback and Perry, 1993; Swindoll, *et al.* 1988). The non-steady state nature of batch studies may best predict contaminant disappearance in water-saturated soils at soluble plume boundaries (Buscheck, *et al.*, 1993; Salanitro, 1993), and thus the extent of contaminant movement for a given groundwater velocity.

Benzene biodegradation in the subsurface is typically controlled by oxygen (Anid, *et al.*, 1993; Barker and Patrick, 1985; Robertiello, *et al.*, 1994; Salanitro, 1993). In aqueous solution at pH 7, an oxygen concentration of ca. 4 mg/L is necessary to completely mineralize a benzene concentration of 1 mg/L; and the solubility of oxygen in water is ca. 10 mg/L at 25 °C. *In situ*, both natural and anthropogenic organic matter as well as minerals compete with benzene for oxygen. Reported first-order rate constants for "aerobic" benzene biodegradation range from field values of 0.007 to 0.024 day^{-1} but laboratory values range from 0.05 to 0.5 day^{-1} (Salanitro, 1993). The latter range is consistent with first order decay constants of 0.02 to 1.0 day^{-1} observed in the laboratory experiments described in the following chapters. Under aerobic conditions, benzene can be completely mineralized to carbon dioxide and water by soil bacteria with and without cell growth (Burback and Perry, 1993; Gibson, 1968), co-metabolized with toluene (Burback and Perry, 1993), or partially metabolized (Grbic-Galic and Vogel, 1987; Keener and Arp, 1994). Intermediate products may include catechol, hydroquinone or phenol (Burback and Perry, 1993; Gibson, 1968; Keener and Arp, 1994). Although anaerobic degradation of benzene by sulfate-reducing (Lovely, *et al.*, 1995) and iron-reducing (Lovely, *et al.*, 1994) bacteria has been demonstrated under laboratory conditions, the comparably low benzene loss rates under anaerobic field conditions make it difficult to separate biodegradation from competing disappearance mechanisms such as partitioning, dilution and volatilization (Borden, *et al.*, 1994; Buscheck, *et al.*, 1993; Wilson, *et al.*, 1994). Under aerobic conditions and at benzene concentrations of 10 mg/L or greater, macronutrients such as nitrogen and phosphorus which are necessary for cellular metabolism and growth may limit benzene biodegradation in soil (Allen-King, *et al.*,1994; McCloskey, 1995; Poor, *et al.*,1995). *In situ* bioremediation may be natural or intrinsic, or may be enhanced using engineered systems which deliver oxygen, alternate electron acceptors and nutrients to the subsurface.

Benzene biodegradation observed in soil microcosms was described using an applicable kinetic model (Chapter II), and equations which predicted the model rate constant based on uncontaminated soil

properties were developed using multiple linear regression (Chapter III). In anticipation of a potential role for soil nitrogen and phosphorus in the predictive equations, a comparison was also made of benzene biodegradation in unamended soil microcosms and in soil microcosms amended with ammonium and potassium phosphates (Chapter IV). The ability to predict benzene biodegradation rate and extent in the subsurface is key to preparing reasonable risk assessments, evaluating potential remediation strategies and establishing attainable clean-up criteria.

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II. Application Of Logistic, Zero-, First- And 3/2-Order Kinetic Models To Benzene Disappearance In Soil Microcosms

ABSTRACT

Aerobic microcosm experiments were prepared with previously-uncontaminated subsurface soils at initial benzene concentrations of 1, 10 and 50 mg/L. For each soil, benzene disappearance versus time data were fit by regression analysis to logistic, zero-, first-, and 3/2-order kinetic models and model kinetic parameters determined for each data set using SAS® REG or NLIN procedures, as appropriate. Models were compared (1) by visual inspection of fitted regression lines to the data and by residual analysis, (2) by unexplained variability (residual sum of squares) and F-tests, and (3) by predictions of total time for benzene to biodegrade below a concentration of 0.005 mg/L. No one model fit the data well over the entire range of initial benzene concentrations. The 3/2-order and logistic models fit the data better than zero- and first-order models at initial benzene concentrations of 1 and 10 mg/L. At these concentrations, predictions of total time for benzene to biodegrade below 0.005 mg/L were the worst for the first-order model. At an initial benzene concentration of 50 mg/L, data were often better fit by zero- or first-order kinetic models. Parameter estimates for 3/2-order and logistic models were not always statistically significant, *i.e.*, the estimates had high standard errors. For the logistic model, reducing the number of model parameters by approximating the concentration of benzene required to produce the initial biomass improved rate constant estimates with little or no deterioration of fit for data obtained at initial benzene concentrations of 1 and 10 mg/L. With this approximation, for a known initial benzene concentration the entire S-shaped benzene disappearance versus time curve could be described by the logistic rate constant. For two soils, microcosm experiments were prepared at initial benzene concentrations of 0.05, 0.10, 0.50, 1, 5, 10 and 50 mg/L and parameter estimates obtained at each concentration for all four models. Rate constants exhibited a dependence upon both soil type and initial benzene concentration.

INTRODUCTION

The biotic or abiotic disappearance in soil of benzene and its aromatic co-contaminants toluene, ethylbenzene and the xylenes, known collectively as BTEX, is of interest to those regulators, scientists and engineers involved in decision-making regarding environmental clean-up of gasoline underground storage tank leaks or migrating landfill leachate. Predictions of contaminant phases and movement over time are essential for developing remediation strategies which minimize both risk and cost. Contaminant fate and transport modeling upon which predictions may be made require knowledge of not only the contaminant properties and concentrations, but also on local geology, weather, groundwater and soil properties (Bonazountas, 1988). *In situ* biodegradation is often modeled as first-order (exponential), based on field observations of contaminant disappearance over time and distance (Borden, *et al.*, 1994; Buscheck, *et al.* 1993; Hutchins and Wilson, 1994). Batch microcosm studies, however, reveal a wider range of applicable kinetic models (Simkins and Alexander, 1984). While batch microcosm studies may have limited applicability to field-scale efforts, they do reveal critical information on the (1) biodegradability of contaminant(s), (2) dependence of biodegradation on contaminant concentration, (3) sequence of biodegradation in contaminant mixtures, (4) lag or acclimation periods, and (5) biodegradation rates and reaction order (Alvarez and Vogel, 1991; Burback and Perry, 1993; Swindoll, *et al.* 1988). The non-steady state nature of batch studies may best predict contaminant disappearance in water-saturated soils at soluble plume boundaries (Buscheck, *et al.*, 1993; Salanitro, 1993), and thus the extent of contaminant movement for a given groundwater velocity.

Models which are more commonly used to describe organic mineralization or disappearance in soil are presented and discussed in detail by Alexander and Scow (1989), Brunner and Focht (1984), and Simkins and Alexander (1984). For this investigation, models were chosen because they have a theoretical basis, their shapes were consistent with data sets, they required only measures of benzene and time, and their use is relevant to soil remediation. The Monod models, with and without growth, were not considered because their underlying assumptions of steady-state growth, freely-diffusible substrate and nutrients, and non-limiting nutrient levels (Alexander and Scow, 1989) were not met under the described experimental conditions. The concept of a half-saturation constant K_s to describe a bacterial affinity for the added substrate is not suitable for the diverse microbial ecology of soil, because the substrate concentration, exposure history or mixed substrates can select for different species of degraders or select for different biodegradation pathways (Alexander and Scow, 1989; Brunner and Focht, 1984; Burback and Perry, 1993). Furthermore, the Monod non-linear models are over-parameterized with respect to the

measured values of substrate S and time t, thus non-linear regression would yield poor and possibly non-singular parameter estimates (Robinson, 1985; Robinson and Tiedje, 1983). The model comparisons described in this paper differ from earlier comparisons in that benzene is the added contaminant; benzene disappearance was observed in subsurface soils instead of a liquid culture medium, lake water or sewage; the concentrations of benzene ranged up to 50 mg/L, representing aromatic concentrations of environmental concern; benzene disappearance over time and not carbon dioxide production was monitored; and comparisons were made for as many as 12 different soils. Because no measurements of bacterial growth, biomass or activity versus time were made, regressions of the selected models to data were empirical. The primary objective of this analysis was to describe observed benzene disappearance versus time in soil microcosms with an applicable kinetic model. Rate constants obtained from this analysis were used to build a predictive model for benzene biodegradation in soil (Chapter III).

The zero-order (linear) model, Equation 1, may apply to saturation kinetics for bacteria living on organic substrates (Alexander and Scow, 1989), and is characterized by a *constant* substrate depletion rate. For saturation kinetics to occur, the amount of substrate present greatly exceeds the ability of a population to mineralize the substrate, and the growth rate of the bacterial population is very low or has reached steady-state. Zero-order kinetics may also appear when a growth factor other than carbon, for example, oxygen, nitrogen, or phosphorus, is limiting.

The first-order (exponential) model, Equation 2, may apply to substrate-limited kinetics for bacteria living on organic substrates (Alexander and Scow, 1989), and is characterized by a *constant percentage* substrate depletion rate. First-order kinetics may best describe pure-culture bacterial growth by binary fission with a constant doubling rate on a single substrate in a liquid medium with no nutrient deficiencies (Alexander and Scow, 1989). The first-order model fit mineralization data for a *Pseudomonas* sp. growing on benzoate concentrations of 0.01, 0.032 and 0.32 mg/L in a liquid medium (Simkins and Alexander, 1984).

The 3/2-order model, Equation 3, has been proposed to describe the biodegradation of organic compounds in soil with microbial growth (Brunner and Focht, 1984). The S-shape curve of the 3/2-order model allows for a lag time or acclimation period, and no estimate of biomass concentration is required. For this model, microbial growth is assumed to be constant (linear) with time, a reasonable assumption since microbial growth may be limited by diffusion of oxygen and nutrients to cells and carbon dioxide from cells, predation or competition, substrate toxicity or build-up of toxic by-products. Microbial growth is also assumed to be independent of the substrate concentration, a poor assumption for low-organic matter soils. This model fit data for mineralization of low concentrations of phenol in a silt loam (Scow, *et al.*, 1986).

The S-shaped curve of the logistic model, Equation 4, is used by ecologists to describe population growth of species whose growth is limited by a carrying capacity K, influenced by environmental factors such as weather and predation, or environmental resources such as food or space. Assumptions inherent to this model are (1) all individuals in the population are equal, for example, in terms of size, age, and reproductive capability, (2) environmental changes produce an immediate effect in growing populations, and (3) the carrying capacity, K, is constant, and has no associated threshold phenomena (McNaughton and Wolf, 1973). Laboratory manipulation of bacterial cultures and their growth medium can minimize violations of these assumptions, which would not hold in the microbially-diverse soil microcosms. The logistic model has more recently been used to describe S-shaped substrate depletion curves observed in batch studies of benzoate (Simkins and Alexander, 1984) and phenol (Jones and Alexander, 1986) disappearance. This model may be most applicable when the added carbon substrate greatly exceeds the concentration required to build-up the initial population of degraders, but low enough that population growth does not exceed environmental resources (Alexander and Scow, 1989), conditions under which logistic microbial growth is expected to occur.

METHODOLOGY

Soil Sampling, Transportation and Storage

Sampling sites throughout Virginia were chosen primarily for the expected soil characteristics, based on published soil surveys or previous research. Subsurface soil samples (ca. 2 kg) were obtained from depths of 1 to 4 meters by a pre-cleaned, 3"-diameter, manually-driven auger; identified and described by an accompanying soil scientist; and transferred directly to sterile polyethylene bags. The polyethylene bags were placed on ice in coolers and returned to the laboratory the same day. In the laboratory, soil samples were mixed by combining portions from each bag into new bags, which were then stored at 8°C in the dark for biological characterization and microcosm preparation. A subsample was air-dried for 48 hours and sieved through a No. 10 (2 mm) sieve for chemical and physical analyses. Soil microcosm experiments were prepared within a week, and soil characterization completed within 2 weeks, of soil sampling. Most of the soils used in this research were moist, acidic, highly weathered, red and yellow-brown soils commonly found in southeastern United States. Soils were taken from both forest and agricultural lands. Two soils were obtained from below the local water table.

Microcosm Preparation

Soil microcosms containing 5 g soil and 5 g sterile, distilled water spiked with benzene concentrations of 1 mg/L (1.2 ± 0.2 mg/L), 10 mg/L (10.4 ± 0.7 mg/L) or 50 mg/L (48 ± 6 mg/L) were prepared in 22-mL acid-washed headspace vials and sealed with aluminum crimp-top caps and Teflon®-lined rubber septa. Benzene (>99%, Sigma-Aldrich) was added by microliter syringe to sterile, distilled water in a volumetric flask, mixed by shaking for 15 minutes and immediately pipetted into soil microcosms. Microcosms were then shaken for 10 seconds to mix the soil and solution, and incubated in the dark under static conditions at a constant temperature of 20 °C. Controls were prepared identically except solutions were amended with 200 mg $(NH_4)_2HPO_4$-NH_4^+ and 25 mg K_2HPO_4-K^+ per liter and soil was autoclaved 5 times over one week. (Nutrients were added to controls to compare benzene disappearance versus time in control microcosms with both unamended and nutrient-amended active microcosms, as described in Chapter IV.) Soil for controls, and solutions, glassware, and plastics were sterilized by autoclaving for 20 minutes at 121 °C and 1.05 kg/cm^2. An experiment consisted of 24 identically-prepared soil microcosms. For each experiment, microcosms were sampled in triplicate daily or less frequently, depending upon the anticipated or observed benzene disappearance rates.

Soil microcosms were water-saturated and incubated at 20 °C to simulate aquifer conditions in a moderate climate and to remove these factors from subsequent analyses. The initial dissolved oxygen concentration was ca. 10 mg/L, an amount sufficient enough to completely mineralize a benzene concentration of 1-2 mg/L. Sufficient oxygen was available in the headspace to degrade a benzene concentration of >50 mg/L in 5 mL of water.

Analytical Technique

The headspace sampling analytical method was selected based upon a detection level of 0.005 mg/L benzene concentration and the ability to sample microcosms with minimum benzene loss and soil disruption. A Hewlett-Packard 19395A headspace sampler and a Hewlett-Packard 5880A gas chromatograph (GC) equipped with a flame ionization detector were used for headspace sampling and sample analysis, respectively. The GC column was 6-ft of 1/8-in OD stainless steel packed with 5% SP 1200 and 1.75% Bentone-34. Injector and detector temperatures were 130 °C and 200 °C, respectively. The initial column temperature was 50 °C, ramped at 6 °C/minute to a final temperature of 90 °C. Nitrogen at a total flow rate of 40 mL/min was the carrier gas. Microcosms were heated in the headspace

sampler for 1 hour at 60 °C prior to sampling. A GC response versus aqueous benzene concentration standard curve was developed from headspace sampling of vials containing 5 ml of water-dissolved benzene at concentrations from 0.005 to 100 mg/L. The standard curve did not change significantly when prepared with microcosms containing nutrient solution or 5 g sterile, sieved sand.

Soil Analysis

Results of selected soil analyses are summarized in Table 1. Refer to Appendix A for a complete listing of the soil properties analyzed. To measure bulk soil pH, 20 g of air-dried and sieved soil were combined with 20 mL of de-ionized, distilled water in a 50 ml beaker, stirred, and allowed to sit for 20 minutes. The pH was measured with a pH meter equipped with a glass electrode and calibrated with buffered solutions at pH 4 and 7. Organic matter content was determined by measuring the weight loss in 2-g soil samples dried first for 24 hours at 110 °C to remove water, and then dried for an additional 24 hours at 430 °C to volatilize organic matter (Davies, 1974). A particle size analysis was done on each soil by the pipette method (Day, 1965). Cation exchange capacity (CEC) was determined by the barium chloride method described by Rhoades (1982). Magnesium concentration in the extractant, diluted as necessary, was determined with a Perkin-Elmer Model 703 atomic adsorption spectrophotometer, calibrated from 0 to 0.5 mg/L using a lanthanum-amended magnesium chloride solution.

Heterotrophic plate counts (HPCs) were prepared from soil within 1 day of sampling. Ten grams of soil were mixed with 95 mL of sterile distilled water in a Waring blender jar and homogenized for 2 minutes on low speed. Subsequent serial dilutions were made by pipetting 10 mL of homogenized soil into sterile dilution jars containing 90 mL of sterile distilled water. Jars were then shaken by hand for one minute. Agar spread plates were prepared in triplicate as described by Wollum (1982) and incubated in the dark at 35 °C for five days. Agar media contained per liter: 15 g Bacto-Agar, 1.0 g Bacto-Peptone, 1.0 g yeast extract, 0.4 g K_2HPO_4, 0.5 g $(NH_4)_2HPO_4$, 0.05 g $MgSO_4 \cdot 7H_2O$, 0.01 g $FeCl_3$, 0.1 g $CaCl_2$, 250 mL of inorganic soil extract and 750 mL distilled water. To make the soil extract, soil was baked 24 hours at 430 °C to remove soil organic matter. Fifty grams baked soil per 500 mL of distilled water were combined in a 1-liter Erlenmeyer flask, autoclaved for 20 minutes at 1.05 kg/cm^2 and 121 °C, and the soil extract decanted and filtered through a 0.2 micron filter.

Oxygen concentration in selected soil microcosms was measured just prior to GC sampling by inserting a 2-½" oxygen (hypodermic) needle electrode (Diamond General, Inc.) through the headspace vial septum into the undisturbed soil-water interface. The oxygen electrode was calibrated using two

water cells, one purged with nitrogen (<0.02 % oxygen) and one purged with breathing air (21% oxygen). In soil microcosms containing either sand or clay spiked to an initial benzene concentration of 10 mg/L, direct oxygen measurements were made when benzene disappearance rates were highest. For both soils, the oxygen content at the soil-water interface ranged from 16-19%, near saturation. Additional oxygen measurements made within the soil sediments, which required removal of the headspace vial cap, were 8-12%, indicating soils were aerobic. Similar measurements made in clay soil microcosms spiked to an initial benzene concentration of 50 mg/L revealed depletion of oxygen in the sediment but not the soil-water interface after 5 days of incubation.

Data Analysis

Models, shown in Equations 1 through 4, were chosen because they have a theoretical basis, their shapes were consistent with data sets, they required only measures of benzene and time, and their use is relevant to soil remediation (Simkins and Alexander, 1984). Because no measurements of bacterial growth, biomass or activity versus time were made, regressions of selected models to the data were empirical. Equation 1 and Equations 2, 3 and 4 were fitted to the experimental data by the method of least squares using SAS® REG and NLIN procedures, respectively. Non-linear models were fit using a modified Gauss-Newton iterative method (SAS®, 1990). To obtain initial parameter estimates for Equations 2, 3 and 4, models were plotted with varying parameters (Appendix B), matched with benzene disappearance versus time data and initial values selected. SAS® NLIN programs and sample outputs are shown in Appendix C. The logistic model was fitted with and without X_0 included in the pararmeter estimates. X_0 was approximated as 0.01 mg/L based on 1 x 10^{-13} g of benzene to meet the carbon requirements of one cell. Soil heterotrophic plate counts (HPCs) ranged from 10^4 to 10^5 CFU/g dry soil (Table 1). HPCs could represent between 1% and 50% of the viable cells in the soil sample, not all heterotrophic bacteria plated necessarily degrade benzene, both bacteria and fungi not enumerated by HPCs may also degrade benzene, and benzene metabolized by cells may be used for both growth and maintenance (Steffan, *et al.*, 1988; Torsvik, *et al.*, 1990). Thus, this estimate of X_0 could easily range an order of magnitude in either direction.

$$\text{Zero-Order} \quad S = S_0 - k_1 t \tag{1}$$

$$\text{First-Order} \quad S = S_0 \exp(-k_2 t) \tag{2}$$

3/2-Order $S = S_0 \exp[(-k_2 t)-(k_3 t^2/2)]$ (3)

Logistic $S = S_0+X_0/\{1+(X_0/S_0)\exp[k(S_0+X_0)t]\}$ (4)

where

S = benzene concentration, mg/L

S_0 = initial benzene concentration, mg/L

t = time, days

X_0 = benzene concentration required to produce the initial population density, mg/L

k = rate constant, $(mg/L \cdot day)^{-1}$

k_1 = rate constant, (mg/L)/day

k_2 = rate constant, day^{-1}

k_3 = rate constant, day^{-2}

Models were compared (1) by visual inspection of the fitted regression lines to the data and by residual analysis, (2) by unexplained variability (residual sum of squares) and F-tests, and (3) by predictions of total time for benzene to biodegrade below a concentration of 0.005 mg/L. By definition, a residual is the difference between the observed response and the response predicted ($S_{observed}$-$S_{predicted}$) by a regression of the model to the data. Ideally, residuals are small and randomly scattered around zero. Plotting residuals revealed trends which could suggest model improvements, as can be seen in Figures 1 and 2.

In a regression using the method of least squares, the total data variability is partitioned into variability explained by the regression and variability not explained (Myers, 1990; Ott, 1993). The latter is the residual sum of squares (RSS) and is calculated as shown in Equation 5. RSS is minimized to find the best fit. For a given data set, a better model yields a smaller RSS. Figures 3, 4 and 5 show changes in RSS as a given soil microcosm experiment data set is modeled by Equations 1 through 4.

If the residual analysis indicates a more complex model is needed, for example, an additional higher order term illustrated by the progression from first-order to 3/2-order, an F-test can be used to compare models (Ott, 1993). With each additional model term, more variability is explained by the regression but the increase in RSS may not be statistically significant. The estimate of the regression variance σ is the mean square error (MSE) and is calculated as shown in Equation 6, where p is the number of parameters estimated in the regression. In Equations 1 and 2, for example, the regressions for

zero- and first-order models estimate 2 parameters: S_0 an intercept, and rate constants k_1 or k_2. The F statistic is formed by the ratio shown in Equation 7, where RSS_2 and MSE_2 are values for the more complex model. The F statistic is compared with values in an F distribution table, where $df_1 = \Delta p$ between models and df_2 = n-p, p for the more complex model. The hypothesis under test is that there is no decrease in variability between models; the hypothesis can be rejected if the F statistic is larger than the tabulated value at the desired confidence level (*e.g.*, p=0.05). The F-test was used to compare first-order with 3/2-order model regressions and logistic model regressions with and without X_0 included in the parameter estimations for experimental data obtained at initial benzene concentrations of 1 mg/L and 10 mg/L. *The latter model will be referred to as the* logistic (X_0=0.01) *model*. Results of the logistic model F-tests are summarized in Tables 2 and 3.

$$RSS = \sum_{n=1}^{i} (S_{i\ observed} - S_{i\ predicted})^2 \quad (5)$$

$$MSE = RSS/(n-p) \quad (6)$$

$$F_{\Delta p,\, n-p,\, 0.05} = [(RSS_1 - RSS_2)/\Delta p]/MSE_2 \quad (7)$$

Models were also compared on their ability to predict the total time require for benzene to degrade below a concentration of 0.005 mg/L in soil microcosm experiments. The model predictions and observed values are shown in Tables 4 and 5 for initial benzene concentrations of 1 mg/L and 10 mg/L, respectively. The observed time is the earliest time in which the average remaining benzene concentration in triplicate microcosms was below 0.005 mg/L. In some cases, microcosm sampling was too frequent and the total time was not observed.

For Alaga sand and Cullen clay loam, microcosm experiments were prepared at initial benzene concentrations of 0.05, 0.10, 0.50, 1, 5, 10 and 50 mg/L and parameter estimates obtained at each concentration for all four models. Six data sets for each soil are plotted with a logistic (X_0=0.01) model fitted regression line, as shown in Figures 6 and 7. To see if estimated rate constants varied predictably with benzene concentration, rate constants for zero-order, first-order and logistic (X_0=0.01) models were graphed as a function of S_0, $\log(S_0)$, and $1/S_0$, respectively, for these two soils (Figure 8). Rate constant estimates for zero-, first- and 3/2-order models for all soils were plotted by initial benzene concentration to identify trends (Figures 9, 10 and 11).

RESULTS AND DISCUSSION

Zero-, first-, 3/2-order and logistic fitted regression lines for benzene disappearance versus time data from microcosms prepared with a sandy loam are shown in Figure 1. Benzene disappearance over time in active soil microcosms was significantly greater than disappearance in microcosms prepared with sterilized soil, indicating a biotic benzene removal mechanism. This was true for all microcosm experiments prepared with an initial benzene concentration of 10 mg/L or less. Immediate sorption of benzene reduced the aqueous benzene concentration in soil microcosms below that of the stock solution. In many experiments the aqueous benzene concentration increased slightly within a day or two after preparation, as can be seen in Figure 1. Data suggest a lag time or acclimation period of up to 4 days, followed by rapid benzene disappearance for 3 days, and finally a period of more gradual benzene disappearance or "tailing" for 4 more days. The observed lag period may be a result of biochemical changes within bacterial or fungal cells to produce benzene-degrading enzymes, or a result of initial cell numbers too low to cause a discernable decrease in benzene concentration. The S-shape trend of these data are representative of benzene disappearance versus time curves for experiments prepared with initial benzene concentrations of 1 mg/L and 10 mg/L. This S-shape can be explained as a mirror image of logistic microbial growth. For logistic growth in a batch culture or microcosm, large increases in cell numbers occur early when substrate concentration is high relative to cell numbers, but growth rate reaches a maximum and slows as substrate concentration falls. In soil microcosms, factors such as oxygen and nutrient availability, predation and competition, substrate toxicity, and build-up of toxic by-products may limit microbial growth before substrate depletion affects growth. The assumption of logistic growth may be valid for low-organic matter, aerobic soils receiving a pulse of biodegradable organic substrate.

The zero-order (linear) model typically over-estimated the observed initial benzene concentration and under-estimated the total time for complete benzene biodegradation, as can be seen in Figure 1. The tendency for lag time data to lie above, and the "tailing" data to lie below, the regression line is more clearly seen in a plot of the residuals, Figure 2. The first-order (exponential) model typically over-estimated the observed initial benzene concentration and total time for complete benzene biodegradation, as can be seen in Figure 1. Again, the mis-fit of the first order model is reflected in a plot of the residuals, Figure 2, which shows lag time and "tailing" data below, and maximum rate data above, the best fit line. The 3/2-order model (Figure 1) typically under-estimates initial benzene concentration, and finds the slight increase in benzene concentration if it is present. This "hump" is indicated by a negative k_2, the more negative k_2 the more pronounced is the hump (Appendix B). Too few data points or

high scatter in the data especially during the "tailing" period caused the 3/2-order model fit to over-estimate the total time for benzene biodegradation. The residuals of this model, plotted in Figure 2, are more evenly scattered above and below zero, although with a hint of a sinusoidal trend. The logistic model (Figure 1) is symmetric about its inflection point. With X_0 held constant for a given data set, increasing k increases the curvature or non-linearity of the logistic S-shape. The sensitivity of the model to changes in k increases as k becomes smaller (Appendix B). Poorer fits of this model were characterized by under-estimates of both initial benzene concentration and total time for benzene biodegradation as can be seen in Figure 6 and 7. As can be seen in Figure 2, the residuals are randomly scattered above and below zero. Visual examination of the regression lines to actual data, as well as residual analyses, indicate that the 3/2-order and the logistic models generally provided the best fit to data from experiments prepared at initial benzene concentrations of 1 mg/L and 10 mg/L. For 50 mg/L initial benzene concentration experiments, logistic (X_0=0.01) models had the worst regressions as illustrated in Figure 6; and with X_0 included in the parameter estimation, initial parameter estimates were not accurate enough to obtain regressions.

By comparing the RSS for each model by soil, Figures 3, 4 and 5 give information on model fit for a greater number of soils. For a given data set, a smaller RSS indicates a better fit, with the understanding that increasing the number of model parameters will also reduce the RSS. Tests for significance of this increase are discussed in the following paragraph. In general, the 3/2-order and logistic models provided a better fit to the data than did zero- and first-order models, except when data scatter was high, as exemplified by the near constant RSS for Weikert soil in Figure 3 and Roanoke and McGary soils in Figure 4. At 1 mg/L and 10 mg/L benzene concentrations, the RSS values for both the 3/2-order and logistic models were similar, visible in Figures 3 and 4. In the logistic (X_0=0.01) model only S_0 and k are included in parameter estimation. Compared with zero- and first-order models, also 2 parameter models, the logistic (X_0=0.01) model generally had lower RSS values, indicating a better fit (Figures 3 and 4). At initial benzene concentrations of 50 mg/L, the RSS values for the logistic (X_0=0.01) model were higher than those of the remaining three models. Although the 3/2-order model has a lower RSS for several soils, for three soils the estimate of k_3 was negative, which plots as an increasing benzene concentration with time, as shown in Appendix B.

F-tests were performed between logistic (X_0=0.01) and logistic models as shown in Tables 1 and 2, and between the first-order and 3/2-order models. The results of F-tests between first- and 3/2-order model regressions for experimental data obtained at initial benzene concentrations of 1 mg/L and 10 mg/L can be surmised from Figures 3 and 4. The improvement (decrease) in RSS for the 3/2-order over the first-order models was statistically significant (p=0.05) for all the soils except Weikert prepared with

1 mg/L, and Roanoke and McGary prepared with 10 mg/L, initial benzene concentrations. At 50 mg/L initial benzene concentration, of the soils with a positive k_3 estimate for the 3/2-order model, less than one-half had a RSS significantly lower than that of the first-order model.

For the logistic model, the values of X_0 varied widely between soils, and in many cases, for the same soil (Tables 2 and 3). By definition, X_0 is the amount of substrate required to grow the degrader population to its initial density. In theory, X_0 should be the same for separate subsamples of a given soil, although the higher applied benzene concentration may effectively select a smaller benzene-degrader population. For example, Burback and Perry (1993) found *Mycobacterium vaccae* grown in liquid culture degraded benzene at an initial concentration of 50 mg/L but not 100 mg/L when benzene was added as a single substrate. An approximation of X_0 was made as described previously, and the regressions obtained with X_0 removed from the parameter estimation. To visually inspect the fit, fitted regression lines for both the logistic (X_0=0.01) and logistic models were plotted with experimental data, as shown in Figure 1. From Tables 2 and 3, these two regressions were significantly different for Alaga, Altavista and Cullen soils prepared with an initial benzene concentration of 1 mg/L, and Alaga, Braddock, Cullen, Riverview and Weikert soils prepared at an initial benzene concentration of 10 mg/L. Figures 6 and 7 show plots of both Alaga sand and Cullen clay loam microcosm data fitted to the logistic (X_0 = 0.01) model over a range of initial benzene concentrations. By visual inspection, the fits are reasonable for all S_0 except 50 mg/L benzene. For both 3/2-order and logistic models, with three parameters being estimated the standard errors on k were higher, as can be seen in Tables 2 and 3 for the logistic model, and in several cases estimates of k were not significantly significant, *i.e.*, the estimates had high standard errors. Both of these models had highly correlated parameter estimates, which can be seen in the asymptotic correlation matrix (Appendix C) for the parameter estimates. Correlations > 0.9 can worsen the parameter estimates (Robinson, 1985). For the logistic model, one solution was to remove X_0 from the parameters estimated and accept a minor degree (if any) of model deterioration in the fit for a statistically-significant rate constant.

From Tables 4 and 5, which compare model predictions of total time for benzene biodegradation below 0.005 mg/L, the over-estimates of the first-order and under-estimates of the zero-order models are clearly seen. Predictions by the 3/2-order and logistic models are not so easily categorized, although the predictions worsen as data scatter increases.

For Alaga sand and Cullen clay loam, microcosms were prepared at initial benzene concentrations of 0.05, 0.10, 0.50, 1, 5, 10 and 50 mg/L, and regressions obtained at each concentration for all four models. The experimental data and fitted regression lines for the logistic (X_0=0.01) model are shown in Figures 6 and 7. Note that for both soils, the benzene disappearance versus time curves become

increasingly non-linear up to an initial benzene concentration of 10 mg/L, but at 50 mg/L these curves are comparatively more linear. One explanation is that at the lower benzene concentrations less bacterial growth is necessary before benzene disappearance is discernible, and nutrient or oxygen availabilities are not limiting. At an initial benzene concentration of 50 mg/L, diffusion of nutrients or oxygen and may limit bacterial growth. In Alaga sand microcosms prepared with an initial benzene concentration of 50 mg/L, for example, 10 mg/L benzene disappeared after approximately 5 days, consistent with the time for that amount to degrade in microcosms prepared with an initial benzene concentration of 10 mg/L. The benzene disappearance rate then slows, suggesting cellular metabolism is inhibited, perhaps due to growth factor deficiencies, benzene toxicity or build-up of toxic by-products. For Cullen clay loam prepared with an initial benzene concentration of 50 mg/L, the logistic regression suggests an increasing benzene disappearance rate after 30 days. A linear fit, however, was not significantly different from the abiotic controls. Ammonium phosphate and potassium phosphate addition stimulated benzene biodegradation in soil microcosms prepared with initial benzene concentrations of 10 mg/L and 50 mg/L (Chapter IV). These data suggest that at higher aqueous benzene concentrations, inorganic nutrients may limit benzene biodegradation in subsurface soil.

