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Modeling Transport and Biodegradation of Benzene and Toluene in Sandy Aquifer Material: Comparisons With Experimental Measurements

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A one-dimensional numerical model is developed for simulating the biodegradation and transport of benzene and toluene in the subsurface environment. Modeled processes include mass exchange between the constituent phases (solid, liquid, gas, and biomass), advective and dispersive transport, and biotransformation, as well as microbial biomass production. Two substrates, two electron acceptors, one trace nutrient, and two microbial populations are modeled. Resulting governing equations include five nonlinear partial differential equations describing component transport in the bulk pore fluids, five nonlinear algebraic equations governing interphase mass exchange, and two ordinary differential equations governing microbial growth. These equations are solved through application of a Galerkin finite element method and a set iterative solution scheme. The utility and validity of the modeling approach is explored through comparisons with laboratory column experiments. Model parameters were estimated independently through laboratory batch experiments, aquifer slurry studies, or from the literature. Simulations are found to provide reasonable agreement with measurements of benzene and toluene biodegradation in saturated continuous-flow columns packed with aquifer material. Sensitivity analyses and comparisons with column data suggest that model predictions are highly dependent upon the microbial parameters, particularly the initial active biomass concentration, the maximum specific substrate utilization rate, and the half-saturation coefficient. The importance of the accurate estimation of these microbial parameters is emphasized.

INTRODUCTION

The widespread release of petroleum hydrocarbons in the environment has led to subsurface contamination by the toxic and water soluble components such as benzene, toluene, and xylenes (BTX) [*Lee et al.*, 1988]. These compounds are highly mobile in subsurface systems, and their occurrence in aquifer drinking water supplies has motivated research into methods for their removal. Several physical, chemical, and biological methods for subsurface remediation have been developed, many of which are expensive or marginally effective [*Environmental Engineering Research Council*, 1990]. However, in situ bioremediation, which involves the use of indigenous microbes for contaminant biodegradation within the aquifer, is potentially less expensive and more effective. The existence of microbes capable of degrading BTX compounds has been demonstrated in laboratory experiments under aerobic [*Alvarez and Vogel*, 1991; *Barker et al.*, 1987; *Lee et al.*, 1988], denitrifying [*Kuhn et al.*, 1985, 1988; *Major et al.*, 1988; *Zeyer et al.*, 1986, 1990], dissimilatory iron-reducing [*Lovely and Lonergan*, 1990], sulfate-reducing [*Edwards et al.*, 1991], and methanogenic [*Grbić-Galić and Vogel*, 1987; *Vogel and Grbić-Galić*, 1986; *Wilson et al.*, 1986] conditions. These studies support the notion that indigenous subsurface microbes may be capable of BTX degradation. Indeed, in situ bioremediation has been successfully applied to the "clean up" of BTX-contaminated aquifers [*Battermann and Werner*, 1984; *Chiang et al.*, 1989; *Hutchins et al.*, 1989; *Lee et al.*, 1988; *Thomas et al.*, 1990; *Verheul et al.*, 1988; *Ward et al.*, 1989]. The major difficulties in implementation of in situ bioremediation of BTX-contaminated aquifers appear to be related to the delivery of appropriate electron acceptors (e.g., oxygen), bioavailability of substrate (e.g., hydrocarbon), and presence and expression of appropriate microbial catabolic capabilities. Unfortunately, documented successful remediations have not provided sufficient information to assess the degree to which each process and parameter influence the performance of bioremediation.

Since relatively little is known about the degree of influence of each process, a conceptually correct predictive model which incorporates these processes and has been verified independently could prove invaluable. The present work is offered as one step toward the attainment of this goal. Mathematical modeling of in situ bioremediation is potentially useful in the assessment of the transport and fate of contaminants, in the optimization and design of cleanup operations, and in the estimation of the duration of such restoration operations. Development of a reasonable model requires quantification of the abiotic processes controlling the transport and availability of the pollutants and nutrients, as well as an understanding of the kinetics of biotransformation and microbial growth and the ecology of active microorganisms. In the past few years a number of mathematical models have been developed that incorporate microbial growth, the transport of a biodegradable pollutant, and the biological uptake of inorganic nutrients in groundwater systems [e.g., *Borden and Bedient*, 1986; *Frind et al.*, 1990; *Kinzelbach et al.*, 1991; *MacQuarrie et al.*, 1990; *Molz et al.*, 1986; *Semprini and McCarty*, 1989; *Sykes et al.*, 1982; *Widdowson et al.*, 1988]. Some of these developed models have not yet been employed in comparisons with independent laboratory or field measurements [e.g., *Molz et al.*, 1986; *Widdowson et al.*, 1988; *Kindred and Celia*, 1989]. In other cases, involving model application to contaminated

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field sites, microbial kinetic parameters have generally been obtained by model fitting [e.g., *MacQuarrie et al.*, 1990; *Semprini and McCarty*, 1989] or literature estimation [e.g., *Kinzelbach et al.*, 1991; *Frind et al.*, 1990]. A major contribution of the work presented herein is that model formulation, parameter estimation, and model predictions are independent of the measurements used to evaluate model applicability.

The first part of this paper describes the development and mathematical verification of a one-dimensional numerical simulator which models the subsurface transport and biodegradation of benzene and toluene. These two compounds are assumed to act as primary substrates and are treated separately in the model because of their different human toxicity, sorption [*Zuane*, 1990], and biodegradability characteristics. The developed model is based upon a microcolony microbial growth concept [*Molz et al.*, 1986; *Widdowson et al.*, 1988] and incorporates component gas phase diffusion and liquid-gas mass exchange. Two electron acceptors, oxygen and nitrate, are considered. The second part of the paper briefly describes the methods employed to estimate model parameters. Model validation is sought through comparisons of model predictions with laboratory data obtained from saturated, continuous-flow, column experiments. Further insight into system behavior is gained in the final section which explores model prediction sensitivity to parameters for the experiments under investigation.

Model Development

Component Mass Balance Equations in the Bulk Fluid Phases

A general macroscopic equation governing the transport of a component i in phase α in a porous medium may be written as [*Abriola*, 1989]:

$$\frac{\partial}{\partial t}(\rho^\alpha \varepsilon_\alpha \omega_i^\alpha) + \nabla \cdot (\rho^\alpha \varepsilon_\alpha v^\alpha \omega_i^\alpha) - \nabla \cdot (\rho^\alpha \varepsilon_\alpha D_i^\alpha \cdot \nabla \omega_i^\alpha) - E_i^\alpha = G_i^\alpha \qquad (1)$$

where

ρ^α mass density of phase α;
ε_α volume fraction of phase α;
ω_i^α mass fraction of component i in phase α;
v^α mass average velocity of phase α;
D_i^α hydrodynamic dispersion coefficient with respect to phase α;
G_i^α internal source/sink of component i in phase α;
E_i^α external mass supply of component i to the α phase due to interphase exchange.

The first term in (1) is the accumulation of component i in phase α. The second and third terms represent the advective and nonadvective fluxes of component i in phase α. The nonadvective flux is here assumed to be of Fickian form. The fourth term describes the mass exchange of component i between the constituent phases. For the system under consideration the last term accounts for the sink/source of component i due to microbial activity. Equation (1) is constrained by the following relations:

$$\sum_i \omega_i^\alpha = 1 \qquad (2)$$

$$\sum_\alpha \varepsilon_\alpha = 1 \qquad (3)$$

$$\sum_\alpha E_i^\alpha = 0 \qquad (4)$$

The model developed in this work is intended to describe biodegradation of the dissolved hydrocarbon plume. A multiphase description of transport is employed, however, because most petroleum hydrocarbons (e.g., benzene and toluene) are less dense than water and tend to be distributed in dissolved form near and above the water table and the capillary fringe zone. Thus unsaturated zone processes, such as oxygen and vapor diffusion, might play a significant role in controlling the biotransformation of these compounds and should be incorporated in any general model. Thus, for the unsaturated/saturated soil system, three phases are considered ($\alpha = w$(water), g(gas), or s(soil)). In the water phase the external mass supply of component i(E_i^w) includes the mass exchange between liquid/gas phases (volatilization E_i^{wg}) and liquid/soil phases (sorption E_i^{ws}), such that $E_i^w = E_i^{wg} + E_i^{ws}$.

A number of assumptions may be employed to simplify the system of equations (1). In this work the air phase is assumed immobile, that is, the gas phase is assumed to be at atmospheric pressure and density gradients are neglected, resulting in $v^g = 0$. Since the model is primarily applied to the saturated zone and to the unsaturated zone in the immediate vicinity of the water table where the relative humidity is high, water is assumed to act as the wetting fluid in the aquifer system. This implies that no direct contact will occur between the gas and solid phases or gas and biomass. Thus it is assumed that no sorption occurs between the gas and solid phases (i.e., $E_i^{gs} = 0$ and $E_i^g = E_i^{gw}$) and that contaminants in the vapor phase are not available to the microorganisms (i.e., $G_g^i = 0$). The suitability of the water-wet matrix assumption is supported by its use in numerous unsaturated zone transport models (see, for example, *van Genuchten and Jury* [1987]), and recent vapor phase sorption studies (see, for example, *Chiou and Shoup* [1985]). The external mass supply of component i in the soil phase is assumed to result from sorption/desorption (i.e., $E_i^s = E_i^{sw}$). In addition, it is assumed that no other chemical reactions occur in the soil phase (i.e., $G_i^s = 0$).

