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Coupling transport and biodegradation of toluene and trichloroethylene in unsaturated soils

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Abstract. Trichloroethylene (TCE), a common groundwater pollutant generally resistant to aerobic biodegradation, can be cometabolized in the presence of another compound such as toluene. The coupled transport and biodegradation of TCE and toluene was investigated and modeled in laboratory soil columns. Toluene biodegradation was linked to microbial growth using Monod kinetics, while TCE degradation was described using Michaelis-Menten kinetics modified to account for changing enzyme levels. Biodegradation of TCE was modeled as a mass fraction of the toluene degradation rate. Both growth and decay were incorporated into the equations to model microbial population dynamics. With the exception of the initial biomass, a single set of parameters to describe both degradation functions was obtained from independent soil batch experiments. Physical parameters were obtained from sterile soil columns. The initial biomass declined from the inlet to the outlet side of the chamber. Toluene was fully degraded in the soil column with the majority occurring closest to the inlet chamber. A substantial amount of TCE was not degraded because it diffused faster and was transformed at a lower rate than toluene.

1. Introduction

The fate of volatile organic compounds (VOCs) in the subsurface is influenced by the simultaneous occurrence of biotic and abiotic processes that together determine the distribution and transformation of these compounds. The behavior of these chemicals in soils is particularly complex because these substances are distributed among the solution, gas, and solid components of the soil matrix. The interactions among the various processes make it difficult to accurately describe the fate of VOCs in unsaturated soils. For instance, the biodegradation rate of VOCs is often dependent on chemical concentrations in the soil solution. This solution concentration is governed by the chemical distribution between the gas and liquid phases (Henry's law coefficient) and between soil solids and solution (sorption partition coefficient). Transport on the pore scale (diffusion) or on the column scale (diffusion and advection) also determines the chemical concentrations over time and space. In turn, these physical processes, which are influenced by chemical concentration, can be altered by biodegradation, depending on how and at what rates a chemical is distributed among the components of soil. In order to predict the fate of VOCs in soil, a thorough understanding is needed of the complex interactions among the different processes that affect chemical concentrations.

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Under aerobic conditions, microbial degradation of TCE occurs only in the presence of another compound, such as toluene, that can support microbial growth. This cometabolic interaction between these two chemicals has been extensively studied over the past decade [e.g., *Wackett and Gibson*, 1988; *Nelson et al.*, 1988; *Fan and Scow*, 1993]. Many of the previous studies have been conducted under batch or steady state flow conditions. Batch systems de-emphasize interactions between biodegradation and physical processes [*Fan and Scow*, 1993; *Chang and Alvarez-Cohen*, 1995a, b] because they consider the net change in chemical concentration over time, most of which is attributed to consumption by microorganisms. On the other hand, in steady-flow systems, chemical concentrations remain constant leading to biodegradation functions which are overly simplified and are often not reflective of environmental conditions [*Baehr and Baker*, 1995; *Lahvis and Baehr*, 1996; *Bekins et al.*, 1993]. There have been theoretical considerations of pollutant transport under transient conditions where model parameters were estimated from parallel experiments [e.g., *Chen et al.*, 1992] or obtained from the literature [e.g., *Kindred and Celia*, 1989].

The focus of this work is to assess and model the biodegradation kinetics of toluene and the cometabolism of TCE under transient transport conditions. Microbial population kinetics previously characterized in soil batch experiments [*El-Farhan*, 1997] were incorporated into a model describing the coupled diffusive transport and biodegradation of toluene and TCE vapors in unsaturated soil columns. Experimental results describing this coupled system were compared to numerical simulations with independently obtained parameters.

2. Methods for Column Experiments

The apparatus used in this study was a two-chamber diffusion cell, shown in Figure 1 [*Glauz and Rolston*, 1989]. The apparatus consists of a soil column with air chambers attached at each end. The inlet chamber is equipped with a piston