Parameter estimates for all four models were dependent upon both soil type and initial benzene concentration. Rate constants were lower for the clay loam than for the sand. Values of k_1 for the zero-order model, k_2 for the first-order model, and k for the logistic (X_0=0.01) model are plotted in Figure 8 as a function of S_0, $\log(S_0)$, and $1/S_0$, respectively, for Alaga sand and Cullen clay loam. For the zero-order model, k_1 increases with increasing initial benzene concentration up to 5 mg/L for Alaga sand and 10 mg/L for Cullen clay loam, then k_1 decreases with increasing benzene concentration. For the first-order model, k_2 is scattered about an average value of 0.8 for Alaga sand and 0.4 for Cullen clay loam with increasing initial benzene concentration to 5 mg/L for both soils, then k_2 decreases with increasing benzene concentration. The zero-order and first-order rate constants are not constant and constant percentage, respectively, but change significantly for initial benzene concentrations from 1 mg/L to 50 mg/L. This analysis indicates how inappropriate these models may be for wide range of *in situ* initial benzene concentrations.

For the logistic (X_0=0.01) model, k rapidly decreases up to 1 mg/L benzene concentration, then decreases much more slowly with increasing benzene concentration. These curves can be fit with a power curve of the form $k=a/S_0$, where a is a constant which changes with soil. The rapid deceleration of biodegradation rate occurs when $a=S_0$, or initial benzene concentrations of approximately 1.5 mg/L for Alaga sand and 0.7 mg/L for Cullen clay loam. These concentrations may correspond to the amount of benzene which can be metabolized in these soils before oxygen diffusion rates become important.

Figures 9, 10 and 11 are plots of the rate constants for zero-, first- and 3/2-order models by initial benzene concentration and soil. Zero-order parameter estimates increased dramatically between experiments prepared at initial benzene concentrations of 1 mg/L to 10 mg/L; but in almost one-half of the soils, k_1 decreased between experiments prepared at 10 mg/L and 50 mg/L (Figure 9). For Roanoke, McGary, Altavista and Cullen soils the biotic disappearance rates were not significantly different from the abiotic disappearance rates at initial benzene concentrations of 50 mg/L. First-order parameter estimates (k_2) decreased between experiments prepared at benzene concentrations of 1 mg/L and 10 mg/L, and between 10 mg/L and 50 mg/L (Figure 10). Note that for both models, the lowest parameter estimates for initial benzene concentrations of 50 mg/L occurred in the five soils with the highest clay content (Table 1). Parameter estimates for the 3/2-order model do not show as clearly a substrate-dependence (Figure 11). The trends between experiments prepared at initial benzene concentrations of 1 mg/L and 10 mg/L are obscured by sign changes in k_2; an increasing positive k_2 corresponds with a decreasing lag time, and a negative k_2 indicates an initial "hump" in the data (Appendix B). The shortest lag times occurred in experiments with an initial benzene concentration of 1 mg/L benzene. More than one-half of the soils had a decrease in k_2 between experiments prepared at initial benzene concentrations of 1 mg/L and 10 mg/L, but k_2 decreased (longer lag time) in all soils between 10 mg/L and 50 mg/L (Figure 11a). Trends in k_3 were easier to identify (Figure 11b). This parameter estimate decreased for most soils between experiments prepared at initial benzene concentrations of 1 mg/L and 10 mg/L, and for all soils between 10 mg/L and 50 mg/L. Lower k_3 reflects longer biodegradation times. Note, too, that three soils had negative estimates of k_3 at 50 mg/L initial benzene concentration. From Tables 2 and 3, the logistic model parameter estimates (k) decreased between experiments prepared at initial benzene concentrations of 1 mg/L and 10 mg/L. The trends for each model indicate a strong dependence of biodegradation on initial benzene concentration.

No one model adequately described benzene disappearance over time in soil for a wide range of initial benzene concentrations. Similar results were reported by Jones and Alexander (1986) for phenol mineralization in batch studies with a lake water. The best fitting models were first-order, Monod (no growth), logistic and logarithmic for phenol concentrations of 0.5, 1.0, 2.0, and ≥5.0 μg/L, respectively, when phenol was assumed to be the sole carbon source for mineralizing bacteria. The observed kinetics changed for pre-incubated samples, for samples collected seasonally, and for samples with suspended particulate matter removed by filtration, and these changes were explained as probable differences in the numbers and distribution of dominant species of phenol-mineralizing bacteria.

Table II. 1. Summary of measured soil properties.

Soil Series	pH	% Clay	CEC (meq/100 g)	% OM	HPC (cfu/g dry soil)
Alaga	4.5	0.3	0.2	0.3	18,000
Altavista	4.6	29	11.8	5.9	17,000
Braddock	4.9	44	3.1	5.5	34,000
Cecil	4.5	1.7	3.1	5.4	6,000
Cullen	5.0	37	3.7	7.6	12,000
Groseclose	7.6	24	10.2	3.0	25,000
McGary	6.6	36	7.3	4.5	23,000
Riverview	6.0	9.4	2.8	2.0	31,000
Roanoke	7.7	30	14.0	3.2	30,000
Ross	4.9	11	5.3	8.9	50,000
Weikert	5.0	1.1	0.6	1.4	78,000
Wheeling	6.0	13	6.5	3.0	90,000

Table II. 2. Comparison of logistic models for experimental data obtained at an initial benzene concentration of 1 mg/L.

Soil Series	n	S_0[a] ± SE[b]	X_0[c] ± SE	k[d] ± SE	Model RSS	Model MSE	F statistic
Alaga	14	1.07 ± 0.04	0.01	5.4 ± 0.2	0.036	0.0030	18†
		1.08 ± 0.02	0.13 ± 0.04	2.1 ± 0.3	0.014	0.0012	
Altavista	25	1.20 ± 0.03	0.01	0.60 ± 0.02	0.21	0.0090	6.8†
		1.17 ± 0.03	0.002 ± 0.001	0.8 ± 0.1	0.16	0.0074	
Braddock	26	0.72 ± 0.02	0.01	1.94 ± 0.05	0.068	0.0028	2.2
		0.75 ± 0.03	0.03 ± 0.02	1.5 ± 0.3	0.062	0.0027	
Cullen	25	0.81 ± 0.02	0.01	1.82 ± 0.04	0.051	0.0022	18†
		0.86 ± 0.02	0.05 ± 0.02	1.2 ± 0.1	0.028	0.0013	
Groseclose	14	0.97 ± 0.06	0.01	2.5 ± 0.2	0.20	0.017	2.9
		1.06 ± 0.08	0.1 ± 0.2	1.2 ± 0.5	0.16	0.014	
McGary	22	0.94 ± 0.02	0.01	0.97 ± 0.03	0.098	0.0049	0.8
		0.96 ± 0.03	0.02 ± 0.01	0.8 ± 0.2	0.094	0.0049	
Roanoke	26	1.13 ± 0.02	0.01	0.40 ± 0.01	0.168	0.0070	0.3
		1.13 ± 0.03	0.01 ± 0.01	0.37 ± 0.06	0.166	0.0072	
Ross	23	0.57 ± 0.04	0.01	3.3 ± 0.2	0.172	0.0082	0.6
		0.62 ± 0.06	0.03 ± 0.04	2.4 ± 0.9	0.167	0.0083	
Weikert	23	0.84 ± 0.08	0.01	2.4 ± 0.3	0.88	0.049	3.5
		1.0 ± 0.1	0.9 ± 3	0.4 ± 0.8	0.73	0.043	

†Regressions are different. For a significance level of 0.05, $F_{1,14} = 4.6$; $F_{1,26} = 4.23$

[a]units are mg/L; [b]standard error; [c]units are mg/L; [d]units are $(mg/L*day)^{-1}$

Table II. 3. Comparison of logistic models for experimental data obtained at an initial benzene concentration of 10 mg/L.

Soil Series	n	S_0[a] ± SE[b]	X_0[c] ± SE	k[d] ± SE	Model RSS	Model MSE	F statistic
Alaga	26	9.1 ± 0.4	0.01	0.31 ± 0.01	22	0.92	5.1†
		9.7 ± 0.5	0.2 ± 0.2	0.17 ± 0.04	18	0.79	
Altavista	25	9.6 ± 0.4	0.01	0.061 ± 0.003	61	2.7	0.7
		9.8 ± 0.5	0.07 ± 0.1	0.04 ± 0.02	59	2.7	
Braddock	24	7.5 ± 0.3	0.01	0.13 ± 0.007	22	1.0	14†
		8.0 ± 0.4	0.4 ± 0.3	0.05 ± 0.01	13	0.62	
Cecil	24	7.7 ± 0.5	0.01	0.13 ± 0.009	56.87	2.6	0.03
		7.8 ± 0.7	0.02 ± 0.04	0.12 ± 0.05	56.79	2.7	
Cullen	20	8.6 ± 0.2	0.01	0.17 ± 0.005	7.5	0.41	33†
		9.3 ± 0.2	0.18 ± 0.07	0.10 ± 0.01	2.6	0.15	
Groseclose	17	7.4 ± 0.2	0.01	0.33 ± 0.007	2.8	0.19	0.5
		7.3 ± 0.2	0.005 ± 0.006	0.37 ± 0.06	2.7	0.19	
McGary	25	7.7 ± 0.6	0.01	0.045 ± 0.005	173	7.5	0.3
		8 ± 1	4 ± 27	0.005 ± 0.03	171	7.8	
Riverview	25	7.8 ± 0.3	0.01	0.20 ± 0.006	11.5	0.50	18†
		8.3 ± 0.3	0.2 ± 0.1	0.11 ± 0.02	6.3	0.29	
Roanoke	25	9.7 ± 0.7	0.01	0.067 ± 0.005	135	5.9	3.1
		10 ± 1	2 ± 4	0.01 ± 0.01	118	5.4	
Ross	26	5.4 ± 0.2	0.01	0.36 ± 0.02	13.21	0.55	0.21
		5.4 ± 0.3	0.003 ± 0.008	0.4 ± 0.1	13.09	0.57	
Weikert	23	8.5 ± 0.2	0.01	0.22 ± 0.006	8.4	0.40	5.5†
		8.8 ± 0.3	0.1 ± 0.08	0.15 ± 0.03	6.6	0.33	
Wheeling	26	8.5 ± 0.2	0.01	0.14 ± 0.004	10.491	0.44	0.009
		8.5 ± 0.2	0.01 ± 0.01	0.15 ± 0.03	10.487	0.46	

†Regressions are different. For a significance level of 0.05, $F_{1,14} = 4.6$; $F_{1,26} = 4.23$
[a]units are mg/L; [b]standard error; [c]units are mg/L; [d]units are $(mg/L*day)^{-1}$

Table II. 4. Observed and predicted total time for benzene to degrade below a concentration of 0.005 mg/L in microcosms prepared with 1 mg/L initial benzene concentration; by soil series and model. Sorted by increasing time for logistic model.

Soil Series	Logistic	Logistic, X_0*=0.01	3/2-Order	Zero-Order	First-Order	Observed
			Time, days			
Alaga	3.0	1.7	2.7	3.2	6	3.7
Ross	5.1	4.6	4.9	5.0	10	6.1
Groseclose	5.1	4.0	5.4	3.5	9.1	3.6
Braddock	7.3	6.7	7.0	7.1	15	9.0
Cullen	7.3	6.4	7.3	6.3	14	8.0
Weikert	8.6	4.7	7.0	5.3	11	6.5
McGary	11	11	11	10	28	12
Altavista	12	14	15	12	40	13
Roanoke	24	23	25	19	72	17

*units are mg/L

Table II. 5. Observed and predicted total time for benzene to degrade below a concentration of 0.005 mg/L for microcosms prepared with 10 mg/L initial benzene concentration; by soil series and model. Sorted by increasing time for logistic model.

Soil Series	Logistic	Logistic, X_0*=0.01	3/2-Order	Zero-Order	First-Order	Observed
			Time, days			
Groseclose	5.4	5.7	6.5	4.7	19	5.1
Ross	6.7	6.8	8.0	6.8	22	8.9
Alaga	6.9	5.1	6.6	6.1	18	7.7
Weikert	9.0	7.6	8.7	7.0	23	8.4
Wheeling	11	12	13	9.9	43	8.4
Riverview	12	9.0	11	8.9	29	>11
Cullen	12	9.7	11	8.2	28	8.9
Cecil	14	14	16	13	45	15
Braddock	25	14	20	15	54	16
Altavista	30	25	32	23	110	25
McGary	69	40	100	45	280	>19
Roanoke	78	22	36	26	110	>25

*units are mg/L

SUMMARY AND CONCLUSIONS

The results of this analysis clearly show that based on visual inspection of fitted regression lines to data and residual analysis, lower RSS values and F-tests, for the zero-, first-, 3/2-order and logistic kinetic models, no one model adequately described benzene disappearance over time in soil for a wide range of initial benzene concentrations. The 3/2-order and logistic models fit the data better than zero- and first-order models at initial benzene concentrations of 1 and 10 mg/L. At these concentrations, predictions of total time for benzene to biodegrade below 0.005 mg/L were the worst for the first-order model. At an initial benzene concentration of 50 mg/L, data were often better fit by zero- or first-order kinetic models. All models examined showed a dependence of their respective rate constants on initial benzene concentration. The apparent substrate-dependence of biodegradation kinetics is important for interpretation of substrate disappearance data from contaminant spill monitoring. At the aerobic, solube plume boundary of a gasoline spill, for example, non-steady state conditions of contaminant mineralization like those modeled in batch studies may exist. For contaminant levels which stimulate bacterial growth without exceeding available oxygen or nutrients, an assumption of exponential decay may lead to a gross overestimate of degradation time.

Between the S-shaped 3/2-order and logistic kinetic models which allow for bacterial or fungal cell growth with time, the 3/2-order model has the following advantages and disadvantages. The assumption of linear microbial growth in soil is realistic, especially with higher substrate concentrations, but its assumption of growth independent of substrate concentration was not observed. This model does not require an estimate of benzene-degrading biomass, easily obtainable in pure cultures in liquid media but not in a heterogeneous medium such as soil. Non-linear regression of this model to experimental data yielded two highly correlated parameters k_2 and k_3. Estimates of k_3 were often not statistically significant (the standard errors of the estimates were high), and took on a negative value in slowly degrading microcosm experiments, predicting an increase in benzene concentration with time. In other words, the 3/2-order model fit the earlier portion of the substrate depletion curve better than the later portion.

The logistic model has the following advantages and disadvantages. The assumption of logistic microbial growth may be valid for soil, but not necessarily as described for pure cultures growing on organic substrate in liquid media. Non-linear regression of this model to experimental data yielded two highly correlated parameters, k and X_0, but estimates of these parameters were not always statistically significant. Regression estimates of X_0 were not constant but varied unpredictably over several orders of magnitude for a given soil spiked at different benzene levels. By approximating X_0 and removing it from the regression, the rate constant k estimates improved with little or no deterioration in model fit for initial

benzene concentrations between 0.05 mg/L to 10 mg/L. With this approximation, for a known initial benzene concentration the entire S-shaped benzene disappearance versus time curve could be described by the logistic rate constant.

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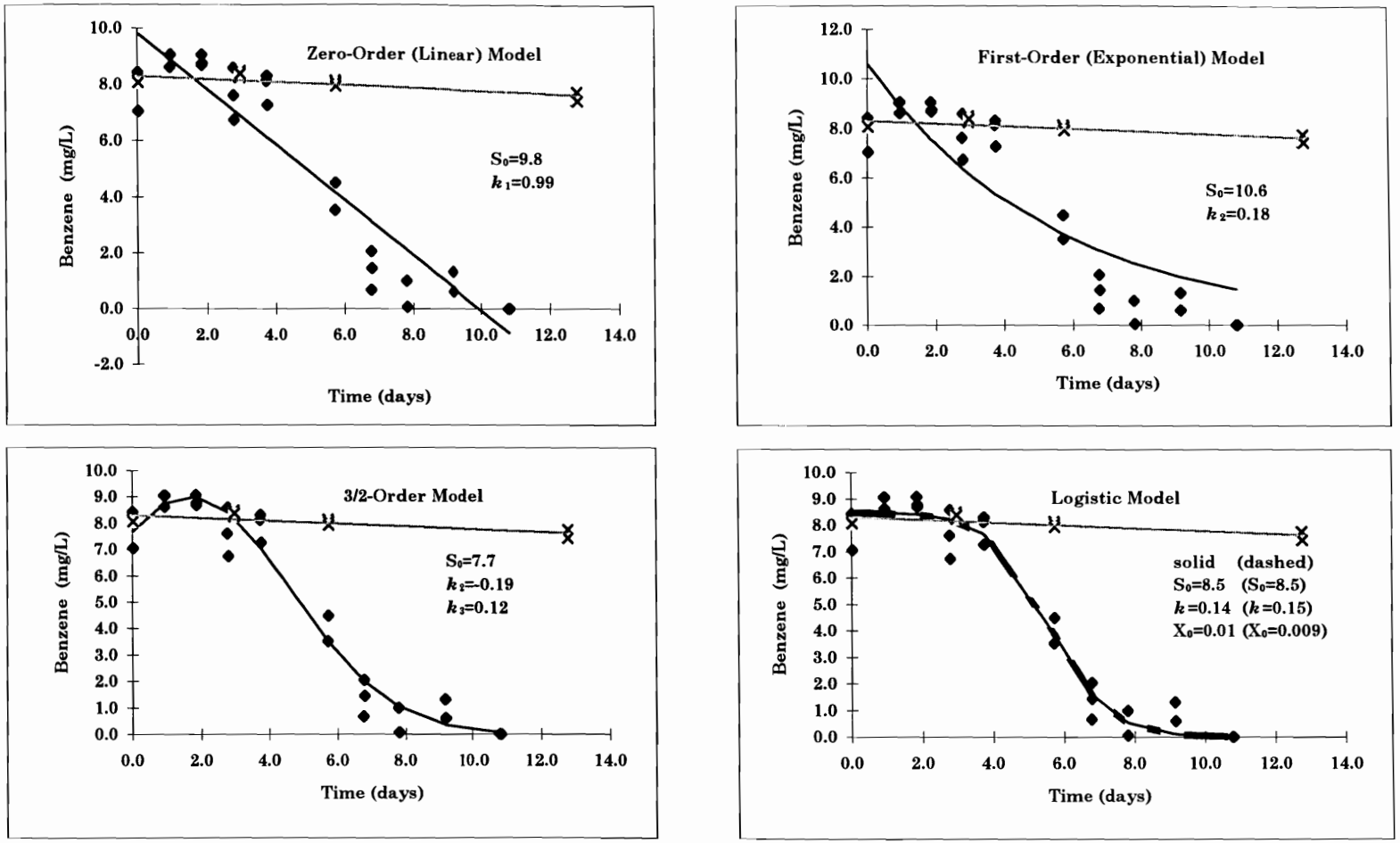

Figure II. 1. Zero-, first-, 3/2-order and logistic fitted regression lines for benzene disappearance versus time in Wheeling sandy loam microcosms. Microcosms were prepared with 5 g soil and 5 g sterile, distilled water spiked at initial benzene concentrations of 10 mg/L. Each datapoint ♦ represents one active microcosm; each **x** represents one sterile microcosm (controls). Two overlapping plots are shown for the logistic model.

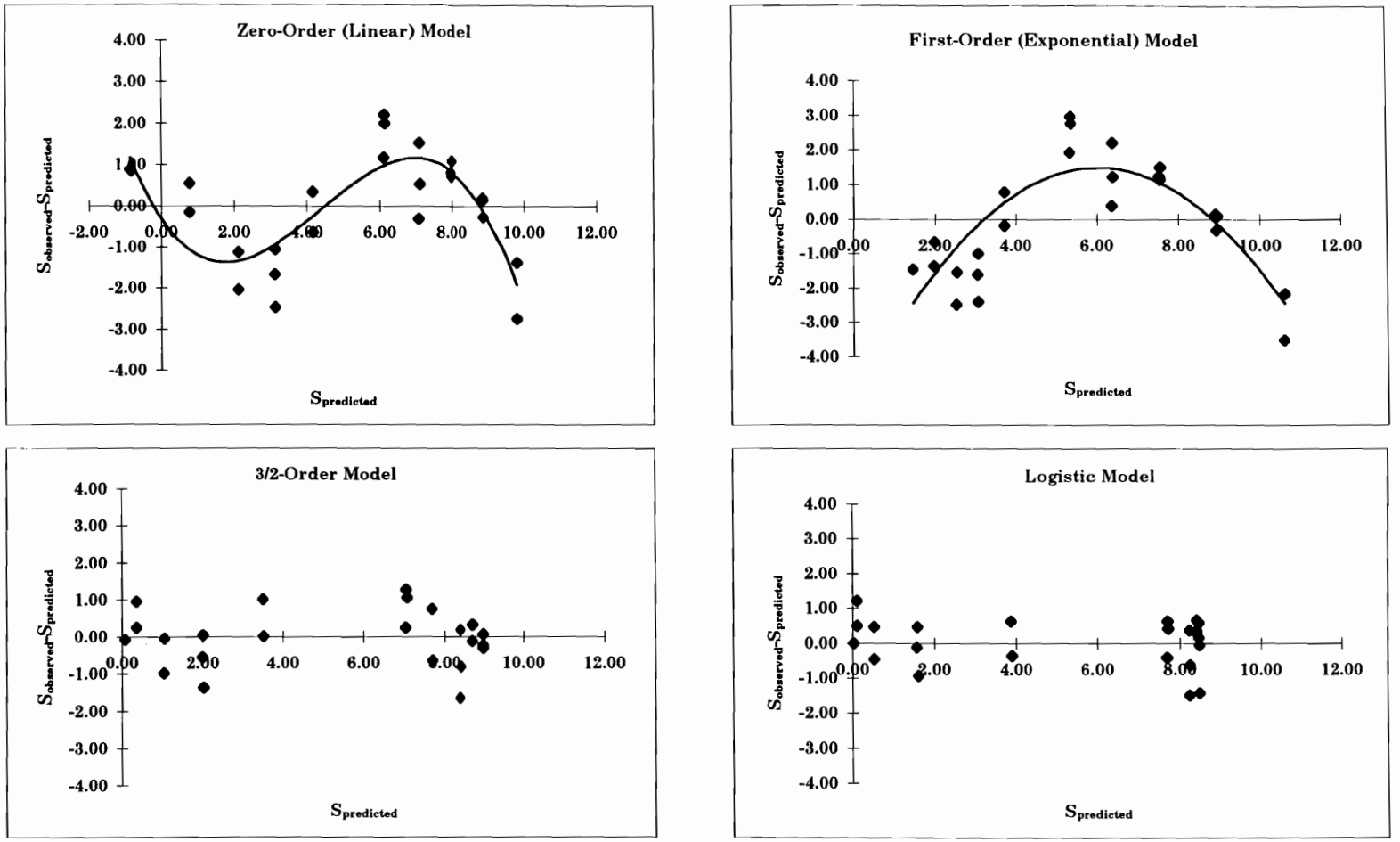

Figure II. 2. Residual analysis for zero-, first-, 3/2-order and logistic regressions of Wheeling sandy loam microcosm data. Each datapoint ♦ represents one active microcosm. Ideally, residuals are randomly scattered randomly above and below and close to zero; if the fit were perfect all of the residuals would be zero. Above residual plots were typical of experimental data for initial benzene concentrations of 1 mg/L and 10 mg/L.

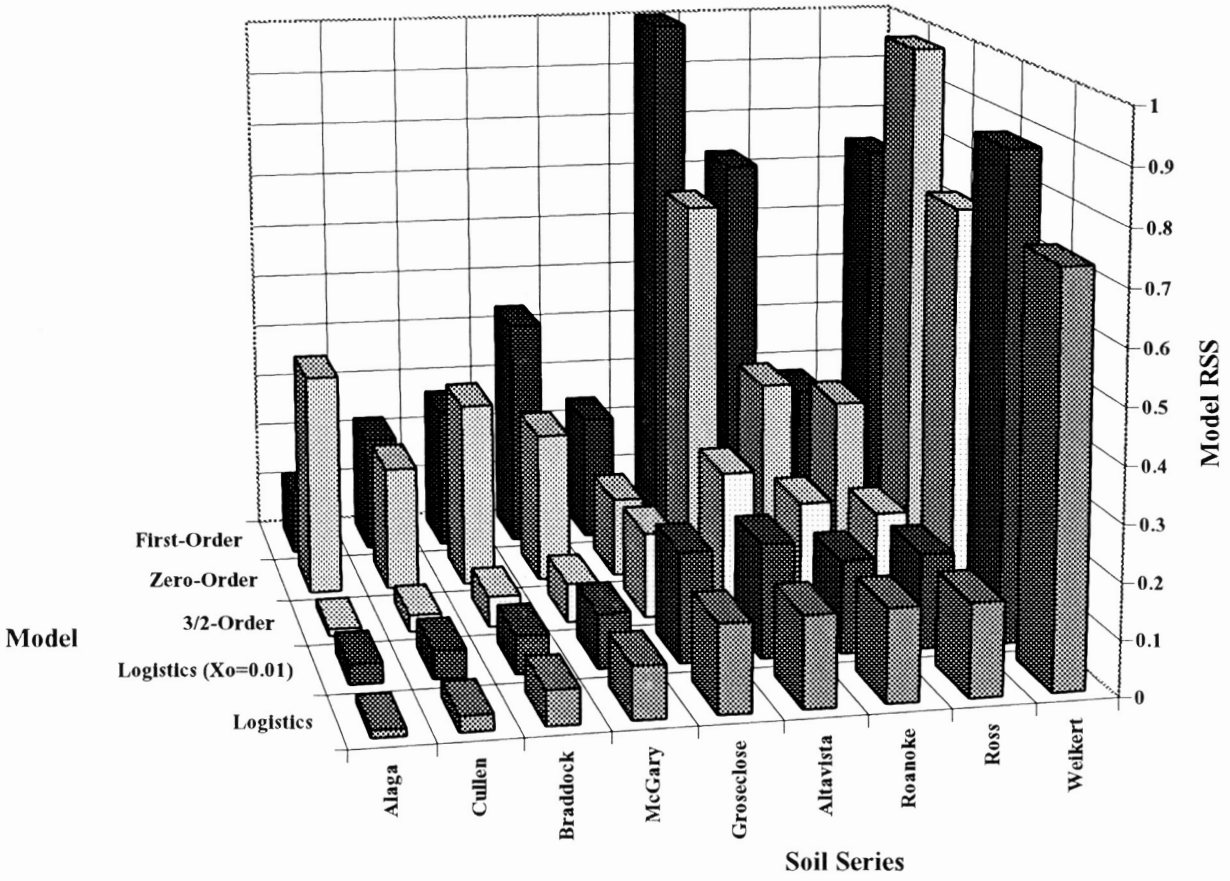

Figure II. 3. Residual sum of squares (RSS) by soil and kinetic model for microcosm experiments prepared at an initial benzene concentration of 1 mg/L. Data are sorted by increasing RSS values for the logistic model. The RSS is the function minimized in a regression using the method of least squares.

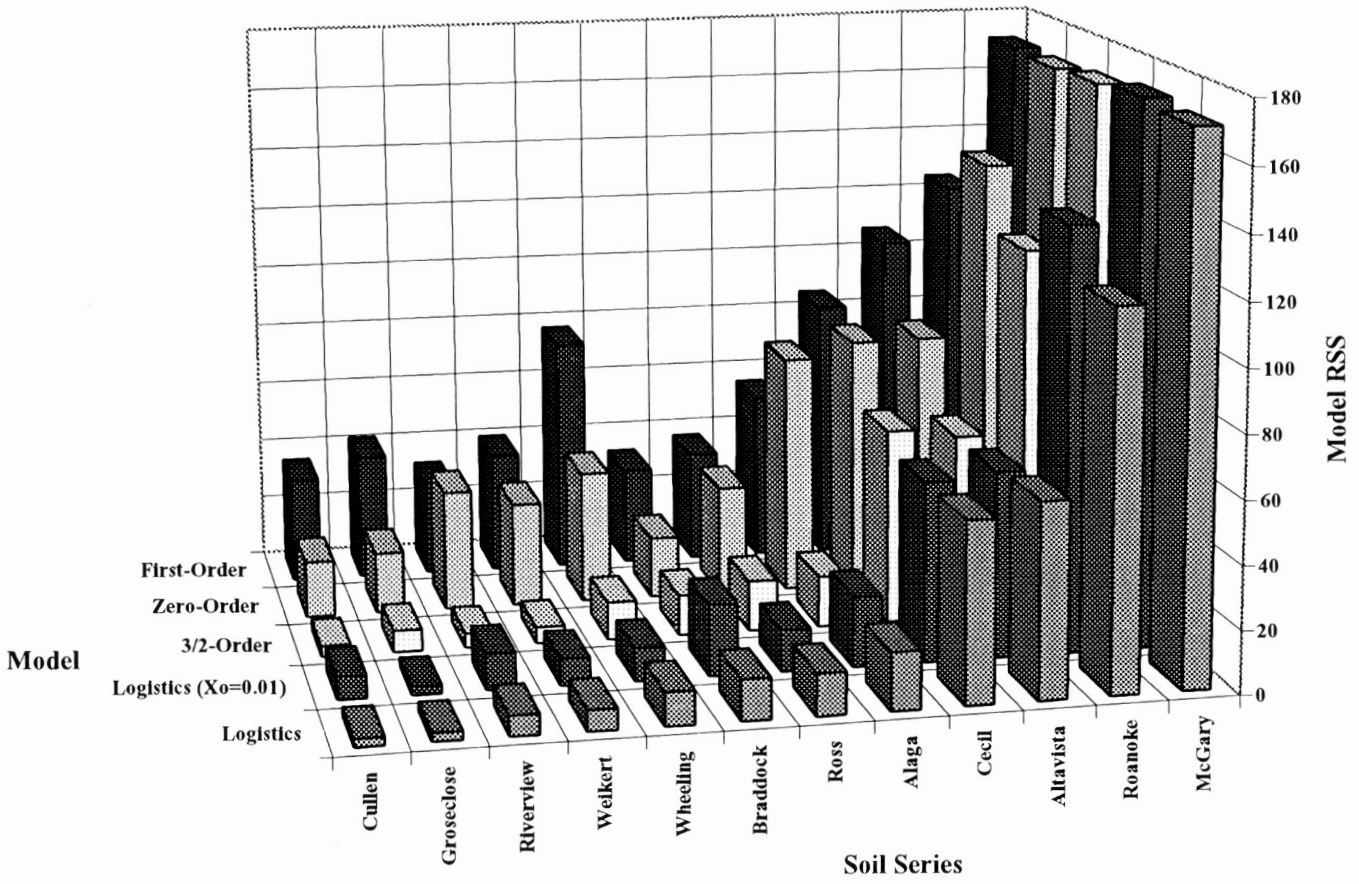

Figure II. 4. Residual sum of squares (RSS) by soil and kinetic model for microcosm experiments prepared at an initial benzene concentration of 10 mg/L. Data are sorted by increasing RSS values for the logistic model. The RSS is the function minimized in a regression using the method of least squares.