Given the previous assumptions, a component mass balance equation for bulk fluid phase transport may be obtained by summing equation (1) over all the phases and employing the constitutive constraint (4):

$$\frac{\partial}{\partial t}[(\rho^w \varepsilon_w + \rho^g \varepsilon_g K_{H'i} + \rho_b \rho^w K_{Di})\omega_i^w] + \nabla \cdot (\rho^w \varepsilon_w v^w \omega_i^w) - \nabla \cdot [(\rho^w \varepsilon_w D_i^w + \rho^g \varepsilon_g D_i^g K_{H'i}) \cdot \nabla \omega_i^w] = G_i^w \qquad (5)$$

where $\rho = \rho^s \varepsilon_s$ is soil bulk density. Equation (5) incorporates a local equilibrium relationship, Henry's law ($\omega_i^g = K_{H'i}\omega_i^w$, where $K_{H'i}$ is the partitioning coefficient of component i between the liquid and gas phases), to describe the gas/liquid partitioning of oxygen and organic components for a dilute aqueous system. Here the liquid/solid phase parti-

tioning process is treated as a linear equilibrium process (i.e., $\omega_i^s = K_{Di}\rho^w\omega_i^w$, where K_{Di} is the "distribution coefficient" for component i between solid and liquid phases). The assumption of linear sorption is commonly used for dilute solutions in sandy soils of low organic content and was judged appropriate for the present application. It should be noted, however, that sorption behavior in many natural aquifer systems may be nonlinear [*Weber et al.*, 1991].

In general, two approaches for representing the microbial activity (the G_i^w term) in the transport equation could be used. The first approach is to incorporate the microbial consumption of component i, which follows a given microbial kinetics, as a "macroscopic" sink term in the transport equation. This approach presumes that bacterial growth is limited by the bulk concentration of component i (i.e., microbial consumption rate is a function of bulk component concentration). To date, most biodegradation models have employed this assumption [e.g., *Borden and Bedient*, 1986; *Corapcioglu et al.*, 1991; *Frind et al.*, 1990; *Kindred and Celia*, 1989; *MacQuarrie et al.*, 1990; *Sykes et al.*, 1982]. The second approach, adopted in our model, recognizes that contaminant biodegradation in the subsurface environment may be limited by diffusional transport into the biophase [e.g., *Bouwer and Cobb*, 1987; *Molz et al.*, 1986; *Widdowson et al.*, 1988; *Kinzelbach et al.*, 1991]. Component transport to the biophase, a microscopic process, is assumed herein to take place through a liquid film layer adjacent to the biomass. This process is incorporated in the transport equation by employing Fick's first law to represent G_i^w.

Several studies have shown that most of the attached soil bacteria in subsurface environments tend to aggregate in colonies of 10 to 100 cells [*Gray et al.*, 1968; *Campbell*, 1977; *Bryers*, 1988; *Harvey et al.*, 1984, 1989]. Biomass is therefore assumed to be present in the form of attached microcolonies [*Molz et al.*, 1986]. It is further assumed that these microcolonies behave as fully penetrated biofilms [*Rittmann and McCarty*, 1980] due to their small thickness. Potential microbial transport mechanisms, such as deposition, declogging, or chemotaxis motion, have not been incorporated in the present model, due to the inadequacy of information regarding these mechanisms and their importance. Thus the modeling focus is on the consumption of benzene and toluene by attached indigenous microorganisms.

Equation (5) may now be written as

$$\frac{\partial}{\partial t}[(\rho^w\varepsilon_w + \rho^g\varepsilon_g K_{H'i} + \rho_b\rho^w K_{Di})\omega_i^w] + \nabla\cdot(\rho^w\varepsilon_w v^w\omega_i^w) - \nabla\cdot[(\rho^w\varepsilon_w D_i^w + \rho^g\varepsilon_g D_i^g K_{H'i})\cdot\nabla\omega_i^w] = -\kappa_i\frac{A_c X_m}{m_c}(\rho^w\omega_i^w - C_i) \quad (6)$$

where

κ_i mass transfer coefficient of component i across the boundary layer;
X_m total biomass concentration;
m_c biomass of one microcolony;
A_c contact area of one microcolony for the mass diffusion process;
C_i concentration of component i with the biophase.

Note that the right-hand side of (6), which describes mass transfer across the boundary layer, incorporates two microscopic parameters, A_c and m_c. An alternative modeling approach would be to lump the mass transfer coefficient and microbial geometry into some effective macroscopic parameter [*Baveye and Valocchi*, 1989]. The first approach is chosen in this work because independent estimates of the mass transfer coefficient are sought.

Equation (6) may be written for each system component. In the present study, five components are considered: two primary substrates, benzene (B) and toluene (T); two potential electron acceptors, oxygen (o) and nitrate (n); and one limiting nutrient (A). Most previous models have incorporated microbial kinetics to describe microbial consumption. This is the approach adopted in the present study. Some investigators have proposed that under circumstances in which the biodegradation rate is faster than the bulk advective transport in the aquifer material, the biodegradation of that organic compound may be treated as "instantaneous." Its degradation may then be computed from a stoichiometric reaction, and no microbial kinetic parameters need be employed [*Borden and Bedient*, 1986; *Rifai and Bedient*, 1987; *Rifai et al.*, 1988]. Although microbial degradation of BTX is relatively fast, a more complete kinetic representation was deemed appropriate for model-laboratory comparisons in the present work. This point is discussed more fully in the sensitivity analysis portion of this paper.

Biodegradation Kinetics

Laboratory experiments with the natural aquifer materials employed in this study revealed that benzene and toluene were degraded as primary substrates under aerobic conditions by a mixed population of indigenous microorganisms [*Alvarez et al.*, 1991]. Other laboratory evidence supports the existence of separate benzene and toluene degraders in this population [*Alvarez and Vogel*, 1991]. Furthermore, xylene was not degraded within the duration of the column experiments described in this paper. For modeling purposes this mixed microbial community is treated as two populations. One species is assumed to be a benzene (B) degrader and the other a toluene (T) degrader. In addition, only toluene was observed to degrade under denitrifying conditions in the laboratory. For this reason the toluene degrader is assumed to be capable of employing nitrate as an alternative electron acceptor. Dissimilatory denitrification has been shown to be regulated by an oxygen threshold concentration [*Coyne and Tiedje*, 1990]. Therefore the model includes a nitrate utilization inhibitory function for the toluene degrader that is related to oxygen concentration. Incorporation of Monod-type microbial kinetics [*Williamson and McCarty*, 1976] yields the following set of equations which govern biophase utilization of substrates, electron acceptors, and any other limiting nutrients:

$$\kappa_i\frac{A_c X_m}{m_c}[\rho^w\omega_i^w - C_i] = \sum_j k_j^i\left(\frac{C_i}{K_{S_{ij}} + C_i}\right)\left(\frac{C_j}{K_{a_{ij}} + C_j}\right)\left(\frac{C_A}{K_{A_{ij}} + C_A}\right)II^{ij}(C_o)X_i \quad (7)$$

$$\kappa_j \frac{A_c X_m}{m_c} [\rho^w \omega_j^w - C_j] = \sum_i E_j^i k_j^i \left(\frac{C_i}{K_{S_{ij}} + C_i}\right)\left(\frac{C_j}{K_{a_{ij}} + C_j}\right) \cdot \left(\frac{C_A}{K_{A_{ij}} + C_A}\right) II^{ij}(C_o) X_i \quad (8)$$

$$\kappa_A \frac{A_c X_m}{m_c} [\rho^w \omega_A^w - C_A] = \sum_i \sum_j F_j^i k_j^i \cdot \left(\frac{C_i}{K_{s_{ij}} + C_j}\right)\left(\frac{C_j}{K_{a_{ij}} + C_j}\right)\left(\frac{C_A}{K_{A_{ij}} + C_A}\right) II^{ij}(C_o) X_i \quad (9)$$

where

$i \in \{T, B\}$
$j \in \{o, n\}$

k_j^i maximum specific substrate utilization rate of component i under j-based respiration;
$K_{S_{ij}}$ half-saturation coefficient of substrate i under j-based respiration;
$K_{a_{ij}}$ half-saturation coefficient of electron acceptor j for degrader i;
$K_{A_{ij}}$ half-saturation coefficient of the limiting component A under j-based respiration for degrader i;
E_j^i electron acceptor use coefficient of degrader i under j-based respiration;
F_j^i limiting nutrient use coefficient with respect to substrate i under j-based respiration;
X_i biomass concentration of degrader i;
C_A concentration of any other limiting component in the system.

A hyperbolic oxygen inhibition function [*Stryer*, 1988] has been employed:

$$II^{ij}(C_o) = 1 - I^i(C_o) \qquad j = 0$$

$$II^{ij}(C_o) = I^i(C_o) \qquad j = n$$

where $I^i(C_o) = K_c^i/(K_c^i + C_o)$ and K_c^i is the inhibition coefficient.

Note that the above equations assume a quasi-steady profile across the liquid film and a uniform concentration profile (i.e., a fully penetrated biofilm or a quasi-steady consumption of the available substrate) within the biophase at each transport time step.

The system of governing equations is completed with the ordinary differential equations describing the growth and decay of the attached biomass populations:

$$\frac{dX_i}{dt} = \sum_j \left\{ k_j^i Y_j^i \left(\frac{C_i}{K_{s_{ij}} + C_i}\right) \cdot \left(\frac{C_j}{K_{a_{ij}} + C_j}\right)\left(\frac{C_A}{K_{A_{ij}} + C_A}\right) II^{ij}(C_o) - k_{dj}^i \right\} X_i \quad (10)$$

$$i \in \{T, B\} \qquad j \in \{o, n\}$$

where Y_j^i is the yield coefficient of bacteria i under j-based respiration, and $k_{d_j}^i$ is the decay coefficient of bacteria i under j-based respiration.

