ELSEVIER

Journal of Contaminant Hydrology 50 (2001) 209–223

JOURNAL OF Contaminant Hydrology

www.elsevier.com/locate/jconhyd

Biodegradation during contaminant transport in porous media: 3. Apparent condition-dependency of growth-related coefficients

Li Li [a], Irfan Yolcubal [b], Susannah Sandrin [a], Max Q. Hu [c], Mark L. Brusseau [a,b,*]

[a] *Department of Soil, Water and Environmental Science, The University of Arizona, 429 Shantz Boulevard, Tucson, AZ 85721, USA*

[b] *Department of Hydrology and Water Resources, The University of Arizona, 429 Shantz Boulevard, Tucson, AZ 85721, USA*

[c] *Lawrence Berkeley National Laboratory, The University of Arizona, 429 Shantz Boulevard, Tucson, AZ 85721, USA*

Received 11 August 1999; received in revised form 8 February 2001; accepted 5 March 2001

Abstract

The biodegradation of organic contaminants in the subsurface has become a major focus of attention, in part, due to the tremendous interest in applying in situ biodegradation and natural attenuation approaches for site remediation. The biodegradation and transport of contaminants is influenced by a combination of microbial and physicochemical properties and processes. The purpose of this paper is to investigate the impact of hydrodynamic residence time, substrate concentration, and growth-related factors on the simulation of contaminant biodegradation and transport, with a specific focus on potentially condition-dependent growth coefficients. Two sets of data from miscible-displacement experiments, performed with different residence times and initial solute concentrations, were simulated using a transport model that includes biodegradation described by the Monod nonlinear equations and which incorporates microbial growth and oxygen limitation. Two variations of the model were used, one wherein metabolic lag and cell transport are explicitly accounted for, and one wherein they are not. The magnitude of the maximum specific growth rates obtained from calibration of the column-experiment results using the simpler model exhibits dependency on pore-water velocity and initial substrate concentration (C_0) for

* Corresponding author. University of Arizona, Department of Soil, Water and Environmental Science, 429 Shantz Boulevard, 38, Tucson, AZ 85721, USA. Tel.: +1-520-621-1646; fax: +1-520-621-1647.

E-mail address: brusseau@ag.arizona.edu (M.L. Brusseau).

PII: S0169-7722(01)00111-5

most cases. Specifically, the magnitude of μ_m generally increases with increasing pore-water velocity for a specific C_0, and increases with decreasing C_0 for a specific pore-water velocity. Conversely, use of the model wherein observed lag and cell elution are explicitly accounted for produces growth coefficients that are similar, both to each other and to the batch-measured value. These results illustrate the potential condition-dependency of calibrated coefficients obtained from the use of models that do not account explicitly for all pertinent processes influencing transport of reactive solutes.

Keywords: Biodegradation; Numerical simulation; Growth-related parameters; Contaminant transport

1. Introduction

Contamination of soil and groundwater by organic chemicals has been widely recognized as a major environmental problem. An understanding of the pertinent physical, chemical, and microbiological processes and their interactions is critical for accurate prediction of contaminant transport in the subsurface. In systems wherein biodegradation is coupled with transport, the magnitude and rate of biodegradation is influenced not only by microbial (activity, cell density) and chemical (substrate bioavailability, nutrient availability) factors, but also by physical factors, such as hydraulic residence time, dispersion/diffusion processes, and material heterogeneity. For example, the impact of residence time on the magnitude of biodegradation during transport has been examined by several researchers (Wookeun et al., 1990; Angley et al., 1992; Estrella et al., 1993; Kelsey and Alexander, 1995; Langner et al., 1998; Brusseau et al., 1999a,b). The coupling of hydrodynamic and chemical/microbiological processes results in additional complexity compared to the batch-type systems often used to investigate biodegradation processes in the laboratory.

A large number of mathematical models have been developed to simulate biodegradation and transport of contaminants in porous media. The majority of models incorporate either the first order or Monod equation to describe biodegradation. The latter approach is generally used when a mechanistic representation of biodegradation is desired, although the use of the Monod equation is constrained by a suite of assumptions (e.g., Alexander and Scow, 1989). With the development of mathematical models for coupled-processes transport, several numerical studies have been conducted to investigate the effect of selected factors on biodegradation and transport (e.g., Ying and Weber, 1979; Sykes et al., 1982; Borden et al., 1986; Widdowson et al., 1988; MacQuarrie and Sudicky, 1990; Molz et al., 1990; Chen et al., 1992; Wood et al., 1995; Brusseau et al., 1999a).