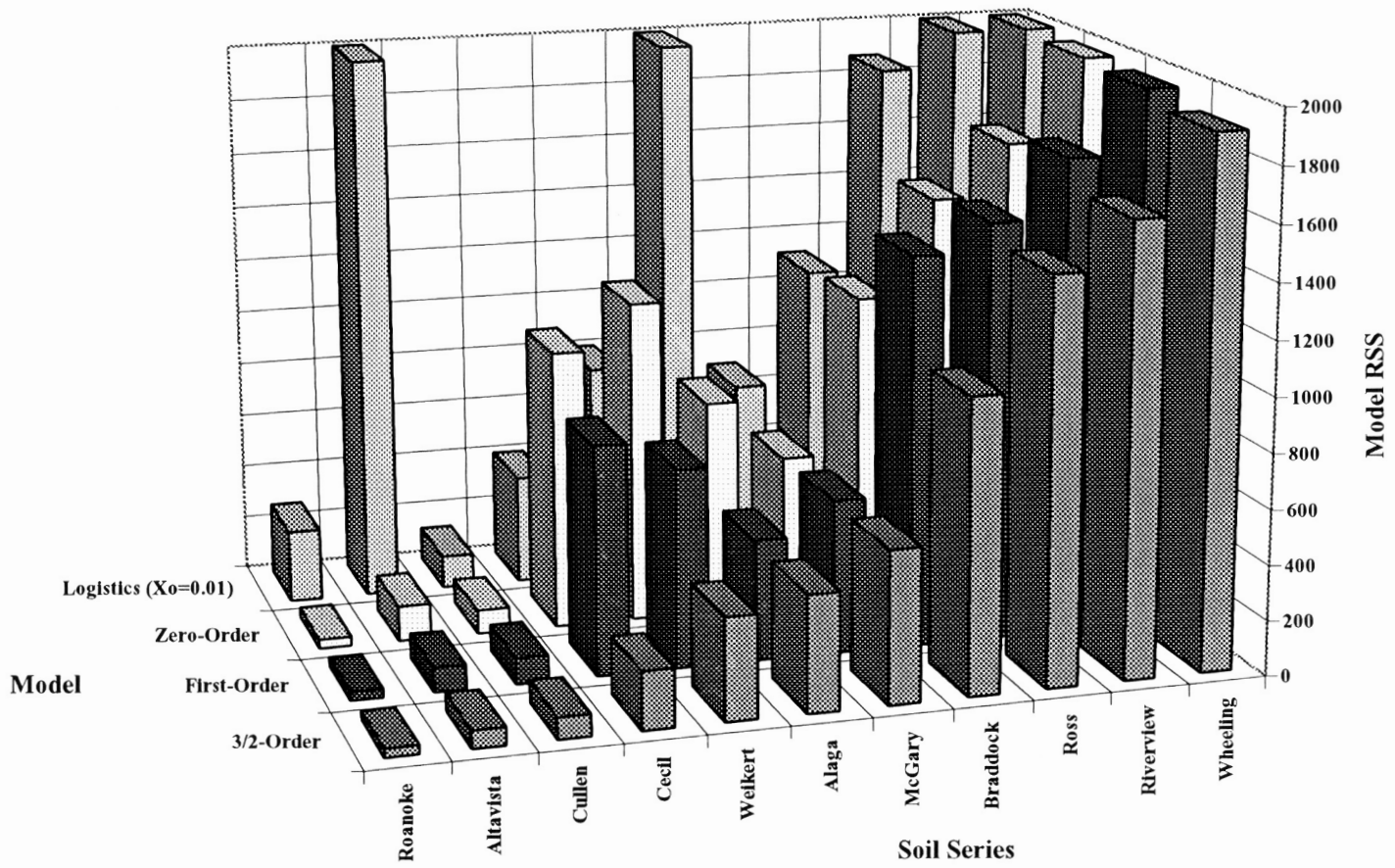

Figure II. 5. Residual sum of squares (RSS) by soil and kinetic model for microcosm experiments prepared at an initial benzene concentration of 50 mg/L. Data are sorted by increasing RSS values for the 3/2-order model. The RSS is the function minimized in a regression using the method of least squares.

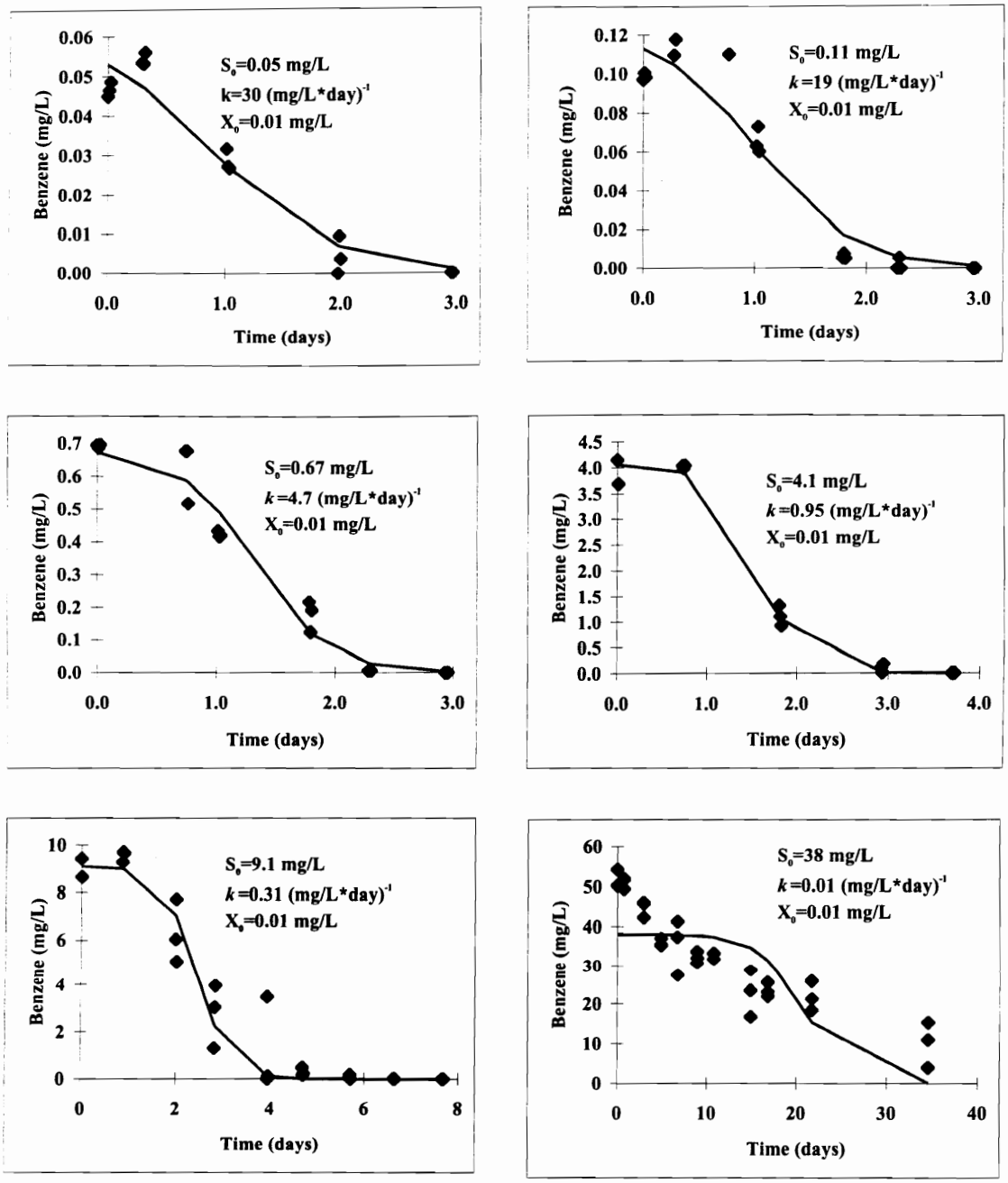

Figure II. 6. Benzene disappearance versus time in Alaga sand microcosms. Microcosms were prepared with 5 g soil and 5 g sterile, distilled water spiked at initial benzene concentrations from 0.05 to 50 mg/L. Each datapoint ♦ represents one active microcosm. Solid lines are fitted regression lines of the logistic model, with X_0 = 0.01 mg/L, to the data.

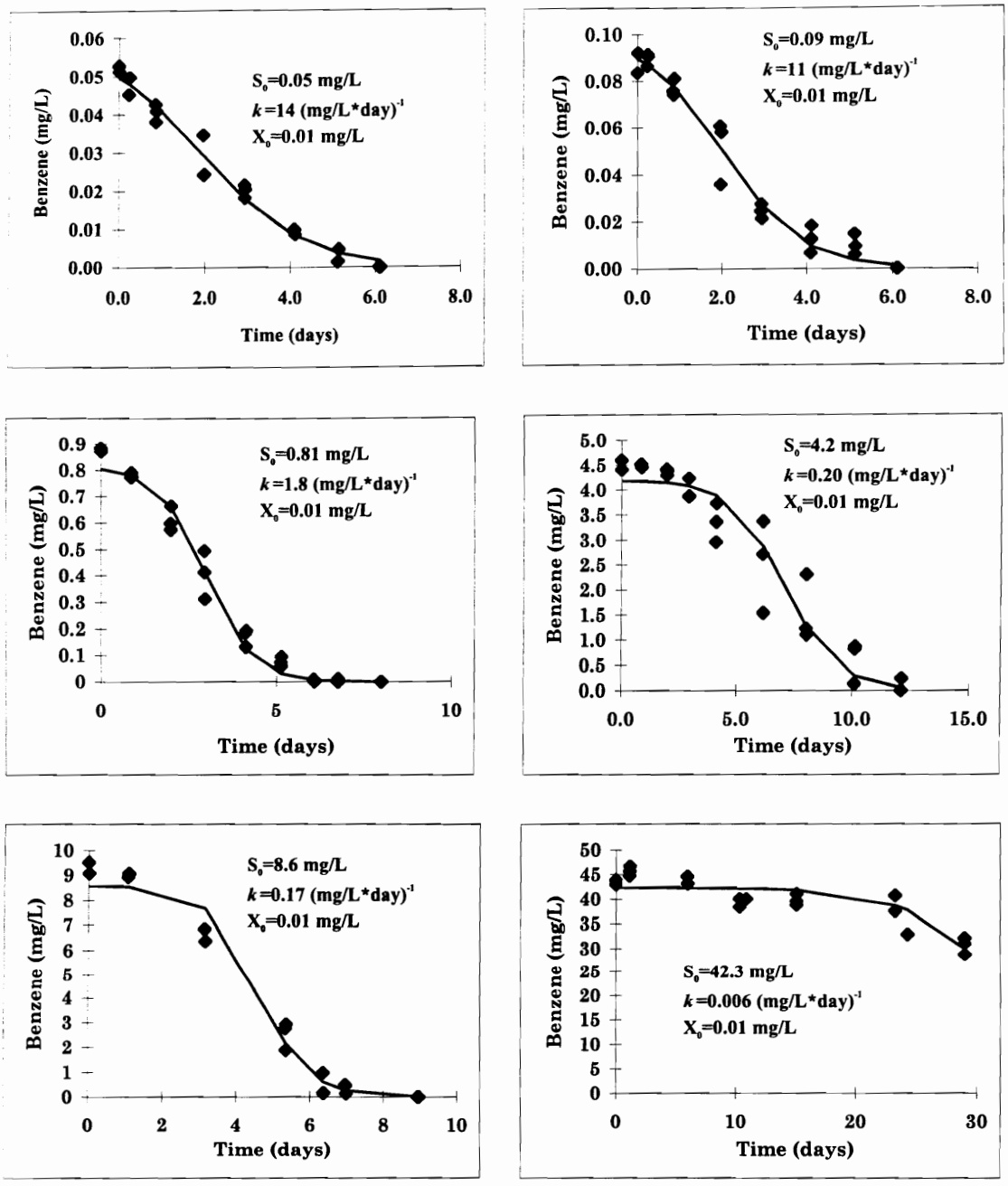

Figure II. 7. Benzene disappearance versus time in Cullen clay loam microcosms. Microcosms were prepared with 5 g soil and 5 g sterile, distilled water spiked at initial benzene concentrations from 0.05 to 50 mg/L. Each datapoint ♦ represents one active microcosm. Solid lines are fitted regression lines of the logistic model, with X_0 = 0.01 mg/L, to the data.

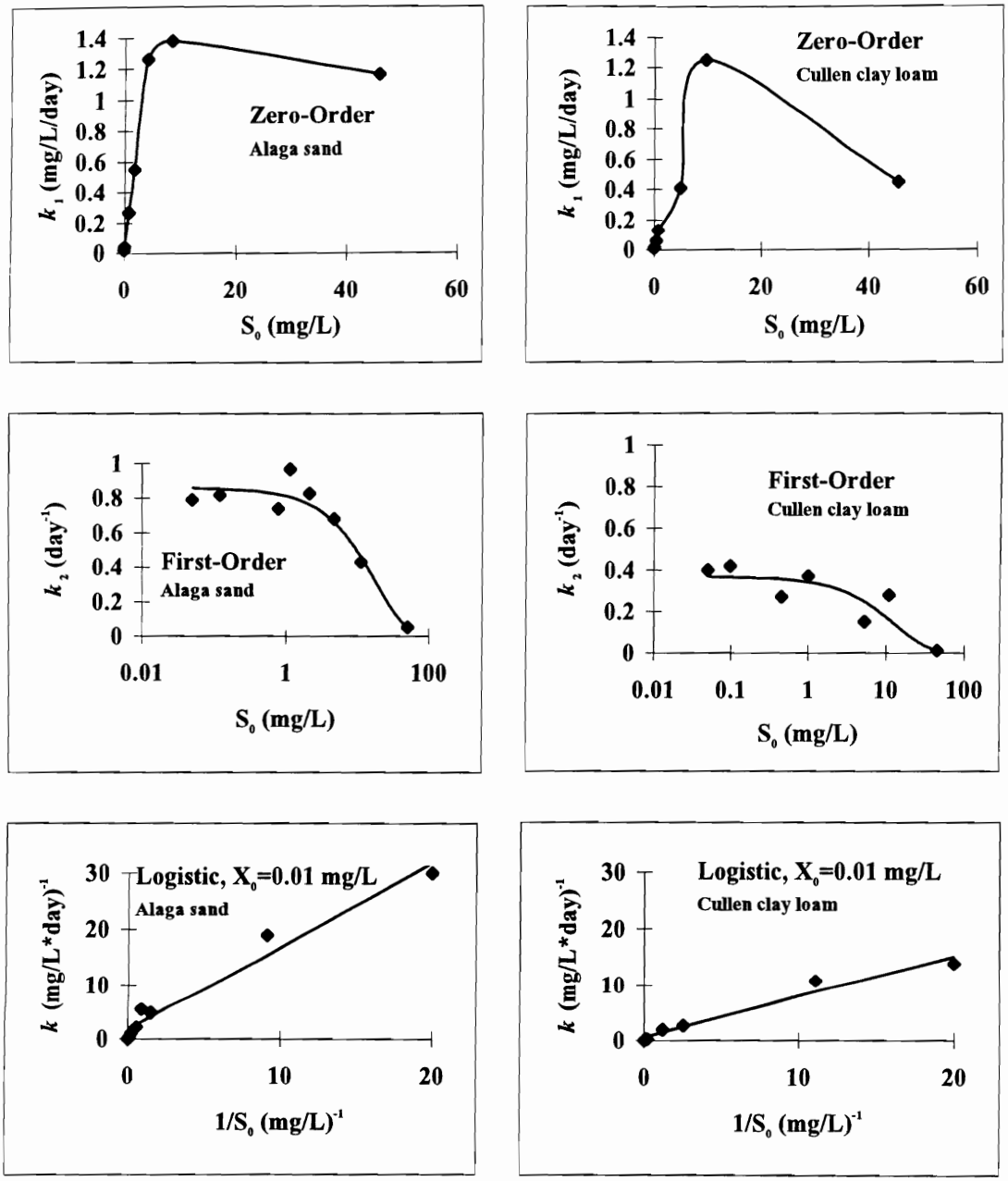

Figure II. 8. Kinetic model rate constants versus initial benzene concentration (S_0) for Alaga sand and Cullen clay loam.

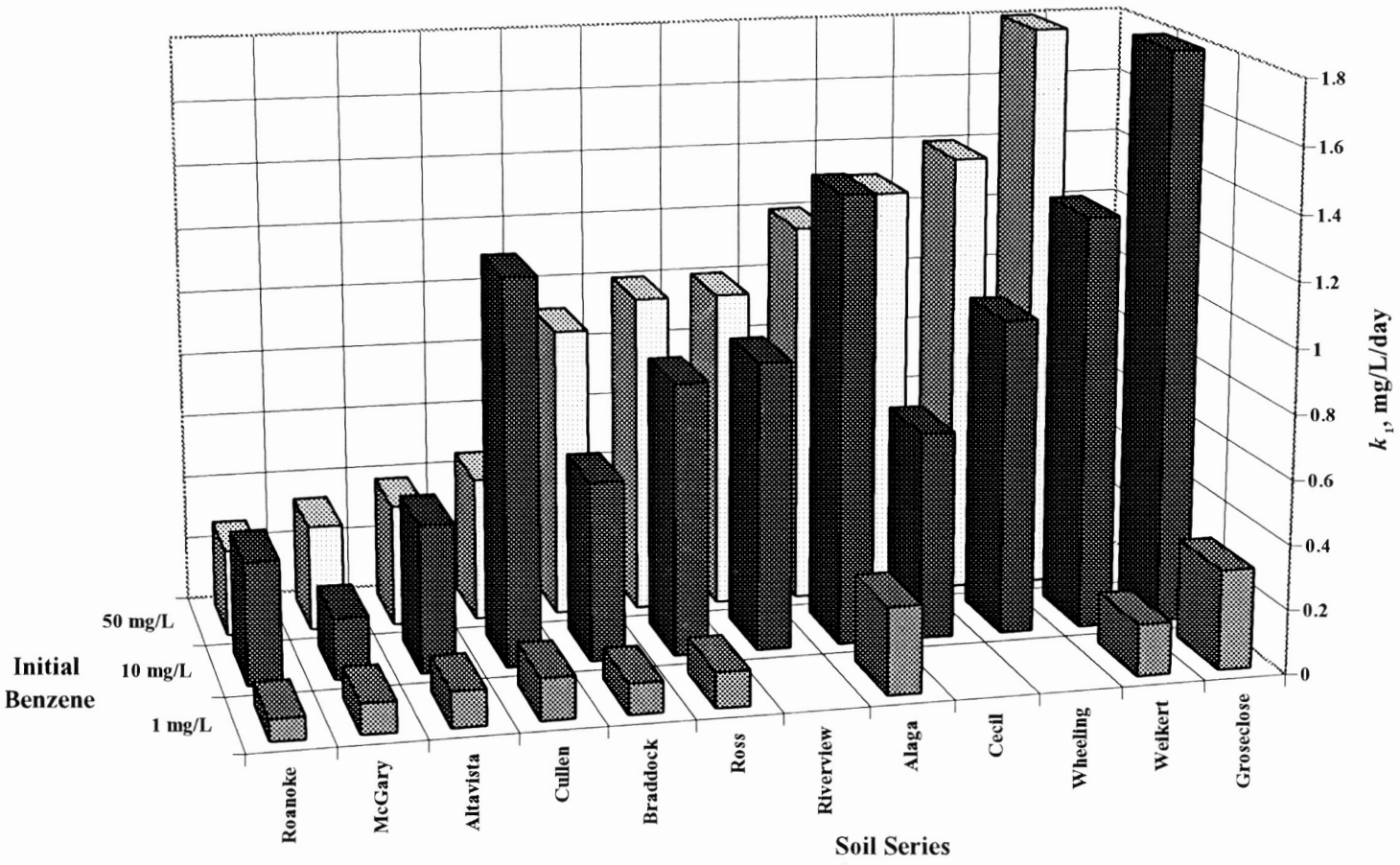

Figure II. 9. Zero-order (linear) model rate constants k_1 by soil and initial benzene concentration. Data are sorted by increasing k_1 values for 50 mg/L initial benzene concentration. Data at 1 mg/L initial benzene concentration were not available for Cecil, Riverview and Wheeling soils and at 50 mg/L for Groseclose soil. Rates for Altavista, Cullen, McGary and Roanoke soils were nearly equal to their abiotic benzene disappearance rates.

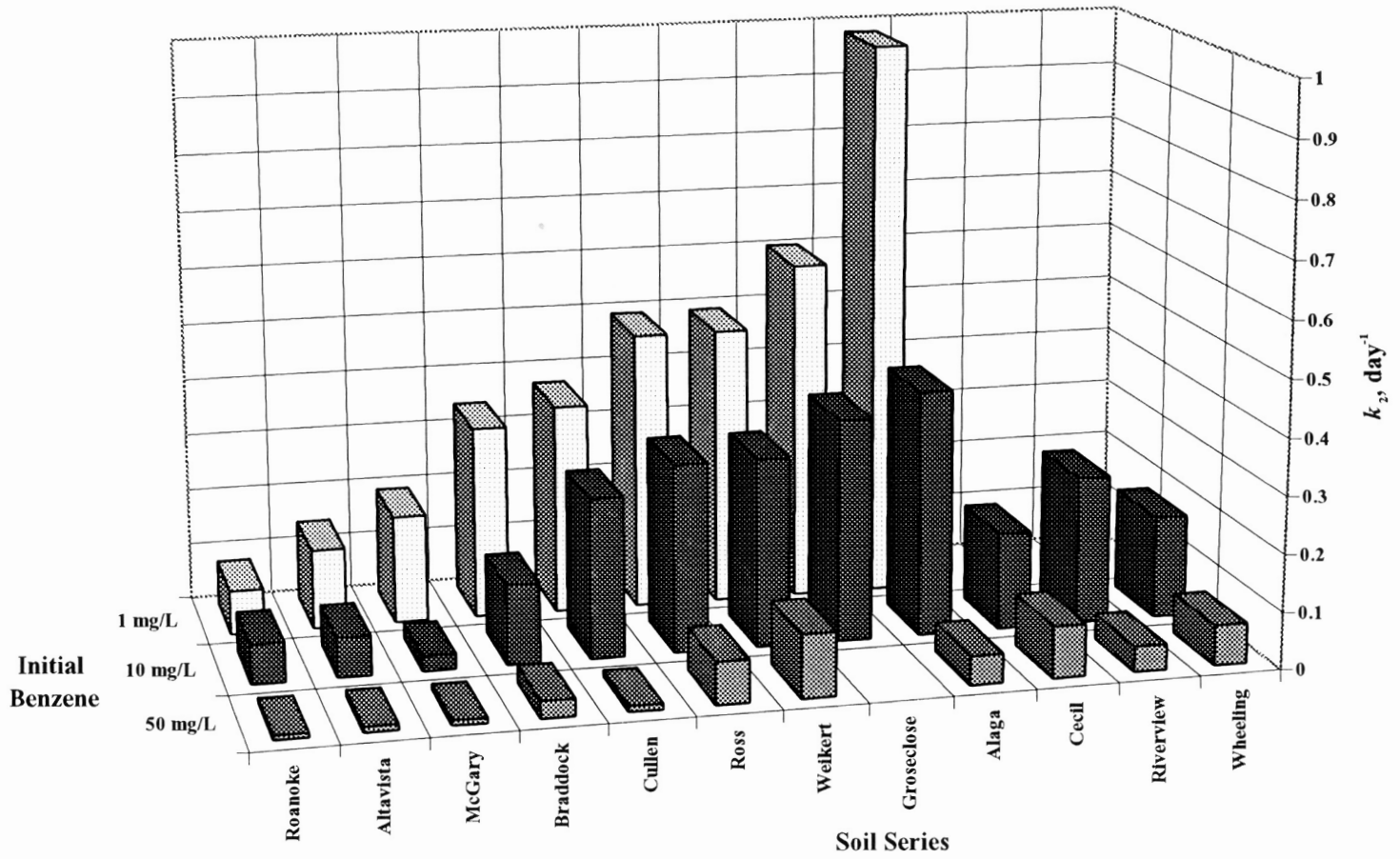

Figure II. 10. First-order (exponential) model rate constants k_2 by soil and initial benzene concentration. Data are sorted by increasing k_2 values for 1 mg/L initial benzene concentration. Data at 1 mg/L initial benzene concentration were not available for Cecil, Riverview and Wheeling soils and at 50 mg/L for Groseclose soil. Rates for Altavista, Cullen, McGary and Roanoke soils were nearly equal to their abiotic benzene disappearance rates.

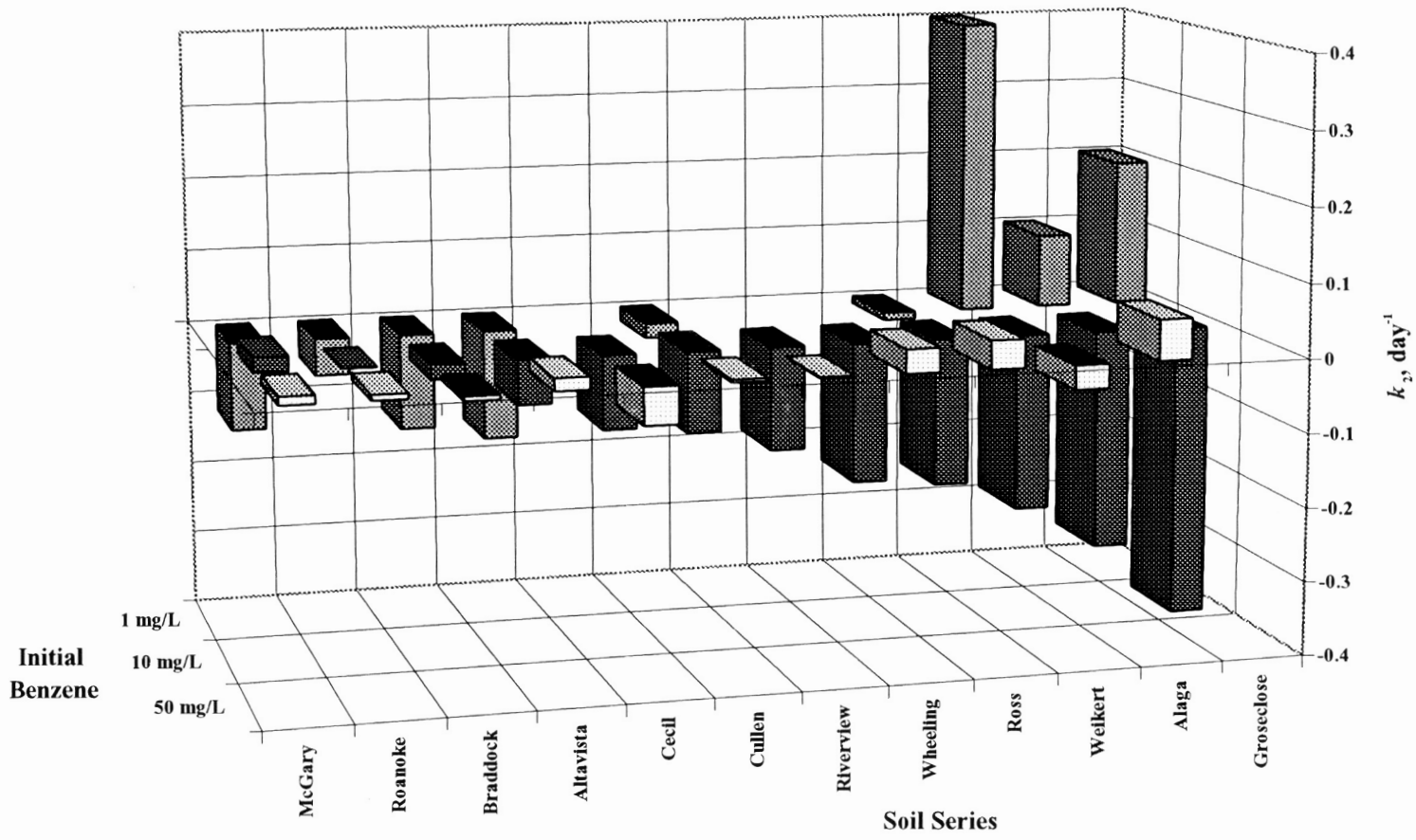

Figure II. 11a. 3/2-order model rate constants k_2 by soil and initial benzene concentration. Data are sorted by increasing k_2 values for 10 mg/L initial benzene concentration. Data at 1 mg/L initial benzene concentration were not available for Cecil, Riverview and Wheeling soils and at 50 mg/L for Groseclose soil. Rates for Altavista, Cullen, McGary and Roanoke soils were nearly equal to their abiotic benzene disappearance rates.

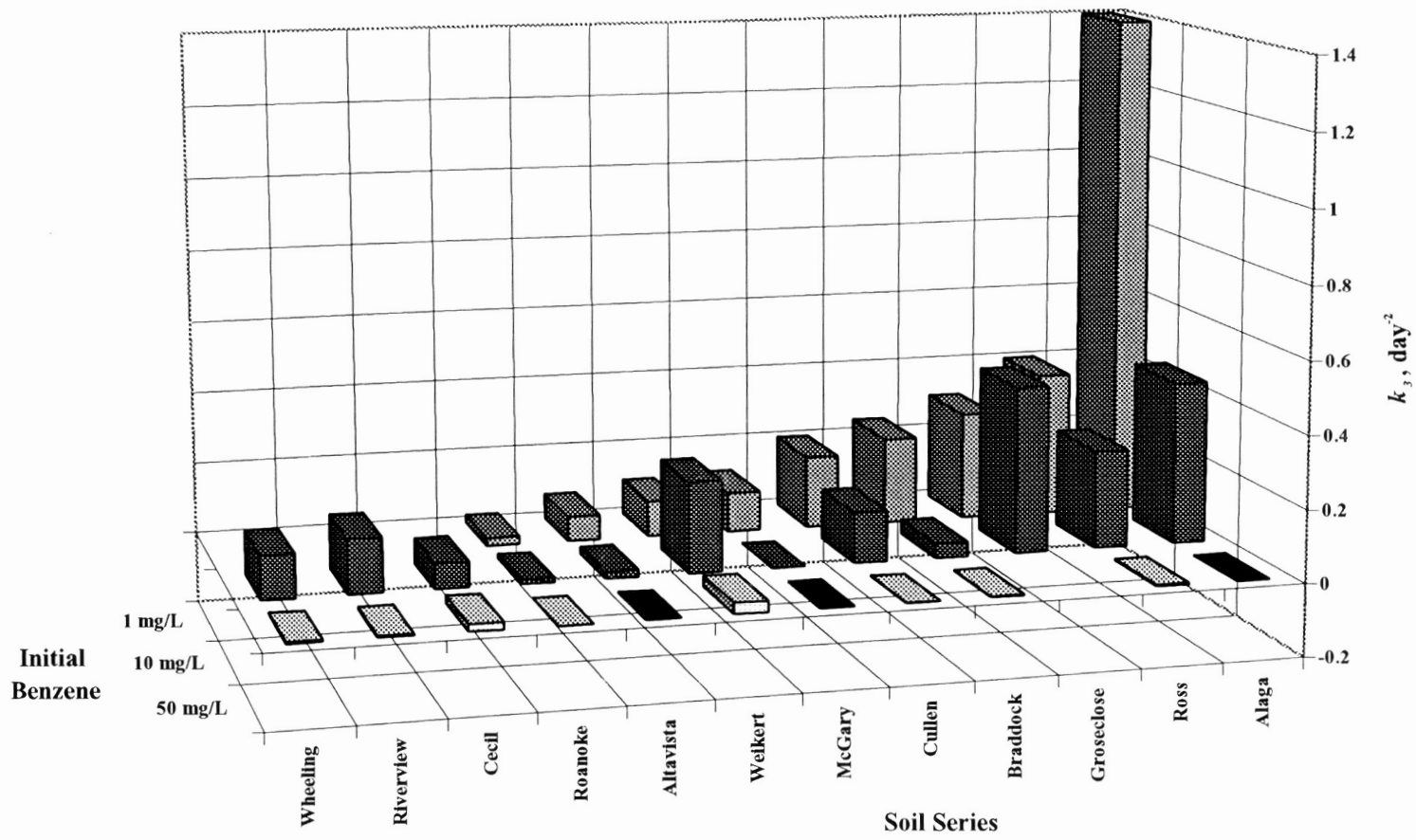

Figure II. 11b. 3/2-order model rate constants k_3 by soil and initial benzene concentration. Data are sorted by increasing k_3 values for 1 mg/L initial benzene concentration. Data at 1 mg/L initial benzene concentration were not available for Cecil, Riverview and Wheeling soils and at 50 mg/L for Groseclose soil. Rates for Altavista, Cullen, McGary and Roanoke soils were nearly equal to their abiotic benzene disappearance rates.

III. A Multiple Linear Regression Model Predicts Benzene Biodegradation In Subsurface Soils

ABSTRACT

Predictive equations were developed to describe benzene biodegradation observed in soil microcosm experiments. Aerobic microcosm experiments were prepared from 16 different previously-uncontaminated subsurface soils spiked with initial benzene concentrations of 1 mg/L or 10 mg/L. A logistic kinetic model was fit to benzene disappearance versus time data using non-linear regression analysis, and kinetic rate constants determined for each soil. Rate constants were related to soil properties in a multiple linear regression (MLR) model. Soil physical, chemical and biological properties tested in the model included calcium, iron, magesium, nitrate, phosphorus, and potassium concentrations; and pH, cation exchange capacity (CEC), chemical oxygen demand (COD), % sand, % clay, heterotrophic plate counts (HPCs) and benzene-degrader counts (BDCs). The "best" MLR models and their regression coefficient estimates were statistically significant at $p \leq 0.05$. For experiments prepared with an initial benzene concentation of 1 mg/L, the resulting predictive equation contained soil phosphorus concentration and CEC. For experiments prepared with an initial benzene concentation of 10 mg/L, the predictive equation contained soil CEC, % sand, and phosphorus, copper, and nitrate concentrations. These models establish for the first time explicit relationships between benzene biodegradation and soil properties.