The model assumes that the number of microcolonies increases with biomass growth but that the size of colonies remains constant. For natural oligotrophic subsurface environments and bacteria using relatively low concentrations of xenobiotics as primary substrates, the accumulation of biomass is assumed not to exceed the amount where the quasi-steady consumption assumption is valid. Within the model, under zero substrate conditions, the microbes are not permitted to die off completely, but are maintained at a minimum biomass concentration level.

In summary, five nonlinear partial differential equations of the form (6) are used to describe the component transport/transformation in the bulk fluid phase; five nonlinear algebraic equations (two of the form (7), two of the form (8), and one of the form (9)) are used to describe component utilization in the biophase; and two ordinary differential equations of the form (10) are used to describe the biomass growth and decay of the two microorganism populations.

ONE-DIMENSIONAL SIMULATOR

To assess the applicability of the mathematical model to biodegradation in a column reactor, a one-dimensional simulator was developed on the basis of the governing equations described above. In the one-dimensional simulator the transport equation (6) is discretized in space through application of a standard Galerkin finite element method. Nodal variables ω_i^w, C_i, and X_m are approximated using linear trial functions. Coefficients ρ^a, ε_a, ρ_b, D_i^a, κ_i, K_{Di}, $K_{H'i}$, V^a, A_c, and m_c are assumed to be elementwise constant. A variably weighted finite difference operator is employed for time discretization. Since a number of alternative discretizations of the sink term in (6) are possible, Appendix A contains the explicit form used in the model.

Equation (6) is nonlinear, due to the implicit dependence of C_i and X_m on the bulk phase mass fractions (through equations (7)–(10)). Five discretized transport equations are incorporated in the simulator. These equations are implicitly coupled through the variables C_i and X_m but are solved sequentially using a Picard iterative procedure. Following solution of the transport equations, the five nonlinear algebraic equations representing component uptake in the biophase are solved using a Newton-Raphson iterative scheme. Finally, biomass concentration is obtained by solution of the growth equations. To maintain consistency with the transport equations, these two ordinary differential equations are discretized by expanding variables in terms of the trial functions and applying a variably weighted finite difference operator in time (see Appendix B). Initial conditions for C_i in the biophase are obtained through solution of the component uptake equations (7)–(9). After all unknowns are determined at the new time level the transport equations are again solved. Thus the solution algorithm is implicit in that the current time level (previous iterate) values of C_i and X_m are employed in the solution of the bulk phase transport equations. Iterations continue within each time step until the following convergence criterion is satisfied:

$$\frac{\|\{\omega_i^w\}_{\eta+1} - \{\omega_i^w\}_\eta\|_\infty}{\|\{\omega_i^w\}_{\eta+1}\|_\infty} < \xi \quad (11)$$

for each component. Here the subscripts η and $\eta + 1$ represent the old and new iteration levels, respectively. A value of 10^{-4} for ξ was used for all presented simulations. The simulator can accommodate first-, second-, or third-type boundary conditions on the component mass fractions in the bulk phase.

In order to limit numerical dispersion in the simulations the nodal spacing Δz and temporal spacing Δt were determined using grid Peclet number, $Pe = v\Delta z/D \leq 2$, and Courant number, $Cr = v\Delta t/\Delta z \leq 1$, criteria [*Huyakorn and Pinder*, 1983]. All solutions presented herein were also tested heuristically for convergence by varying time step size and grid spacing.

The one-dimensional simulator was initially verified through comparison with the *Ogata and Banks* [1961] solution for solute transport under steady state flow conditions in a homogeneous water-saturated soil-packed column. The model was further tested by comparison to solutions obtained in other biotransformation modeling studies. Figure 1 shows a simulation comparison with an example presented by *Molz et al.* [1986]. The model of Molz et al. is based on the microcolony concept and considers a single microbial population and a single substrate. It employs an Eulerian-Lagrangian solution scheme. The example simulation illustrates single-substrate hydrocarbon biodegradation under oxygen-limiting conditions (Figure 8 of *Molz et al.* [1986]). The parameters and assumed boundary and initial conditions used in the comparison are shown in Table 1 [*Molz et al.*, 1986, p. 1212]. For this comparison the present simulator was modified to employ the time weighting scheme used by Molz et al., and the number of governing equations was reduced. In Figure 1 the discrete symbols represent the Molz et al. solution. The solid curves show model simulations from the present study. Reasonable agreement was obtained for all simulation times.

EXPERIMENTAL METHODOLOGY

Once mathematical verification of the simulator was completed, an experimental study was undertaken to provide information for conceptual model validation. As a first step, the experiments were designed to investigate biodegradation

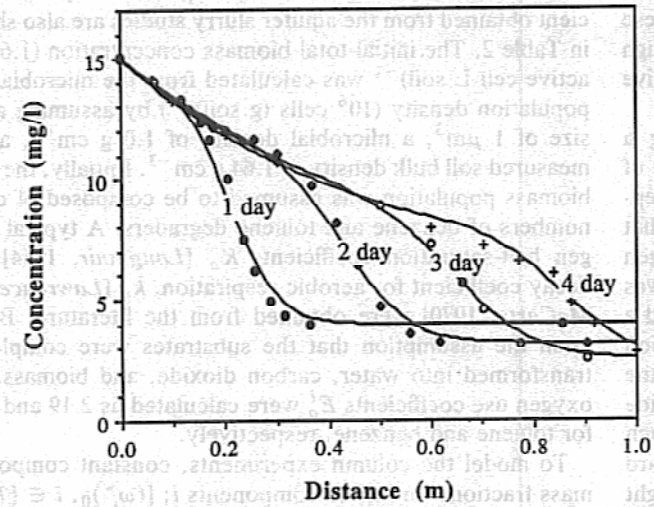

Fig. 1. Model verification simulation (symbols, predictions from *Molz et al.* [1986]; curves, our model).

TABLE 1. Parameters Used in Model Verification

Parameter	Value
Physicochemical Parameters	
Porosity n	0.30
Pore velocity v, m d^{-1}	0.25
Soil bulk density ρb, g cm^{-3}	1.67
Dispersivity α, cm	0.50
Liquid film diffusion coefficient	
D_{sb}, cm^2 d^{-1}	0.06
D_{ob}, cm^2 d^{-1}	0.71
Retardation factor Ra	
substrate	1.12
oxygen	1.00
Liquid film thickness δ, cm	0.05
Mass transfer coefficient $\kappa_i = D_{ib}/\delta$	
κ_s, cm d^{-1}	1.2
κ_0, cm d^{-1}	14.2
Microbial Parameters	
Initial biomass, cell (g soil)$^{-1}$	10^6
Colony population density, cells per colony	100
Colony mass m_c, g per colony	10^{-10}
Maximum specific substrate utilization rate k, L d^{-1}	15.61
Yield coefficient Y, g cell per g substrate	0.278
Colony contact area A, m^2	7.85×10^{-11}
Oxygen use coefficient E, mg O_2 (mg S)$^{-1}$	1.4
Substrate half-saturation coefficient K_s, mg L^{-1}	120.0
Oxygen half-saturation coefficient K_a, mg L^{-1}	0.77
Dacay coefficient k_d, L d^{-1}	0.02

in continuous flow, water saturated, soil columns. To facilitate model-experimental comparisons, efforts were made to measure or quantify all required model parameters independently. Sandy aquifer material from a site underlying a gas plant facility in Kalkaska, Michigan, was employed in all experiments. This material has a low organic carbon content (0.01–0.08%) [*Chiang et al.*, 1989]. The aquifer core material was sampled aseptically from the shallow sandy aquifer using plastic coring tubes. These samples contained no detectable levels of BTX, although the aquifer had been contaminated at a level of approximately 200 μg L^{-1} total BTX [*Chiang et al.*, 1989]. Filled sample tubes were sealed and transported to the laboratory, where they were stored at 4°C until used.

Parameter Measurement

Monod coefficients (the maximum specific substrate utilization rate k and the half-saturation coefficient K_s), the yield coefficient (Y), and the initial soil microbial population were measured from aquifer slurry experiments. For the measurement of Monod coefficients, benzene and toluene were added concurrently as the sole carbon sources to 120-mL serum bottles containing 16 g of aquifer material (10 mL bulk volume) and 50 mL of a minimal mineral medium. The medium provided essential inorganic nutrients to the indigenous aquifer microbes. Samples containing the following initial concentrations of both benzene and toluene were prepared: 1, 10, 50, 100, and 250 mg/L^{-1}. Removal rates were measured over this wide range of concentrations in order to encompass the transition from first- to zero-order kinetics with respect to substrate concentration utilization. Measurements were performed in duplicate. Each slurry experiment was used to obtain an initial specific substrate utilization rate measurement (i.e., the measured initial degradation rate divided by the microbial concentration) corre-

sponding to an initial substrate concentration. This initial utilization rate, in turn, yielded a point in the Hanes linearization [*Dowd and Riggs*, 1965; *Grady and Lim*, 1980]. The k and K_s were calculated on the basis of the slope and intercept of the Hanes linearization. The yield coefficient was estimated by measuring the increase in microbial number as a result of benzene and toluene biodegradation in the batches. The initial aquifer microbial population was estimated by a direct microbial count method, the acridine orange direct count (ADOC) procedure. Further details of the analytical techniques and experimental procedures used in this study are given by *Alvarez et al.* [1991] and *Alvarez and Vogel* [1991].

Adsorption isotherm experiments for the aquifer materials were conducted using completely mixed batch reactors (the bottle-point technique) [*Randtke and Snoeyink*, 1983]. Soil samples from the Kalkaska aquifer were sterilized by autoclaving and sodium azide addition. A fixed weight of aquifer material and various initial solution concentrations of benzene and toluene were combined in a series of batch reactors. After 2 weeks of mechanical shaking, samples were centrifuged and analyzed to calculate the disappearance of solutes. Plots of sorbed phase versus aqueous phase concentration yielded estimates for the partition coefficients. To explore the hydraulic effect on adsorption isotherms, partitioning coefficients were also obtained from short-bed continuous flow reactors packed with aquifer material [*Smith and Weber*, 1989]. Note that sorption studies were conducted on aquifer material obtained from the same contaminated site but from a different sample core than that employed in the microbial studies.