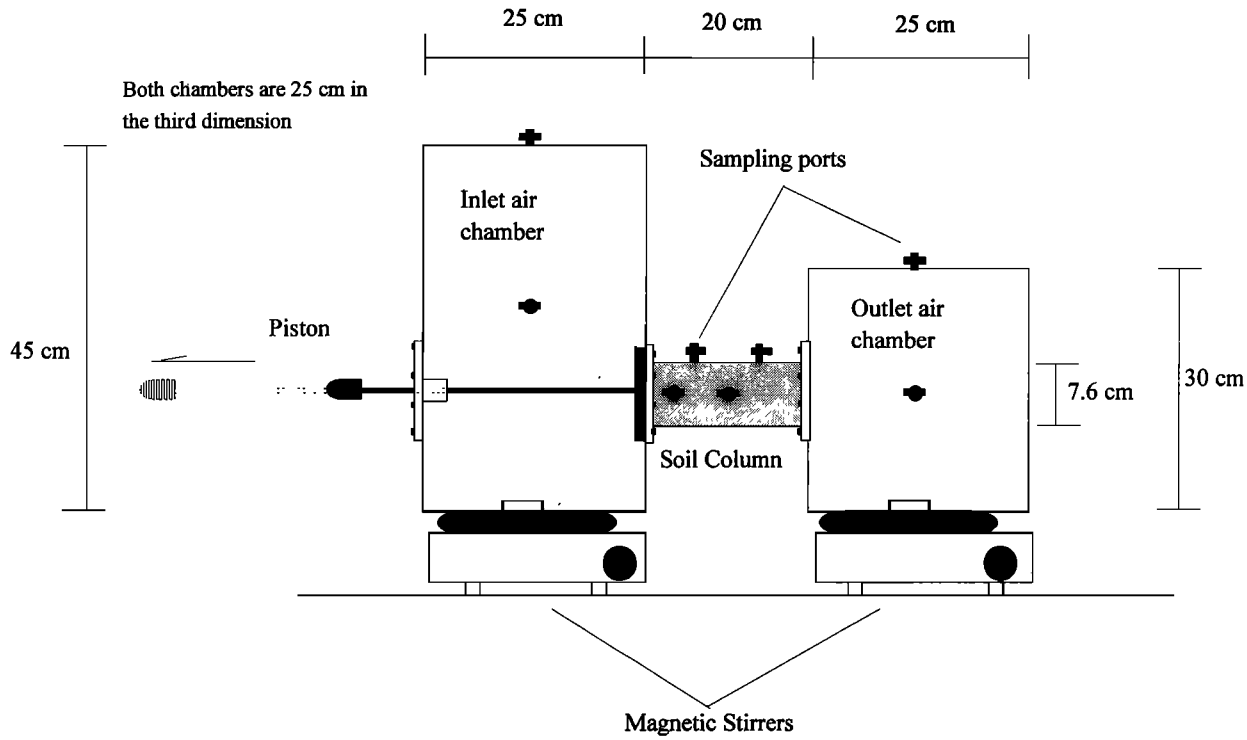

Figure 1. A schematic of the two-chamber diffusion cell.

mechanism to isolate the chamber from the soil column to permit establishment of initial conditions. Magnetic stirrers inside both chambers maintain them at well-mixed conditions throughout the experiment. Initially, the piston is pushed against the soil column and chemical vapors are injected into the inlet chamber. The vapors are allowed to mix and equilibrate to reach an initial concentration (C_0) in this chamber. The experiment is started by pulling the piston back and allowing the gas to diffuse through the soil column towards the outlet chamber. Gas samples are taken from the inlet and outlet chambers and from column sampling ports placed 2.5, 5, 10, and 15 cm from the inlet chamber. Gas samples from the end chambers are taken using a 1-mL gas-tight syringe (Hamilton Company, Reno, Nevada) and analyzed using a Hewlett-Packard 5890A gas chromatograph (GC) (Hewlett Packard Company, Palo Alto, California) equipped with a flame ionization detector (FID) and a 10-foot (~3m) packed column 20% SP-2100, 0.1% Carbowax 1500, on 100/120 mesh Supelcoport (Supelco Incorporated, Bellefonte, Pennsylvania) run isothermally at 140°C. Gas samples from the soil column are taken using a 50-μL Hamilton gas-tight syringe and analyzed with an SRI GC (SRI Company, Torrance, California), equipped with an FID and a 30-m fused silica capillary column, 0.53 mm inside diameter, 3.0 mm film thickness (Supelco), run isothermally at 100°C.

The effective inlet and outlet chamber lengths are approximately 720 and 380 cm, respectively (effective length is equal to chamber volume divided by the cross-sectional area of the soil column). Chamber sizes were selected using the error analysis from *Glauz and Rolston* [1989] with the goal of having chemical concentrations gradually change in the system over the duration of the experiment.

Each soil column, 20 cm in length and 7.6 cm in internal diameter, was prepared by attaching 7-cm extensions to both ends and packing the entire length with air-dry Yolo silt loam (fine-silty, mixed, nonacid, thermic Typic Xerorthent) to a bulk density of 1.33 g/cm^3. The indigenous microbial community in this soil readily degrades toluene and cometabolizes TCE, as summarized in previous batch studies [*Fan and Scow*, 1993]. Water was added uniformly to either end of the packed soil to bring it to a volumetric water content (θ) of approximately 0.25 cm^3 water/cm^3 soil, giving an air-filled porosity of 0.25 cm^3 air/cm^3 soil. The column was allowed to equilibrate horizontally with periodic reorientation in a cold room (4°C) for 12–16 weeks, after which the two 7-cm end columns were cut off and discarded, while the middle column was used in the experiment. Uniformity of packing was checked using gamma attenuation methods [*Grismer et al.*, 1986], and similarity of water content in the discarded columns was checked at the end of the equilibration period, as was the soil column itself at the end of experiments.

To minimize the lag period associated with microbial growth and enzyme induction, the columns (attached to the chambers) were exposed to a continuous, 100% relative humidity air flow of 0.1 L/min containing a toluene concentration of 40 mg/L for a period of 5–7 days, which is the time when degradation started. This preacclimation time period is slightly longer than the lag periods of approximately 80 hours observed in the batch experiments studying biodegradation kinetics in this soil [*Fan and Scow*, 1993; *Mu and Scow*, 1994]. At the conclusion of preacclimation the inflow toluene concentration was 40 mg/L, whereas the outflow toluene concentration was approximately 35 mg/L. To start an experiment, toluene was flushed out from the soil column with clean air, and the desired chemical ratio was injected in the vapor phase into the inlet chamber; then the experiment started. The effectiveness of the flushing process was checked by taking gas samples from the soil column 12 hours after flushing was stopped.

Duplicate experiments were conducted for three chemical concentration ratios of toluene to TCE: 20:1, 40:1, and 60:1. The 60:1 experiments were conducted in an apparatus that did not contain a sampling port 2.5 cm away from the inlet chamber. Chemical concentrations in the gas phase reflecting these ratios were established in the inlet chamber before starting each experiment. The different ratios were obtained by maintaining the TCE concentration at 1 mg/L air and changing the gas toluene concentration (approximately 14, 29, and 40 mg toluene/L for the 20:1, 40:1, 60:1 experiments, respectively). These toluene and TCE gas concentrations result in the desired ratios in the liquid phase. These ratios were selected because batch studies showed these concentrations to represent a broad range in the kinetics of the degrading microbial populations [*Fan and Scow*, 1993].

Certified toluene and TCE were obtained from Fisher Scientific (Fair Lawn, New Jersey). Soil-liquid and liquid-vapor partition coefficients for toluene and TCE on Yolo silt loam were obtained using the equilibrium partitioning in closed systems method (EPICS) [*Petersen et al.*, 1994].