INTRODUCTION

Benzene is a colorless, volatile, flammable and chemically unreactive hydrocarbon component of gasoline fuels. Benzene is a minor fuel constituent by volume, 0.8% for gasoline fuels (AWWA, 1990). The global storage, distribution and consumption of gasoline has made benzene a common

pollutant (Miller, et al., 1990; Salanitro, 1993). Partially soluble in water, benzene not volatilized can move from the site of a spill through the soil with percolating water to contaminate groundwater. Typical co-contaminants are toluene, ethyl benzene, and the xylenes, known collectively as the BTEX fraction. Once in the subsurface environment, benzene can volatilize and diffuse through the soil atmosphere, partition into soil organic matter, leach with moving groundwater or remain trapped in soil pore spaces. Benzene does not undergo significant adsorption reactions with soil minerals (Perlinger and Eisenreich, 1991) and is not hydrolyzed (Sittig, 1991). Benzene concentrations in the vicinity of a contaminant plume typically range up to 10 mg/L, with higher concentrations found in unweathered spills and nearer to spill sites (Aelion and Bradley, 1991; Borden, *et al.*, 1994; Hallbourg, *et al.*, 1992; Salanitro, 1993). Benzene is a known carcinogen based on both animal studies and studies of occupationally-exposed workers (Sittig, 1991). The United States Environmental Protection Agency (USEPA) drinking water maximum contaminant level (MCL) for benzene is 0.005 mg/L with a goal of zero. The low MCL for benzene can dictate subsurface remediation goals.

The biodegradation of benzene and its co-contaminants in the subsurface by indigeneous microorganisms is dependent not only upon the contaminant properties and concentrations, but also on local geology, weather, and soil physical, chemical and biological properties. Data from laboratory studies with soil microcosms and soil columns, field plot studies and pilot scale experiments, and *in situ* monitoring at contaminant spill sites have been used to define a general range of conditions for which *in situ* biodegradation may be considered a viable remediation strategy (Allen-King, *et al.*, 1994; Borden, 1994; Buscheck, *et al.*, 1993; USEPA, 1994). *In situ* bioremediation may be natural or intrinsic, or may be enhanced using engineered systems which deliver oxygen, alternate electron acceptors and nutrients to the subsurface. Soil vapor extraction, air sparging, biosparging and bioventing are examples of enhanced *in situ* bioremediation technologies. The EPA provides a checklist for a cursory evaluation of these technologies based on local geology and groundwater flow, contaminant volatility and soil properties such as permeability, water content, pH, buffering capacity, and carbon:nitrogen:phosphorus ratio, a checklist developed from field experiences with these technologies (USEPA, 1994).

Benzene biodegradation in the subsurface is typically controlled by oxygen (Anid, *et al.*, 1993; Barker and Patrick, 1985; Robertiello, *et al.*, 1994; Salanitro, 1993). In aqueous solution at pH 7, an oxygen concentration of ca. 4 mg/L is necessary to completely mineralize a benzene concentration of 1 mg/L; the solubility of oxygen in water is ca. 10 mg/L at 25 °C. *In situ*, both natural and anthropogenic organic matter, as well as minerals, compete with benzene for oxygen. Reported first-order rate constants for "aerobic" benzene biodegradation range from field values of 0.007 to 0.024 day^{-1} but laboratory

values range from 0.05 to 0.5 day^{-1} (Salanitro, 1993). The latter range is consistent with first-order rate constants of 0.02 to 1.0 day^{-1} determined for experimental data described in Chapter II. Under aerobic conditions, benzene can be completely mineralized to carbon dioxide and water by soil bacteria with and without cell growth (Burback and Perry, 1993; Gibson, 1968). Nitrifying bacteria can partially oxidize benzene with concurrent inhibition of ammonium oxidation (Keener and Arp, 1994). Benzene can be co-metabolized with toluene (Burback and Perry, 1993). Benzene can undergo partial mineralization under anaerobic conditions (Grbic-Galic and Vogel, 1987). Although anaerobic degradation of benzene by sulfate-reducing (Lovely, 1995) and iron-reducing (Lovely, 1994) bacteria has been demonstrated under laboratory conditions, the comparably low benzene disappearance rates under anaerobic field conditions make it difficult to separate biodegradation from competing disappearance mechanisms such as partitioning, dilution and volatilization (Borden, *et al.*,1994; Buscheck, *et al.*, 1993; Wilson, *et al.*, 1994). Under aerobic conditions and at benzene concentrations of 10 mg/L or greater, macronutrients such as nitrogen and phosphorus necessary for cellular metabolism and growth may limit benzene biodegradation in soil (Allen-King, *et al.*,1994; McCloskey, 1995; Poor, *et al.*, 1995).

Given the heterogenity of soil and the diversity of soil microfauna and flora, the purpose of this research was to find which soil properties are important to benzene biodegradation, and to determine if biodegradation can be predicted based on these properties. The ability to predict benzene biodegradation rate and extent in the subsurface is key to preparing reasonable risk assessments, evaluating potential remediation strategies and establishing attainable clean-up criteria.

METHODOLOGY

Soil Sampling, Transportation and Storage

Sampling sites throughout Virginia were chosen primarily for the expected soil characteristics, based on published soil surveys or previous research. Subsurface soil samples (ca. 2 kg) were obtained from depths of 1 to 4 meters by a pre-cleaned, 3"-diameter, manually-driven auger; identified and described by an accompanying soil scientist; and transferred directly to sterile polyethylene bags. The polyethylene bags were placed on ice in coolers and returned to the laboratory the same day. In the laboratory, soil samples were mixed by combining portions from each bag into new bags, which were then stored at 8 °C in the dark for biological characterization and microcosm preparation. A subsample was air-dried for 48 hours and sieved through a No. 10 (2 mm) sieve for chemical and physical analyses.

Soil microcosm experiments were prepared within a week, and soil characterizations completed within 2 weeks, of soil sampling. Most of the soils used in this research were moist, acidic, highly weathered, red and yellow-brown soils commonly found in southeastern United States. Soils were taken from both forest and agricultural lands. Two soils were obtained from below the local water table.

Microcosm Preparation

Soil microcosms containing 5 g soil and 5 g sterile, distilled water spiked with initial benzene concentrations of 1 mg/L (1.2 ± 0.2 mg/L) or 10 mg/L (10.4 ± 0.7 mg/L) were prepared in 22-ml acid-washed headspace vials and sealed with aluminum crimp-top caps and Teflon®-lined rubber septa. Benzene (>99%, Sigma-Aldrich) was added by microliter syringe to sterile, distilled water in a volumetric flask, mixed by shaking for 15 minutes and immediately pipetted into soil microcosms. Microcosms were then shaken for 10 seconds to mix the soil and solution, and incubated in the dark under static conditions at a constant temperature of 20 °C. Controls were prepared identically except solutions were amended with 200 mg $(NH_4)_2HPO_4$-NH_4^+ and 25 mg K_2HPO_4-K^+ per liter and soil was autoclaved 5 times over one week. (Nutrients were added to controls to compare benzene disappearance versus time in control microcosms with both unamended and nutrient-amended active microcosms, as described in Chapter IV.) Soil for controls, solutions, glassware, and plastics were sterilized by autoclaving for 20 minutes at 121 °C and 1.05 kg/cm^2. An experiment consisted of 24 identically-prepared soil microcosms. For each experiment, microcosms were sampled in triplicate daily or less frequently, depending upon the anticipated or observed benzene disappearance rates.

Soil microcosms were water-saturated and incubated at 20 °C to simulate aquifer conditions in a moderate climate and to remove these factors from subsequent analyses. The initial dissolved oxygen concentration was ca. 10 mg/L, an amount sufficient enough to completely mineralize a benzene concentration of 1-2 mg/L. Sufficient oxygen was available in the headspace to degrade a benzene concentration of >50 mg/L in 5 mL of water.

Analytical Technique

The headspace sampling analytical method was selected based upon a detection level of 0.005 mg/L benzene concentration and the ability to sample microcosms with minimum benzene loss and soil disruption. A Hewlett-Packard 19395A headspace sampler and a Hewlett-Packard 5880A gas

chromatograph (GC) equipped with a flame ionization detector were used for headspace sampling and sample analysis, respectively. The GC column was 6-ft of 1/8-in OD stainless steel packed with 5% SP 1200 and 1.75% Bentone-34. Injector and detector temperatures were 130 °C and 200 °C, respectively. The initial column temperature was 50 °C, ramped at 6 °C/minute to a final temperature of 90 °C. Nitrogen at a total flow rate of 40 mL/min was the carrier gas. Microcosms were heated in the headspace sampler for 1 hour at 60 °C prior to sampling. A GC response versus aqueous benzene concentration standard curve was developed from headspace sampling of vials containing 5 mL of water-dissolved benzene at concentrations from 0.005 to 100 mg/L. The standard curve did not change significantly when prepared with microcosms containing 5 g sterile, sieved sand.

Soil Analysis

Results of selected soil analyses are summarized in Table 1. Refer to Appendix A for a complete listing of the soil properties analyzed. To measure bulk soil pH, 20 g of air-dried and sieved soil were combined with 20 mL of de-ionized, distilled water in a 50 mL beaker, stirred, and allowed to sit for 20 minutes. The pH was measured with a pH meter equipped with a glass electrode and calibrated with buffered solutions at pH 4 and 7. Bulk soil density was determined by weighing a standard volume of air-dried and sieved soil. Phosphorus and copper were extracted from 4 cc of air-dried and sieved soil with Mehlich No. 1 extractant (0.05 N hydrochloric acid and 0.025 N sulfuric acid) in a 60-mL straight-walled plastic beaker by shaking for 5 minutes at 180 rpm on a reciprocating shaker (3.8 cm stroke length) and filtered through Whatman No. 1 filter paper. Phosphorus and copper concentrations in the extractants were determined with a Jarell Ash ICAP 9000 inductively coupled plasma spectrophotometer, calibrated over the expected range of concentrations using spectrally pure, plasma-quality reference solutions. Concentrations measured in the extractant were converted to mg/kg in soil by a dilution factor calculated using the bulk density for each soil. The detection limits for this method were 0.2 mg/kg for phosphorus and 0.008 mg/kg for copper. The standard deviations of duplicate soil samples ranged from 0.3% to 10% for phosphorus and 0.04% to 6% for copper. Nitrate was extracted by equilibrating 20 cc of air-dried sieved soil with 50 mL of 0.02 N copper sulfate for 10 minutes in a 125-mL Erlenmeyer flask on a wrist action shaker. The sample was then filtered through Whatman No. 1 filter paper and the filtrate nitrate measured with an Orion SA 720 Ionanalyzer equipped with an Orion nitrate ion specific electrode. The method detection limit was below 3 mg/kg nitrate-N, and the standard deviation based on 13 soil replicates was 10%. Soil water content was determined by drying 2 g of soil in pre-weighed aluminum

pans for 24 hours at 110 °C and measuring the weight loss. Soil water content was determined for both air-dried sieved soils and wet, refrigerated soils used for the preparation of microcosms. A particle size analysis was done on each soil by the pipette method (Day, 1965). Standard deviations of a duplicate sand, silt and clay sample were 0.1%, 3% and 6%, respectively, increasing with decreasing sand content. Cation exchange capacity (CEC) was determined by the barium chloride method described by Rhoades (1982). Magnesium concentration in the extractant, diluted as necessary, was determined with a Perkin-Elmer Model 703 atomic adsorption spectrophotometer, calibrated from 0 to 0.5 mg/L using a lanthanum-spiked magnesium chloride solution. The standard deviation of duplicate soil samples ranged from 0.02% to 40%, with higher variability observed at lower CECs.

Heterotrophic plate counts (HPCs) and benzene degrader counts (BDCs) were prepared from soil within 1 day of sampling. The same dilutions were used for both HPCs and BDCs. Ten grams of soil were mixed with 95 mL of sterile distilled water in a Waring blender jar and homogenized for 2 minutes on low speed. Subsequent serial dilutions were made by pipetting 10 mL of homogenized soil into dilution jars containing 90 mL of sterile, distilled water. Jars were then shaken by hand for one minute. For HPCs, agar spread plates were prepared in triplicate as described by Wollum (1982) and incubated in the dark at 35 °C for five days. Agar media contained per liter: 15 g Bacto-Agar, 1.0 g Bacto-Peptone, 1.0 g yeast extract, 0.4 g K_2HPO_4, 0.5 g $(NH_4)_2HPO_4$, 0.05 g $MgSO_4 \bullet 7H_2O$, 0.01 g $FeCl_3$, 0.1 g $CaCl_2$, 250 mL of inorganic soil extract and 750 mL distilled water. To make the soil extract, soil was baked 24 hours at 430 °C to remove soil organic matter. Fifty grams of baked soil per 500 mL of distilled water were combined in a 1-liter Erlenmeyer flask, autoclaved for 20 minutes at 1.05 kg/cm^2 and 121 °C, and the soil extract decanted and filtered through a 0.2 micron filter. Standard deviations of HPCs ranged from 6% to 44%.

BDCs were made using a most probable number (MPN) method described by Alexander (1982). A basal salts medium was used for MPN tubes, a medium identical to the one for HPCs except that the Bacto-Agar and Bacto-Peptone were omitted and benzene was the sole carbon source. The autoclaved medium was cooled and transferred to a volumetric flask. Benzene was added neat to 10 mg/L, the flask shaken by hand for 15 minutes, and 4.5 ml of medium transferred by pipetter into 25 22-mL sterile headspace vials. Five tubes were prepared at each soil dilution from 10^{-6} to 10^{-3} by pipetting 0.5 mL of the above-described soil dilutions (10^{-5} to 10^{-2}) into the headspace vials. The vials were sealed with teflon-lined silicon septa and aluminum crimp tops and incubated in the dark at 35 °C for three weeks. Analysis was done by the headspace sampling method previously described. A positive was scored for vials with benzene concentrations below 1 mg/L.

Oxygen concentration in selected soil microcosms was measured just prior to GC sampling by inserting a 2-½" oxygen (hypodermic) needle electrode (Diamond General, Inc.) through the headspace vial septum into the undisturbed soil-water interface. The oxygen electrode was calibrated using two water cells, one purged with nitrogen (<0.02 % oxygen) and one purged with breathing air (21% oxygen). In soil microcosms containing either sand or clay spiked to an initial benzene concentration of 10 mg/L, direct oxygen measurements were made when benzene disappearance rates were highest. For both soils, the oxygen content at the soil-water interface ranged from 16-19%, near saturation. Oxygen measurements made within the soil sediments, which required removal of the headspace vial cap, were 8-12%, indicating soils were aerobic.

Data Analysis

A logistic kinetic model, shown in Equation 1 (Simkins and Alexander, 1984), was fit to experimental data by the method of least squares method using the SAS® NLIN procedure. Model intercept S_0 and rate constant k were estimated by the non-linear regression analysis, and X_0 was made a constant equal to 0.01 mg/L for all soils. Resulting regressions provided a reasonable fit to the data (Chapter II) as can be seen in Figures 1 and 2. The logistic model may be most applicable when the added carbon substrate greatly exceeds the concentration required to build-up the initial population of degraders, but low enough that population growth does not exceed environmental resources (Alexander and Scow, 1989), conditions under which logistic microbial growth is expected to occur. The logistic kinetic model (Equation 3) did not account for the observed dependence of benzene biodegradation on initial benzene concentration, therefore separate multiple linear regression (MLR) models were necessary to develop predictive equations for experiments prepared at initial benzene concentrations of 1 and 10 mg/L.

$$S = S_0 + X_0/\{1+(X_0/S_0)\exp[k(S_0+X_0)t]\} \quad (1)$$

where

S = benzene concentration, mg/L

S_0 = initial benzene concentration, mg/L

t = time, days

X_0 = benzene concentration required to produce the initial population density, mg/L

k = rate constant, $(mg/L \cdot days)^{-1}$

The MLR models were developed using SAS® REG to fit the general model shown in Equation 2. The "best" models were determined by selecting combinations of up to 13 different soil properties, including higher order and interaction terms, and transformations on both properties and kinetic parameters, running all possible regressions (2^{13}) with these model terms, and sorting the results by Cp, a statistic which evaluates the bias (underfit) and variance (overfit) of subset models, compared to that of the full model. The top models based on lowest Cp were examined in detail. Model performance criteria such as the coefficient of determination (R^2), mean squared error (MSE), coefficient of variation (CV), F-test, partial-t tests for model coefficients, variance inflation factors (VIFs), and prediction intervals were compared among the top models. Detailed explanations of these statistics can be found in Ott (1993) and Myers (1990). R^2 is the ratio of the amount of variability in the data explained by the regression to the total variability, and is a measure of the fit of model to the data. The MSE is an estimate of the model variance. The F statistic is the ratio of the variance of the regression to the variance of the error. Large ratios indicate small probabilities (prob>F) that the variability is due to chance. The partial-t test is the ratio of the individual coefficient to its standard error, and large ratios indicate small probabilities (prob>t) that the variability is due to chance. In MLR, a partial-t test on a coefficient is valid only in the presence of all the model parameters. The CV is the ratio of the square root of the MSE (or standard error) to the average value of the response, expressed as a percentage. A lower CV means a lower percent of the average response is due to standard error. VIFs are a measure of collinearity between model parameters, a real concern when modeling soil properties. A VIF of zero indicates no collinearity, and VIFs greater than 10 indicate high collinearity.

$$\ln(y) = \beta_0 + \sum_{n=1}^{i} \beta_i x_i + \sum_{n=1}^{i} \beta_i x_i^2 + \sum \sum \beta_{ij} x_i x_j \qquad (2)$$

where

y = logistic kinetic model rate constant k (response)

$x_i \ldots x_n$ = soil properties (regressor variables)

β_0 , $\beta_i \ldots \beta_n$ = regression coefficients

Models presented in Tables 2 and 3 were selected based on the following criteria: (1) highest R^2 and F statistic for the fewest MLR model regressors, and (2) models and their regression coefficient estimates (except intercept) were statistically significant at $p \leq 0.05$ with all of the soils in the model *and*

with 4 soils removed from the model. The latter criteria proved the most difficult to meet. The soils removed from the model to evaluate its predictive capability are highlighted in Table 4. Data for these soils were obtained from McCloskey (1994). The natural logarithm of k was used as the response to avoid the possibility of negative values.

Model performance is affected by multiple collinearity. Pearson product-moment correlations (r_{xy}) can reveal sources of collinearity and indicate the strength of a linear relationship between two variables. If two variables are not correlated, r is zero. If two variables are perfectly correlated, r is +1 for a positive, and -1 for a negative, correlation (SAS®, 1988). A correlation matrix was run on all model regressor variables and correlations were used to both select model regressor variables and to analyze the role of the regressor variables in the MLR model.

RESULTS AND DISCUSSION

Model Performance

The following general observations can be made based on the MLR models summarized in Tables 2 and 3. Predictive equations (Equations 3 and 4) were different for experiments prepared at initial benzene concentrations of 1 mg/L and 10 mg/L: at the lower benzene concentration the predictive equation contained fewer parameters and had poorer statistics than the equation for the higher benzene concentration. Both models and their regression coefficient estimates were statistically significant at $p \leq 0.05$. The statistics deteriorated with models built with 13 soils instead of 9 soils for 1 mg/L benzene concentration (R^2=0.61, p=0.0086), and 16 soils instead of 12 soils for 10 mg/L benzene concentration (R^2 = 0.93, p=0.0001) but regression coefficient estimates changed little and remained statistically significant at $p \leq 0.05$. Due to soil sorption of benzene, initial aqueous concentrations for ranged from 60% to 100% of the applied benzene concentration. Thus, Equation 3 may be valid for initial aqueous benzene concentrations from 0.6 to 1 mg/L and Equation 4 from 6 to 10 mg/L.

Predicted k values for soils in the models were within a factor of 1.7 and 1.1 for 1 mg/L and 10 mg/L initial benzene concentration models, respectively (Table 4). Predictions for soils not in the models, highlighted in Table 4, were all within a factor of 3. Together these MLR models contained only 5 individual soil properties: % sand, cation exchange capacity (CEC), and phosphorus, nitrate, and copper concentrations. Phosphorus, nitrate and copper concentrations were positively correlated with k. CEC was negatively correlated with k. At 10 mg/L initial benzene concentration, % sand was a quadratic function

of k. At 1 mg/L initial benzene concentration, no particle size term (*e.g.*, % sand, % silt or % clay) appeared in the model. Neither model contained pH, heterotrophic plate counts (HPCs) or benzene degrader counts (BDCs).

For an initial benzene concentration of 1 mg/L:

$$k = \exp(0.9 + 0.04\ P - 0.1\ CEC) \quad (3)$$

For an initial benzene concentration of 10 mg/L:

$$k = \exp(-4 - 0.0009\ \%Sand^2 + 0.09\ \%Sand + 0.0007\ \%Sand \cdot P + 0.05\ NO_3\text{-}N - 0.2\ CEC + 0.4\ Cu) \quad (4)$$

where

% sand = weight % of sand in air-dried, sieved (No. 10 mesh) soil

CEC = cation exchange capacity, meq/100 g

Cu = Mehlich 1-extractable copper concentration, mg/kg

NO_3-N = 0.02 N $CuSO_4$-extractable nitrate-nitrogen, mg/kg

P = Mehlich 1-extractable soil phosphorus concentration, mg/kg

For initial benzene concentrations of 1 mg/L and 10 mg/L and for $S_0 >> X_0$, the logistic model can be rewritten as shown in Equation 5, with k determined from either Equation 3 or 4, as appropriate. Equation 5, based on the predicted values of k from Equations 3 and 4, is plotted in Figure 3 with benzene disappearance versus time data for Purcellville sandy loam. This soil was *not* in the MLR model.

$$S = S_0/[1 + X_0/S_0 \exp(S_0 k t)] \quad (5)$$

where

S = aqueous benzene concentration, mg/L

S_0 = initial aqueous benzene concentration, mg/L

Model Interpretation

At an initial benzene concentration of 1 mg/L, biodegradation depended upon soil cation exchange capacity (CEC) and phosphorus concentration. The relationship between these soil factors is shown in Figure 4, a plot of k versus CEC for varying levels of phosphorus. As CEC increases, an greater amount of phosphorus is necessary to produce the same biodegradation rate. CEC is a traditional measure

of nutrient availability, particularly exchangeable magnesium, calcium, potassium and sodium. CEC was significantly correlated with magnesium (r=+0.71) and calcium (r=+0.56) measured in the soils listed in Table 1. Phosphates readily form insoluble precipitates with Ca^{2+} above pH 5.5 as soil calcium activity increases, and measured soil phosphorus was significantly correlated with calcium (r=+0.51). This suggests that CEC may be a surrogate for calcium in the MLR models.

These soils had relatively low CECs, from 0.2 meq/100g for Alaga sand (3% clay) to 14 meq/100 g for Roanoke clay loam (30% clay). These low CECs suggest that kaolinite, a 1:1 layer silicate, is the predominant clay. The permanent charge of clay soils arises from isomorphic substitution of silica atoms with aluminum, for example, in the crystalline structure. Kaolinite, however, has little or no isomorphic substitution and the permanent charge is near zero (Bohn, *et al.*, 1985). The cation and anion exchange charges associated with kaolinite are pH-dependent and arise from 3 sources: protonation (or deprotonation) of hydroxide groups at the exposed clay edges, clay coatings or minerals of aluminum or iron oxides and hydroxides, and clay coatings of organic matter. As pH decreases, protonation of these surfaces increases and the anion exchange capacity increases; and conversely, the CEC increases with increasing pH. CEC was significantly correlated with sand (r=-0.65) and pH (r=+0.62); in other words, as soil pH and the silt plus clay fraction increased, so did CEC. CEC, therefore, could also be a surrogate for either pH or particle size (*e.g.*, % sand, % silt and % clay) in Equation 3.

Iron oxides and hydroxides, such as hematite (Fe_2O_3) and geothite (FeOOH) which give soils their characteristic red and yellow-brown colors, respectively, and aluminum oxides and hydroxides such as gibbsite ($Al_2(OH)_6$) and boehmite (AlOOH), account for most of the pH-dependent charge in highly weathered soils (Bohn, *et al.*, 1985). Phosphorus is retained in soil by specific adsorption to these surfaces or minerals. Thus, another plausible explanation of the interaction of phosphorus with CEC is that for the soils under consideration, CEC is proportional and exchangeable phosphorus inversely proportional to iron and aluminum oxide and hydroxide content.

Phosphates also readily form insoluble precipitates with Fe^{3+} and Al^{3+} below pH 5.5 (Bohn, *et al.*, 1985), and yet correlations between phosphorus and iron (r=+0.10) and aluminum (r=-0.12) were low. Soil solution concentrations of total iron and aluminum in temperate region soils are low, typically <0.005 mg/L and <0.01 mg/L, respectively (Bohn, *et al.*, 1985), but iron and aluminum extracted from soil by Mehlich-1 solution, a dilute acid, ranged from 2 to 740 mg/kg for iron and 37 to 430 mg/kg for aluminum. The highest concentrations of iron and aluminum cations were associated with Ross, a high organic matter content soil, and with Altavista, Braddock and Cullen, acidic clay soils. Extractable phosphorus was correspondingly low for the latter three soils (Table 1). Not only pH but particle size and organic matter content influence iron and aluminum, and thus phosphorus, availabilities in soils.

Although Equation 3 contained only two regressor variables, phosphorus concentration and CEC, these two soil properties were interrelated with calcium, pH, particle size and possibly organic matter, factors which may control phosphorus availability. The simplest and most plausible interpretation of this model is that under aerobic and water-saturated soil conditions for an initial benzene concentration of 1 mg/L, microbial biodegradation of benzene is controlled by available phosphorus.

At an initial benzene concentration of 10 mg/L, biodegradation depended upon soil CEC, % sand, and phosphorus, nitrate and copper concentrations. The % sand term in Equation 4 is quadratic: rate constant k is positively correlated with sand below a content of about 40%, and negatively correlated with sand above a content of about 80%. That sand content influenced biodegradation is no surprise. Higher permeabilities permit increased water, oxygen and nutrient delivery rates and carbon dioxide removal rates in subsurface soils. Sandier soils with low organic matter content exhibit lower benzene sorption and consequently benzene is more bioavailable. The water retention capacity, however, diminishes with increasing grain size, and natural microbial processes in highly drained subsurface soils are dependent on rainfall or water table fluctuations. Sands low in organic matter content store fewer nutrients, reflected in low cation and anion exchange capacities, and under natural conditions biodegradation of significant amounts of added organic carbon may become nutrient-limited. Sandy soils with low pH are often poorly buffered and carbon dioxide produced during mineralization of benzene can drive the soil pH even lower reducing the biodegradation efficiency. For natural *in situ* bioremediation, an optimum sand content may exist, as suggested by a plot of k versus sand content for varying levels of phosphorus shown in Figure 5.

Phosphorus and nitrogen are essential nutrients for cell growth and metabolism, and ratios of C:N:P measured in soil bacteria are on the average 31:5:1 (Paul and Clark, 1989). Soil microbiota cycle phosphorus between organic and inorganic forms. Organic forms include nucleic acids, nucleotides, phosphoproteins and phospholipids, phytins (plants), and intracellular polyphosphate granules. In biological systems, adenosine triphosphate (ATP) is the energy currency which sustains life. In the absence of specific enzymes, organic phosphorus is remarkably resistant to hydrolysis (Ehrlich, 1990). Inorganic phosphorus is most commonly orthophosphate (H_3PO_4). Orthophosphate introduced into soil is strongly adsorbed to soil iron and aluminum oxides or precipitated with calcium, magnesium and iron cations, reactions which strongly retard its migration through soil and limits its bioavailability. Under natural conditions, soil solution phosphorus rarely exceeds 1 mg/L (Bohn, *et al.*,1985). Soil microflora and fauna release insoluble soil phosphorus by the productions of acids, enzymes or chelators and through their reduction of iron in iron phosphate. A plot of k versus sand content for varying levels of phosphorus reveals the interaction between sand and phosphorus (Figure 5). High sand content and elevated extractable phosphorus levels together magnify the kinetic response.

Nitrogen is also cycled between organic and inorganic forms by soil microbiota. Organic forms include proteins (amino acids), nucleic acids and microbial cell wall constituents; inorganic forms include ammonium, nitrite, nitrate, and nitrogen gases. The ammonium cation can be volatilized as ammonia (not predominant in acidic soils, pKa = 9.3), immobilized by reactions with soil organic matter and clays, and transformed by nitrifiers into nitrate. Nitrate is held in soil solution by electrostatic attraction to adsorbed soil cations (Bohn, *et al.*, 1985), evidenced by its high correlation with soil cations calcium (r=+0.80) and magnesium (r=+0.83). Consequently, nitrate is easily leached by water moving the soil column. Efficiencies of nitrogen transformations depend upon soil oxygen, pH, moisture, temperature and organic matter content. For example, production of nitrate in soil through the biologically-mediated oxidation of the ammonium ion is thought to be pH-dependent in agricultural soils, with an optimum pH between 6.6 and 8 and negligible production below pH 4. This may explain the correlation of nitrate to pH (r=+0.53). Soil bacteria species can also use nitrate as an electron acceptor, alternate to oxygen, under the anoxic conditions associated with hydrocarbon-contaminated soil. Benzene biodegradation, however, proceeds slowly if at all under anoxic conditions (Allen-King, *et al.*, 1994; Anid, *et. al.*, 1993; Hutchins, 1991). As with soil phosphorus (phosphate), nitrate as a cellular nutrient best describes its role in Equation 4.

Copper is an essential cell micronutrient, present in oxidase enzymes and important in protein and carbohydrate metabolism (Brady, 1990). Copper, with its three stable states as neutral atom, cuprous or cupric ion, is both a strong chelating agent and a highly effective catalyst (Frieden, 1968). The solubility of copper in soil solution is higher than that of iron, and its solution concentration in temperate region soils is 0.03 to 0.3 mg/L (Bohn, *et al.*,1985). Weak acid or EDTA-extractable copper levels range from 0.1 to 3 mg/kg (Adriano, 1986), consistent with those values reported in Table 1. Copper deficiencies in plants can occur at extractable copper levels below 1 mg/kg (Adriano, 1986). Copper can be specifically adsorbed by layer silicate clays, by soil organic matter, and by iron, aluminum and manganese oxides and hydroxides. Copper was highly correlated with zinc (r=+0.83), and significantly correlated with silt (r=+0.66), iron (r=+0.68), manganese (r=+0.65), and chemical oxygen demand (r=+0.68). Chemical oxygen demand (COD) is a measure not only of soil organic matter, but of the electron donor capacity of soil. Unsurprisingly, COD was also highly correlated with iron (r=+0.99), manganese (r=+0.99), and zinc (r=+0.95). These metals are not only strongly complexed with soil organic matter, but in acid or water-logged soils may exist in reduced states. The role of copper in Equation 4 may be either as an essential cellular nutrient or as a measure of soil organic matter. Soil organic matter retains water and nutrients, forms stable aggregates which increase water and air permeability, acts as a buffering agent, and is itself a nutrient source for microbiota (Stevenson, 1982).

Hydrophobic benzene, however, readily partitions into soil organic matter (Chiou, 1979) or is adsorbed at organic surfaces such as organic coatings on clay (Chiou, 1985), reducing its bioavailability.

The models shown in Equations 3 and 4 are consistent with field experience with organic contaminant bioremediation, exemplified by the recently published EPA guidelines. EPA recommends for *in situ* natural remediation, for example, soil moisture content between 40 to 85% of field holding capacity, a moderate climate (5° to 45° C), and an aerated soil with a dissolved oxygen concentration of >1 mg/L. Soil microcosms were water-saturated and incubated at 20° C, to simulate aquifer conditions in a moderate climate and to remove these factors from subsequent model development. The initial dissolved oxygen concentration was ca. 10 mg/L, an amount sufficient to completely mineralize a benzene concentration of 1-2 mg/L. Sufficient oxygen was available in the headspace to degrade a benzene concentration of >50 mg/L in 5 ml of water. Diffusion of oxygen from headspace to soil solution at the higher benzene concentration may in part explain the appearance of sand in Equation 4. EPA also recommends that soils with intrinisic permeabilities $>10^{-10}$ cm^2 (hydraulic conductivities $>10^{-5}$ cm/sec) characteristic of silt or larger grain soils as a prerequisite for enhanced *in situ* bioremediation (USEPA, 1994). Higher soil permeabilities mean increased fluid transport rates in subsurface soils, a benefit to either natural or engineered aerobic bioremediation.