Continuous-Flow Columns

Two pairs of sterile glass columns (56 cm long, 2.2 cm inner diameter, and narrowed on the bottom) equipped with six sampling ports were used in the experiments [*Anid and Vogel*, 1990]. Both ends of the columns and the sampling ports were sealed with Teflon tape and closed with rubber stoppers. The aquifer material was filled according to a method described by *Siegrist and McCarty* [1987]. Tracer (bromide) studies were first conducted in all the columns to characterize transport properties [*Anid and Vogel*, 1990]. On the basis of these measurements the average porosity for all the columns was estimated at 0.38, the average pore velocity at 0.33 m d^{-1}, and the dispersivity at 2.24 cm. These parameters were estimated by fitting tracer breakthrough concentrations to a solution of the advective-dispersive transport equation [*van Genuchten and Parker*, 1984].

Biodegradation experiments were begun by feeding a constant composition solution to the columns. One pair of columns received hydrogen peroxide as an electron acceptor, a second pair received nitrate. Two control columns that had been autoclaved for 4 hours at 120°C received hydrogen peroxide, nitrate, oxygen, and sodium azide, which was added to inhibit microbial activity. All columns were fed a mixture of benzene, toluene, and xylenes at a concentration of 20 mg L^{-1} each. These were added along with the electron acceptors and a sterile basal media to provide inorganic nutrients essential for microbial growth. Each column was fed at a flow rate of 2 mL h^{-1} using a Harvard syringe infusion pump 22 equipped with a 50 mL gastight syringe (Hamilton Company, Reno, Nevada). Effluent samples (0.5 mL) from a switching valve located on the steel tubing connected to the effluent end of the column were collected with gastight syringes and were analyzed for concentrations of benzene, toluene, and xylenes by a HP 5890 gas chromatograph (GC). Dissolved oxygen was also measured with a biological oxygen monitor YSI 530 after 32 days. Experimental procedures for the column experiments are described in more detail by *Anid and Vogel* [1990].

MODEL COMPARISONS TO LABORATORY EXPERIMENTS

The mathematical model presented in this paper was used to predict the performance of the saturated aquifer column experiments. Two simulation cases were considered corresponding to the previously described column experiments involving (1) hydrogen peroxide addition and (2) nitrate addition. The required physicochemical parameters and microbial parameters used in the mathematical model are summarized in Table 2. The numerical parameters and boundary and initial conditions are given in Table 3. Note that, under the column experimental conditions described above, only a single fluid (aqueous) phase was present, and there were no nutrient limitations. Thus, for model simulations, the gas phase volume fraction was set to zero ($\varepsilon_g = 0$) in transport equations (6), and solution of (9) was not required.

Values for porosity, pore velocity, and dispersivity obtained from the bromide tracer data are tabulated in Table 2. Partitioning coefficients evaluated from the batch sorption studies are also listed. The hydrodynamic dispersion coefficient D_i^w is expressed as $D_i^w = \alpha|v^w| + D_i^m$ [*Freeze and Cherry*, 1979], where α is the longitudinal dispersivity, v^w is the pore velocity in the column, and D_i^m is the effective molecular diffusivity of component i in the aqueous phase. D_i^m values were estimated using a literature correlation [*Wilke and Chang*, 1955]. The film mass transfer coefficient κ_i was estimated by the *Wilson and Geankoplis* [1966] correlation:

$$\kappa_i = 1.09 v^w Re^{-2/3} Sc^{-2/3} \tag{12}$$

where Re is the Reynolds number ($Re = d_p \varepsilon_w v^w \rho^w / \mu_w$; d_p is the soil particle diameter) and Sc is the Schmidt number ($Sc = \nu / D_i^*$; ν is the kinematic viscosity). This correlation is valid for $0.0016 < Re < 55$ and $0.35 < \varepsilon_w < 0.75$.

The Monod coefficients (k and K_s) and the yield coefficient obtained from the aquifer slurry studies are also shown in Table 2. The initial total biomass concentration (1.64 mg active cell L soil)$^{-1}$ was calculated from the microbial soil population density (10^6 cells (g soil)$^{-1}$) by assuming a cell size of 1 μm^3, a microbial density of 1.0 g cm^{-3}, and a measured soil bulk density of 1.64 g cm^{-3}. Initially, the total biomass population was assumed to be composed of equal numbers of benzene and toluene degraders. A typical oxygen half-saturation coefficient, K_a [*Longmuir*, 1954] and decay coefficient for aerobic respiration, k_d [*Lawrence and McCarty*, 1970] were obtained from the literature. Based upon the assumption that the substrates were completely transformed into water, carbon dioxide, and biomass, the oxygen use coefficients E_o^i were calculated as 2.19 and 2.15 for toluene and benzene, respectively.

To model the column experiments, constant component mass fractions for all the components i; $[(\omega_i^w)_0, i \in \{T, B, o, n\}]$, were assumed as initial conditions within the column.

TABLE 2. Parameters Used in Model Simulations

Parameter	Value
Physicochemical Parameters	
Pump flow rate Q(M), mL h^{-1}	2
Porosity n(M)	0.38
Pore velocity v(M), m d^{-1}	0.33
Soil bulk density ρb(M), g cm^{-3}	1.64
Dispersivity α(M), cm	2.24
Molecular diffusivity D^*(L)	
T, m^2 d^{-1}	7.34×10^{-5}
B, m^2 d^{-1}	8.24×10^{-5}
O, m^2 d^{-1}	1.86×10^{-4}
n, m^2 d^{-1}	1.39×10^{-4}
Partitioning coefficient kd(M)	
T, cm^3 g^{-1}	0.139
B, cm^3 g^{-1}	0.093
O, cm^3 g^{-1}	0.0
n, cm^3 g^{-1}	0.0
Mass transfer coefficient κ_i(L)	
T, m d^{-1}	6.38×10^{-1}
B, m d^{-1}	6.89×10^{-1}
O, m d^{-1}	1.19×10^{-1}
n, m d^{-1}	9.78×10^{-1}
Microbial Parameters	
Initial biomass (M), cell (g soil)$^{-1}$	10^6
Colony population density (L), cells per colony	100
Oxygen use coefficient	
E_o^t(C), mg O_2 (mg T)$^{-1}$	2.19
E_o^b(C), mg O_2 (mg B)$^{-1}$	2.15
Nitrate use coefficient F_o^t(C), mg NO_3 (mg T)$^{-1}$	3.39
Maximum specific substrate utilization rate	
K_o^t(M), d^{-1}	9.9
k_n^t(M), d^{-1}	9.9
k_o^b(M), d^{-1}	8.3
Yield coefficient	
Y_o^t(M), g cell (g T)$^{-1}$	0.5
Y_n^t(M), g cell (g T)$^{-1}$	0.5
Y_0^b(M), g cell (g B)$^{-1}$	0.5
Colony mass m_c(C), g per colony	10^{-10}
Contact area of one colony A_c(L), m^2 per colony	1.19×10^{-10}
Half-velocity constant of toluene	
$K_s to$(M), ng L^{-1}	17.4
$K_s tn$(M), mg L^{-1}	17.4
Half-velocity constant of benzene $K_s bo$(M), mg L^{-1}	12.2
Half-velocity constant of oxygen	
$K_a to$(L), mg L^{-1}	0.1
$K_s bo$(L), mg L^{-1}	0.1
Half-velocity constant of nitrate Ks_{tn}(L), d^{-1}	2.6
Decay coefficient	
$k_{d_o}^t$(L), d^{-1}	0.1
$k_{d_n}^t$(L), d^{-1}	0.1
$k_{d_o}^b$(L), d^{-1}	0.1

M, measured; L, literature; C, calculated.

A constant composition influent was continuously injected into the column during the experiments. This was modeled as a third- or flux-type boundary condition at $z = 0$. A zero concentration gradient (second type) boundary condition was employed at the outlet (see Table 3).

Hydrogen Peroxide–Amended Column

The first simulation case involved the prediction of the effluent breakthrough curves of benzene and toluene under sufficient oxygen conditions. Substrate (B and T) effluent breakthrough data and uncalibrated model predictions are shown in Figures 2*a* and 2*b*. The model simulation for the toluene breakthrough curve is in reasonably close agreement with the laboratory data. Unlike the toluene breakthrough curve, the benzene prediction does not match the laboratory data. The predicted peak of the normalized concentration is lower, and the predicted breakthrough curve is more narrow. However, the predicted trends are similar to the laboratory data. A model sensitivity analysis revealed that breakthrough curves were sensitive to the initial active biomass of benzene and toluene degraders. Unfortunately, benzene and toluene degraders could not be differentiated with the ADOC procedure used in this study. Moreover, this procedure can only really give order of magnitude estimates of biomass [*Alvarez et al.*, 1991]. Thus the initial distribution of biomass between benzene and toluene degraders is known

with less certainty than the other measured parameters. On the basis of this reasoning the initial biomass distribution was adjusted, within the range of experimental uncertainty, to produce a better model fit. When the benzene degrader population was reduced to 25% of its initial value, model predictions improved, as shown in Figures 2*a* and 2*b*. The model simulation of the benzene breakthrough curve now provides a better match with the laboratory data, while the model simulation of the toluene breakthrough curve is not significantly affected. This adjusted biomass distribution (X_T = 0.81 mg L^{-1} and X_B = 0.21 mg L^{-1}) was employed in all subsequent model simulations.