3. Theoretical Development and Application of Numerical Model

Chemical vapors diffusing through the soil column undergo sorption, dissolution, and biodegradation. Data collected under these conditions will reflect the simultaneous occurrence of these processes. Assuming that TCE and toluene behave singularly as trace gases, their diffusion in soil can be described by Fick's law. The one-dimensional equation for soil [*Petersen et al.*, 1994] with sinks is

$$\varepsilon \frac{\partial C}{\partial t} = D_p \frac{\partial^2 C}{\partial x^2} \pm \left(\frac{dC}{dt}\right)_{\text{Sink Terms}} \tag{1}$$

where C is the gas-phase concentration (mg VOC/L air), ε is the air-filled porosity (cm^3 air/cm^3 soil), D_p is the effective soil-gas diffusivity ((cm^3 air) (cm soil)$^{-1}$ (day)$^{-1}$), t is time (day), and x is distance (cm). The sink terms in the above equation include sorption, dissolution, and biodegradation.

Sorption is calculated using an equilibrium, linear adsorption isotherm given by

$$C_{\text{solid}} = K_d C_{\text{liquid}} \tag{2}$$

where K_d is the solid/aqueous partition coefficient (cm^3/g). For TCE and toluene, K_d is equal to 0.58 and 0.87 [*Petersen et al.*, 1994], respectively, for the Yolo silt loam used in the experiments. The linearity of the isotherm and the equilibrium condition were verified in batch and sterile soil column experiments [*Petersen et al.*, 1994].

Partitioning into water is calculated by equilibrium Henry's law partitioning using

$$C_{\text{gas}} = HC_{\text{liquid}} \tag{3}$$

where H is Henry's law dimensionless coefficient, with values equal to 0.261 and 0.397 for toluene and TCE, respectively [*Petersen et al.*, 1994].

Using (2) and (3), (1) was rewritten in the following form

$$R\varepsilon \frac{\partial C}{\partial t} = D_p \frac{\partial^2 C}{\partial x^2} - \left(\frac{dC}{dt}\right)_{\text{Biodegradation}} \tag{4}$$

where R is the retardation factor, calculated according to [*Petersen et al.*, 1994]

$$R = 1 + \frac{\theta}{\varepsilon H} + \frac{\rho_b K_d}{\varepsilon H} \tag{5}$$

where θ is the soil-water content (cm^3 water/cm^3 soil) and ρ_b is the bulk density (g soil/cm^3).

During the first part of this study, sterilized soil columns were used to establish a relationship between the effective diffusion coefficient (D_p) and the air-filled porosity for the Yolo silt loam used in our experiments. The resulting relationship is given by [*Troeh et al.*, 1982]

$$D_p = \left(\frac{\varepsilon - u}{1 - u}\right)^v D_0 \tag{6}$$

where D_0 is the diffusion coefficient in air, $u = 0.12$, and $v = 1.23$ [*Petersen et al.*, 1994]. For TCE, $D_0 = 7214$ cm^2/d, and for toluene $D_0 = 6955$ cm^2/d.

Toluene degradation was calculated by modeling microbial growth across the column using Monod kinetics and linking that to chemical consumption. The set of equations used to achieve this are

$$R^{\text{tol}}\varepsilon \frac{dC^{\text{tol}}}{dt} = D_p^{\text{tol}} \frac{\partial^2 C^{\text{tol}}}{\partial x^2} - \frac{\theta}{Y^{\text{tol}}}\left(\frac{dB}{dt}\right)_{\text{growth}} \tag{7}$$

and

$$\left(\frac{dB}{dt}\right)_{\text{growth}} = \frac{\mu_{\max}^{\text{tol}} C^{\text{tol}}}{H^{\text{tol}} K_s^{\text{tol}} + C^{\text{tol}}} B \tag{8}$$

where the superscript tol designates using values for toluene, B is the biomass (mg/L liquid), $\mu_{\max}^{\text{tol}}$ is the maximum specific growth rate (1/day), K_s^{tol} is the Monod half-saturation constant for toluene (mg toluene/L liquid), and Y^{tol} is the yield coefficient (mg biomass/mg toluene). Note that (8) is the Monod equation written in terms of the gas-phase concentration using Henry's law.

Because TCE is not a growth compound, its degradation was modeled using a Michaelis-Menten equation modified to incorporate changes in microbial populations (and thus enzyme levels) [*Alvarez-Cohen and McCarty*, 1991; *Criddle*, 1993] according to

$$R^{\text{TCE}}\varepsilon \frac{dC^{\text{TCE}}}{dt} = D_p^{\text{TCE}} \frac{\partial^2 C^{\text{TCE}}}{\partial x^2} - \theta \frac{v_{\text{eff}} C^{\text{TCE}}}{H^{\text{TCE}} K_m^{\text{TCE}} + C^{\text{TCE}}} \tag{9}$$

where the superscript TCE designates using values for TCE, and K_m^{TCE} is the Michaelis-Menten half-saturation constant for TCE (mg/L liquid). v_{eff} is the maximum effective rate of TCE degradation ((mg TCE) (L liquid)$^{-1}$ (day)$^{-1}$) which can be directly related to toluene degradation and biomass growth by a variety of models [e.g., *Anderson and McCarty*, 1994; *Chang and Alvarez-Cohen*, 1995a; *Criddle*, 1993]. The model proposed by *Criddle* [1993] was used here (equation (10)) because it required the least number of parameters, which were already determined from batch experiments [*El-Farhan*, 1997].

$$v_{\text{eff}} = \frac{1}{Tr_{\text{TCE}}}\left[\frac{1}{Y^{\text{tol}}}\left(\frac{dB}{dt}\right)_{\text{growth}}\right] \tag{10}$$

where Tr_{TCE} is the transformation coefficient (mg toluene/mg TCE). According to (10), TCE degradation will stop with the depletion of toluene from the apparatus.

Table 1. A Summary of the Parameters Used to Describe Toluene Degradation

Parameter	Value	n*
μ_{max}^{tol}, 1/day	3.22 (0.76)	20
K_s^{tol}, mg toluene/L liquid	1.95 (1.71)	8
K_i, mg TCE/L liquid	2.73 (1.89)	16
Y^{tol}, mg biomass/mg toluene†	0.35	...
b, 1/day	1.43 (0.8)	20

The standard deviation is given in parenthesis.
*Number of batch experiments used to obtain the parameter values and the standard deviation (taken from *El-Farhan* [1997]).
†From *Mu and Scow* [1994].