Another EPA guideline for successful bioremediation requires that the ratios of soluble C:N:P to be from 100:10:5 to 100:1:0.5. The weak acid-extractable nitrogen and phosphorus concentrations reported in Table 1 were higher than their corresponding water-extractable concentrations, which ranged from 0.1 to 9.6 mg/L for nitrate-N and were <0.05 mg/L for phosphate-P. From these data and using the C:N:P ratios as a guideline, all soils in Table 1 had sufficient solution nitrogen but not phosphorus to degrade a benzene concentration of 1 mg/L, but both solution nitrogen and phosphorus deficiencies occurred at 10 mg/L initial benzene concentration. Weak acid-extractable nutrients, however, are generally considered bioavailable. Reapplying the above C:N:P ratios to nutrient concentrations listed in Table 1, sufficient nitrogen and phosphorus existed in all soils to completely anabolize a benzene concentration of 10 mg/L into cell mass. Differing microbial nutrient needs and extraction efficiencies, nutrient diffusion rates, and the extent of benzene catabolism and anabolism may explain the apparent influence of nitrate and phosphorus concentrations on the logistic rate constant k predicted by Equations 3 and 4.

Another important soil factor which does not appear in Equations 3 and 4 is pH. The EPA guideline recommends a soil pH of 6-8 for *in situ* bioremediation (USEPA, 1994), and reflects the need for buffering capacity to counteract potentially toxic acidity produced by carbon dioxide generation. In

this pH range, typical soil buffers include hydroxide and carbonate ions, as well as silicates, borates, ammonia, phosphates and organic bases. Soil pH, however, is not the only measure of buffering capacity. Low pH soils with high iron and aluminum hydroxides and oxide, mineral surface or organic matter content also buffer excess acidity. Thus, in highly weathered, low pH subsurface soils increased clay content means increased buffering capacity but decreased carbon dioxide removal rates because of correspondingly lower permeabilities. In this investigation, benzene biodegradation was observed in soil microcosms from pH 4.3 to 7.7 at or below an initial benzene concentration of 10 mg/L. Soil bulk pH in unamended microcosms actively degrading benzene were typically unchanged over the biodegradation period. At low organic contaminant concentrations even low pH soils may contain enough buffering capacity to maintain their bulk pH.

Microbial activity in soil is necessary for organic contaminant biodegradation, and yet neither HPCs nor BDCs, traditional measures of bioactivity, regressed well in MLR models. Bacteria and fungi in soil are abundant and diverse, and both are known to degrade petroleum hydrocarbons including benzene. While viable bacteria numbers in soil range from 10^7 to 10^9 cells/gram of soil (Bossert and Bartha, 1984), typically less than 1% of these are culturable (Torsvik, *et al.*, 1990). Problems with agar culturing include no bacterial growth; slow or preferential growth due to the quality, concentration or toxicity of the substrate; nutrient limitations or excess; and interference from adjacent colonies. Liquid cultures have similar constraints. Many techniques suffer from the inability to efficiently extract intact cells from soil particles. Measures of cell activity such as glucose metabolism, oxygen consumption and carbon dioxide production can be made from soil samples but may not correlate well with cell counts (Sharabi and Bartha, 1993; Steffan, *et al.*, 1988). Benzene-degraders counts (BDCs) ranged from 0 to 10^5 cells/g dry soil, and yet benzene biodegradation was observed in every soil. Culture media may have been deficient in growth factors for some bacterial or fungal species, or soil microbes may have co-metabolized benzene with natural organic compounds in soils with low BDCs. The EPA recommends a relatively low total heterotrophic plate count (HPC) >1,000 cfu/g dry soil (USEPA, 1994) as an adequate measure of soil bioactivity. Soils listed in Table 1 had HPCs of 0.6×10^4 to 9×10^4 cfu/g dry soil, above the EPA guideline.

Similar approaches to modeling have been previously reported. Knaebel, *et al.*, (1994) related organic surfactant mineralization kinetic parameters to soil characteristics using regression analysis. They measured carbon dioxide production in woodlot silt loam amended with 1% of surfactant-clay or surfactant-humic mixtures. Mineralization data were fit best by a 3/2-order kinetic model, assuming no microbial growth. Kinetic parameters were significantly correlated with soil desorption coefficients but not microbial activity or biomass. Dobbins, *et al.*, (1987) measured short-term mineralization rates based

on carbon dioxide production in four soils spiked with phenol concentrations from 0.005 mg/L to 1 mg/kg. The resulting kinetic data were fit with a Monod-type hyperbolic saturation model assuming no microbial growth. They related the maximum substrate utilization velocity to soil depth, silt content, microbial biomass and activity individually and in combinations using regression analysis, but found only poor predictions with these characteristics. The saturation constant was not significantly correlated with soil CEC, clay content or organic matter content.

Table III. 1. Summary of measured chemical and physical soil properties for 16 previously-uncontaminated subsurface soils.

Soil Series	Texture Class	pH	%Sand	Phosphorus (mg/kg soil)	CEC (meq/100 g)	Nitrate-N (mg/kg soil)	Copper (mg/kg soil)
Alaga	sand	4.5	96	27	0.2	3	0.11
Altavista	clay loam	4.6	44	0	11.8	3	3.1
Bojac	sand	5.3	90	3.6	0.8	5	0.54
Braddock	clay	4.9	21	0.75	3.1	15	0.92
Catpoint	sand	4.6	99	7.7	0.2	3	0.64
Cecil	loamy sand	4.5	72	2.3	3.1	3	0.95
Cullen	clay loam	5.0	37	1.1	3.7	5	0.95
Groseclose	loam	7.6	38	24	10.2	43	0.26
Leedsville	silt loam	4.3	27	0	4	3	0.94
McGary	clay loam	6.6	26	2.6	7.3	5	1.10
Purcellville	sandy loam	4.5	58	1.2	6	23	0.64
Riverview	sandy loam	6.0	72	1.4	2.8	5	1.3
Roanoke	clay loam	7.7	20	0.73	14	13	4.8
Ross	silt	4.9	20	8.8	5.3	3	5.6
Weikert	loamy sand	5.0	74	0.75	0.6	3	0.78
Wheeling	sandy loam	6.0	61	5.1	6.5	5	1.3

Table III. 2. Analysis of variance and regression coefficient estimates for a multiple linear regression model of logistic rate constant k with 9 soils in the model for experiments prepared at an initial benzene concentration of 1 mg/L.

Source	DF	Sum of Squares	Mean Square	F Statistic	Prob>F
Model	2	5.01	2.50	28.4	0.0009
Error	6	0.53	0.089		
Total	8	5.54			
Root MSE	0.297		**R^2**	0.905	
Dep Mean	0.494		**Adjusted R^2**	0.873	
C. V.	60.1				
Regressor Variable	**DF**	**Regression Coefficient Estimate**	**Standard Error**	**Prob>| t |**	**VIF**
Intercept	1	+0.94	0.19	0.0028	0
CEC	1	-0.12	0.022	0.0016	1.04
P	1	+0.041	0.010	0.0063	1.04

Table III. 3. Analysis of variance and regression coefficient estimates for a multiple linear regression model of logistic rate constant *k* with 12 soils in the model for experiments prepared at an initial benzene concentration of 10 mg/L.

Source	DF	Sum of Squares	Mean Square	F Statistic	Prob>F
Model	4	5.03	0.838	48.9	0.0003
Error	7	0.08	0.017		
Total	11	5.11			
Root MSE	0.131		**R^2**	0.983	
Dep Mean	-1.90		**Adjusted R^2**	0.963	
C. V.	-6.88				
Regressor Variable	**DF**	**Regression Coefficient Estimate**	**Standard Error**	**Prob>| t |**	**VIF**
Intercept	1	-4.0	0.30	0.0001	0
Cu	1	+0.44	0.039	0.0001	3.07
$Sand^2$	1	-0.00085	0.00015	0.0023	112
Sand	1	+0.090	0.014	0.0013	84.0
CEC	1	-0.22	0.016	0.0001	3.14
NO_3-N	1	+0.050	0.0054	0.0002	2.44
Sand*P	1	+0.00071	0.00013	0.0026	5.76

Table III. 4. Observed and predicted logistic rate constant *k* values for experiments prepared at initial benzene concentrations of 1 and 10 mg/L. Bojac, Catpoint, Leedsville and Purcellville soils, highlighted in the below table, were *not* in the models.

	1 mg/L benzene		10 mg/L benzene	
Soil Series	**Observed *k*[a]**	**Predicted *k***	**Observed *k***	**Predicted *k***
Alaga	5.4	7.5	0.31	0.31
Altavista	0.60	0.63	0.06	0.07
Braddock	1.9	1.8	0.13	0.14
Cecil	not available	not available	0.13	0.15
Cullen	1.8	1.7	0.17	0.14
Groseclose	2.5	2.0	0.33	0.34
McGary	0.97	1.2	0.045	0.048
Riverview	not available	not available	0.20	0.20
Roanoke	0.40	0.50	0.06	0.06
Ross	3.3	1.9	0.36	0.38
Weikert	2.4	2.4	0.22	0.21
Wheeling	not available	not available	0.14	0.13
Bojac	4.6	2.7	0.09	0.11
Catpoint	1.1	3.4	0.03	0.08
Leedsville	0.57	1.6	0.10	0.08
Purcellville	1.7	1.3	0.21	0.24

[a]units are $(mg/L*day)^{-1}$

SUMMARY AND CONCLUSIONS

Benzene biodegradation in soil can be predicted from soil properties. Using non-linear regression analysis, a logistic kinetic model was fit to experimental data from batch microcosm studies, and rate constants obtained for 16 previously-uncontaminanted subsurface soils. The rate constants were related to soil properties using a multiple linear regression (MLR) model. The soil factors which control benzene biodegradation depended upon initial benzene concentration. At an initial benzene concentration of 1 mg/L, benzene biodegradation was controlled by soil phosphorus availability. At an initial benzene concentration of 10 mg/L, benzene biodegradation was controlled by soil sand content, nutrient availability (cation exchange capacity), and nitrate-nitrogen, phosphorus and copper concentrations.

Although additional work is needed to evaluate the utility of these models for remedial efforts in the field, limited application may be possible. These batch soil microcosm studies may mimic *in situ* natural or stimulated conditions of water-saturated soil with oxygenated, relatively slow-moving groundwater with benzene concentrations below 10 mg/L, as might be found at the boundaries of a hydrocarbon contaminate plume. The soils tested for model development were sampled from below forested and cropped lands, with heterotrophic plate counts of ca. 10^4, and are more typical of weathered subsurface soils found in the southeastern United States. Model use should be restricted to soils with properties within those listed in Appendix A. The predictive equations presented in this chapter establish for the first time an explicit relationship between benzene biodegradation and soil properties. Moreover, the seemly endless list of important and interacting soil properties are reduced to a manageable few.

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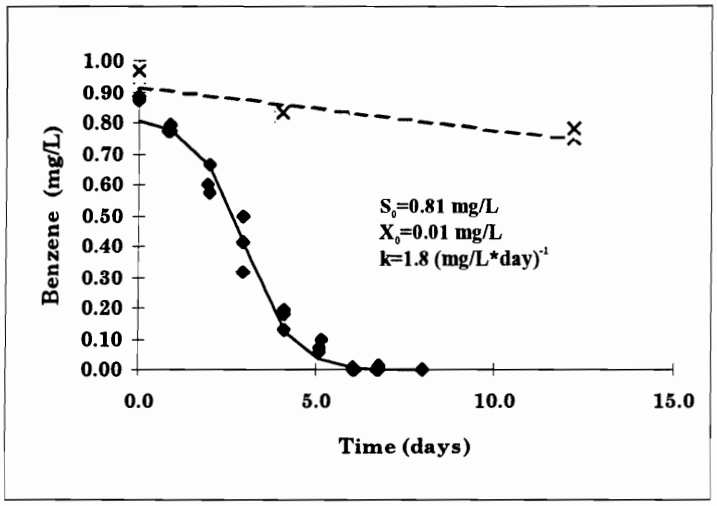


Figure III. 1. Benzene disappearance in Cullen clay loam spiked at an initial benzene concentration of 1 mg/L. Aqueous benzene remaining ♦, in active soil microcosm; x, in sterile soil microcosms. Each data point represents one microcosm. Solid line plots the logistic model (Equation 1) regression.

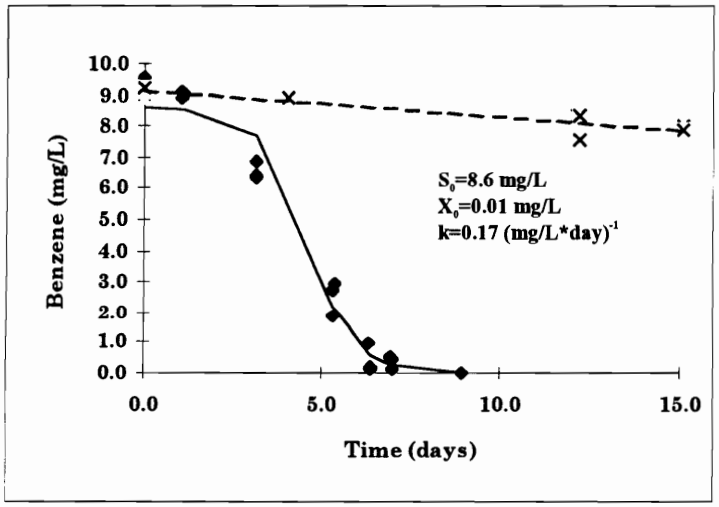
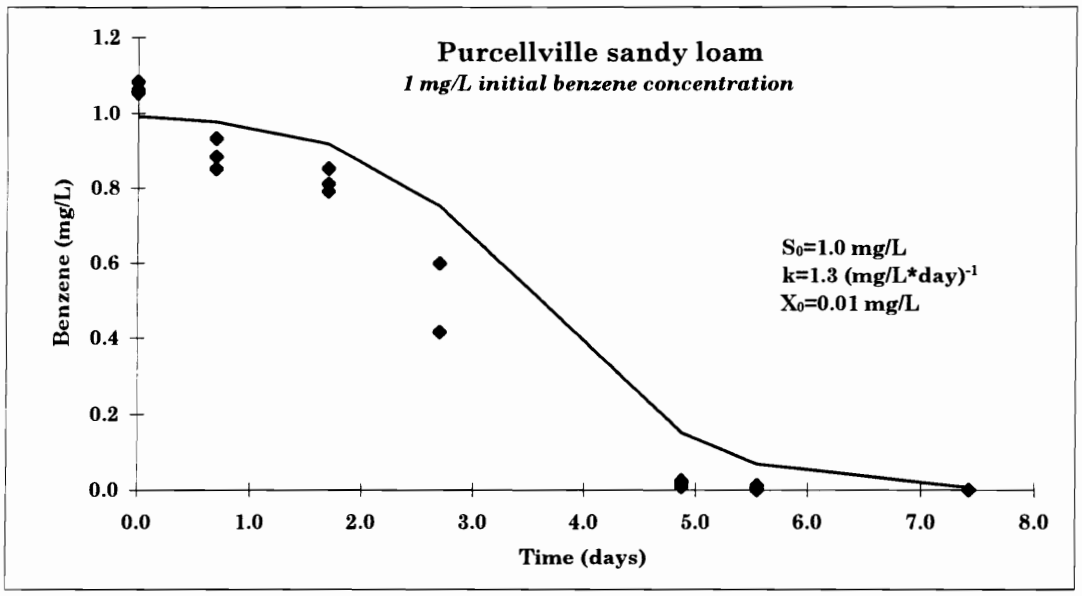


Figure III. 2. Benzene disappearance in Cullen clay loam spiked at an initial benzene concentration of 10 mg/L. Aqueous benzene remaining ♦, in active soil microcosm; x, in sterile soil microcosms. Each data point represents one microcosm. Solid line plots the logistic model (Equation 1) regression.



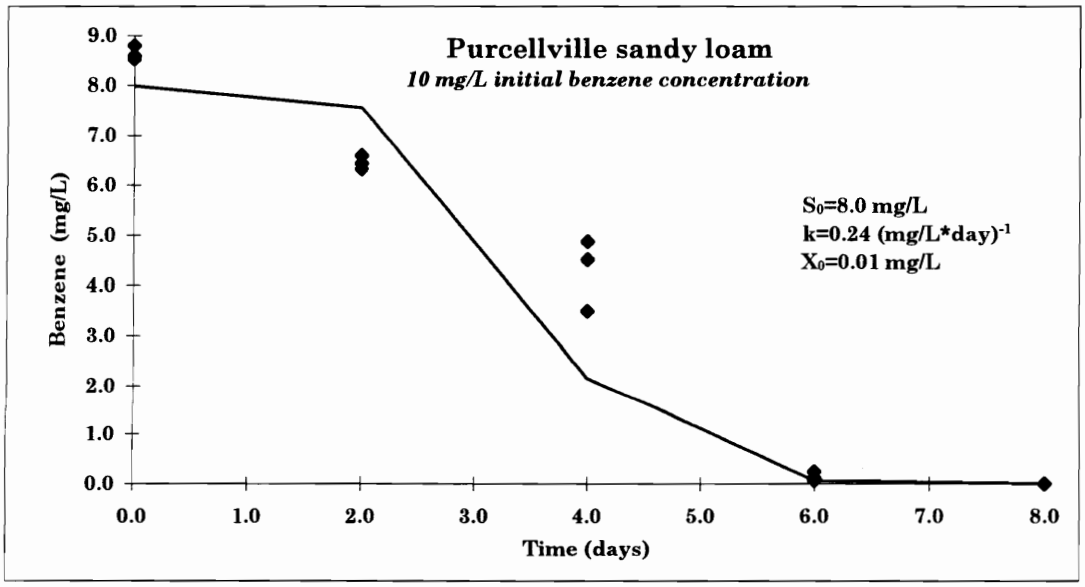


Figure III. 3. Benzene disappearance in Purcellville sandy loam spiked at an initial benzene concentrations of 1 and 10 mg/L. Aqueous benzene remaining ♦, in active soil microcosms. Each data point represents one microcosm. Solid lines are *predicted* benzene concentrations based on uncontaminated soil properties. Purcellville sandy loam was *not* included in the multiple linear regression (MLR) models.

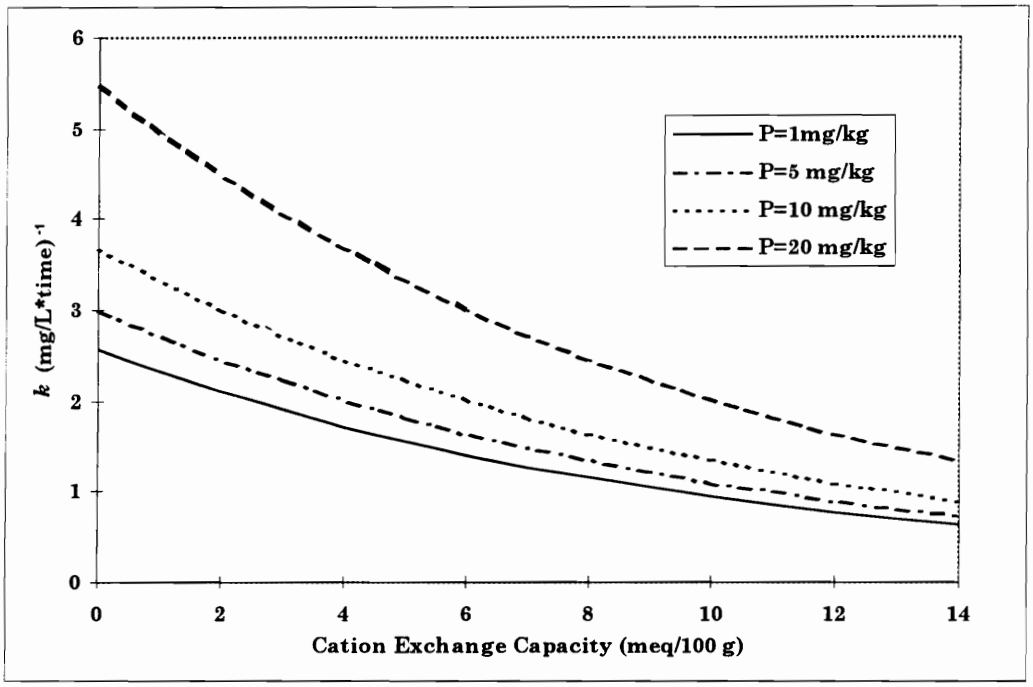


Figure III. 4. Plot of logistic rate constant *k* versus CEC for varying levels of phosphorus (Equation 3).

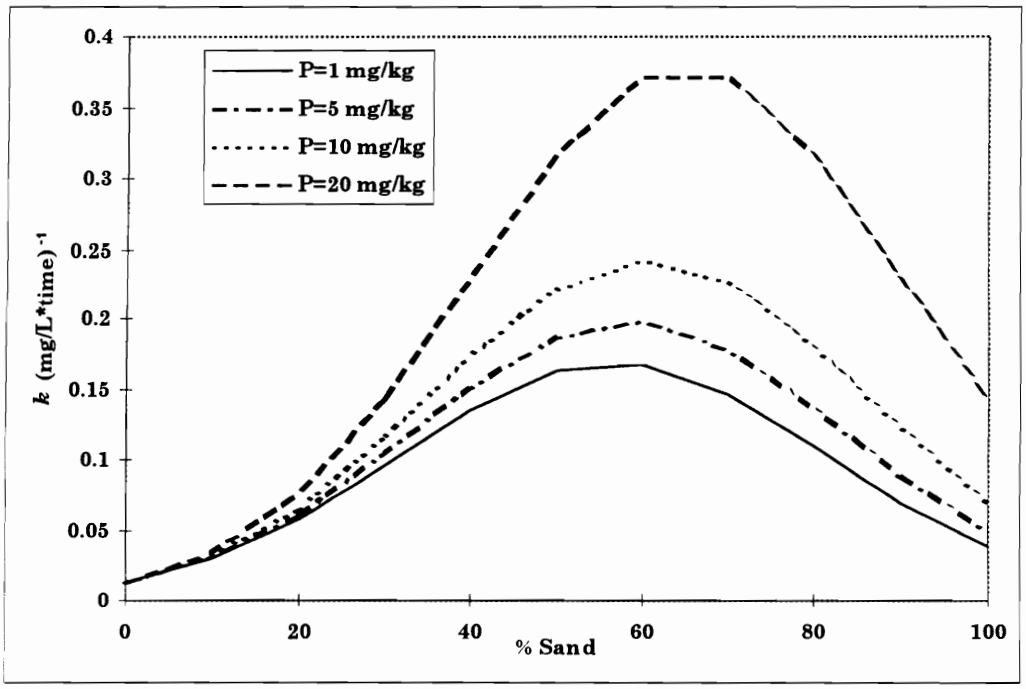


Figure III. 5. Plot of logistic rate constant *k* versus sand content for varying levels of phosphorus (Equation 4). Values for copper and nitrate concentrations were 1 mg/kg and 5 mg/kg, respectively, and CEC was 5 meq/100 g.

IV. Ammonium And Potassium Phosphates Stimulate Benzene Disappearance In Soil Microcosms

ABSTRACT

Aerobic microcosm experiments were prepared with previously-uncontaminated subsurface soils spiked with initial benzene concentrations of 1, 10 and 50 mg/L, with and without added ammonium and potassium phosphates (11 mM nitrogen, 6 mM phosphorus and 0.6 mM potassium). For each experiment, zero-order (linear) and logistic kinetic models were fit to benzene disappearance versus time data and model kinetic parameters determined for each data set using SAS® REG or NLIN procedures, respectively. An F-test was used to test the hypothesis that the regressions between unamended and nutrient-amended experiments were the same. Benzene disappearance was stimulated by nutrient addition in one (11%), 6 (50%), and 5 (45%) soils at initial benzene concentrations of 1, 10 and 50 mg/L, respectively. The logistic model improved the resolution of regressions over the linear model for experiments prepared at initial benzene concentrations of 1 and 10 mg/L. In general, nutrient addition had the greatest affect on benzene disappearance in low pH soils.

INTRODUCTION

The objective of this research was to investigate the role of inorganic nutrients on benzene disappearance in soil. Benzene is readily biodegraded under aerobic conditions, is relatively soluble in water (1770 mg/L at 25 °C; Lide, 1994), and is an EPA-designated priority pollutant and known human carcinogen. Benzene is a minor fuel constituent by volume, 0.8% for gasoline fuels (AWWA, 1990), but the global storage, distribution and consumption of gasoline has made benzene a common pollutant.

Ammonium and potassium phosphates may expedite treatment of contaminant spills by providing degrading bacteria or fungi with essential nutrients for cell growth and metabolism.

Oil or gasoline spills which threaten the environment can be sudden and dramatic, exemplified by the *Exxon Valdez* accident, or gradual and unnoticed for years, typified by leaking underground storage tanks. The number of leaking underground storage tanks could range above 1 million (Miller, *et al.*, 1990). The costs of extensive spills may be tangible, for example, unuseable or unsaleable property, disappearance of employment, and higher taxes; and intangible, for example, damage to population health or destruction of a natural ecosystem. In one case, a 38,000 L (10,000 gallon) gasoline spill in Bellview, Florida, rendered the city's entire drinking water supply unuseable due to soil benzene, toluene, ethyl benzene and xylene (BTEX) concentrations as high as 890 mg/L (Miller, *et al.*, 1990). Contaminated soil can be excavated for disposal, isolated with physical barriers, or remediated for reuse by accelerating natural processes which degrade, transform or transport organic compounds. There are two broad categories of remediation options: *ex situ* and *in situ* treatment. Soils removed from the subsurface for bioremediation, washing, or incineration are treated *ex situ*. Soils treated in place by warming, or by gas (*e.g.*, air) and water circulation, with or without added emulsifiers, inorganic salts, hydrogen peroxide, or acclimated bacteria, are treated *in situ*. *In situ* treatment offers the advantage of minimal material handling with its attendant lower costs and health risks. As of 1993, the EPA was considering or implementing *in situ* remediation of soil, sediment, sludge or groundwater contaminated with gasoline, jet fuel, lube oil or BTEX at 19 military installations, 9 industrial sites, 2 service stations, and 2 landfills (EPA, 1994a).

Carbon, oxygen, nitrogen, phosphorus and potassium are essential nutrients for cell growth and metabolism. Prior to a contaminant spill, subsurface soils may be oligotrophic. With the influx of hydrocarbons, soil microbes metabolize degradable carbon with a concomitant consumption of oxygen. Benzene biodegradation in the subsurface is typically controlled by oxygen (Salanitro, 1993). In aqueous solution at pH 7, an oxygen concentration of ca. 4 mg/L oxygen is necessary to completely mineralize a benzene concentration of 1 mg/L; the solubility of oxygen in water is ca. 10 mg/L at 25 °C. As oxygen is depleted and soil conditions become anoxic, denitrifying bacteria can use nitrate as an electron acceptor alternate to oxygen. Nitrate is reduced to nitrite, then nitrous oxide or nitrogen gases. Nitrate can also be biotically reduced to ammonium under anoxic conditions (Paul and Clark, 1989). Benzene biodegradation proceeds slowly if at all under denitrifying conditions (Hutchins, 1991; Allen-King, *et al.*, 1994; Anid, *et al.*, 1993).

Nitrogen is a major component of cellular proteins, nucleic acids and cell wall constituents. Cellular phosphorus compounds include nucleic acids and nucleotides, phosphoproteins and phospholipids, and phytins. In biological systems, adenosine triphosphate (ATP) is the energy currency which sustains life. Potassium is associated with the active site of protein synthesis enzymes. Based on an elemental analysis of the dry mass of a bacterial cell, $C_{60}H_{87}O_{32}N_{12}P$ is an empirical ratio of these nutrients in the cell (Neidhardt, 1990; EPA, 1994b). Inorganic forms of nitrogen include ammonium, nitrite, nitrate, and nitrogen gases. In soil, the ammonium cation can be volatilized as ammonia (not predominant in acidic soils, pKa = 9.3), immobilized by reactions with soil organic matter and clays, and transformed by nitrifiers into nitrate. Nitrate is held in soil solution by electrostatic attraction to adsorbed soil cations (Bohn, *et al.*, 1985). Nitrate is easily leached by percolating water. Efficiencies of nitrogen transformations depend upon soil oxygen, pH, moisture, temperature and organic matter content. For example, production of nitrate in soil through the biologically-mediated oxidation of ammonium is thought to be pH-dependent in agricultural soils, with an optimum between pH 6.6 and 8 and negligible production below pH 4. Denitrification has a similar pH-dependency, with an optimum between pH 6 and 8 (Paul and Clark, 1989).

Inorganic phosphorus is most commonly orthophosphate (H_3PO_4). Orthophosphate introduced into soil is strongly adsorbed to soil iron and aluminum oxides and hydroxides or precipitated with calcium, magnesium and iron cations, reactions which strongly retard its migration through soil and limit its bioavailability. Under natural conditions, soil solution phosphorus rarely exceeds 1 mg/L (Bohn, *et al.*,1985). Soil microflora and fauna release insoluble soil phosphorus by the productions of acids, enzymes or chelators and through their reduction of iron in iron phosphate. Potassium in soil solution in temperate region soils is normally from 1-10 mg/L (Bohn, *et al.*,1985). Like ammonium, potassium can be specifically adsorbed into the interlayers of 2:1 layer silicates, a fixation process which significantly reduces its bioavailability (Bohn, *et al.*, 1985).

Enhanced *in situ* bioremediation may include circulation of water and oxygen, addition of inorganic nitrogen and phosphate as nutrients, or nitrate and sulfate as alternate electron acceptors, to stimulate microbial mineralization of contaminants. Laboratory microcosm and column experiments, field plot, and full-scale remediation efforts indicate mixed results for the addition of inorganic nitrogen and phosphate to soil to stimulate hydrocarbon biodegradation. Comparisons of research results is challenging due to the wide spectrum of contaminant and nutrient combinations and concentrations, experimental and analytical methodologies, and research objectives.

Bragg, *et al.*, (1994) found that following the wreck of the *Exxon Valdez* oil tanker, which heavily contaminated miles of rocky intertidal Alaskan shoreline, nitrogen fertilizer increased apparent

biodegradation rates in treatment plots of relatively unweathered crude oil where nitrogen was present at elevated levels in the shallow subsurface. In areas where fertilizer was rapidly removed from the treatment area by the wave action or groundwater flows, biodegradation was reduced.

Li, *et al.*, (1994) measured remaining product in 18 soil field plots contaminated with 1-48% Bunker C oil and amended with air, air and fertilizer, or air and fertilizer and acclimated bacteria. They found that the biodegradation rates were not significantly different between the aerated unamended and amended plots. Based on low dissolved oil and grease, high dissolved oxygen and low biomass concentrations, they concluded that hydrocarbon desorption from soil controlled the removal rate, and low desorption rates did not support high biomass concentrations, thus nutrient requirements were low.

During the first 20 weeks of pH-controlled, aerated and nutrient-amended field plot experiments, Huesemann (1994) observed a nearly complete removal of low molecular weight aromatic compounds (<20 carbons) in soil contaminated with 50 g/kg crude oil. After 20 weeks neither soil composition nor nutrient formulations but contaminant molecular structure appeared to control contaminant biodegradation. He explained these results not as a desorption phenomena but as an decrease in the biodegradability of the remaining weathered hydrocarbons.

Low concentrations of an oily waste containing 15% monoaromatics were added weekly to nutrient-amended soil slurries to compare the effectiveness of different nutrient salts on biodegradation (Rasiah, *et al.*, 1992). Calcium nitrate, followed by sodium and potassium nitrates caused the highest removal rates, and ammonium nitrate and ammonium chloride caused the lowest removal rates. When phosphorus was combined with the nitrogen salts, rates increased significantly for all but the calcium nitrate-amended soil. Rasiah, *et al.*, (1992) explained these results as reduced bioavailability of ammonium by soil fixation, and the ability of calcium to increase the bioavailability of the waste.