Nitrate-Amended Column

A simulation of column operation with nitrate addition was compared with actual column laboratory data. This laboratory column was fed with a solution containing oxygen at 8.5 mg L^{-1}, a concentration insufficient for complete mineralization of benzene and toluene, and nitrate at 330 mg L^{-1} a concentration sufficient for complete mineralization of benzene and toluene (effluent nitrate levels never dropped below 30 mg L^{-1}). The model kinetic parameters (k, Ks, Y, kd) for toluene under nitrate-based respiration in the column reactor were assumed the same as those under aerobic respiration [*Alvarez et al.*, 1991; *Evans et al.*, 1991]. Batch experiments revealed that, unlike toluene, benzene degradation under oxygen limiting conditions was not enhanced by the addition of nitrate. Similar results regarding the recalcitrance of benzene under denitrifying conditions has been reported by several researchers [*Anid and Vogel*, 1990; *Evans et al.*, 1991; *Kuhn et al.*, 1988; *Hutchins et al.*, 1989]. Thus the specific substrate utilization rate of benzene by nitrate oxidation was set to zero for model simulations. The inhibition coefficient for toluene degradation by nitrate oxidation due to the presence of molecular oxygen was estimated as 0.01 mg L^{-1} of O_2, which represents the value where the denitrification rate is reduced to 50% of its maximum rate [*Parkin and Tiedje*, 1984].

After long times, toluene was completely degraded within the column, while the benzene concentration level was reduced by only about 25%, due to the inability of benzene degraders to employ nitrate as an alternative electron acceptor (Figures 3*a* and 3*b*). Here, as in the aerobic case, model predictions of toluene effluent concentrations are in reasonable agreement with the experimental data. The initial benzene breakthrough behavior and the long-term pseudo steady state benzene effluent concentration are also predicted by the model. Benzene biodegradation during intermediate times, however, is not accurately simulated. The benzene breakthrough profile exhibits a local minimum at about 6 days. The increase in the benzene effluent concentration appears to correspond to the point of complete utilization of toluene. Possible explanations for this behavior include the following: (1) the assumed independence of benzene and toluene degradation in the model may not be appropriate under oxygen limiting conditions, (2) an incomplete mineralization of substrates occurred, that is, some of the benzene was transformed to intermediates, which resulted in a smaller oxygen use coefficient than that employed in the model, or (3) the kinetic constant employed for benzene in the presence of nitrate may not be appropriate. Further experimental measurements will be undertaken to choose from among these alternatives.

The laboratory experiments demonstrated that benzene

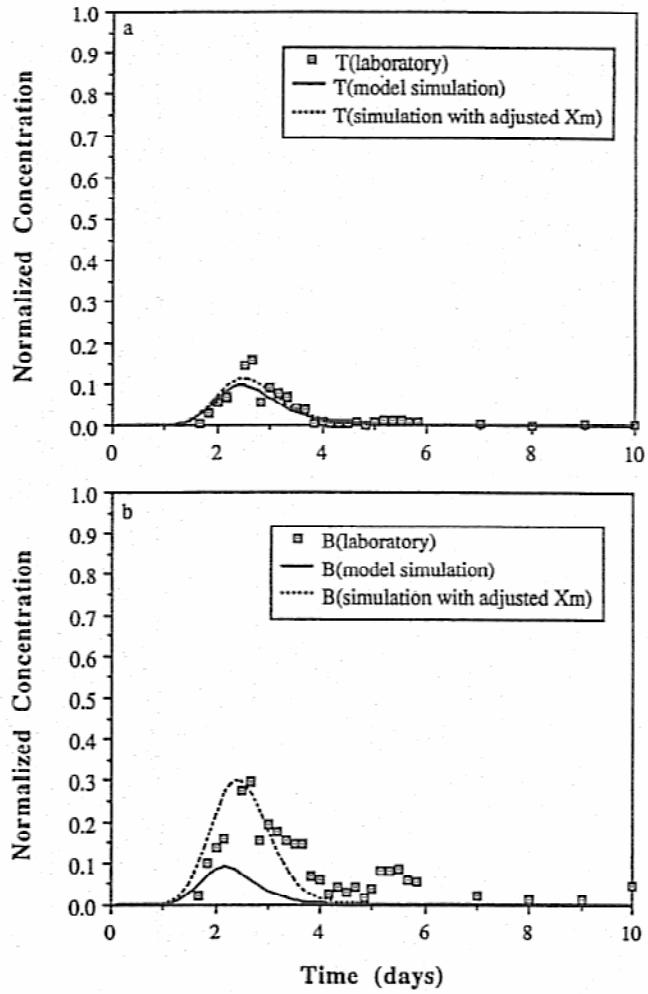

Fig. 2. Comparison of model simulations and measured effluent concentrations under aerobic conditions (*a*) toluene and (*b*) benzene (solid curve, initial biomass: X_B and X_T = 0.82 mg/L; dotted curve, adjusted biomass: X_B = 0.21 mg/L and X_T = 0.82 mg/L).

TABLE 3. Initial and Boundary Conditions Used in Model Simulations

	H_2O_2-Amended Case	NO_3^--Amended Case
Δz	0.014 cm	0.014 cm
Δt	0.01 day	0.01 day
	Initial Conditions	
$\omega_t^w(0, z)$	0.0 mg L^{-1}	0.0 mg L^{-1}
$\omega_b^w(0, z)$	0.0 mg L^{-1}	0.0 mg L^{-1}
$\omega_o^w(0, z)$	8.5 mg L^{-1}	8.5 mg L^{-1}
$\omega_n^w(0, z)$	0.0 mg L^{-1}	0.0 mg L^{-1}
	Boundary Conditions	
$\omega_t^w(t, z = 0^-)$	20.0 mg L^{-1}	20.0 mg L^{-1}
$\omega_b^w(t, z = 0^-)$	20.0 mg L^{-1}	20.0 mg L^{-1}
$\omega_o^w(t, z = 0^-)$	132.7 mg L^{-1}	8.5 mg L^{-1}
$\omega_n^w(t, z = 0^-)$	0.0 mg L^{-1}	330.0 mg L^{-1}

Initial conditions, $t = 0$, $\omega_i^w = \omega_i^w(0, z)$; boundary conditions, $t > 0$, $z = 0$, $V^w\omega_i^w|z = 0^- = (V^w\omega_i^w - D_i^w\partial/\partial_z\omega_i^w)|z = 0^+$, $t > 0$, $z = 56$ cm, $\partial/\partial_z\omega_i^w = 0$.

and toluene degradation occurred continuously in the presence of oxygen and nitrate and that nitrate was utilized as an electron acceptor. This suggests that using nitrate as an adjunct electron acceptor in the application of in situ bioremediation at a benzene and toluene contaminated site might reduce the total oxygen demand or the required amount of hydrogen peroxide.

PARAMETER SENSITIVITY

Subsequent to simulation of the column experiments, the specific parameters used in modeling the microbiological and physicochemical fate of benzene and toluene were varied to explore their influence on the predicted breakthrough concentration profile. This analysis served two purposes: to investigate model sensitivity to these parameters and to explore the influence of parameter measurement error or system variability on the predicted outcome of bioremediation applications. The one-dimensional simulation of the hydrogen peroxide–amended aquifer column experiments presented in Figure 2 served as the base scenario for this sensitivity analysis. Simulation parameters for this base scenario are given in Table 2. All computations were performed with the adjusted initial active biomass concentration. Table 4 summarizes the ranges of parameter values that were employed in model sensitivity simulations. Ranges were obtained from a literature survey, because measured values for these parameters are often unavailable for specific applications. These parameters included the microbial parameters: the Monod constants, the yield coefficient, and the cell decay coefficient. In contrast, the retardation factor Ra was varied only over the range of values obtained from experimental measurements with aquifer material. The mass transfer coefficient for the liquid boundary layer was varied over a range of values computed from several correlation expressions [*Wilson and Geankopolis*, 1966; *Williamson et al.*, 1963; *Friedlander*, 1957]. A summary and discussion of the results of this sensitivity analysis is given below.

TABLE 4. Range of Parameters Used in Analysis of Sensitivity

Parameter	Low Value	Base Value	High Value
k_o^t, d^{-1}	0.1	9.9	11.0*
k_o^b, d^{-1}	0.04	8.3	8.3
K_{so}^t, mg L^{-1}	0.044†	17.4	38.0‡
K_{so}, mg L^{-1}	12.2	12.2	34.0‡
Y_o^t, Y_o^b, mg cell per mg substrate	0.4	0.5	0.6
k_{do}^t, k_{do}^b, d^{-1}	0.01§	0.1	0.2§
X_b, mg L^{-1}	0.21	0.21	0.82
Ra_o^t	1.6‖	1.6	2.1‖
Ra_o^b	1.4‖	1.4	1.6‖

*Button [1985].
†Roberston and Button [1987].
‡Goldsmith and Balderson [1988].
§Lawrence and McCarty [1970].
‖H. Corseuil (personal communication, 1991).

The maximum specific substrate utilization rate k controls the maximum rate of B and T degradation. In Table 4 the lower values of k for benzene/toluene were obtained from kinetic studies with microbial populations derived from the same contamination site [*Alvarez et al.*, 1991]. These studies were conducted on two pure cultures. The toluene degrader was isolated from an on-site activated carbon groundwater treatment system [*Kukor and Olsen*, 1989]. The benzene degrader was isolated from the contaminated aquifer material. Employing these data, the model predicts a superior performance of the mixed culture over that of the pure culture (Figure 4). If a similar column study were conducted using pure culture isolates, the model predicts that only 1% of the influent toluene would be degraded. The value for k derived from the aquifer slurry studies with toluene (9.9 d^{-1}) is fairly close to the highest reported value of 11.1 d^{-1} [*Button*, 1985], and model simulations using these two values are in close agreement. Predictions for benzene concentrations using high and low k estimates exhibited trends similar to those for toluene.