A major effect of TCE concentration on toluene degradation is competitive inhibition when the toluene-to-TCE ratio approached 1 [*Fan and Scow*, 1993; *Mu and Scow*, 1994]. The competitive inhibition effect was modeled by defining an apparent Monod half-saturation constant ($K_s'^{tol}$) to replace the K_s^{tol} in (8) [*Criddle*, 1993]:

$$K_s'^{tol} = K_s^{tol}\left(1.0 + \frac{C^{TCE}}{H^{TCE}K_i}\right) \tag{11}$$

where K_i is the TCE inhibition coefficient (mg TCE/L liquid).

The net biomass change was described by the formulation used by *Bekins et al.* [1993], which is given in (12), below. The first term accounts for growth as a result of toluene consumption. The second term accounts for biomass decay. In order to determine if TCE deactivation effects on biomass are significant, the rate of deactivation was estimated for the 20:1b experiment and compared to the biomass decay rate [*Chang and Alvarez-Cohen*, 1995a]. In the 20:1b experiment, approximately 560 mg toluene and 9 mg TCE were transformed over 400 hours. Using the Y^{tol} and Tr_{TCE} values given in Tables 1 and 2, the specific biomass deactivation rate (i.e., rate of biomass deactivation/biomass) was estimated to be on the order of 0.002 per day. This rate is insignificant in comparison to the biomass decay rate b, shown in Table 1. Consequently, biomass deactivation was not incorporated in (12):

$$\frac{dB}{dt} = \frac{\mu_{max}^{tol}C^{tol}B}{H^{tol}K_s^{tol} + C^{tol}} - bB \tag{12}$$

where b is the biomass decay rate (1/day).

Equations (4)–(12) were used to model coupled TCE and toluene transport and biodegradation in the unsaturated soil columns using an implicit, finite difference iterative scheme. The space step was 0.1 cm, and the time step was varied to maintain mass conservation. The boundary conditions for the soil column are the well-mixed end chambers. Model simulations maintained mass balance within 0.01% for every time step; boundary fluxes were smooth over time; and simulation results were independent of time and space step sizes. Parameters for describing biodegradation of toluene and TCE are shown in Tables 1 and 2, respectively. These parameters were obtained from batch experiments [*Fan and Scow*, 1993] where similar degradation functions were used to model toluene and TCE degradation [*El-Farhan*, 1997]. Column data were modeled by fitting the initial biomass (B_0) and by allowing biodegradation parameters obtained from batch experiments to vary within their standard deviation. A general-purpose optimization code utilizing the Levenberg-Marquardt algorithm to minimize the root mean square error (RMSE) between model and measurements was used to fit the data [*Clausnitzer and Hopmans*, 1991].

Table 2. A Summary of the Parameters Used to Describe TCE Degradation

Parameter	Value	n*
K_m^{TCE}, mg TCE/L liquid	2.8 (1.66)	7
Tr_{TCE}, mg toluene/mg TCE	27.73 (10.89)	6

The standard deviation is given in parenthesis.
*Number of batch experiments used to obtain the parameter values and the standard deviation (taken from *El-Farhan* [1997]).

4. Results

4.1. Toluene

Toluene data collected from three toluene to TCE concentration ratios (20:1, 40:1, and 60:1) are shown in Figures 2, 3, and 4, respectively. Duplicate experiments (a and b) for each concentration ratio were generally similar, with the exception of the 20:1 experiments, where the toluene column concentrations in Figure 2a are lower than those in Figure 2b. This difference can be explained by different preacclimation procedure for the two columns. The column used for Figure 2a was pre-exposed to toluene for 7 days, whereas the column in Figure 2b had only 5 days of pre-exposure. Though both experiments at the 20:1 ratio started with similar concentrations,

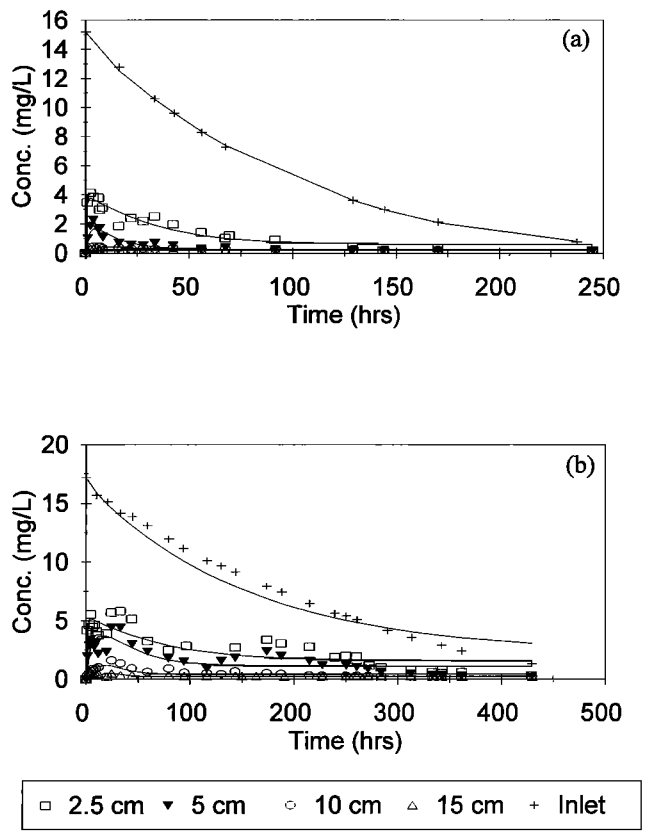

Figure 2. Replicates of the 20:1 experiment showing toluene concentrations at different locations in the apparatus. The symbols represent data and the lines represent model predictions.

they differed in the time it took for toluene to diffuse out of the inlet chamber. Such a difference would be expected if there were differences in the degradation rates in the column, which may well have resulted from the different preacclimation times.