Bioventing of a jet fuel-contaminated site (30 to 23,000 mg/kg soil) was not stimulated by application of water containing dissolved sodium trimetaphosphate, ammonium chloride, and potassium nitrate. Researchers (Miller, *et al.*,1990) concluded that the sand contained sufficient natural nutrients. In jet fuel-contaminated sediment with 650 mg/kg of weathered total petroleum hydrocarbons (TPH), 90% and 70% of the measurable hexane disappeared in nitrate-amended and nitrate plus phosphate-amended aerobic soil microcosms, respectively (Aelion and Bradley, 1991). In controls amended with sodium azide (which shuts down aerobic but not anaerobic metabolic pathways) 30% of the measurable hexane disappeared in the same time period. Reported methane production and denitrifier counts were increased in amended microcosms. These data suggest anoxic or anaerobic soil conditions existed, and nitrate functioned as an electron acceptor.

Nitrate and phosphate were circulated at a field scale groundwater extraction and infiltration system designed to remediate a gasoline-contaminated site. When the nitrate-amended water finally overtook the BTX plume, the BTX concentration in groundwater, stable for two years at 25-50 mg/L, dropped to less than 0.02 mg/L in 3 months (EPA, 1994a). Here again, nitrate may have been functioning as an electron acceptor. In a field plot experiment, a BTEX concentration of ca. 10 mg/L was completely degraded in an aerobic sandy subsurface soil only after the addition of ammonium nitrate (Allen-King, *et al.*,1994).

In laboratory experiments prepared with the same soil, biodegradation of ca. 25 mg/L toluene was greater in soil microcosms amended with ammonium sulfate. Thorton-Manning, *et al.*, (1987) reported increased biodegradation extent in 20 mL of a subsurface clay soil slurry spiked with 0.006 mg of radiolabeled phenol and amended with ammonium chloride; but increased rate and extent with added sodium phosphate or both ammonium chloride and sodium phosphate. In a similar experiment with a subsurface loam, the biodegradation extent was increased by sodium phosphate addition with or without ammonium chloride. They observed first-order kinetics with no apparent lag time, suggesting microbial population growth was not a factor. Swindoll, *et al.*, (1988) examined the influence of ammonium nitrate and potassium phosphate on biodegradation of ca. 0.1 mg/kg soil phenol and toluene in a fine sand. They observed no lag times for either substrate and no effect from nutrient addition on total amounts of phenol and toluene mineralized. In soil microcosms amended with mineral salts, glucose or amino acids, toluene disappearance was inhibited compared to an unamended control. Based on an increase in cell numbers, they explained these results as the stimulation of non-degrading organisms present in the soil.

The findings summarized above have common links. First, the need for supplemental nitrogen and phosphorus nutrients is dictated by the biologically available hydrocarbon concentration, which may be very low for insoluble and highly weathered hydrocarbon mixtures. Second, to stimulate biodegradation of hydrocarbons, added nutrients must remain bioavailable. In the subsurface, fixation of potassium and ammonium by clay, adsorption and precipitation of phosphate to soil minerals, and transport of nitrate by water through the soil are all processes which may reduce the bioavailability of these nutrients. Nutrient addition may also stimulate non-hydrocarbon degrading segments of the microbial population, especially in the presence of naturally-occurring soil organic matter. This would not necessarily reduce the nutrient bioavailabililty, as nutrient recycling may play a significant role in sustaining high microbial populations. Third, nitrate has dual functions as cellular nutrient and electron acceptor under anoxic conditions. Even in aerobic soil, anoxic microsites can exist within microbial colonies (*e.g.*, biofilms) or soil aggregates. Heavily contaminated subsurface is often characterized by decreased oxygen and increased carbon dioxide, nitrogen, nitrous oxide and methane gases. (The latter

are associated with anoxic or anaerobic conditions.) Biodegradation may be limited primarily by the presence of a suitable electron acceptor, such as oxygen or nitrate, and not by cellular macronutrients. Fourth, the nutrient demands are likely to be different between growing and steady-state microbial populations; between aerobic and anoxic or anaerobic populations; and between populations with different acclimation histories (*e.g.*, low pH vs. high pH), and thus can vary both spatially and temporally in a contaminated subsurface.

METHODOLOGY

Soil Sampling, Transportation and Storage

Sampling sites throughout Virginia were chosen primarily for the expected soil characteristics, based on published soil surveys or previous research. Subsurface soil samples (ca. 2 kg) were obtained from depths of 1 to 4 meters by pre-cleaned, 3"-diameter, manually-driven auger; identified and described by an accompanying soil scientist; and transferred directly to sterile polyethylene bags. The polyethylene bags were placed on ice in coolers and returned to the laboratory the same day. In the laboratory, soil samples were mixed by combining portions from each bag into new bags, which were then stored at 8°C in the dark for biological characterization and microcosm preparation. A subsample was air-dried 48 hours and sieved through a No. 10 (2 mm) sieve for chemical and physical analyses. Soil microcosm experiments were prepared within a week, and soil characterization completed within 2 weeks, of soil sampling. Most of the soils used in this research were moist, acidic, highly weathered, red and yellow-brown soils commonly found in the Southeast. Soils were taken from both forest and agricultural lands. Two soils were obtained from below the local water table.

Microcosm Preparation

Unamended and nutrient-amended soil microcosms containing 5 g soil and 5 g sterile, distilled water spiked with initial benzene concentrations of 1 mg/L (1.4 ± 0.4 mg/L), 10 mg/L (10.4 ± 0.7 mg/L) or 50 mg/L (47 ± 7 mg/L) were prepared in 22-mL acid-washed headspace vials and sealed with aluminum crimp-top caps and Teflon®-lined rubber septa. Nutrient solutions contained 200 mg $(NH_4)_2HPO_4$-NH_4^+ and 25 mg K_2HPO_4-K^+ per liter (11 mM nitrogen, 6 mM phosphorus and 0.6 mM potassium). Benzene (>99%, Sigma-Aldrich) was added by microliter syringe to sterile, distilled solution

in a volumetric flask, mixed by shaking for 15 minutes and immediately pipetted into soil microcosms. Microcosms were then shaken for 10 seconds to mix the soil and solution, and incubated in the dark under static conditions at a constant temperature of 20 °C. Controls were identically prepared with nutrient-amended solution and soil which had been autoclaved 5 times over one week. Soil for controls, and solutions, glassware, and plastics were sterilized by autoclaving for 20 minutes at 121 °C and 1.05 kg/cm^2. An experiment consisted of 24 identically-prepared soil microcosms. For each experiment, microcosms were sampled in triplicate daily or less frequently, depending upon the anticipated or observed benzene disappearance. Soil microcosms were water-saturated and incubated at 20 °C to simulate aquifer conditions in a moderate climate and to remove these factors from subsequent analyses. The initial dissolved oxygen concentration was ca. 10 mg/L, an amount sufficient enough to completely mineralize a benzene concentration of 1-2 mg/L. Sufficient oxygen was available in the headspace to degrade a benzene concentration of >50 mg/L in 5 mL of water.

Analytical Technique

The headspace sampling analytical method was selected based upon a detection level of 0.005 mg/L benzene concentration and the ability to sample microcosms with minimum benzene loss and soil disruption. A Hewlett-Packard 19395A headspace sampler and a Hewlett-Packard 5880A gas chromatograph (GC) equipped with a flame ionization detector were used for headspace sampling and sample analysis, respectively. The GC column was 6-ft of 1/8-in OD stainless steel packed with 5% SP 1200 and 1.75% Bentone-34. Injector and detector temperatures were 130 °C and 200 °C, respectively. The initial column temperature was 50 °C, ramped at 6 °C/minute to a final temperature of 90 °C. Nitrogen at a total flow rate of 40 mL/min was the carrier gas. Microcosms were heated in the headspace sampler for 1 hour at 60 °C prior to sampling. A GC response versus aqueous benzene concentration standard curve was developed from headspace sampling of vials containing 5 mL of water-dissolved benzene at concentrations from 0.005 to 100 mg/L. The standard curve did not change significantly when prepared with microcosms containing nutrient solution or 5 g sterile sieved sand.

Soil Analysis

The results of selected soil analyses are summarized in Table 1. Refer to Appendix A for a complete listing of soil analyses. To measure bulk soil pH, 20 g of air-dried and sieved soil was combined with 20 mL of de-ionized, distilled water in a 50 mL beaker, stirred, and allowed to sit for 20 minutes. The pH was measured with a pH meter equipped with a glass electrode, calibrated with buffered solutions at pH 4 and 7. Bulk soil density was determined by weighing a standard volume of air-dried and sieved soil. Phosphorus, calcium and potassium were extracted from 4 cc of air-dried and sieved soil with Mehlich No. 1 extractant (0.05 N hydrochloric acid and 0.025 N sulfuric acid) in a 60-mL straight-walled plastic beaker by shaking for 5 minutes at 180 rpm on a reciprocating shaker (3.8 cm stroke length) and filtered through Whatman No. 1 filter paper. Cation concentrations in the extractants were determined with an Jarell Ash ICAP 9000 inductively coupled plasma spectrophotometer, calibrated over the expected range of concentrations using a spectrally pure, plasma-quality reference solution. Concentrations measured in the extractant were converted to mg/kg in soil by a dilution factor calculated using the bulk density for each soil. The detection limits for this method were 0.2 mg/kg for phosphorus, 0.4 mg/kg for calcium and 1 mg/kg for potassium. The standard deviations of duplicate soil samples ranged from 0.3% to 10% for phosphorus, 0.3% to 30% for calcium and 1% to 70% for potassium. For the soils tested, these standard deviations increased with decreasing cation soil concentrations. Dilute acid-extractable phosphorus, calcium and potassium are considered bioavailable (Paul and Clark, 1989). Nitrate was extracted by equilibrating 20 cc of air-dried sieved soil with 20 mL of filter-sterilized water in a centrifuge tube for one hour on a Lab-Quake rotating shaker. The sample was then centrifuged and the supernatant filtered through a 0.2 μm filter and the filtrate nitrate measured with a Dionex ion chromatograph equipped with an Ionpac AS4A analytical column. The method detection limit was below 0.1 mg/kg nitrate-N. Soil water content was determined by drying 2 g of soil in pre-weighed aluminum pans for 24 hours at 110 °C and measuring the weight disappearance. Soil water content was determined for both air-dried sieved soils and wet, refrigerated soils used for the preparation of microcosms. A particle size analysis was done on each soil by the pipette method (Day, 1965). Standard deviations of a duplicate sand, silt and clay sample were 0.1%, 3% and 6%, respectively, increasing with clay content. Cation exchange capacity (CEC) was determined by the barium chloride method described by Rhoades (1982). Magnesium concentration in the extractant, diluted as necessary, was determined with an atomic adsorption spectrophotometer, calibrated from 0 to 0.5 mg/L using a lanthanum-spiked magnesium

chloride solution. The standard deviation of duplicate soil samples ranged from 0.02% to 40%, with higher variability observed at lower CECs. Chemical oxygen demand (COD) was determined by the method described by Eaton, *et al.* (1995) using 0.05 to 0.25 grams of air-dried, sieved soil in 5 mL distilled water. Standard deviations of duplicate soil samples ranged from 0.4% to 10%, except for the high organic content soil which had a standard deviation of 28%.

Oxygen content in selected soil microcosms was measured before GC sampling by inserting a 2-½" oxygen (hypodermic) needle electrode (Diamond General, Inc.) through the headspace vial septum into the undisturbed soil-water interface. The oxygen electrode was calibrated using two water cells, one purged with nitrogen (<0.02 % oxygen) and one purged with breathing air (21% oxygen). In soil microcosms containing either sand or clay spiked to an initial benzene concentration of 10 mg/L, direct oxygen measurements were made when benzene disappearance rates were highest. For both soils, the oxygen content at the soil-water interface ranged from 16-19%, near saturation. Oxygen measurements made within the soil sediments, which required removal of the headspace vial cap, were 8-12%, indicating soils were aerobic. Similar measurements made in a clay soil at 50 mg/L benzene revealed depletion of oxygen in the sediment but not the soil-water interface after 5 days of incubation.

Data Analysis

Models, shown in Equations 1 and 2, were chosen because they have a theoretical basis, their shapes were consistent with data sets, they required only measures of benzene and time, and their use is relevant to soil remediation. Equation 1 and Equation 2 were fitted by the method of least squares to experimental data using SAS® REG and NLIN procedures, respectively. To obtain initial parameter estimates for Equation 2, the model was plotted with varying parameters matched with benzene disappearance data and initial values selected. The logistic model was fitted without X_0 included in the parameter estimation. X_0 was approximated as 0.01 mg/L based on 1×10^{-13} g benzene to meet the carbon requirements of one cell. Soil heterotrophic plate counts (HPCs) ranged from 10^4 to 10^5 CFU/g dry soil. HPCs could represent between 1% and 50% of the viable cells in the soil sample, not all heterotrophic bacteria plated necessarily degrade benzene, both bacteria and fungi not enumerated by HPCs may also degrade benzene, and benzene metabolized by cells may be used for both growth and maintenance (Steffan, *et al.*, 1988; Torsvik, *et al.*, 1990). Thus, this estimate of X_0 could easily range an order of magnitude in either direction. The logistic model with this estimate of X_0 resulted in better regressions and parameter estimates than did the linear model for microcosm experiments prepared at initial benzene concentrations of 1 mg/L and 10 mg/L (Chapter II).

Zero-Order $S = S_0 - k_1 t$ (1)

Logistic $S = S_0 + X_0/\{1+(X_0/S_0)\exp[k(S_0+X_0)t]\}$ (2)

where

S = benzene concentration, mg/L

S_0 = initial benzene concentration, mg/L

t = time, days

X_0 = benzene concentration required to produce the initial population density, mg/L

k = proportionality constant, $(mg/L \cdot day)^{-1}$

k_1 = proportionality constant, (mg/L)/day

For each soil, separate regressions using the zero-order (linear) model were made for benzene disappearance versus time data from unamended and nutrient-amended experiments, and the resulting regressions termed the "full" model. These data sets were then combined, one regression made through the data, and this regression termed the "restricted" model. This process was repeated with the logistic kinetic model. The "full" model has a total of four parameters estimated, two rate constants and two intercepts. To obtain the "full" model residual sum of squares (RSS) and mean squared error (MSE), the contributions of the individual regressions are summed. The "restricted" model has a total of two parameters estimated, a rate constant and an intercept. The F statistic is formed by the ratio shown in Equation 3, where RSS_{full} and MSE_{full} are values for the more complex model, and Δp the change in the number of estimated parameters between models. The F statistic is compared with values in an F distribution table, where $df_1 = \Delta p = 2$ and $df_2 = n-p$, where n is the number of observations and p is the number of parameters in the more complex model. The hypothesis under test is that there is no decrease in unexplained variability between the full and restricted models; the hypothesis can be rejected if the F statistic is larger than the tabulated value at the desired confidence level (*e.g.*, p=0.05). In other words, if the unexplained variability increases significantly in the "full" model, then the separate regressions are different. With this approach, the regressions as a whole are being compared and not the individual parameter estimates. Parameter estimates were assumed to be significantly different if their 95% confidence intervals did not overlap.

$$F_{\Delta p,\, n\text{-}p,0.05} = [(RSS_{restricted}\text{-}RSS_{full})/\Delta p]/MSE_{full} \quad (3)$$

RESULTS AND DISCUSSION

Benzene disappearance over time for Alaga sand prepared with initial benzene concentrations of 1, 10 and 50 mg/L, with and without nutrient-amendment, are shown in Figure 1. For each of these experiments, benzene disappearance in active soil microcosms was significantly greater than benzene disappearance in microcosms prepared with sterilized soil, indicating a biotic benzene removal mechanism. This was true for all microcosms prepared at initial benzene concentrations of 10 mg/L or less, however, at an initial benzene concentration of 50 mg/L, four unamended soils had disappearance rates in active microcosms which were not significantly different from abiotic controls. Generally, fewer control microcosms relative to active microcosms were sampled after initial experimentation showed miminal benzene disappearance over a 30-day period in both solution and soil microcosm controls (Appendix D). An increasing impact of added nutrients with increasing initial benzene can be clearly seen in Figure 1. This pattern was not seen for every soil, as is discussed below. For Alaga sand, selected unamended and nutrient-amended microcosm experiments were respiked to a benzene concentration of 20 mg/L after the initial benzene disappeared. In both experiments benzene disappearance occurred without delay, but the disappearance rates observed in the nutrient-amended experiment were four times higher than the rate observed in the unamended microcosm experiment, as can be seen in Figure 2. After 14 days, nutrients added to remaining microcosms in the unamended experiment increased the disappearance rate (Figure 2). These data provided evidence that nutrients and not oxygen limited benzene disappearance in unamended Alaga sand microcosms.

The total times observed for benzene to degrade below 0.005 mg/L in unamended and nutrient amended soil microcosms spiked at initial benzene concentrations of 1, 10 and 50 mg/L are shown in Table 2. These data suggest that supplemental nutrients decreased total degradation time in 78%, 67% and 45% of the soils prepared at initial benzene concentrations of 1, 10 and 50 mg/L, respectively. For Alaga, Altavista, Braddock, Cecil, Ross and possibly Weikert soils the same nutrient amendment had a greater effect with increasing benzene concentration, based on the reduction in time for benzene to degrade below a concentration of 0.005 mg/L (Table 2). In the Ross silt, for example, the total degradation time was shortened by 2.5 days, 4 days, and 14 days for nutrient-amended experiments prepared with initial benzene concentrations of 1, 10 and 50 mg/L benzene, respectively. A comparison of total times alone, however, does not distinguish between significant and non-significant changes in

benzene disappearance rates. For example, Table 2 shows a one day difference in total time between unamended and nutrient-amended experiments at an initial benzene concentration of 1 mg/L for Alaga sand. Is this a significant difference? The data plotted in Figure 1 do not present a convincing argument that a difference exists. This comparison also fails to discern changes in benzene disappearance rates in soils which did not completely degrade during the observation period. One method of evaluating the influence of nutrients on benzene disappearance over time in soil microcosm experiments is to first fit the kinetic model to the data using regression analysis, and second to compare regression lines using an F-test. The better the kinetic model selection, the better the differentiability between regressions, providing the data scatter is not too high. All data sets were modeled with both a linear and a logistic model, as shown in Equations 1 and 2, respectively. Experiments at initial benzene concentrations of 1 and 10 mg/L exhibited an S-shape curve which can be adequately modeled by logistic regression (Chapter II). Data sets at an initial benzene concentration of 50 mg/L had more variability in shape and generally higher data scatter and a linear regression was, in several cases, more appropriate than logistic. Using both models for data comparisons provided an opportunity to see how sensitive the results were to model selection.

Regression and F-test results for soils prepared with an initial benzene concentration of 10 mg/L are presented in detail in Table 3 for the zero-order (linear) model and Table 4 for the logistic model. With the linear model, 6 (50%) of the soils had significantly different regressions between unamended and nutrient-amended experiments (Table 3). Upon closer examination, only 5 (42%) of the soils had significantly higher rates of benzene disappearance (Table 3). Of these soils, Riverview and Weikert had significantly different intercepts as well as slopes, deduced from the intercept estimates with their standard errors. The model intercept depends upon the model selected, as can be seen by comparing both the zero-order and logistic intercept estimates for Riverview soil (Tables 3 and 4). With the logistic model, 8 (67%) of the soils had significantly different regressions between unamended and nutrient-amended experiments (Table 4), but only 5 (42%) had significantly higher rate constants. Groseclose soil had significantly different intercepts which yielded a shorter degradation period in nutrient-amended experiments. Wheeling soil had a significantly lower rate constant but higher intercept which also resulted in a shorter degradation period in nutrient-amended experiments. For both the linear and the logistic model, Cullen soil had lower rate constants and significantly different intercepts in nutrient-amended experiments (Tables 3 and 4), suggesting an inhibitory effect of nutrient addition on benzene disappearance. Figures 3, 4 and 5 illustrate the separate (full model) and combined (restricted model) regressions for both linear and logistic models with three different soils. In Figure 3, a clear difference between separate regressions can be seen, irregardless of the model selected. In Figure 4, the variability

in the data attributed to error for the logistic model was too great to distinguish between the regressions; however, the error variability was lowered when a linear model was used and the two regressions were separated. In Figure 5, although the data suggest two different regressions with either model, high data scatter prevents their separation.

Because both a higher intercept and a higher rate constant can be a result of higher applied benzene (Chapter II), only those soils with a significantly higher rate constant but not intercept were considered to have been stimulated by nutrient addition. Rate constants or intercepts with an overlapping 95% confidence interval were not considered different. One (11%), 6 (50%), and 5 (45%) soils at initial benzene concentrations of 1, 10 and 50 mg/L were stimulated by nutrient addition. The logistic model improved the resolution of regressions over the linear model at initial benzene concentrations of 1 and 10 mg/L as evidenced by closer intercept estimates and higher F-statistics (Tables 3 and 4). Soils with significantly higher rate constants in nutrient-amended microcosm experiments are listed in Table 5.

A summary of selected soil analyses is presented in Table 1, which is sorted by increasing pH. Note that low pH soils at the higher benzene concentrations are among those stimulated by nutrient addition. Was the soil response a result of increased pH when nutrients were added? This could well be true for poorly buffered soils such as sands. The Alaga sand, for example, had a bulk pH of 4.5 (Table 1). For an experiment prepared at an initial benzene concentration of 10 mg/L with no nutrients added the average pH in soil microcosms was initially 4.5 and dropped slowly to 4.3 after 4 days; in nutrient-amended microcosms the pH was initially 6.1 and dropped to 5.8 after 4 days. In the Cullen clay loam, however, the pH in unamended and nutrient-amended soil microcosms remained constant at 5.1 and 5.4, respectively, near the bulk soil pH of 5.0. Novak, *et al.*, (1992), reported increased toluene biodegradation in aerobic microcosms prepared from acidic soil when the pH was increased to near neutral. Increases in biodegradation rates with pH may be the result of either increased nitrogen or phosphorus availability or establishment of an optimum pH range for cellular metabolism.

Several explanations are plausible for no significant increase in benzene disappearance in soil microcosms with the addition of ammonium and potassium phosphates. First, the soil may already contain sufficient nutrients. Data suggest that for experiments prepared at an initial concentration of 1 mg/L or less most of the soils tested were not nutrient-limited. Groseclose loam, which contained relatively high nitrate and phosphorus (Table 1), may have had sufficient nutrients at all levels of initial benzene. Alaga sand, however, had a relatively high phosphorus level but low nitrate and potassium (Table 1).

Second, the added nutrients can react with adsoption sites in the soil or precipitate with soil counter ions. This may explain the failure of nutrients to stimulate benzene disappearance in Roanoke

and Groseclose soils, which had the highest calcium levels and pH values. Above pH 7, phosphate readily precipitates with soil calcium (Bohn, *et al.*, 1985). The changes in water-extractable ammonium, potassium, nitrate amd phosphorus concentrations in Alaga sand and Cullen clay loam are graphed in Figure 6. A 1:1 (by weight) soil to water solution was equilibrated for 6 hours on a rotating shaker, and the solution separated from solids be centrifugation. Extractants were analyzed on a Dionex ion chromatograph equipped with an AS4A column for anions, and a CS12 column for cations. Soil was air-dried and sieved but not sterile. A log plot of concentration was necessary to show the full range. Notice the difference in phosphate ion concentrations for the soils receiving the same initial dose: Alaga sand had 100 mg/L but Cullen clay loam had only 4 mg /L remaining in soil solution after six hours of equilibration time. A similar affect can be seen with the added ammonium and potassium cations, but soil calcium dropped slightly, possibly due to precipitation with added phosphates. The increase in nitrate in Cullen clay loam may be due to displacement of nitrate from adsorption sites by added phosphate. The drop in nitrate observed for the Alaga sand may reflect the measurement variability at that low concentration, or perhaps consumption of nitrate by soil microbes. The high affinity for phosphate by the clay loam may be due to reactive iron and aluminum oxide minerals surfaces which physically adsorb and over time chemically adsorb phosphate (Bohn, *et al.*, 1985).

Third, the added nutrients may stimulate mineralization of naturally occurring soil organic matter. Ross silt had the highest organic matter content, as can be seen from the relatively high COD. Benzene disappearance in Ross silt was stimulated by nutrient addition only at an initial benzene concentration of 50 mg/L, perhaps after naturally-occurring biodegradable organic matter was consumed.

Finally, another essential nutrient may be missing or limiting in the soil. Essential micronutrients such as iron, copper, and manganese are more bioavailable in low pH or high organic matter soils (Bohn, *et al.*, 1985). The bioavailability of these micronutrients may also explain the increased benzene disappearance in nutrient-amended low pH soils.

Table IV. 1. Summary of measured chemical and physical soil properties for 12 previously-uncontaminated subsurface soils. Data are sorted by increasing pH.

Soil Series	pH	CEC (meq/100 g)	Nitrate-N (mg/kg soil)	Calcium (mg/kg soil)	Phosphorus (mg/kg soil)	Potassium (mg/kg soil)	COD (mg O_2/g soil)
Alaga	4.5	0.2	0.1	29	27	2	2.0
Cecil	4.5	3.1	0.6	80	2.3	64	0.9
Altavista	4.6	11.8	1.1	500	0	98	3.1
Braddock	4.9	3.1	9.1	370	0.75	440	4.1
Ross	4.9	5.3	0.8	850	8.8	59	85
Weikert	5.0	0.6	0.1	130	0.75	18	7.6
Cullen	5.0	3.7	0.1	110	1.1	109	4.3
Riverview	6.0	2.8	0.4	410	1.4	7	4.9
Wheeling	6.0	6.5	1.6	810	5.1	16	2.5
McGary	6.6	7.3	0.3	950	2.6	20	4.1
Groseclose	7.6	10.2	9.6	4650	24	41	7.0
Roanoke	7.7	14.0	0.2	1300	0.73	20	2.5

Table IV. 2. Observed total time for benzene to degrade below a concentration of 0.005 mg/L for unamended and nutrient-amended soil microcosms prepared at initial benzene concentrations of 1, 10 and 50 mg/L.

Soil Series	1 mg/L benzene		10 mg/L benzene		50 mg/L benzene	
	Unamended	*Nutrient-Amended*	*Unamended*	*Nutrient-Amended*	*Unamended*	*Nutrient-Amended*
	Time (days)					
Alaga	3.7	2.7	7.7	3.9	>35	8.6
Altavista	13	6.6	25	8.5	>55	41
Braddock	9.0	5.6	16	9.8	>36	16
Cecil	not available	not available	15	5.7	32	14
Cullen	8.0	6.0	8.9	11	>29	27
Groseclose	3.6	3.0	5.1	5.0	not available	not available
McGary	11	>9	>19	>17	>45	>43
Riverview	not available	not available	>11	6.7	>21	27
Roanoke	17	8.5	>25	>16	>55	>41
Ross	6.1	3.6	8.9	4.9	31	17
Weikert	6.5	4.0	8.4	5.1	28	>17
Wheeling	not available	not available	11	9.0	>22	>22

Table IV. 3. Comparison of linear models for experiments prepared at an initial benzene concentration of 10 mg/L. The full model is described by a separate regression for each data set; the restricted model, shown highlighted below, is described by one regression through combined data sets.

Soil Series	n	S_0 [a] ± SE [b]	k [c] ± SE	Model RSS	Model MSE	F statistic	Data Set
Alaga	26	8.5 ± 0.7	1.4 ± 0.1	77	3.2	6.8†	unamended
	14	8 ± 1	2.4 ± 0.4	43	3.6		amended
	40	7.0 ± 0.7	1.2 ± 0.2	212	5.6		combined
Altavista	25	10.5 ± 0.6	0.46 ± 0.05	82	3.6	19†	unamended
	20	10.3 ± 0.6	1.3 ± 0.1	52	2.9		amended
	45	8.9 ± 0.6	0.43 ± 0.07	375	8.7		combined
Braddock	24	8.3 ± 0.3	0.56 ± 0.04	20	0.90	3.4†	unamended
	26	8.1 ± 0.4	0.76 ± 0.09	81	3.4		amended
	50	8.0 ± 0.4	0.61 ± 0.05	130	2.7		combined
Cecil	24	8.4 ± 0.7	0.65 ± 0.09	82	3.7	2.3	unamended
	16	10.5 ± 0.8	1.5 ± 0.3	47	3.3		amended
	40	8.6 ± 0.5	0.71 ± 0.08	161	4.2		combined
Cullen	20	9.8 ± 0.4	1.2 ± 0.08	18	1.0	17*†	unamended
	26	12.0 ± 0.6	1.1 ± 0.09	60	2.5		amended
	46	10.8 ± 0.6	1.09 ± 0.09	197	4.5		combined
Groseclose	17	8.5 ± 0.5	1.8 ± 0.2	20	1.3	0.15	unamended
	17	9.2 ± 0.6	2.1 ± 0.2	31	2.0		amended
	34	8.9 ± 0.4	1.9 ± 0.1	52	1.6		combined
McGary	25	8.5 ± 0.9	0.19 ± 0.09	171	7.4	2.0	unamended
	25	10.7± 0.5	0.52 ± 0.06	56	2.4		amended
	50	9.6 ± 0.5	0.32 ± 0.06	267	5.6		combined
Riverview	25	8.0 ± 0.5	0.90 ± 0.08	39	1.7	3.2†	unamended
	23	9.8 ± 0.6	1.5 ± 0.1	46	2.2		amended
	48	8.5 ± 0.4	1.04 ± 0.08	110	2.4		combined
Roanoke	25	10 ± 0.8	0.38 ± 0.07	139	6.0	0.8	unamended
	26	10 ± 0.8	0.54 ± 0.09	124	5.2		amended
	51	9.9 ± 0.5	0.42 ± 0.05	282	5.8		combined
Ross	26	5.8 ± 0.4	0.85 ± 0.09	35	1.5	3.0	unamended
	17	6.5 ± 0.5	1.4 ± 0.2	20	1.3		amended
	43	5.8 ± 0.3	0.93 ± 0.09	72	1.8		combined
Weikert	23	9.1 ± 0.5	1.3 ± 0.1	34	1.6	8.5†	unamended
	17	9.6 ± 0.5	2.1 ± 0.2	21	1.4		amended
	40	8.7 ± 0.5	1.3 ± 0.1	106	2.8		combined
Wheeling	26	9.8 ± 0.5	0.99 ± 0.08	43	1.8	1.2	unamended
	26	11.3 ± 0.6	1.3 ± 0.1	64	2.7		amended
	52	10.5 ± 0.4	1.11 ± 0.07	118	2.4		combined

†Regressions are different. For a significance level of 0.05, $F_{2,40} = 3.23$; $F_{2,60} = 3.16$

[a]units are mg/L, [b]standard error, [c]units are mg/L/day; *Unamended k is larger than nutrient-amended

Table IV. 4. Comparison of logistic models for experiments prepared at an initial benzene concentration of 10 mg/L. The full model is described by a separate regression for each data set; the restricted model, shown highlighted below, is described by one regression through the combined data sets.