An examination of the Monod expression, $dC/dt = -k[C/(K_s + C)]X_m$, reveals that when the concentrations of benzene and toluene are substantially below the corresponding half-saturation coefficient K_s, biodegradation rates will depend on the maximum rate coefficient and the substrate concentration and will be inversely proportional to K_s. However, when K_s is much smaller than the benzene or toluene concentration, the biodegradation rate becomes zero order, and hydrocarbon degradation rates will increase linearly with k and the active microbial concentration. Simulations with a larger K_s value for toluene degradation

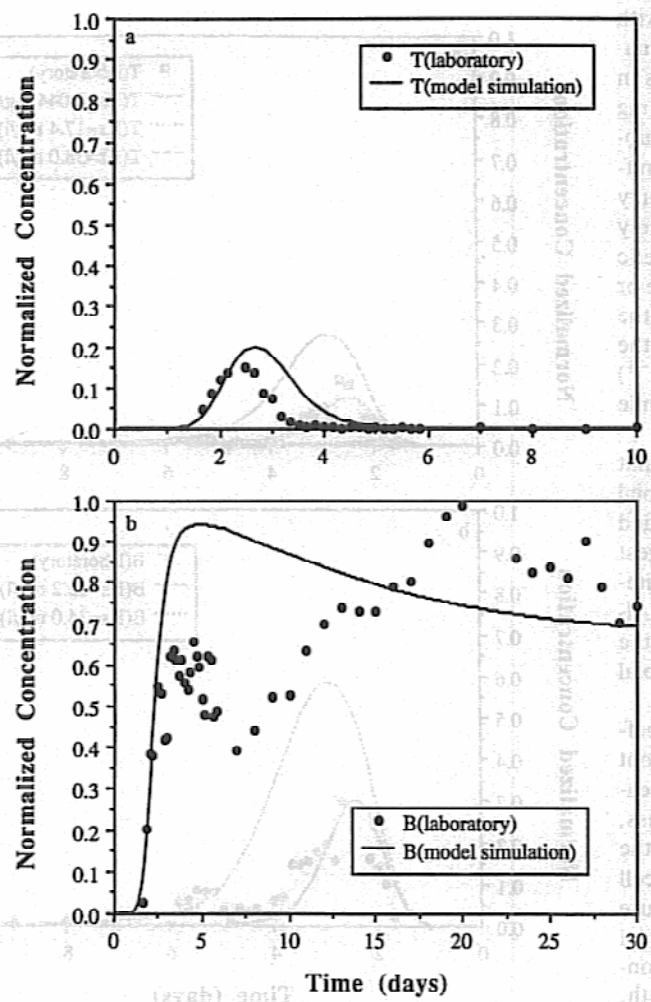

Fig. 3. Comparison of model simulations and measured effluent concentrations for nitrate-amended column for (*a*) toluene and (*b*) benzene.

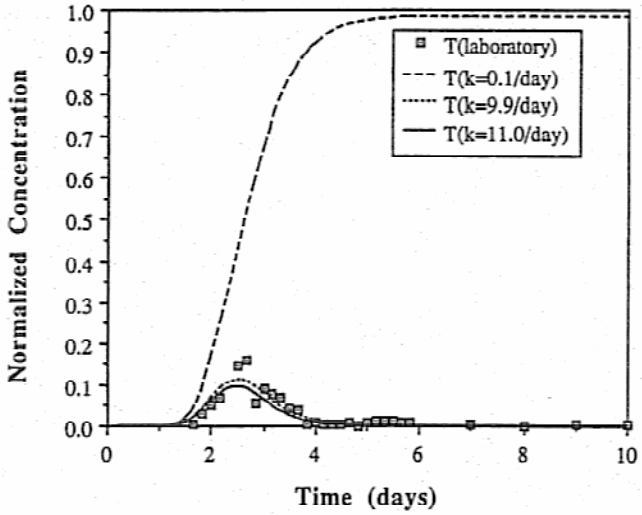

Fig. 4. Sensitivity of model predictions to varying maximum specific substrate utilization rate k.

[*Goldsmith and Balderson*, 1988] illustrate the critical importance of this parameter. When K_s is raised to 38 mg L^{-1}, the model simulation exhibits much higher breakthrough concentrations than were actually observed (Figure 5*a*). The hydrocarbon degradation rate constants for this simulation are approximately k/K_s (in a first-order expression), with relatively low values due to the high K_s values. Alternatively, if K_s values are chosen to be extremely small, as in the minimum literature value for toluene (K_s = 0.044 mg L^{-1}) [*Robertson and Button*, 1987], then the specific substrate utilization rate becomes k (zero order), and no significant breakthrough occurs. Note that the aquifer slurry derived value (17.4 mg L^{-1}) allows the model to accurately simulate the column data. In this situation the Monod kinetic expression cannot be simplified by either a first-order rate or zero-order rate expression, since the K_s value is close to the actual concentration fed to the column (20 mg L^{-1}). In the case of benzene the aquifer slurry value for K_s (12.2 mg L^{-1}) was also found to fit the column data reasonably well, while an extreme literature value did not (Figure 5*b*).

The model simulations described above demonstrate that neither literature values nor measured values from microbial isolates for the biodegradation kinetic coefficients provided good predictions of the column data. These results suggest that biodegradation kinetic coefficients may be very site-specific parameters requiring independent measurement. Alternatively, the improved degradation predicted with the mixed culture kinetics may simply indicate that microbial consortia generally outperform natural isolates.

Another microbial parameter of interest is the yield coefficient (Y). Our aquifer slurry data indicated a value of about 0.5 mg cells per mg hydrocarbon degraded. Accurate measurement of cell yield, like initial cell numbers or biomass, however, could easily vary as much as 50%. Fortunately, the model simulation is relatively insensitive to changes in cell yield for both the toluene and benzene degraders (Figure 6*a*). When cell yields were varied from 0.4 to 0.6, the model simulations did not change significantly. Since yield controls, in part, the relative change in biomass due to growth, it only indirectly affects the subsequent hydrocarbon degradation rate (the more biomass, the more BT degraders in the system). Thus the yield coefficient does not have the influence exhibited by other microbial parameters. As shown in Figure 6*b*, however, cell yield would have more influence if the initial biomass concentration were lower ($X_m = 10^3$ cells (g soil)$^{-1}$). Under such circumstances the yield and the rate of hydrocarbon degradation would partially control the rate of biomass increase until the biomass was significantly high such that other factors would begin to dominate.

Another parameter that affects biomass increase is the microbial decay coefficient (k_d) for which no value was determined from aquifer slurry studies. Literature values ranging from 0.01 d^{-1} [*Lawrence and McCarty*, 1970], typically associated with anaerobic conditions, to 0.2 d^{-1}, typically associated with aerobic conditions [*Lawrence and McCarty*, 1970], were used in the model simulations. Similar to the results observed for cell yield, these values had very little effect on breakthrough curves. Decay coefficients would, however, be expected to influence the minimum hydrocarbon concentration obtained after long times, since cell decay is the only process which reduces biomass accumulation in the model.

One of the reasons cell yield and decay have less influence on the breakthrough curves is the prior existence of biomass capable of degrading benzene and toluene. At pristine sites the number of BT degraders would be expected to be quite

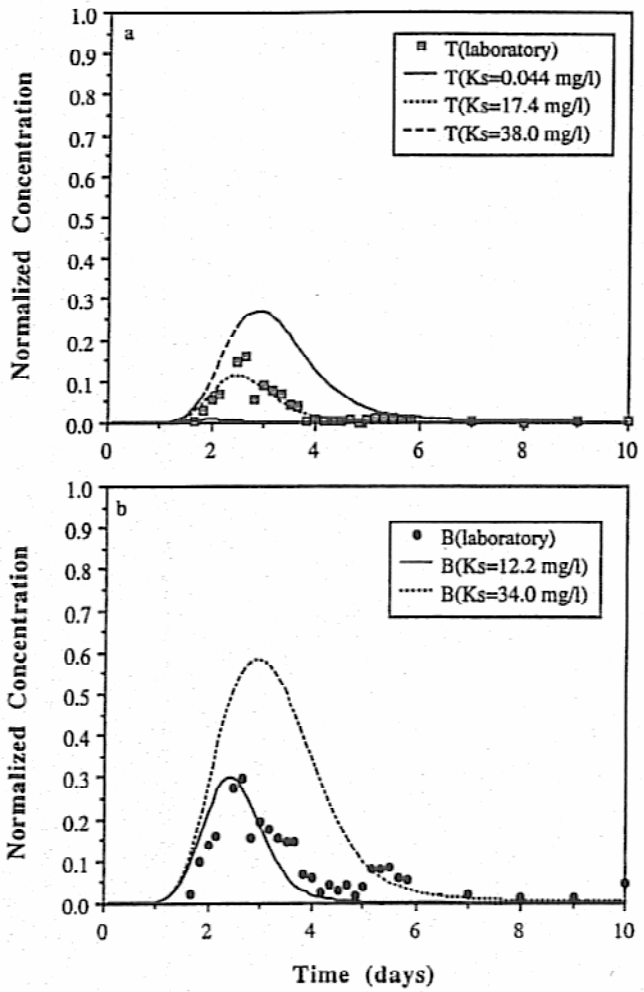

Fig. 5. Sensitivity of model predictions to varying half velocity constant K_s for (*a*) toluene and (*b*) benzene.

low. If, however, as in this case, the aquifer was exposed to benzene and toluene, the number of BT degraders would be relatively high. The biomass in this study, estimated at approximately 10^6 bacteria per gram of aquifer material, has tremendous influence on the breakthrough curve. If the initial biomass concentration were sufficiently high, no benzene or toluene would pass through the column. If the initial biomass were very low, the movement of benzene and toluene through the column would exceed the rate of biodegradation, and high breakthrough concentrations would be anticipated. As illustrated previously (Figure 2*b*), the initial biomass concentration had a significant influence on the breakthrough curves. In addition, this is the one parameter determined from the sediment slurry studies that appears to prevent the model from fitting the benzene breakthrough curve more accurately.