In all the experiments the maximum concentrations of toluene observed at the port closest to the inlet chamber were low in relation to the starting concentration in that chamber, reflecting the high rates at which the soil microbial community could degrade toluene. Some oscillations were observed in the data, especially in the 20:1 experiments. These oscillations became less pronounced as the initial toluene concentrations were increased, suggesting that the oscillations may have been due to the competitive inhibition of toluene degradation by TCE when the ratio of the two compounds approached unity.

Model fits (i.e., (7), (8), and (12)) to data describing the disappearance of toluene are also shown in Figures 2–4. In general, model predictions were equivalent to or underestimated the toluene concentrations in the column. Model simulations were unable to capture the oscillations observed in the data, which were most evident in the 20:1 experiments.

The fitted values to the initial biomass (B_0) at different locations in the column for all experiments are shown in Table 3. The fitted B_0 for the first 20:1 experiment (Table 3, 20:1a) were lower than those for the second 20:1 experiment (Table 3, 20:1b). Values at the first port for the two 20:1 experiments were approximately 550 and 300 mg (L soil solution)$^{-1}$, respectively, which are equivalent to approximately 1.03×10^8 and 6.2×10^7 cells/g soil (assuming a cell density of 1 g/cm^3 and a cell volume of 1 μm^3 and using measured θ and ρ_b values of 0.25 cm^3 water/cm^3 soil and 1.33 g/cm^3, respectively). Again, these differences are consistent with the differences in the pre-exposure histories of the two experiments. The differences in B_0 within each experiment (Table 3) were within 1 order of magnitude, which is small relative to the 5–6 order of magnitude difference between our preacclimated soil and Yolo soil that had not been acclimated to toluene. *Mu and Scow* [1994]

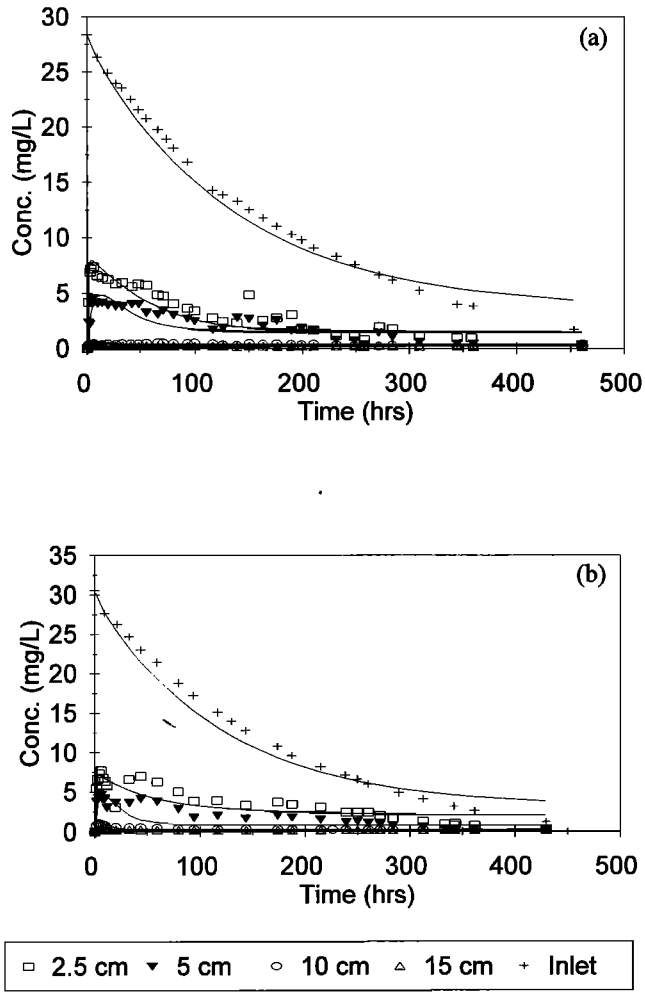

Figure 3. Replicates of the 40:1 experiment showing toluene concentrations at different locations in the apparatus. The symbols represent data and the lines represent model predictions.

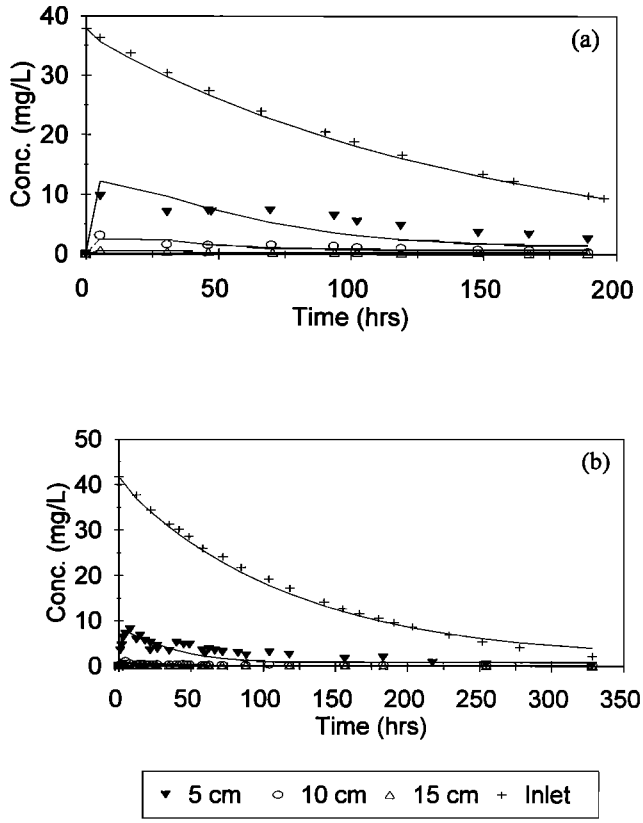

Figure 4. Replicates of the 60:1 experiment showing toluene concentrations at different locations in the apparatus. The symbols represent data and the lines represent model predictions.