Soil Series	n	S_0[a] ± SE[b]	k[c] ± SE	Model RSS	Model MSE	F statistic	Data Set
Alaga	26	9.1 ± 0.4	0.31 ± 0.01	22	0.92	11†	unamended
	14	9 ± 1	0.65 ± 0.06	38	3.2		amended
	40	7.6 ± 0.6	0.41 ± 0.03	148	3.9		combined
Altavista	25	9.6 ± 0.4	0.061 ± 0.003	61	2.7	46†	unamended
	20	9.1 ± 0.4	0.158 ± 0.008	28	1.6		amended
	45	7.5 ± 0.6	0.079 ± 0.009	484	11		combined
Braddock	24	7.5 ± 0.3	0.135 ± 0.007	22	1.0	1.2	unamended
	26	7.3 ± 0.4	0.17 ± 0.01	54	2.3		amended
	50	7.5 ± 0.3	0.150 ± 0.006	84	1.8		combined
Cecil	24	7.7 ± 0.5	0.135 ± 0.009	57	2.6	6.8†	unamended
	16	8.6 ± 0.3	0.180 ± 0.006	7.9	0.56		amended
	40	8.1 ± 0.4	0.160± 0.008	108	2.9		combined
Cullen	20	8.6 ± 0.2	0.170 ± 0.005	7.5	0.41	40*†	unamended
	26	9.9 ± 0.3	0.095 ± 0.003	39	1.6		amended
	46	9.3 ± 0.5	0.130 ± 0.007	205	4.6		combined
Groseclose	17	7.4 ± 0.2	0.333 ± 0.007	2.8	0.19	4.6*†	unamended
	17	8.4 ± 0.2	0.331 ± 0.008	3.9	0.26		amended
	34	7.9 ± 0.2	0.333 ± 0.007	10.8	0.34		combined
McGary	25	7.7 ± 0.6	0.045 ± 0.005	173	7.5	4.7†	unamended
	25	9.6 ± 0.4	0.072 ± 0.004	52	2.3		amended
	50	8.2 ± 0.4	0.049 ± 0.004	318	6.6		combined
Riverview	25	7.8 ± 0.3	0.203 ± 0.006	11.5	0.50	0.4*	unamended
	23	8.2 ± 0.2	0.199 ± 0.005	10.5	0.50		amended
	48	8.1 ± 0.2	0.201 ± 0.004	22.8	0.50		combined
Roanoke	25	9.7 ± 0.7	0.067 ± 0.005	135	5.9	0.5	unamended
	26	9.0 ± 0.5	0.078 ± 0.005	101	4.2		amended
	51	9.3 ± 0.4	0.073 ± 0.004	247	5.0		combined
Ross	26	5.4 ± 0.2	0.36 ± 0.02	13	0.55	1.4	unamended
	17	5.7 ± 0.5	0.44 ± 0.04	28	1.9		amended
	43	5.4 ± 0.3	0.39 ± 0.02	48	1.2		combined
Weikert	23	8.5 ± 0.2	0.222 ± 0.006	8.4	0.40	21†	unamended
	17	8.4 ± 0.009	0.311 ± 0.009	4.0	0.27		amended
	40	8.3 ± 0.3	0.26 ± 0.009	41	1.1		combined
Wheeling	26	8.5 ± 0.2	0.145 ± 0.004	10.5	0.44	3.2*†	unamended
	26	9.4 ± 0.2	0.134 ± 0.003	11.0	0.46		amended
	52	9.0 ± 0.2	0.138 ± 0.003	27.3	0.55		combined

†Regressions are different. For a significance level of 0.05, $F_{2,40} = 3.23$; $F_{2,60} = 3.16$

[a]units of mg/L, [b]standard error, [c]units of $(mg/L*day)^{-1}$; *Unamended k is larger than nutrient-amended

Table IV. 5. Summary of soils which had higher benzene disappearance rates in nutrient-amended soil microcosm experiments. Models shown parenthetically were able to differentiate between unamended and nutrient-amended regressions.

1 mg/L benzene		10 mg/L benzene		50 mg/L benzene	
Alaga	(logistic)	Alaga	(linear & logistic)	Alaga	(linear & logistic)
		Altavista	(linear & logistic)	Altavista	(linear & logistic)
		Braddock	(linear)	Braddock	(linear & logistic)
		Cecil	(logistic)	Cullen	(linear & logistic)
		McGary	(logistic)	Ross	(linear & logistic)
		Weikert	(linear & logistic)		

SUMMARY AND CONCLUSIONS

Benzene disappearance in unamended and nutrient-amended soil microcosms was compared using both linear and non-linear data regressions. The logistic model enabled increased resolution between separate regressions at lower benzene concentrations, where data were most non-linear. Added ammonium phosphate and potassium phosphate nutrients stimulated benzene disappearance in soil microcosms prepared at initial benzene concentrations of 10 and 50 mg/L. At these benzene levels, approximately 50% of the soils tested responded to nutrient addition, with low pH soils exhibiting the greatest response. These results are consistent with current literature, which indicate a need for nitrogen and phosphorus in subsurface surface soils under aerobic conditions at higher concentrations of soluble hydrocarbons, and likewise reflect the difficulties encountered when trying to meet microbial nutrient demands by circulating soluble salts through a highly reactive soil column.

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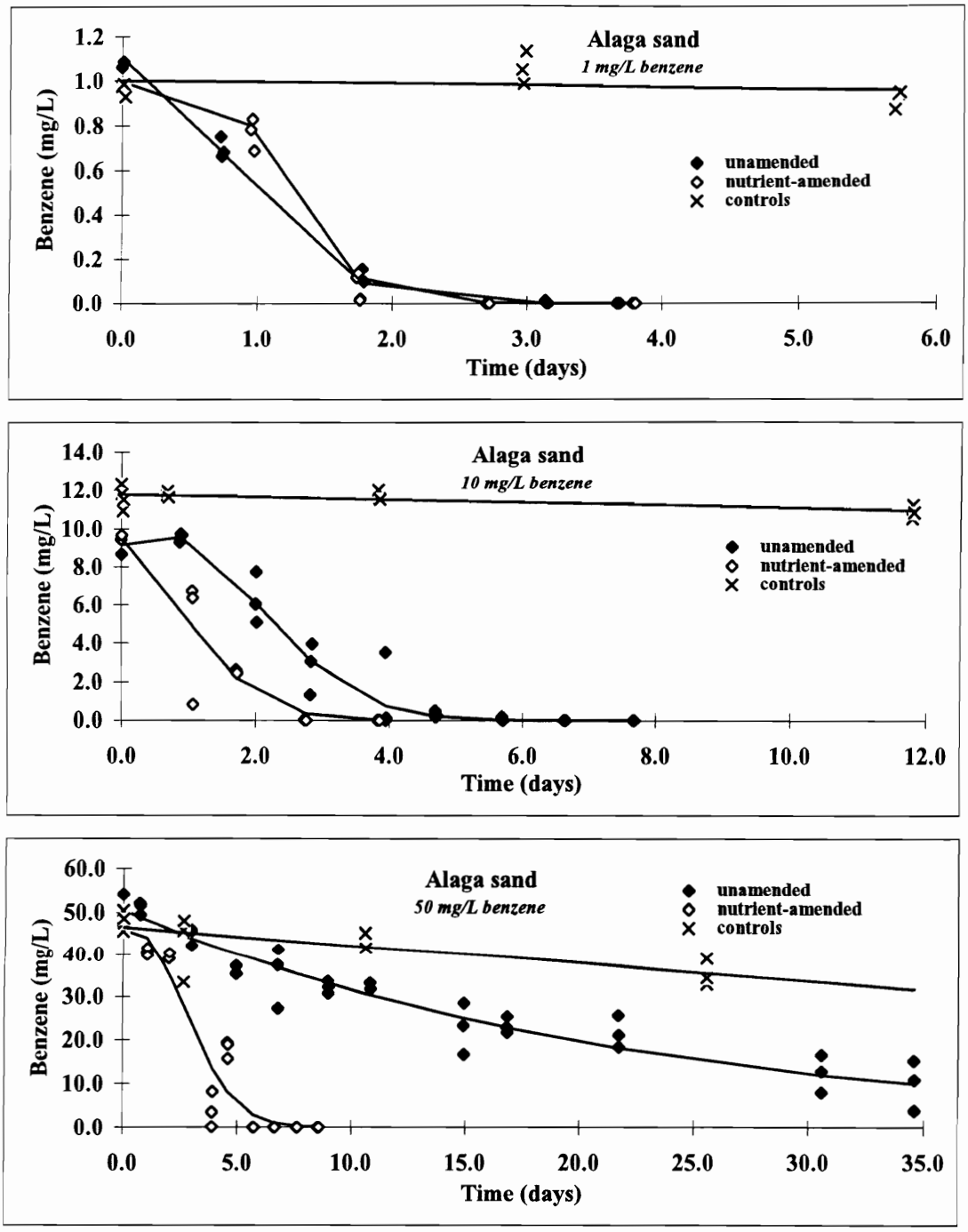


Figure IV. 1. Benzene disappearance versus time in unamended and nutrient-amended soil microcosms prepared with Alaga sand. Microcosms were prepared with 5 g soil and 5 g sterile, distilled water spiked at initial benzene concentrations of 1, 10 or 50 mg/L. Nutrient-amended microcosms contained 200 mg $(NH_4)_2HPO_4$ - NH_4^+ and 25 mg K_2HPO_4 - K^+ per liter. Each data point represents one active or control microcosm.

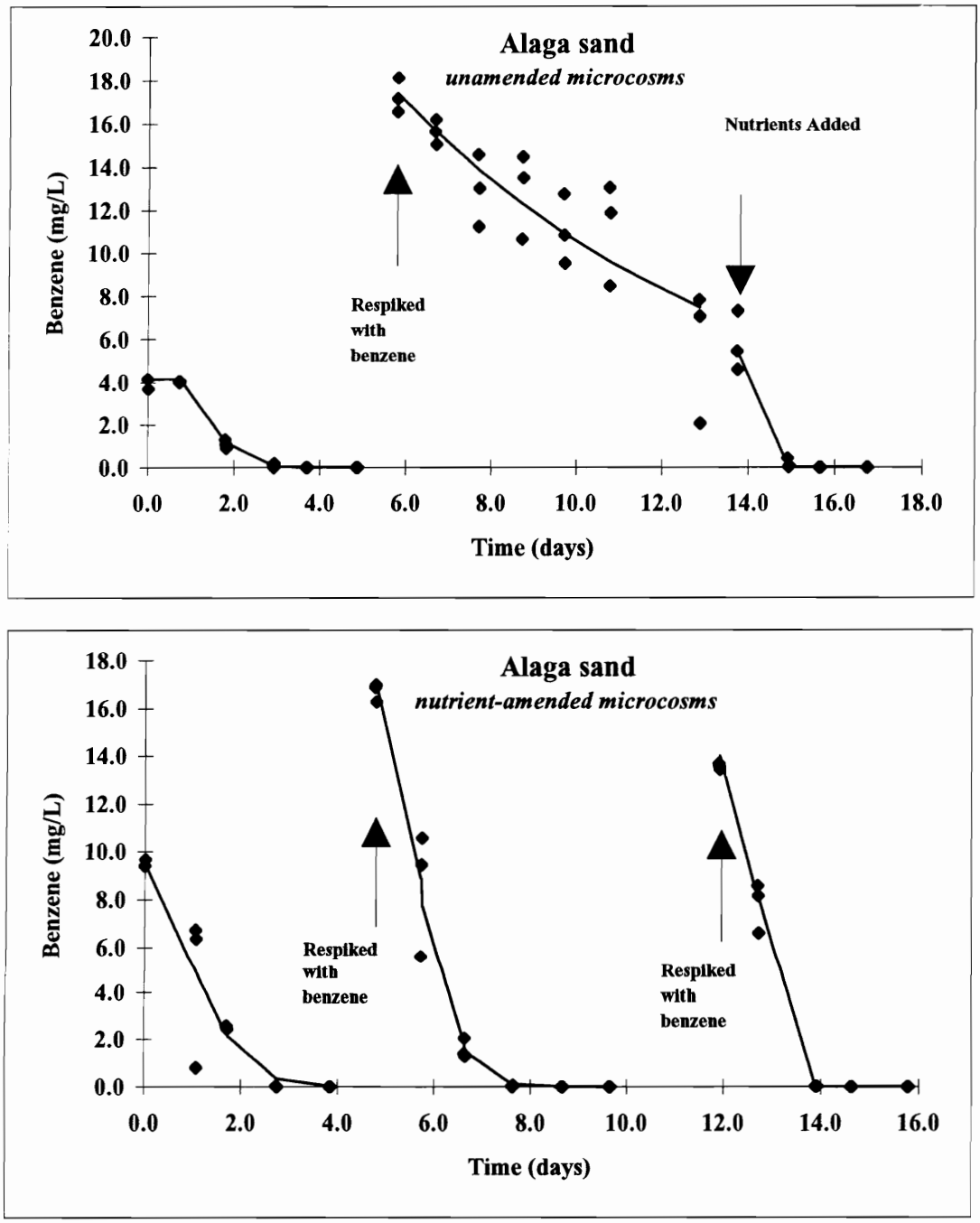


Figure IV. 2. Benzene disappearance versus time in unamended and nutrient-amended soil microcosms prepared with Alaga sand and respiked at a benzene concentration of 20 mg/L after the initial benzene disappearance was observed. Microcosms were prepared with 5 g soil and 5 g sterile, distilled water spiked with benzene, with and without added nutrients. Nutrient-amended microcosms contained 200 mg $(NH_4)_2HPO_4$ - NH_4^+ and 25 mg K_2HPO_4 - K^+ per liter. Each data point represents one active microcosm.

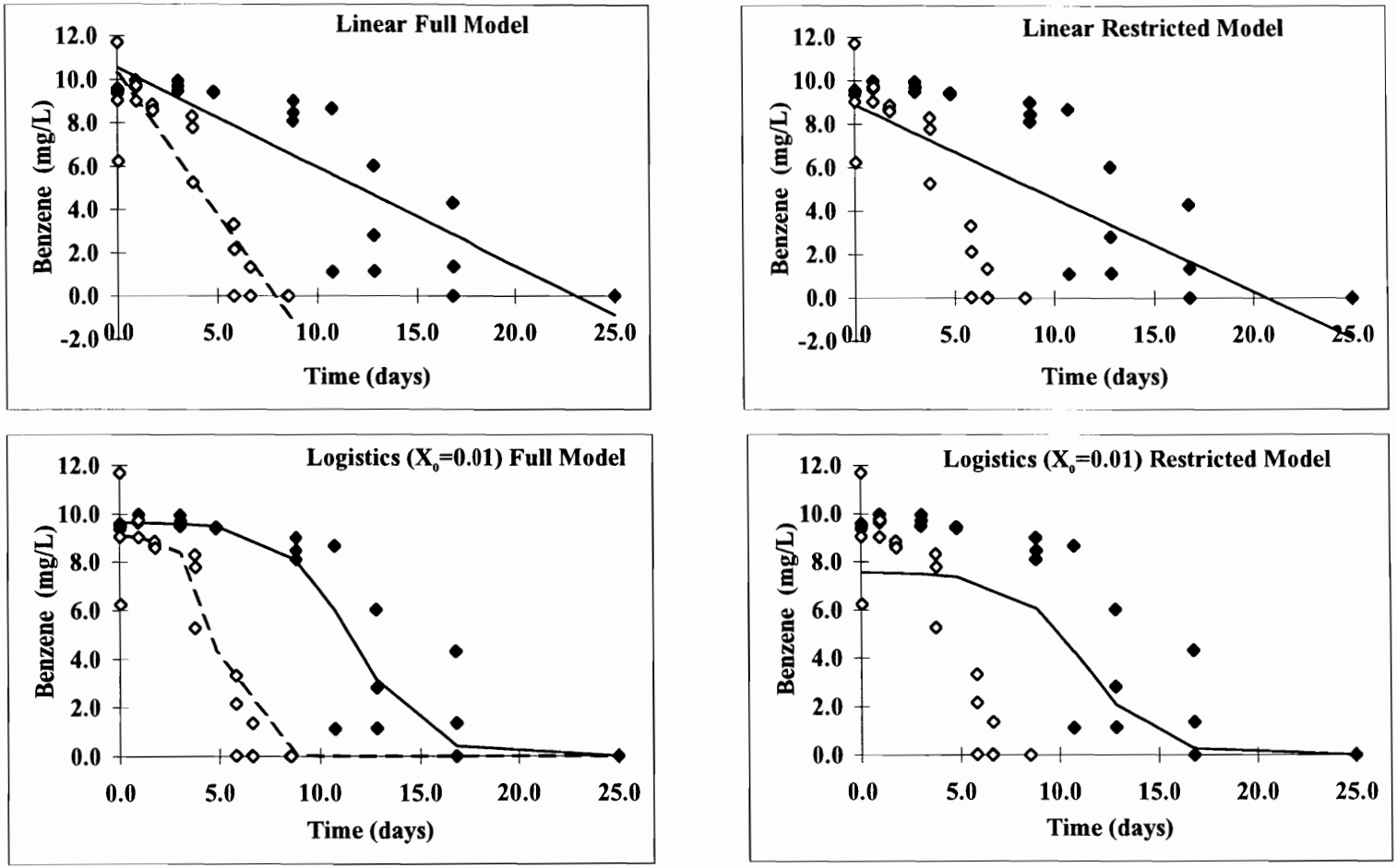


Figure IV. 3. Benzene disappearance versus time in unamended and nutrient-amended soil microcosms prepared with Altavista clay loam. These data show a significant difference in benzene disappearance between unamended (solid data points) and nutrient-amended (open data points) experiments. Microcosms were prepared with 5 g soil and 5 g sterile, distilled water spiked at an initial benzene concentration of 10 mg/L. Nutrient-amended microcosms contained 200 mg $(NH_4)_2HPO_4$-NH_4^+ and 25 mg K_2HPO_4-K^+ per liter. Each data point represents one active microcosm.

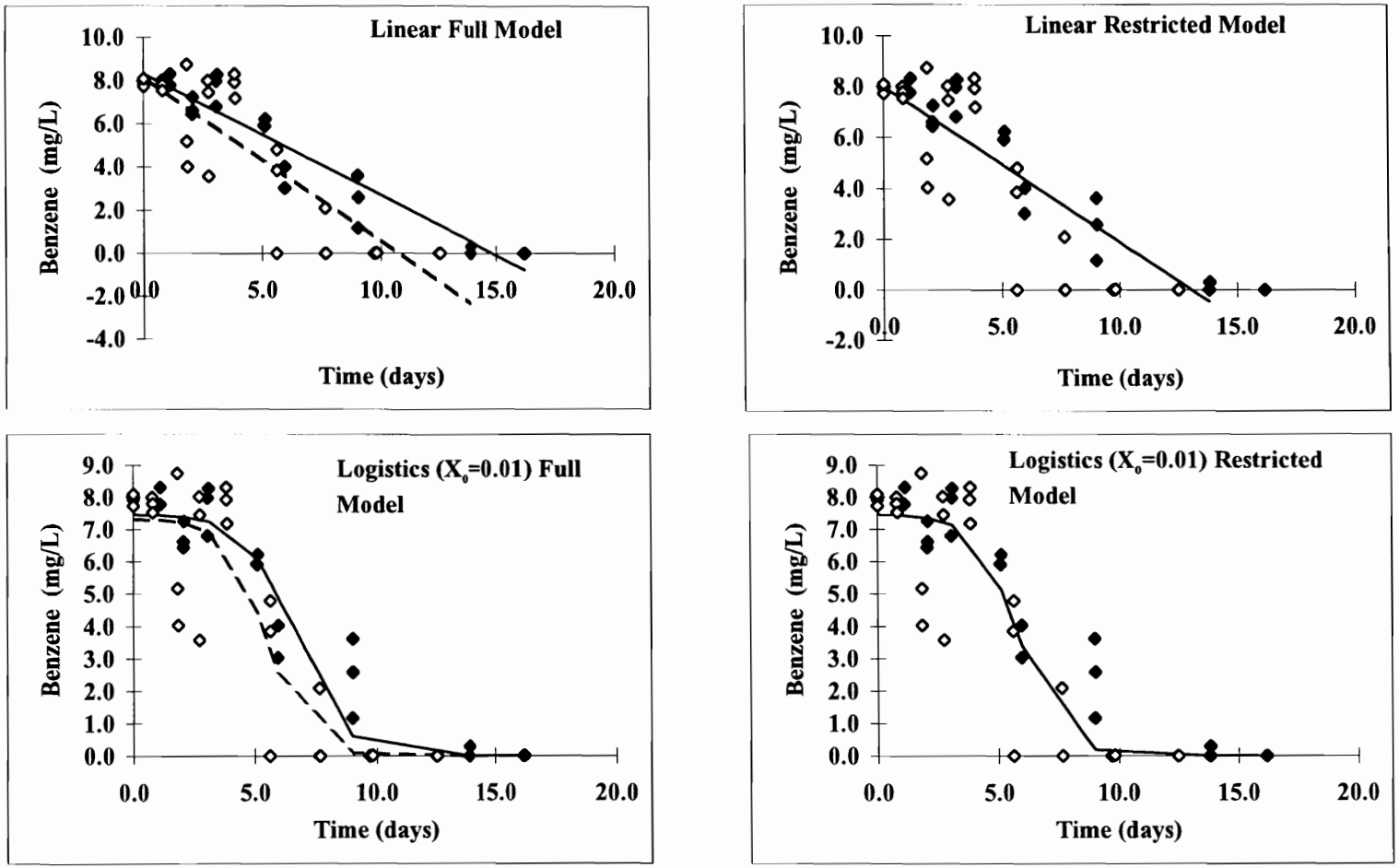


Figure IV. 4. Benzene disappearance versus time in unamended and nutrient-amended soil microcosms prepared with Braddock clay. Linear but not logistics regressions revealed a significant difference in benzene disappearance between unamended (solid data points) and nutrient-amended (open data points) experiments. Microcosms were prepared with 5 g soil and 5 g sterile, distilled water spiked at an initial benzene concentration of 10 mg/L. Nutrient-amended microcosms contained 200 mg $(NH_4)_2HPO_4$-NH_4^+ and 25 mg K_2HPO_4-K^+ per liter. Each data point represents one active microcosm.

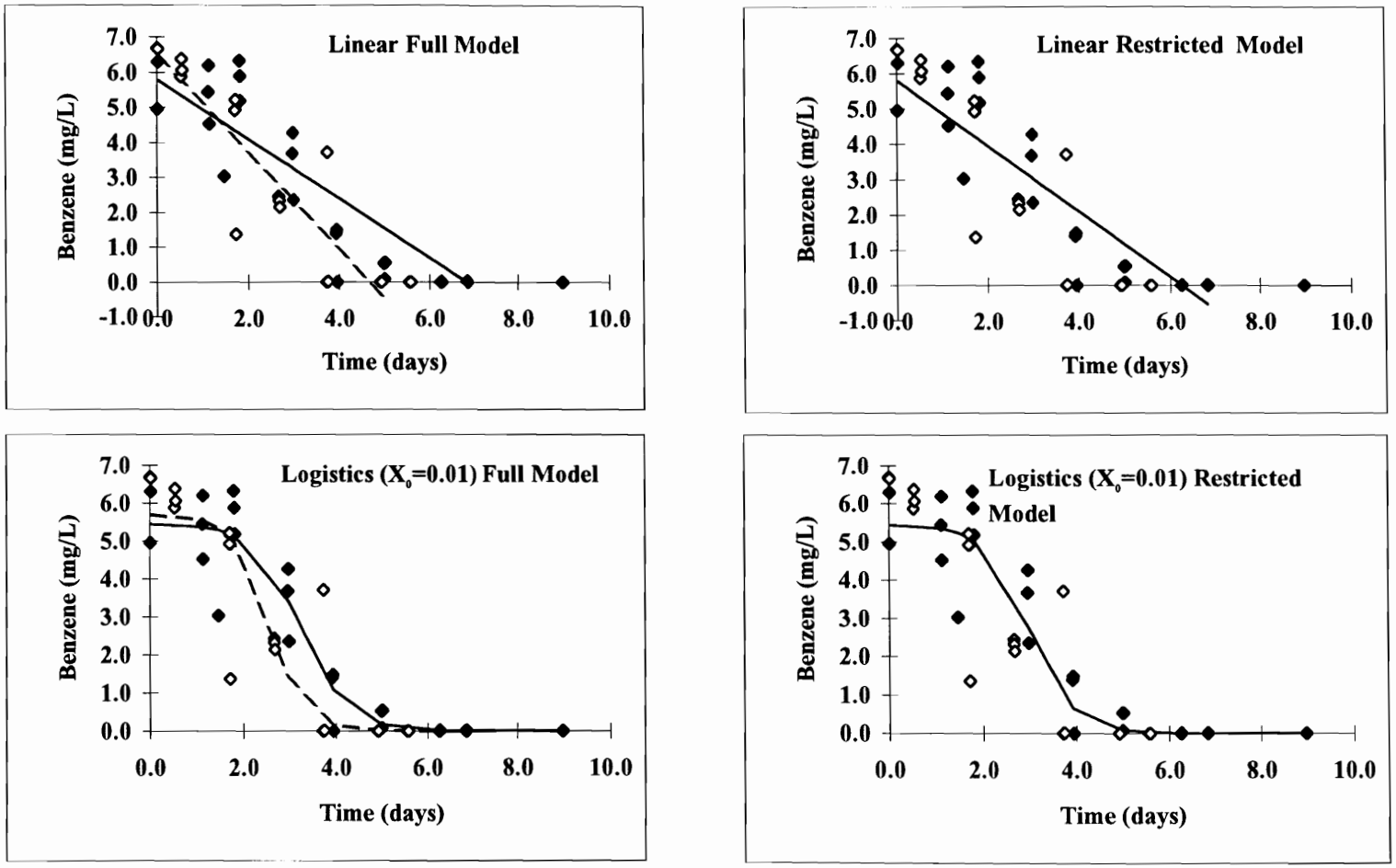


Figure IV. 5. Benzene disappearance versus time in unamended and nutrient-amended soil microcosms prepared with Ross silt. Due to the high data scatter, neither linear and logistics regressions revealed a significant difference in benzene disappearance between unamended (solid data points) and nutrient-amended (open data points) experiments. Microcosms were prepared with 5 g soil and 5 g sterile, distilled water spiked an initial benzene concentration of 10 mg/L. Nutrient-amended microcosms contained 200 mg $(NH_4)_2HPO_4$-NH_4^+ and 25 mg K_2HPO_4-K^+ per liter. Each data point represents one active microcosm.

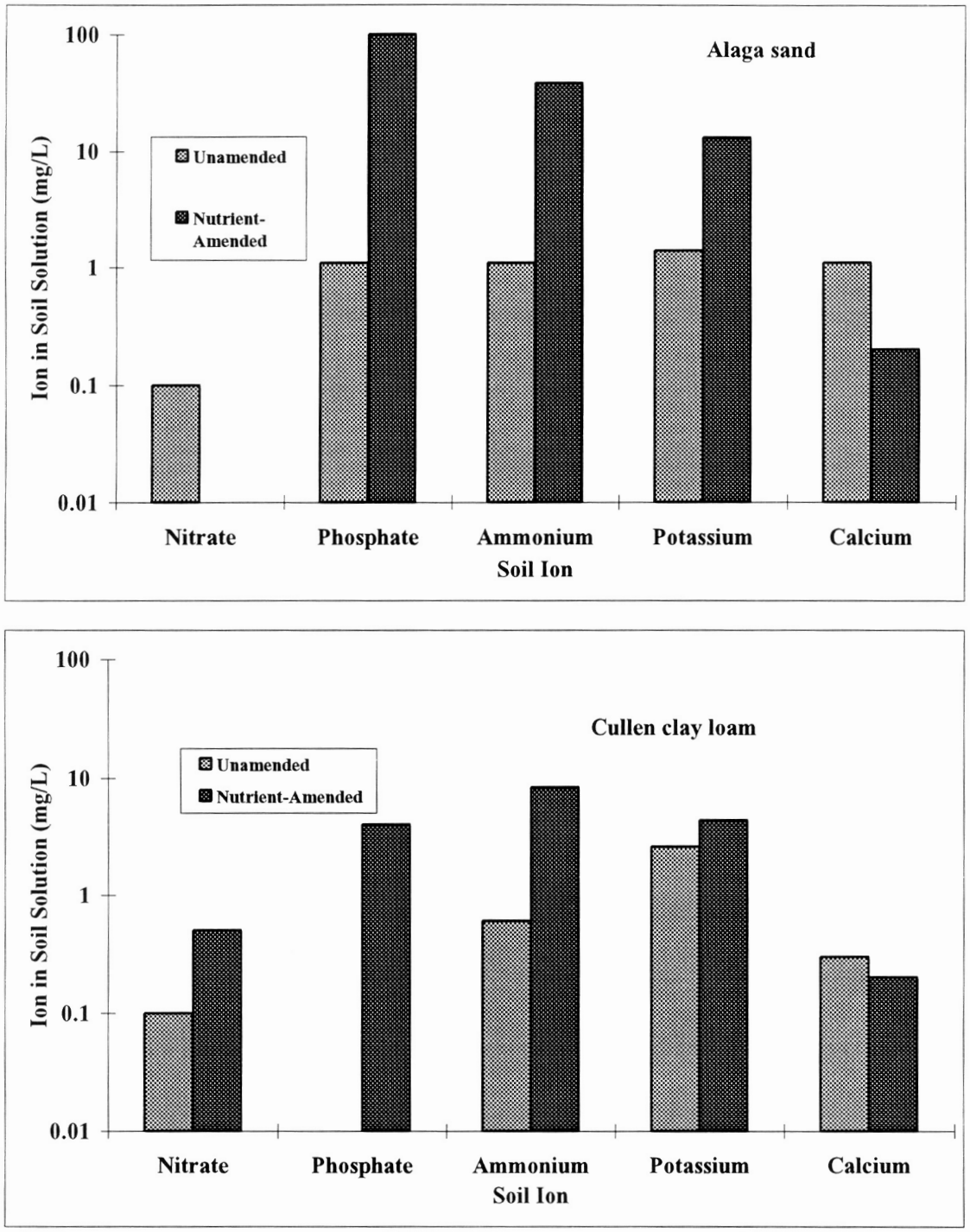


Figure IV. 6. Log plot of water-extractable ion concentrations in unamended and nutrient-amended soils after 6 hours of equilibration in a 1:1 (by weight) soil:water solution. Nutrient-amended soils contained 200 mg $(NH_4)_2HPO_4$ - NH_4^+ and 25 mg K_2HPO_4 - K^+ per liter.

V. Conclusions

The objectives of this research were to correlate benzene biodegradation with physical, chemical and biological properties of soil, to determine if biodegradation could be predicted based on measured or observed soil properties, and to investigate the role of nutrients on benzene biodegradation in soil. Benzene biodegradation observed in soil microcosms was described using an applicable kinetic model (Chapter II), and equations which predicted the model rate constant based on uncontaminated soil properties were developed using multiple linear regression (Chapter III). In anticipation of a potential role for soil nitrogen and phosphorus in the predictive equations, a comparison was also made of benzene biodegradation in unamended soil microcosms and in soil microcosms amended with ammonium and potassium phosphates (Chapter IV). The ability to predict benzene biodegradation rate and extent in the subsurface is key to preparing reasonable risk assessments, evaluating potential remediation strategies and establishing attainable clean-up criteria.

Benzene biodegradation in soil can be predicted from soil properties. Using non-linear regression analysis, a logistic kinetic model was fit to experimental data from batch microcosm studies, and rate constants obtained for 16 previously-uncontaminanted subsurface soils. The rate constants were related to soil properties using multiple linear regression (MLR). The soil factors which were observed to control benzene biodegradation depended upon initial benzene concentration. At an initial benzene concentration of 1 mg/L, benzene biodegradation was controlled by soil phosphorus availability. At an initial benzene concentration of 10 mg/L, benzene biodegradation was controlled by soil sand content, nutrient availability (cation exchange capacity), and nitrate-nitrogen, phosphorus and copper concentrations. Predictive equations are summarized in Equations 1, 2 and 3.

Additional work is needed to evaluate the utility of these models for remedial efforts in the field, to develop a model for contaminant mixtures (*e.g.*, BTEX), and to develop a model which is independent of initial concentration. A limited application of the predictive equations, however, may be possible.

These batch soil microcosm studies may mimic *in situ* natural or stimulated conditions of water-saturated soil with oxygenated, relatively slow-moving groundwater and aqueous benzene concentrations below 10 mg/L, as might be found at the boundaries of a hydrocarbon contaminate plume. The soils tested for model development were sampled from below forested and cropped lands, with heterotrophic plate counts of ca. 10^4, and are typical of the weathered subsurface soils found in the southeastern United States. Model use should be restricted to soils with properties within those listed in Appendix A. These predictive equations establish for the first time an explicit relationship between benzene biodegradation and soil properties. Moreover, the seemly endless list of important and interacting soil properties are reduced to a manageable few.