Under certain conditions the mass diffusion process in the immobile water adjacent to the biomass might be rate limiting. Two parameters, the mass transfer coefficient and the total contact area, control this process. Numerous mass transfer correlations are available to estimate the mass transfer coefficient across a boundary layer. Most were developed, however, for diffusion-limited dissolution of fluid spheres suspended in a laminar flow regime. Three correlations were used to examine the model sensitivity to the mass transfer coefficient. The model simulations using κ values obtained from *Wilson and Geankoplis* [1966] and *Williamson et al.* [1963] did not vary significantly from the column data, but simulations using values from *Friedlander* [1957] did exhibit a greater deviation (Figure 7). The possible reason for this deviation is that correlations developed by *Friedlander* [1957] are based on fluid-fluid interactions and do not account for the tortuous flow in the soil matrix. Such hydraulic conditions result in lower mass transfer coefficients and hence less biomass growth in the column.

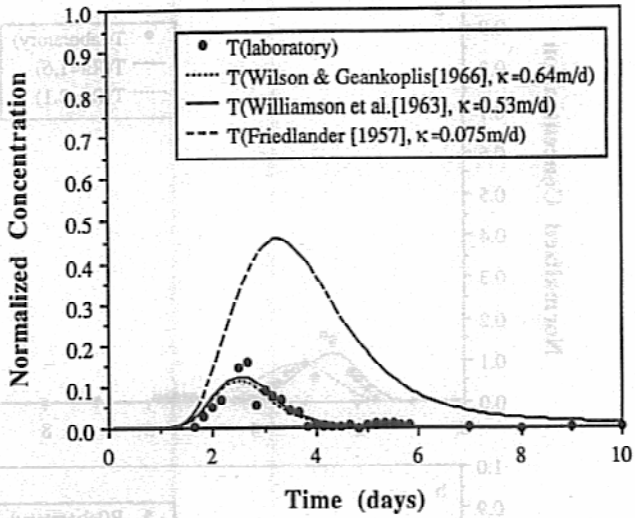

Fig. 7. Sensitivity of model predictions to varying mass transfer coefficient κ.

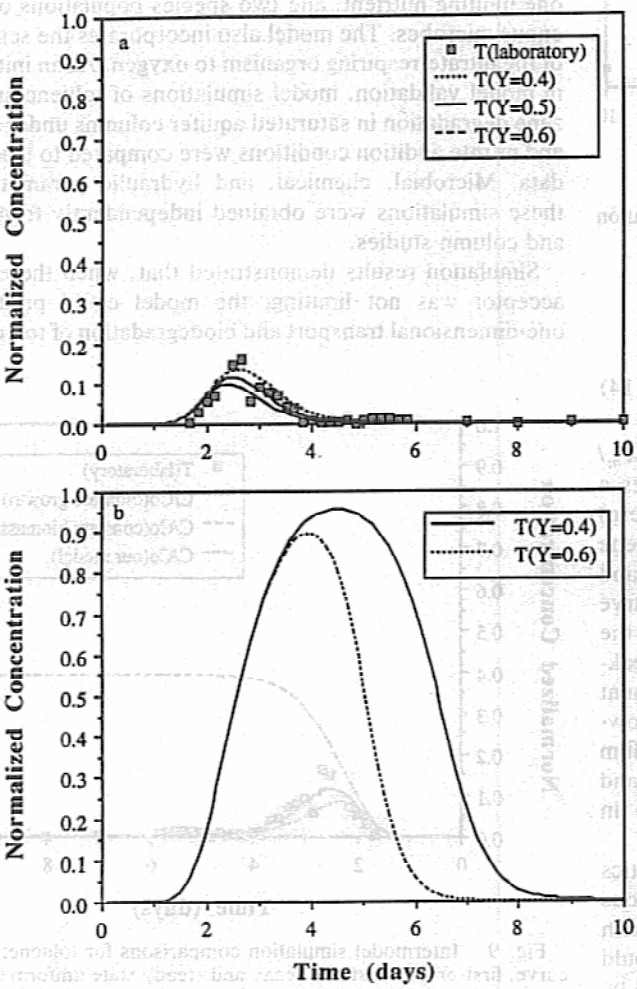

Fig. 6. Sensitivity of model predictions to varying cell yield coefficient Y for (*a*) $X_T = 0.82$ mg L^{-1} and (*b*) $X_T = 0.82 \times 10^{-3}$ mg L^{-1}.

The soil samples used for column packing and for analysis of the partitioning coefficients between soil and BT were obtained from the same BT-contaminated site (Kalkaska), although from different soil core samples. Variation of the retardation factor over the range of measured values had a significant influence on the timing of the breakthrough and a lesser influence on the concentration minimum (Figures 8*a* and 8*b*). Completely mixed batch reactor experiments yielded Ra values of 1.6 for toluene and 1.4 for benzene, and short packed-bed continuous-flow column reactor measurements yielded 2.1 for toluene and 1.6 for benzene.

Whether a simplified modeling approach could capture the dominant processes observed in the column experiments was also investigated. A number of alternative approaches have been proposed to model microbial degradation. First-order substrate utilization, without microbial growth or decay, is perhaps the simplest approach. To illustrate the importance of incorporating biomass growth and decay in these column simulations, a simplified model based on first-order kinetics was developed. Assumptions of this model include no mass transfer resistance, no oxygen-limited conditions, and first-order biodegradation kinetics with respect to the substrate concentration. Under such conditions, (6) and (10) for single substrate biodegradation can be rewritten as

$$\left(1 + \frac{\rho_b K_D}{n S_w}\right) \frac{\partial}{\partial t} (\rho^w \omega^w) + \frac{\partial}{\partial z} (V^w \rho^w \omega^w) - \frac{\partial}{\partial z} \left[D^w \frac{\partial}{\partial z} (\rho^w \omega^w) \right] = -k' \rho^w \omega^w \qquad (13)$$

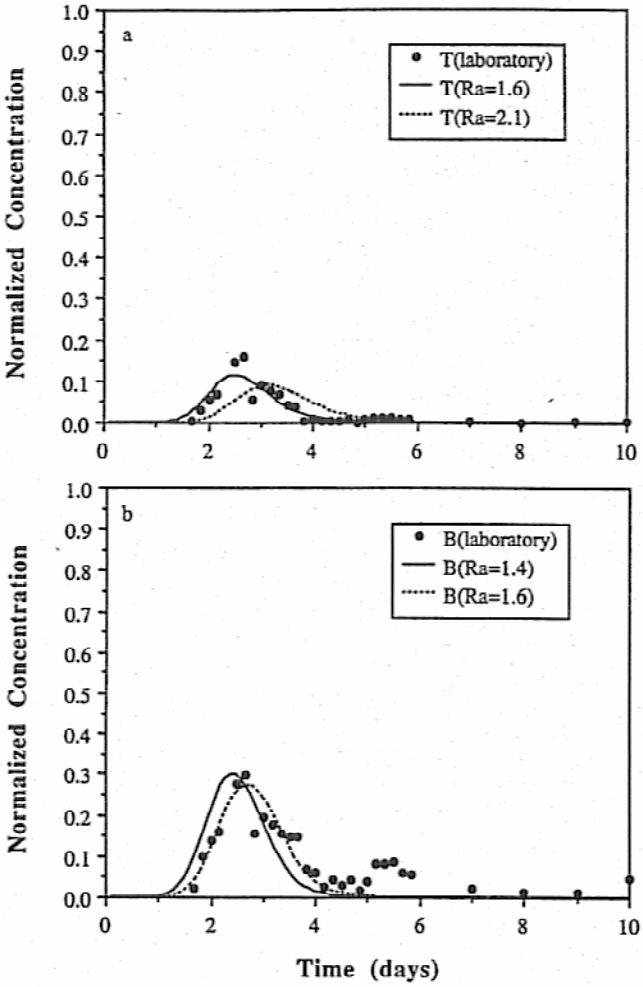

Fig. 8. Sensitivity of model predictions to varying retardation factor Ra for (a) toluene and (b) benzene.

$$\frac{dX_m}{dt} = (k' Y \rho^w \omega^w - k_d) X_m \qquad (14)$$

The first-order decay rate k' can be approximated as kX_m/K_s. Note that this is a function of X_m. If one assumes a steady state biomass concentration (i.e., (14) equal to zero) at the measured value of 0.81 mg L^{-1} for the toluene degrader, (13) underpredicts the toluene degradation and overpredicts the breakthrough concentration (dashed curve in Figure 9). If microbial growth and decay is included in the model (coupled solution of (13) and (14)), the effluent breakthrough simulation for toluene is in much closer agreement with the laboratory data (dotted curve in Figure 9). However, a full model simulation, which includes liquid film resistance and Monod kinetics, matches the long-term and short-term laboratory data more closely (solid curve in Figure 9).

An alternative modeling approach is to neglect kinetics and treat the substrate biodegradation as an instantaneous reaction which follows a stoichiometric relationship with oxygen. When oxygen is not limiting, such a model would predict that any substrate in contact with oxygen would be completely degraded. Using such a model, benzene and toluene would be predicted to be completely degraded at the column inlet. Clearly, such a model could not be used to simulate the observed laboratory effluent data.