Table 3. Fitted B_0 Values at Each Port for the Different Experiments

Experiment	2.5-cm Port	5-cm Port	10-cm Port	15-cm Port
20:1a	551.6	68.25	44.08	NA
20:1b	304.0	4.5	28.8	NA
40:1a	508.85	35.2	123.4	NA
40:1b	1006.8	48.2	62.3	24.0
60:1a	···	315.9	208.1	NA
60:1b	···	691.1	188.0	NA

NA, fitted values were below 10^{-6} mg/L due to low measured toluene concentrations at this port. B_0 values in mg/L.

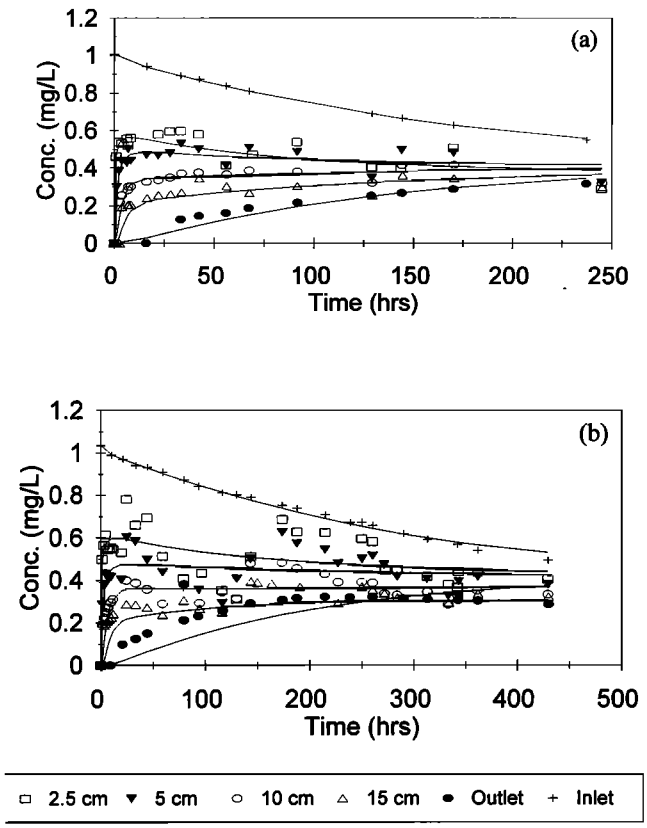

Figure 5. TCE data for the 20:1 experiment at different locations in the apparatus. The symbols represent the data and the lines represent model predictions. (a) Data corresponding to Figure 2a and (b) to Figure 2b.

measured a B_0 value for this soil equal to 4×10^2 cells/g soil prior to exposure to toluene.

The spatial distribution in B_0 for each experiment was used as model input parameters for simulating the toluene concentrations in the inlet chamber. The model dramatically overpredicted toluene disappearance in this chamber, indicating that the average degradation rate in the column was overestimated. This may be the result of representing the B_0 distribution across the column with the single discrete values given in Table 3. These biomass values are averages for each interval of soil (i.e., 0–3.25 cm, 3.25–7.5 cm, 7.5–12.5 cm, and 12.5–20.0 cm), whereas the biomass distribution in the soil column would actually be a continuous function. Consequently, the inlet chamber was simulated by allowing μ_{max}^{tol} to be fitted outside the range of one standard deviation around the mean. The fitted μ_{max}^{tol} values for the two 20:1 experiments in Figures 2a and 2b were 1.32 and 1.48 $(day)^{-1}$, respectively, which are approximately half of the values obtained from batch experiments (Table 1). Similar results were obtained for the 40:1 and 60:1 experiments.

The model generally overpredicted the residual toluene concentrations in the system. This can be attributed to the fact that no mathematical limit was imposed on the microbial decay term in (12) because this limit is not known. This permitted the modeled microbial populations within the column to decline to zero levels, whereas they may have persisted in the column at some steady state concentration greater than zero.

4.2. Trichloroethylene Experiments

Trichloroethylene data collected from the 20:1, 40:1, and 60:1 experiments are shown in Figures 5, 6, and 7, respectively. The data showed oscillations paralleling those observed in the toluene data, especially in the 20:1 experiments. Figures 5–7 show that TCE degradation ceases towards the end of each experiment as toluene is depleted from the system. Trichloroethylene data were modeled with (4)–(12) using the B_0 distribution obtained during the toluene analysis. Parameters obtained from batch experiments describing TCE degradation were allowed to vary within one standard deviation. The model was unable to describe the oscillations exhibited by the port data but was able to capture the increasing and decreasing trends in the data as well as the residual TCE concentration at the termination of the experiment. Data from the inlet and outlet chambers were successfully modeled using parameters from batch experiments, with the model slightly underpredicting TCE concentrations in the outlet chamber. In the 60:1 experiments the model underpredicted TCE concentrations at early times at the 15-cm port and the exit chamber (Figure 7), which reflects an overprediction of TCE degradation in the soil column. Though not as dramatic, this difference between the model and data is also detectable in the 20:1 (Figure 5) and 40:1 (Figure 6) experiments. If the relationship linking TCE degradation to toluene (i.e., (10)) overpredicts TCE degradation, we would expect the TCE model to deviate more from the data with increasing toluene concentrations.