Simplified logistic equation which best described experimental data obtained at initial benzene concentrations of 1 mg/L and 10 mg/L:

$$S = S_0/[1 + X_0/S_0\exp(S_0 k t)] \quad (1)$$

where

S = aqueous benzene concentration, mg/L

S_0 = initial aqueous benzene concentration, mg/L

X_0 = 0.01 mg/L

t = time, days

k = rate constant, $(mg/L \cdot day)^{-1}$

For an initial aqueous benzene concentration of 0.6 to 1 mg/L:

$$k = \exp(0.9 + 0.04\ P - 0.1\ CEC),\ (mg/L \cdot day)^{-1} \quad (2)$$

For an initial benzene aqueous concentration of concentration of 6-10 mg/L:

$$k = \exp(-4 - 0.0009\ \%Sand^2 + 0.09\ \%Sand + 0.0007\ \%Sand \cdot P + 0.05\ NO_3\text{-}N - 0.2\ CEC + 0.4\ Cu),\ (mg/L \cdot day)^{-1} \quad (3)$$

where

% sand = weight % of sand in air-dried, sieved (No. 10 mesh) soil

CEC = cation exchange capacity, meq/100 g

Cu = Mehlich 1-extractable copper concentration, mg/kg

NO_3-N = 0.02 N $CuSO_4$-extractable nitrate-nitrogen, mg/kg

P = Mehlich 1-extractable soil phosphorus concentration, mg/kg

For initial benzene concentrations of 1 and 10 mg/L, benzene disappearance versus time data obtained from unamended soil microcosm studies were non-linear and S-shaped. The logistic or 3/2-order kinetic models fit the data better than zero-order (linear) or first-order (exponential) models at initial benzene concentrations of 1 mg/L and 10 mg/L, but offered little or no improvement over these models at initial benzene concentrations of 50 mg/L. All models examined showed a dependence of their respective rate constants on initial benzene concentration. The apparent substrate-dependence of biodegradation kinetics is important for interpretation of substrate disappearance data from contaminant spill monitoring. At the aerobic, BTEX plume boundary of a gasoline spill, for example, non-steady state conditions of contaminant mineralization like those modeled in batch studies may exist. For contaminant levels which stimulate bacterial growth without exceeding available oxygen or nutrients, an assumption of exponential decay may lead to a gross overestimate of degradation time. Within the core of an anaerobic contaminant plume at elevated contaminant concentrations, where oxygen or inorganic nutrients are most likely limiting biodegradation, zero-order or first-order kinetic models may better describe contaminant disappearance over time.

Added ammonium phosphate and potassium phosphate nutrients increased benzene disappearance rates in soil microcosms prepared at an initial benzene concentration of 10 mg/L, and increased both the rate and extent of benzene disappearance in soil microcosms prepared at an initial benzene concentration of 50 mg/L. At benzene concentrations of 10 mg/L and 50 mg/L, approximately 50% of the soils tested responded to nutrient addition, with low pH soils exhibiting the greatest response. These data indicate a need for nitrogen and phosphorus in subsurface surface soils under aerobic conditions at higher concentrations of soluble hydrocarbons, and likewise reflect the difficulties encountered when trying to meet microbial nutrient demands by circulating soluble salts through a highly reactive soil column.

APPENDIX A: Summary of Soil Properties

Table A. 1. Description of soils and sampling information.

Soil Series	Soil Classification	County in Virginia	Sample Depth (m)	Land Use
Alaga	Thermic, coated Typic Quartzimsammets	Surry	1.5	Forest
Altavista	Fine-loamy, mixed thermic, Aquic Hapludults	Culpeper	1.5	Forest
Braddock	Clayey, mixed, mesic Typic Hapludults	Montgomery	1.5	Agriculture
Cecil	Clayey, kaolinitic, thermic Typic Hapludults	Appomattox	4.0	Commercial
Cullen	Clayey, mixed, mesic, Typic Hapludulf	Appomattox	1.0	Commercial
Groseclose	Fine, mixed, mesic Typic Hapludults	Montgomery	3.0	Agriculture
McGary	Fine, mixed, mesic Aeric Ochraqualfs	Montgomery	1.5	Agriculture
Riverview	Fine-loamy, mixed, thermic Fluventic Dystrochrepts	Appomattox	1.0	Agriculture
Roanoke	Clayey, mixed, thermic Typic Ochraquults	Culpeper	1.5	Agriculture
Ross	Fine-loamy, mixed, mesic Cumulic Hapludolls	Montgomery	1.5	Forest
Weikert	Loamy-skeletal, mixed, mesic Lithic Dystrochrepts	Montgomery	0.5	Forest
Wheeling	Fine-loamy, mixed, mesic Typic Hapludults	Montgomery	3.0	Agriculture

Table A. 2. Physical description of soils.

Soil Series	Texture	Particle Size			Predominant Color[a]		Bulk Density
		% Sand	% Silt	% Clay			(g/cc)
Alaga	Sand	96.4	3.3	0.3	10YR 6/6	brownish yellow	1.32
Altavista	Clay loam	43.6	27.4	29.0	7.5YR 5/6	strong brown	1.08
Braddock	Clay	21.3	34.8	43.9	5.0YR 5/8	yellowish red	0.98
Cecil	Loamy sand	72.2	26.1	1.7	5.0YR 4/4	reddish brown	0.85
Cullen	Clay loam	37.1	25.4	37.4	2.5YR 4/6	red	0.88
Groseclose	Loam	37.9	38.4	23.7	10YR 5/3	brown	1.03
McGary	Clay loam	25.7	38.8	35.5	10YR 5/6	yellowish brown	1.08
Riverview	Sandy loam	72.0	18.6	9.4	10YR 5/4	yellowish brown	1.13
Roanoke	Clay loam	20.1	50.2	29.7	2.5YR 6/3	light reddish brown	1.03
Ross	Silt	19.8	69.7	10.5	7.5YR 3/2	dark brown	0.73
Weikert	Loamy sand	74.1	24.8	1.1	7.5YR 5/6	strong brown	1.14
Wheeling	Sandy loam	60.8	26.7	12.5	7.5YR 4/6	strong brown	1.00

[a]Based on the Munsell color chart.

Table A. 3. Soil chemical properties. Units as shown ± standard deviation of duplicate samples.

Soil Series	pH	CEC[a] (meq/100 g)	COD[b] (mg O_2/g dry soil)	TKN-N[c] (mg TKN-N/kg dry soil)	SS[d] (mg/L)
Alaga	4.5±0.1	0.17±0.07	2.0±0.2	200±80	26
Altavista	4.6±0.1	11.8±0.6	3.1±0.5	1000±100	51
Braddock	4.9±0.1	3.1±0.1	4.1±0.06	300±100	90
Cecil	4.5±0.1	3.1±0.3	0.9±0.2	100±70	26
Cullen	5.0±0.1	3.7±0.2	4.3±0.1	100±100	26
Groseclose	7.6±0.1	10.2±0.002	7.0±0.4	500±100	141
McGary	6.6±0.1	7.3±0.8	4.1±0.04	200±80	38
Riverview	6.0±0.1	2.8±0.3	4.9±0.07	300±30	38
Roanoke	7.7±0.1	14.0±0.8	2.5±0.7	200±70	653
Ross	4.9±0.1	5.3±0.2	90±60	2200±200	115
Welkert	5.0±0.1	0.6±0.2	7.6±0.9	300±70	38
Wheeling	6.0±0.1	6.5±0.5	2.5±0.4	200±40	51

[a]Cation exchange capacity (barium chloride method)
[b]Chemical oxygen demand
[c]Total Kjeldahl nitrogen
[d]Suspended solids (based on soil solution conductivity)

Table A. 4. Soil cation concentrations (mg/kg) ± standard deviation of duplicate samples (mg/kg). Cations were extracted with 0.05 N HCl and 0.025 N H_2SO_4 and extractants analyzed by an inductively-coupled plasma spectrophotometer.

Soil Series	Al	Ca	Cu	Fe	K	Mg	Mn	P	Zn
Alaga	110±2	29±9	0.11±0.01	12±1	2±1	6±1	0.6±0.2	27±0.3	0.4±0.01
Altavista	259±1	500±10	3.1±0.05	11±0.1	98±1	283±2	1.0±0.1	0	1.1±0.03
Braddock	188±1	371±1	0.9±0.2	24±9	440±30	151±5	2±2	0.75±0.04	0.67±0.02
Cecil	96±3	80±15	1.0±0.3	12.4±0.3	64±2	34±0.7	11±0.9	2.3±0.7	0.7±0.2
Cullen	219±3	110±40	0.94±0.05	33.6±0.7	109±7	110±10	1.5±0.5	1.1±0.1	0.56±0.01
Groseclose	50±10	4650±60	0.26±0.08	1.8±0.7	41±1	1130±10	22±1	24±7	0.22±0.06
McGary	70±9	950±50	1.1±0.3	10±1	20±3	213±7	16±4	2.58±0.04	1.0±0.4
Riverview	79±2	410±20	1.30±0.06	38±2	7±1	165±6	8.2±0.2	1.4±0.2	0.71±0
Roanoke	44±3	1300±10	4.8±0.1	6.3±0.1	20±1	542±6	1.8±0	0.73±0.02	1.33±0.02
Ross	430±10	850±40	5.62±0.01	740±50	59±4	111±4	170±10	9±1	4.32±0.05
Welkert	157±1	130±20	0.8±0.2	16.5±0.6	18±1	31±4	16±3	0.75±0.03	0.8±0.3
Wheeling	153±1	810±40	1.3±0.2	33.2±0.2	16±2	208±6	3.3±0.4	5±2	1.09±0.01

Table A. 5. Water and organic matter content, weight % ± standard deviation of 5 replicates.

Soil Series	% Water (Air-Dried, Seived Soil)	% Water (Field Moisture)	% Organic Matter
Alaga	0.10±0.04	4.5±0.1	0.30±0.03
Altavista	10.9±0.7	14±2	5.9±0.2
Braddock	2.20±0.08	32.0±0.6	5.49±0.05
Cecil	0.56±0.04	25±1	5.37±0.09
Cullen	1.49±0.06	25±1	7.6±0.1
Groseclose	1.44±0.08	27.5±0.7	3.0±0.1
McGary	10.8±0.2	16±1	4.53±0.06
Riverview	0.44±0.02	31±2	1.97±0.02
Roanoke	6.4±0.5	19±1	3.23±0.06
Ross	1.4±0.1	66±1	8.9±0.3
Weikert	0.45±0.01	4.6±0.1	1.38±0.03
Wheeling	0.9±0.4	17.9±0.2	3.0±0.1

Table A. 6. Soil nitrate-N concentrations (mg/kg) ± standard deviation of duplicate samples (mg/kg).

Soil Series	Nitrate-N (mg/kg)	
	Water-extracted[a]	0.02 N $CuSO_4$-extracted[b]
Alaga	0.1±0.02	3±0.3
Altavista	1.1±0.02	3±0.3
Braddock	9.1±0.62	15±2
Cecil	0.6±0.06	3±0.3
Cullen	0.1±0.05	5±0.5
Groseclose	9.6±0.09	43±4
McGary	0.3±0.01	5±0.5
Riverview	0.4±0.04	5±0.5
Roanoke	0.2±0.01	13±1
Ross	0.8±0.33	3±0.3
Weikert	0.1±0	3±0.3
Wheeling	1.6±0.3	5±0.5

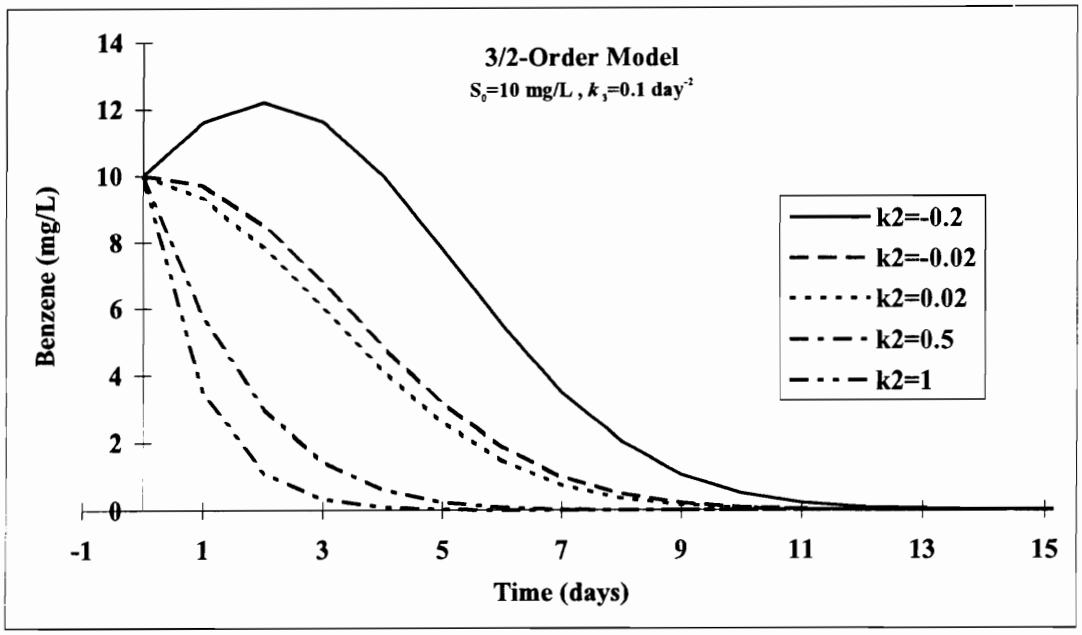
[a]Analyzed with a Dionex ion chromatograph
[b]Analyzed with an Ionanalyzer ion specific electrode

Table A. 7. Biological properties of soil. Plate counts are shown ± standard deviation of duplicate samples. Benzene-degrader enumeration is shown with the upper and lower 95% confidence interval in parentheses.

Soil Series	HPC[a] (cfu/g dry soil)	Fungi & Bacteria[b] (cfu/g dry soil)	Fungi[c] (cfu/g dry soil)	Benzene-Degraders[d] (MPN/g dry soil)
Alaga	18,000±7,000	340,000±50,000	1,000±300	0
Altavista	17,000±4,000	14,000±700	500±500	200 (80-500)
Braddock	34,000±3,000	170,000±80,000	2,000±90	50,000 (20,000-130,000)
Cecil	6,000±800	20,000±1,000	900±300	1,000 (500-3,000)
Cullen	12,000±4,000	23,000±2,000	1,000±300	9,000 (4,000-20,000)
Groseclose	25,000±700	25,000±700	260±0	0
McGary	23,000±8,000	50,000±20,000	700±400	400,000 (100,000-900,000)
Riverview	31,000±7,000	50,000±30,000	790±0	30,000 (10,000-70,000)
Roanoke	30,000±10,000	13,000±800	0	0
Ross	50,000±20,000	60,000±30,000	0	8,000 (3000-20,000)
Weikert	78,000±5,000	260,000±70,000	8,000±1,000	800 (300-2,000)
Wheeling	90,000±20,000	220,000±30,000	2,000±1,000	20,000 (8,000-50,000)

[a]Heterotrophic bacterial plate counts on basal salts with Bactopeptone agar.
[b]Fungal and bacterial plate counts on sucrose agar.
[c]Fungal plate counts on dextrose with rose bengal agar.
[d]Bacterial enumeration by Most Probable Number (MPN). Bacteria grown in liquid culture with basal salt solution and benzene added as the sole carbon source.

APPENDIX B: 3/2-Order and Logistics Kinetic Models



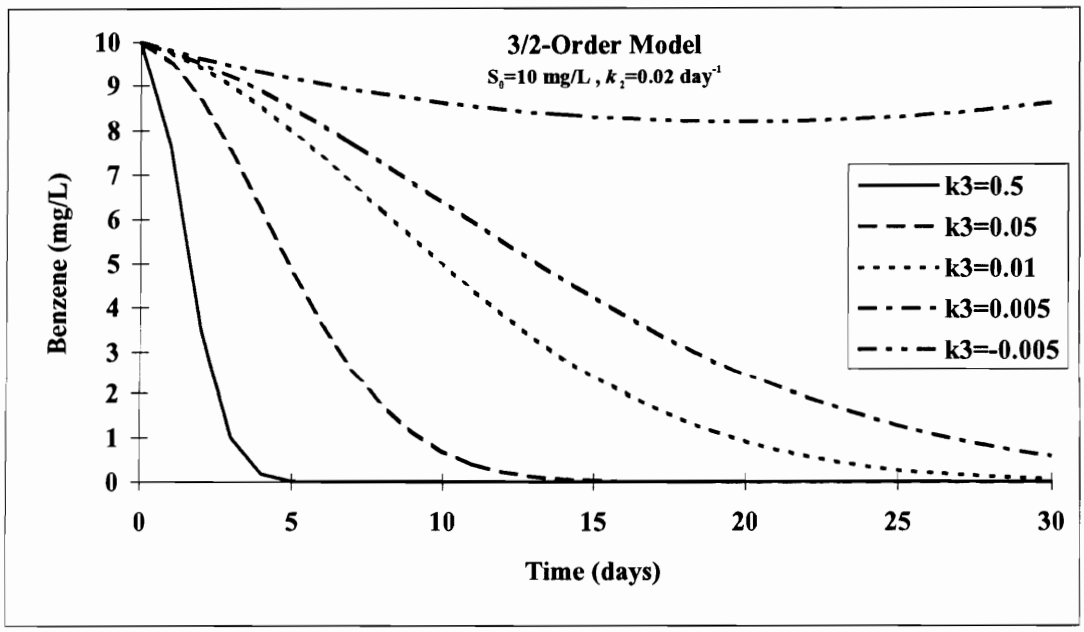
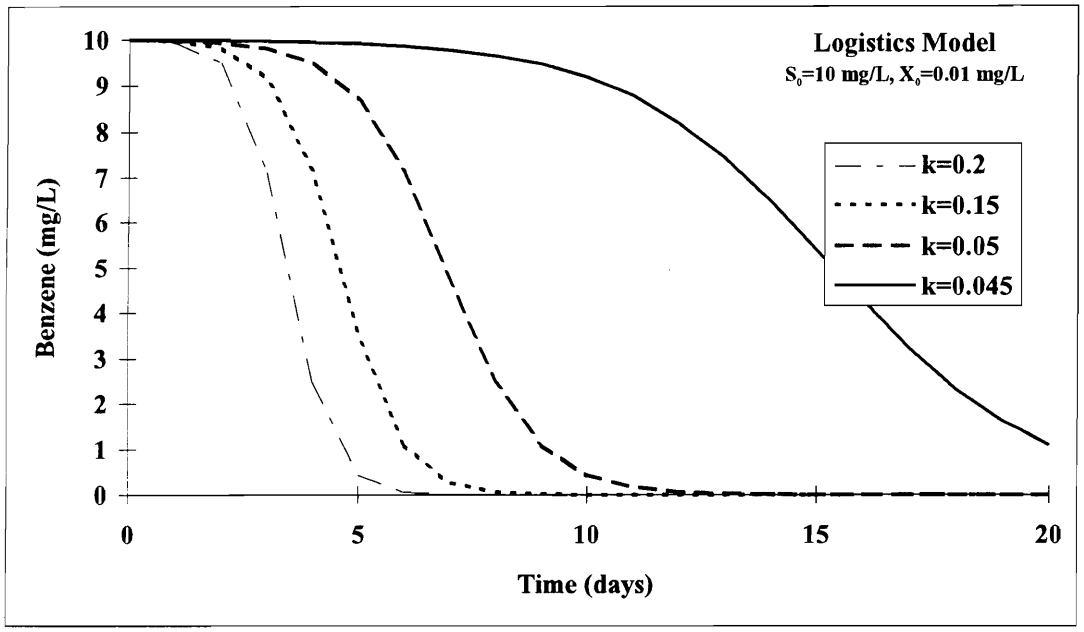


Figure B. 1. Plots of the 3/2-order model for varying k_2 and k_3.



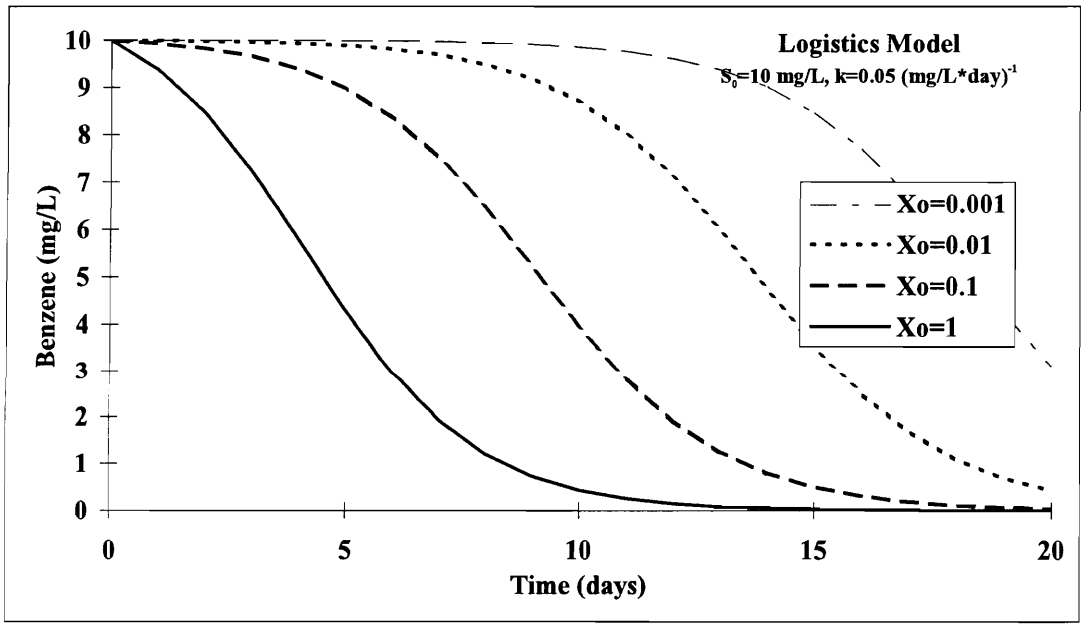


Figure B. 2. Plots of logistic kinetics model for varying X_0 and *k*.

APPENDIX C: SAS® NLIN Example Programs and Printouts

```
options linesize=84 pagesize=48 date number pageno=1;
title 'wheeling, 50 ppm, nutrient-amended, exponential model';
proc nlin data=sasuser.whee50n;
parms k0=50 k1=0.02;
model benzene=k0*exp(-k1*time);
der.k0=exp(-k1*time);
der.k1=-time*k0*exp(-k1*time);
run;
quit;
```

Figure C. 1. SAS® NLIN program for the first-order (exponential) kinetic model.

```
options linesize=85 pagesize=48 date number pageno=1;
title 'Wheeling, 10 ppm, unamended, 3/2-order model';
proc nlin data=sasuser.wheel0;
parms k0=10 k1=-0.2 k2=0.1;
model benzene=k0*exp((-k1*time)-(0.5*k2*(time**2)));
der.k0=exp((-k1*time)-(0.5*k2*(time**2)));
der.k1=-k0*time*exp((-k1*time)-(0.5*k2*(time**2)));
der.k2=-0.5*k0*(time**2)*exp((-k1*time)-(0.5*k2*(time**2)));
run;
quit;
```

Figure C. 2. SAS® NLIN program for the 3/2-order kinetic model.

```
options linesize=84 pagesize=48 date number pageno=1;
title 'Wheeling, 10 ppm, unamended, logistics model';
proc nlin data=sasuser.wheel0;
parms k0=10 k1=0.015 k2=0.009;
id alpha beta;
alpha=k0+k2;
beta=exp(k1*alpha*time);
model benzene=alpha/(1+((k2/k0)*beta));
der.k0=(1+((k2/k0)*beta)-
(k2*beta*alpha*(k1*time-(1/k0)))/k0)/(1+(k2/k0)*beta)**2;
der.k1=-(k2*time*(alpha**2)*beta)/(k0*((1+(k2/k0)*beta)**2));
der.k2=(1+((k2/k0)*beta)-(beta*alpha*(k2*k1*time+1))/k0)/(1+(k2/k0)*beta)**2;
run;
quit;
```

Figure C. 3. SAS® NLIN program for the logistic kinetic model.

```
options linesize=84 pagesize=48 date number pageno=1;
title 'wheeling, 50 ppm, nutrient-amended, logistics model';
proc nlin data=sasuser.whee50n;
parms k0=50 k1=0.002;
id alpha beta k2;
k2=0.01;
alpha=k0+k2;
beta=exp(k1*alpha*time);
model benzene=alpha/(1+((k2/k0)*beta));
der.k0=(1+((k2/k0)*beta)
-(k2*beta*alpha*(k1*time-(1/k0)))/k0)/(1+(k2/k0)*beta)**2;
der.k1=-(k2*time*(alpha**2)*beta)/(k0*((1+(k2/k0)*beta)**2));
der.k2=(1+((k2/k0)*beta)-(beta*alpha*(k2*k1*time+1))/k0)/(1+(k2/k0)*beta)**2;
run;
quit;
```

Figure C. 4. SAS® NLIN program for the logistic (X_0 = 0.01) kinetic model.

Wheeling, 10 ppm, unamended, 3/2-order model
17:54 Tuesday, January 16,

Non-Linear Least Squares Iterative Phase
Dependent Variable BENZENE Method: Gauss-Newton

Iter	K0	K1	K2	Sum of Squares
0	10.000000	-0.200000	0.100000	177.618902
1	7.679745	-0.191860	0.109238	13.036576
2	7.669188	-0.193773	0.115738	11.984644
3	7.676654	-0.192748	0.115530	11.983819
4	7.676927	-0.192722	0.115524	11.983818
5	7.676933	-0.192721	0.115524	11.983818

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics Dependent Variable BENZENE

Source	DF	Sum of Squares	Mean Square
Regression	3	988.8802816	329.6267605
Residual	23	11.9838184	0.5210356
Uncorrected Total	26	1000.8641000	
(Corrected Total)	25	345.9548654	

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval Lower	Upper
K0	7.676933155	0.39810374194	6.8533994442	8.5004668657
K1	-0.192721212	0.04074195313	-0.2770016849	-0.1084407381
K2	0.115524136	0.01424672392	0.0860527791	0.1449954927

Asymptotic Correlation Matrix

Corr	K0	K1	K2
K0	1	0.8285934053	-0.625884753
K1	0.8285934053	1	-0.918724808
K2	-0.625884753	-0.918724808	1

Figure C. 5. Example of SAS® NLIN output for the 3/2-order kinetic model.

Wheeling, 10 ppm, unamended, logistics model
17:54 Tuesday, January 16,

Non-Linear Least Squares Iterative Phase
Dependent Variable BENZENE Method: Gauss-Newton

Iter	K0	K1	K2	Sum of Squares
34	8.846022	0.070406	0.164904	38.551453
35	8.777270	0.093944	0.066046	18.938715
36	8.680355	0.107035	0.036203	16.087015
37	8.559068	0.126870	0.014768	13.373789
38	8.521567	0.135274	0.013869	10.553271
39	8.503842	0.139153	0.011410	10.521861
40	8.484429	0.143853	0.009144	10.497535
41	8.481012	0.144780	0.008954	10.487524
42	8.479866	0.145016	0.008854	10.487482
43	8.479697	0.145055	0.008838	10.487481
44	8.479667	0.145062	0.008835	10.487481

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics Dependent Variable BENZENE

Source	DF	Sum of Squares	Mean Square
Regression	3	990.3766189	330.1255396
Residual	23	10.4874811	0.4559774
Uncorrected Total	26	1000.8641000	
(Corrected Total)	25	345.9548654	

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval Lower	Upper
K0	8.479667140	0.23156973357	8.0006325019	8.9587017782
K1	0.145061624	0.02682888186	0.0895623000	0.2005609484
K2	0.008835112	0.01139583840	-0.0147387860	0.0324090104

Asymptotic Correlation Matrix

Corr	K0	K1	K2
K0	1	-0.630425458	0.5830463748
K1	-0.630425458	1	-0.989979137
K2	0.5830463748	-0.989979137	1

Figure C. 6. Example of SAS® NLIN output for the logistic kinetic model.

APPENDIX D: Summary of Control Microcosm Data

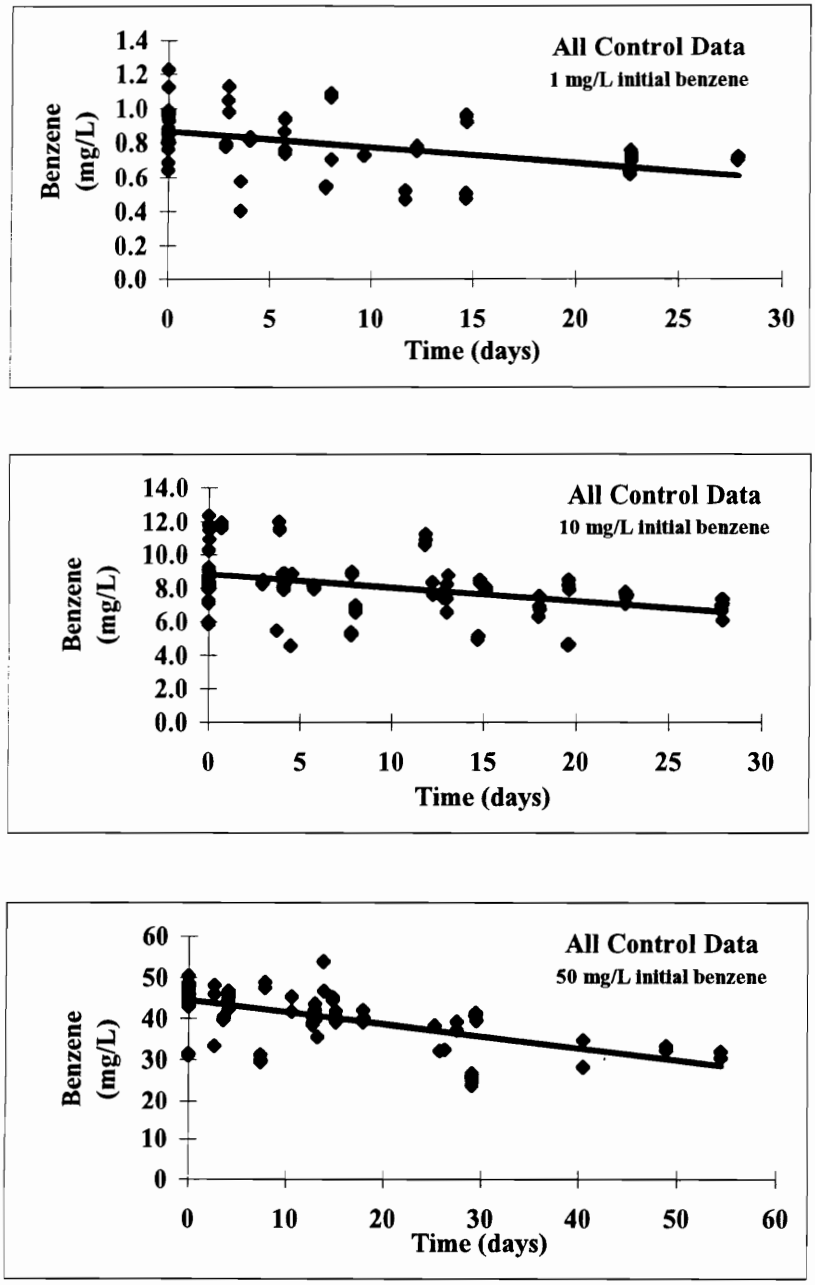


Figure D. 1. Benzene disappearance versus time in control soil microcosms. Microcosms were prepared with 5 g sterile soil and 5 g sterile, distilled water spiked at an initial benzene concentration of 1, 10 or 50 mg/L amended 200 mg $(NH_4)_2HPO_4$ - NH_4^+ and 25 mg K_2HPO_4 - K^+ per liter. Each data point represents one control microcosm. Rate constants (k_1) were 0.009, 0.08 and 0.3 mg/L/day for initial benzene concentrations of 1, 10, and 50 mg/L, respectively. Abiotic rate constants for individual soils ranged from 0.002 to 0.03 mg/L/day, from 0.02 to 0.1 mg/L/day, and 0.2 to 0.4 mg/L/day for initial benzene concentrations of 1, 10 and 50 mg/L, respectively.

Vita

Noreen Dell Poor was born on September 30, 1955, to Victor and Florence Poor. She is a graduate of Winston Churchill High School in San Antonio, Texas. In 1974, she joined the United States Navy to see the world, and served honorably as an Aviation Electronics Technician. While in the Navy, Noreen completed a Bachelor of Arts degree from the University of Maryland University College in August, 1980. She married Montie H. Foster in November, 1980. After finishing her Navy tour, Noreen enrolled at the University of Texas at Austin where she earned a Master of Science in Engineering degree in August, 1985. Her first engineering job was as a mechanical engineer in the Fluids Branch, PRC, Inc., Kennedy Space Center, Florida, from November, 1985 to November, 1986. During this time, she joined the United States Naval Reserves (USNR) as an Engineering Duty Officer. Her participation in the Naval Reserves opened new job opportunities, and she accepted a position as a mechanical engineer in the Seawater System Branch and later the Steam System Branch of the Submarine Monitoring, Maintenance and Support Office, Naval Sea Systems Command, Arlington, Virginia, where she worked from November, 1986, to August, 1992. Noreen is a registered Professional Engineer in the Commonwealth of Virginia. After attending the Virginia Polytechnic Institute and State University in the beautiful mountains of western Virginia, she earned a Doctor of Philosophy degree in Civil Engineering in January, 1996. She specializes in the biodegradation of organic contaminants in the subsurface.

Noreen D. Poor