Model sensitivity to boundary conditions was also explored. Changing the inlet boundary condition from a third-type to a first-type condition did not significantly affect the simulated effluent breakthrough concentration for this example (Figure 10a). Note that the boundary condition, however, has a dramatic impact on the biomass growth at the inlet (Figure 10b). A first-type condition essentially supplies unbounded substrate and electron acceptors for biomass growth at the boundary. The third-type condition, which limits the advective and dispersive flux of mass across the boundary, is clearly more physically accurate. A discrepancy in column breakthrough simulations using these alternative boundary conditions might occur under other circumstances (e.g., a shorter column or a sudden increase in influent concentration). These simulations illustrate the importance of careful evaluation of boundary conditions in applying biodegradation models.

CONCLUSIONS

In this paper a mathematical model describing the transport and biotransformation of BTX compounds in sandy aquifer environments was developed. This model accounts for transport and degradation processes in the unsaturated soil zone and models two substrates, two electron acceptors, one limiting nutrient, and two species populations of indigenous microbes. The model also incorporates the sensitivity of the nitrate-respiring organism to oxygen. As an initial step in model validation, model simulations of toluene and benzene degradation in saturated aquifer columns under aerobic and nitrate addition conditions were compared to laboratory data. Microbial, chemical, and hydraulic parameters for these simulations were obtained independently from batch and column studies.

Simulation results demonstrated that, when the electron acceptor was not limiting, the model could predict the one-dimensional transport and biodegradation of toluene and

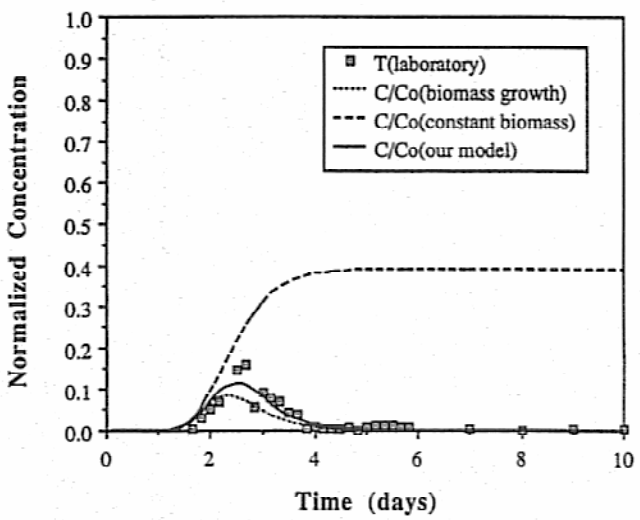

Fig. 9. Intermodel simulation comparisons for toluene: dashed curve, first-order substrate decay and steady state uniform biomass concentration; dotted curve, solution of (13) and (14) includes biomass growth; solid curve, full model (equations (6)–(10)) includes biomass growth and liquid film resistance.

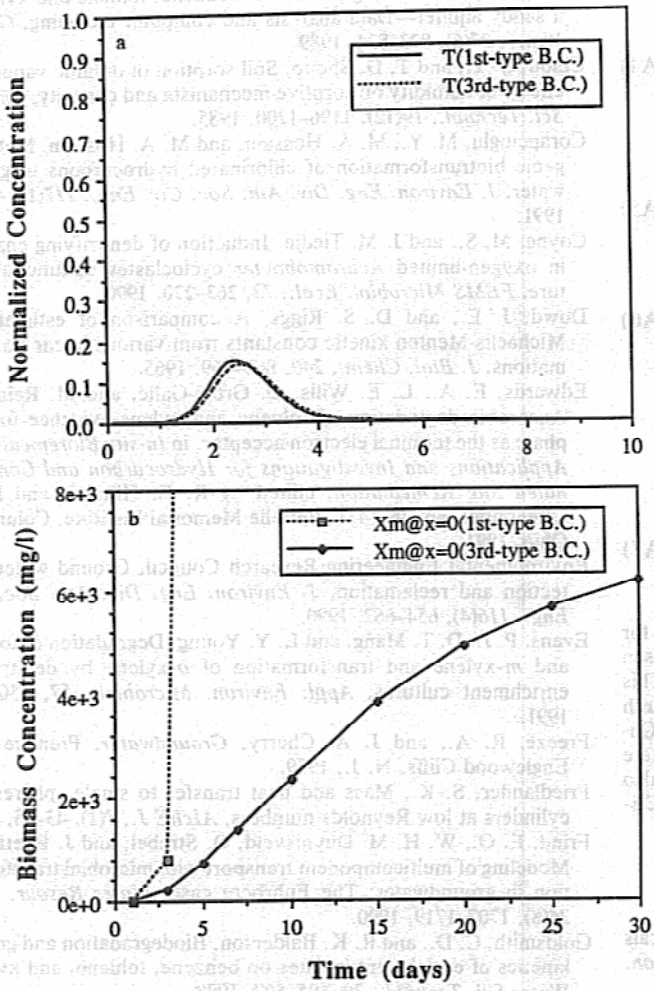

Fig. 10. Simulation comparisons for first- and third-type boundary conditions for (*a*) effluent toluene concentrations and (*b*) biomass growth at $x = 0$.

benzene. This suggests that the mechanisms incorporated in the model can accurately describe the column system under such conditions. Model predictions for toluene concentrations were reasonable for conditions under which nitrate acted as an electron acceptor, even though microbial kinetic parameters had been derived from those measured under aerobic conditions.

Under electron acceptor limiting conditions, however, the model did not provide accurate predictions of concentrations, although overall trends were modeled. Possible reasons for this model inadequacy may be (1) the inappropriateness of the assumption of benzene and toluene degrader independence, (2) incomplete metabolism of benzene, resulting in a lower oxygen use coefficient, and (3) the inappropriateness of the kinetic constants under electron acceptor limiting conditions.

A parameter sensitivity analysis revealed that k and K_s values must be measured from aquifer slurry experiments with mixed indigenous cultures to accurately model the column data. Measuring parameters using isolated cultures does not appear to be a valid approach. In addition, model prediction of the breakthrough concentration was sensitive to the initial active biomass concentration.

Model simulations also showed that neither a simplified model using first-order kinetics nor a model which assumes that the removal of organic occurs as an instantaneous reaction could accurately predict the column behavior. The importance of employing the appropriate boundary condition at the column inlet was also illustrated. Inappropriate application of a first-type condition to a flux-limited situation yielded unlimited (and unrealistic) biomass growth at the inlet.

This work has illustrated the utility of a comprehensive experimental/modeling approach for the enhancement of our understanding of biodegradation processes in porous media. Simulation results have revealed that further conceptual model refinement will be necessary for the accurate prediction of multisubstrate degradation, particularly under electron acceptor limiting conditions. Extension of the model to two dimensions will facilitate the application of the insight gained from these laboratory studies to the exploration of alternative field remediation schemes.

APPENDIX A: DISCRETIZATION OF THE SINK TERM

Application of variable time weighting to the finite element expansion of the sink term in equation (6) yields

$$\sum_r \left\{ \int_{\Omega^e} \frac{k_i A_c}{m_c} \sum_s \left[(\rho^w \omega_{is}^w N_s - C_{is} N_s) \sum_k (X_{mk} N_k) \right] N_r \, d\Omega \right\}$$

$$= \theta \sum_r \left\{ \int_{\Omega^e} \frac{k_i A_c}{m_c} \sum_s \left[((\rho^w \omega_{is}^w)^{t+1} - (C_{is})^{t+1}) N_s \cdot \sum_k (X_{mk}^{t+1} N_k) \right] N_r \, d\Omega \right\} + (1 - \theta) \cdot \sum_r \left\{ \int_{\Omega^e} \frac{k_i A_c}{m_c} \sum_s \left[((\rho^w \omega_{is}^w)^t - (C_{is})^t) N_s \sum_k (X_{mk}^t N_k) \right] N_r \, d\Omega \right\} \quad \text{(A1)}$$

where $r, s, k \in [1, nn]$; $e \in [1, nn - 1]$; nn is the total number of elements; N is the basis function; θ is the time weighting factor; and t and $t + 1$ represent the old and new time levels, respectively.

APPENDIX B: APPLICATION TO THE GROWTH EQUATION OF A VARIABLE-TIME-WEIGHTED FINITE DIFFERENCE OPERATOR

The weak form of the growth equation (10) is

$$\sum_r \int_{\Omega^e} \left\{ \sum_s \left[\frac{dX_{is}}{dt} - M_i \sum_k (C_{ik} N_k X_{is}) \right] N_s \right\} N_r \, d\Omega = 0 \quad \text{(A2)}$$

where

$$M_i = \sum_j \left\{ k_j^i Y_j^i \left(\frac{1}{K_{s_{ij}} + C_i} \right) \left(\frac{C_j}{K_{a_{ij}} + C_j} \right) \cdot \left(\frac{C_A}{K_{A_{ij}} + C_A} \right) II^{ij}(C_o) - k_{dj}^i \right\} \quad \text{(A3)}$$

The matrix form of (A2) is

$$[A]\left\{\frac{dX_i}{dt}\right\} + [B]\{X_i\} = 0 \qquad \text{(A4)}$$

where

$$[A] = \sum_r \sum_s \int_{\Omega^e} N_s N_r \, d\Omega \qquad \text{(A5)}$$

$$[B] = \sum_r \sum_s \int_{\Omega^e} M_i \sum_k C_{ik} N_k N_s N_r \, d\Omega \qquad \text{(A6)}$$

The variably weighted form of (A4) is

$$\frac{[A]^{t+1}}{\Delta t}\{X_i\}^{t+1} - \frac{[A]^t}{\Delta t}\{X_i\}^t + \theta[B]^{t+1}\{X_i\}^{t+1} + (1-\theta)[B]^t\{X_i\}^{t+1} = 0 \qquad \text{(A7)}$$

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