The goodness of fit to TCE data depended greatly on good

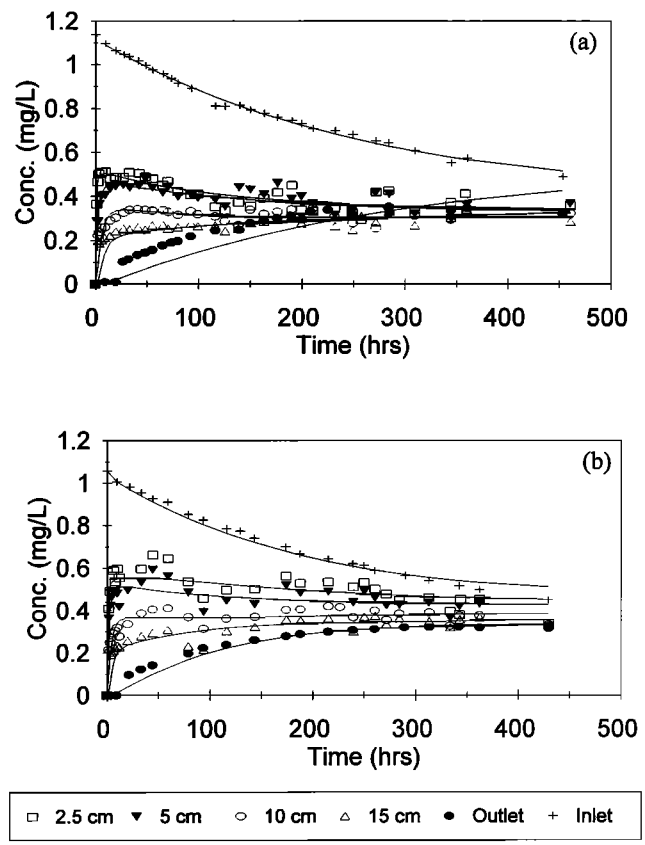

Figure 6. TCE data for the 40:1 experiment at different locations in the apparatus. The symbols represent the data and the lines represent model predictions. (a) Data corresponding to Figure 3a and (b) to Figure 3b.

fits to the toluene data. In the first experiment of the 20:1 ratio (Figure 2a) the model is closer to capturing the behavior of the toluene data and is thus more able to model the TCE data from this experiment (Figure 5a). On the other hand, in the second 20:1 experiment the model underpredicts toluene concentrations (Figure 2b) and thus TCE concentrations (Figure 5b).

The concentration of TCE remaining in the column at the end of the experiment decreased as the initial ratio of toluene to TCE concentration increased. This was expected because the larger mass of toluene present at the higher ratios could support a larger mass of TCE degradation.

5. Discussion

5.1. Toluene

Toluene was readily degraded inside the soil column, and thus the preacclimation process seemed to be sufficient in building up microbial populations and inducing the enzymes needed for toluene degradation. This conclusion is also supported by the relatively high B_0 values fitted for the different experiments. As the initial concentration increased, toluene diffused further into the soil columns but was never detected in the outlet chamber. The increase in toluene concentrations in the soil column at early times indicates that the amount of toluene entering the column by diffusion was greater than the amount that could be biodegraded in sections of the column close in the inlet chamber. With abundant toluene the existing populations were likely saturated with respect to the amount of toluene they could transport across their membranes. Only with additional cell growth in the column sections close to the inlet chamber could more toluene be metabolized before diffusing further in the column.

Another factor contributing to toluene moving past biomass at the inlet side of the column is the high diffusive fluxes at early times in the experiment. Such high fluxes could result in a disequilibrium in chemical distribution between the gas and liquid phases, in which case the toluene concentration in the liquid phase would not reflect the concentrations present in the gas phase. However, this effect would be expected to exist only at very early time periods, when the toluene concentration gradients in the gas phase are very high.

Overprediction of the toluene biodegradation rate in the region near the inlet chamber may be explained by the occurrence of nutrient limitation in that region. Analysis of the nitrate concentrations in soil after the termination of the 40:1 experiment showed that concentrations were lowest in that region (less than 1 μg/g soil). Nutrient limitation diminishes the dependency of the biodegradation function on the carbon source (toluene). *Allen-King et al.* [1996] observed zero-order toluene biodegradation kinetics in sand columns under steady toluene infiltration. The more complex toluene biodegradation kinetics, which they initially observed, collapsed to zero-order kinetics when nitrogen limitations became evident. In our experiments it was not possible to isolate the contribution of nitrogen deficiency to biodegradation kinetics because nitrogen limitation of growth was not explicitly described by the model. Nitrogen effects on toluene biodegradation may be quantified by running soil-batch experiments amended with high toluene concentrations and various nitrate concentrations.

The deviation between model and data should increase from the 20:1 to the 60:1 experiment if the exact parameter values

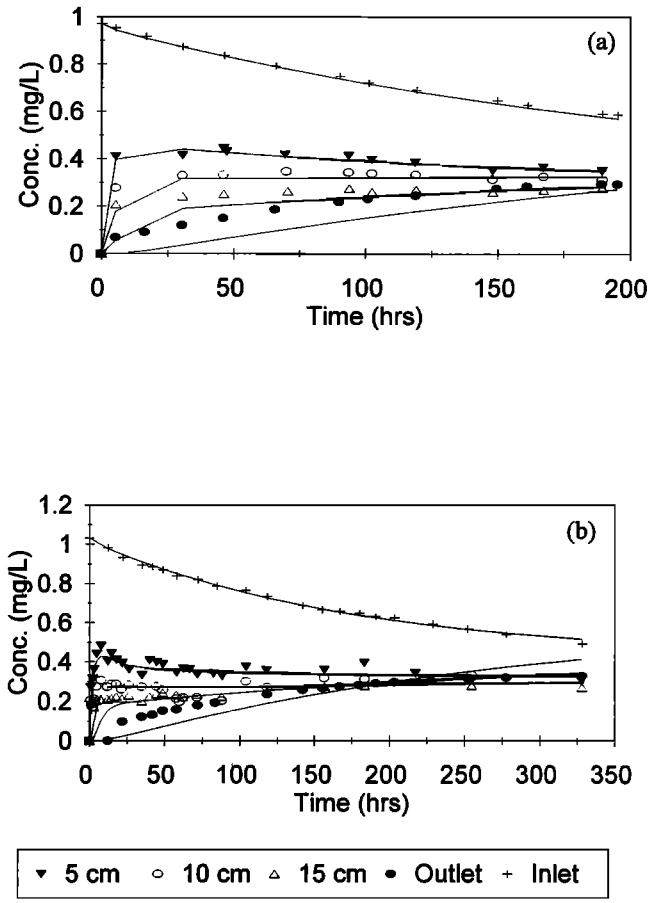

Figure 7. TCE data for the 60:1 experiment at different locations in the apparatus. The symbols represent the data and the lines represent model predictions. (a) Data corresponding to Figure 4a and (b) to Figure 4b.

used to simulate the 20:1 experiment were used to simulate the 60:1 experiment. However, this is not the case. While fitting each experiment, the biodegradation parameters were always allowed to vary within one standard deviation around their averages, while a new initial biomass value was fitted. Thus parameter values used for each experiment were not exactly equal to values used in other experiments. Consequently, we were able to observe that the model generally overpredicts biodegradation in the column but unable to quantitatively observe the increase in the overprediction from the 20:1 to the 60:1 experiment.

Oscillations observed in the toluene column data may have been due to the competitive inhibition effects that TCE has on toluene. When the ratio of the two chemicals approaches unity, toluene degradation has been shown to decrease [*Fan and Scow*, 1993]. When toluene concentrations increase and competitive inhibition becomes less important, then toluene biodegradation begins with consequent decreases in toluene concentrations. In the 20:1 experiments, toluene to TCE concentration ratios inside the soil column often approached unity, which may have led to the oscillations observed. The effects of competitive inhibition would be expected to be greater in the 20:1 than 60:1 experiment, and this was supported by evidence of less oscillations at the higher toluene concentrations. However, the fact that the model does not capture these oscillations may be an indication that competi-

tive inhibition is not a smooth function (as modeled in (11)) but rather a more complex function that involves a threshold toluene-to-TCE concentration ratio where the competitive inhibition mechanism becomes active.

In general, simulations from the model coupling biodegradation, with parameters obtained from batch experiments, to the diffusive transport equations compared well with experimental data. The conceptual model describing biological activity as a series of batch systems in equilibrium with the bulk gas phase seems sufficient for describing the coupled transport and biodegradation under transient conditions in a soil column. Allowing parameters obtained from batch systems to vary within one standard deviation of a mean value was important in the fitting process. To improve the correspondence between μ_{max}^{tol} in the batch and column experiments, nutrient limitation effects on microbial growth kinetics needs to be incorporated into the model. That nutrient-limited growth was present is supported by the fact that the μ_{max}^{tol} values fitted to the inlet chamber data were lower than the average μ_{max}^{tol} value obtained from batch experiments.

The observed residual toluene concentration in the columns dropped below the analytical detection limit by the end of each experiment, yet the model often predicted a finite residual concentration. *Nicol et al.* [1994] noted a similar phenomenon and attributed it to the lack of a lower limit in the model for biomass decay. They set an artificial limit within the model to prevent the biomass from decaying below the initial biomass value. In our work we do not know the lower biomass population limit. Conceptually, the treatment presented by *Nicol et al.* [1994] seems reasonable for representing population decay functions. Their formulation causes the decay term to go to zero as microbial populations approach the lower limit. This results in a steady biomass population that grows as fast as it decays while consuming the growth compound.

5.2. Trichloroethylene

Trichloroethylene port data reflect both the coupled TCE diffusion and biodegradation and the effects of toluene on TCE transformation. Oscillations in the TCE data were typically more evident in the inlet side section of the column, where most of the toluene was degraded, than in regions that toluene never reached. This indicates that these oscillations are linked to the occurrence of toluene degradation.

No measurable TCE degradation occurred once toluene had been depleted throughout the entire column. At first glance, cessation of TCE degradation with removal of its cosubstrate seems contrary to what was observed in pure culture systems [*Alvarez-Cohen and McCarty*, 1991; *Chang and Alvarez-Cohen*, 1995b]. *Alvarez-Cohen and McCarty* [1991] observed continuous TCE transformation in pure culture systems when the cosubstrate (methane) had been removed. *Chang and Alvarez-Cohen* [1995b] were able to study TCE transformation by resting cells induced by, but in the absence of, primary substrates such as methane, propane, toluene, or phenol. In both these studies, high resting-cell populations (and thus high enzyme levels) were used to transform TCE over very short time periods (2–10 hours). In our system the biomass decreased as the toluene was depleted slowly over a period of several weeks. This would result in small induced microbial populations and enzyme levels by the end of each experiment, which would result in a low amount of TCE transformation at the end of each experiment, after toluene is depleted.

The amounts of TCE remaining in our experiments after complete toluene degradation were higher than expected. With the exception of the 20:1 experiments, TCE should have been fully depleted from the apparatus using the average value for the toluene-to-TCE transformation coefficient (Tr_{TCE}). The fact that significant TCE remained in the 40:1 and 60:1 experiment indicates that Tr_{TCE} is dependent on the amount of toluene present. *Chang and Alvarez-Cohen* [1995b] reported competitive inhibition effects of high toluene concentrations on TCE. This would serve to lower the mass of TCE degraded per mass of toluene as toluene concentrations increase. *Fan and Scow* [1993] did not observe significant competitive inhibition effects of toluene on TCE in their soil-batch experiments with similar toluene to TCE ratios. *El-Farhan* [1997] did observe large variations in Tr_{TCE} obtained from those batch experiments.

Any potential effects of competitive or other types of toluene-induced inhibition of TCE degradation fell within a single standard deviation of the estimated biological parameters (from batch experiments) and thus its effects could not be isolated from other sources of variability. To understand whether these inhibition phenomena are important determinants in the kinetics of TCE degradation, it would be necessary to obtain more precise estimates of the biological and physical parameters that determine the transport and biodegradation of TCE.

In conclusion, it was possible to simulate the coupled transport and biodegradation of two interacting VOCs measured under transient conditions in unsaturated soil columns. In all cases a single set of physical and biological parameters, with the exception of initial biomass density, could describe the biodegradation of toluene and TCE at different locations and times in the soil column. All parameters were obtained independently, either from sterile soil columns (for physical parameters) or separate batch experiments (for biological parameters), with the exception of the μ_{max}^{tol} value needed to model the inlet chamber. Model simulations were most successful in describing those experiments when the toluene concentrations were high relative to TCE. As the ratios between the two VOCs decreased, because toluene was degraded more rapidly than TCE, it became increasingly difficult to predict the concentrations of the chemicals in the column. This greater difficulty was due in part to potential interactions between TCE and toluene (as observed in batch systems) and because of analytical limitations.

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