Mathematical models are often used to solve the inverse problem to obtain values for selected coefficients. A major concern in this regard is the robustness of the calibrated coefficients. Ideally, these coefficients should be representative of specific processes and independent of given conditions. However, due to a lack of information or resources, simplified models that do not explicitly describe all pertinent processes are often used to simulate transport problems. In such cases, the calibrated coefficients become composite (lumped) parameters that represent multiple processes, and that can therefore be expected to exhibit condition dependency.

The purpose of this paper is to investigate the condition dependency of calibrated growth-related parameters associated with solute biodegradation under transport conditions. This is accomplished by using a non-dimensionalized transport model, coupled with the modified Monod equations, to simulate the results of a series of miscible-displacement experiments conducted with different residence times and initial solute concentrations. The variability of the calibrated growth-related coefficient as functions of residence time and substrate concentration is examined.

2. Methods

2.1. Mathematical modeling

A one-dimensional steady-state flow model that includes linear, instantaneous sorption, nonlinear biodegradation, biomass growth, decay and transport, and electron–acceptor availability is used for this study. The non-dimensional equations are (Brusseau et al., 1999a)

$$R\frac{\partial C^*}{\partial T} = -\frac{\partial C^*}{\partial X} + \frac{1}{P}\frac{\partial^2 C^*}{\partial X^2} - \varepsilon_c M^* \left(\frac{C^*}{K_c^* + C^*}\right)\left(\frac{O^*}{K_o^* + O^*}\right) \tag{1}$$

$$\frac{\partial M^*}{\partial T} = \varepsilon_m M^* \left(\frac{C^*}{K_c^* + C^*}\right)\left(\frac{O^*}{K_o^* + O^*}\right) - \omega_b(M^* - 1) - M^*_{\text{cell loss}} \tag{2}$$

$$\frac{\partial O^*}{\partial T} = -\frac{\partial O^*}{\partial X} + \frac{1}{P}\frac{\partial^2 O^*}{\partial X^2} - \varepsilon_o M^* \left(\frac{C^*}{K_c^* + C^*}\right)\left(\frac{O^*}{K_o^* + O^*}\right) \tag{3}$$

where the following non-dimensional parameters are defined as

$$X = \frac{x}{L}, T = \frac{vt}{L}, P = \frac{vL}{D} \tag{4}$$

$$C^* = \frac{C}{C_0}, O^* = \frac{O}{O_0}, M^* = \frac{M}{M_0} \tag{5}$$

$$R = 1 + \frac{\rho}{\theta}K_d, K_c^* = \frac{K_c}{C_0}, K_o^* = \frac{K_o}{O_0} \tag{6}$$

$$\varepsilon_c = \frac{\mu_m L M_0}{vYC_0}, \varepsilon_o = \frac{\gamma_o \mu_m L M_0}{vO_0}, \varepsilon_m = \frac{\mu_m L}{v} \tag{7}$$

$$\omega_b = \frac{bL}{v} \tag{8}$$

where, b is first order cell mass decay coefficient [T^{-1}]; C is substrate concentration [ML^{-3}]; C_0 is substrate boundary input concentration [ML^{-3}]; O is electron–acceptor concentration [ML^{-3}]; O_0 is electron–acceptor boundary input concentration [ML^{-3}]; M is biomass concentration [ML^{-3}]; M_0 is initial biomass [ML^{-3}]; $M^*_{\text{cell loss}}$ is eluted cell concentration; x is distance [L]; L is column length; v is average pore-water velocity [LT^{-1}]; D is hydrodynamic dispersion coefficient [L^2T^{-1}]; is bulk density of the porous medium [ML^{-3}]; θ is fractional volumetric water content of the packed column; K_c is the half-saturation constant for the substrate [ML^{-3}]; K_o is the half-saturation constant for the electron acceptor [ML^{-3}]; K_d is the equilibrium sorption constant [L^3M^{-1}]; μ_m is the maximum specific growth rate of the microbial population [T^{-1}]; γ_o is a stoichiometric coefficient equal to the mass of electron acceptor utilized by bacteria per unit mass of substrate degraded; Y is yield coefficient (biomass produced/mass of substrate degraded).

The governing equations are solved with a second order upwind method for solving PDEs and an Adam–Bashforth three-step method for solving ODEs of the system under the following initial and boundary conditions.

$$C^*(X,0) = 0.0 \tag{9}$$

$$O^*(X,0) = 1.0 \tag{10}$$

$$M^*(X,0) = 1.0 \tag{11}$$

$$C^* - \frac{1}{P}\left(\frac{\partial C^*}{\partial X}\right)_{X=0} = C_0^*, \left(\frac{\partial C^*}{\partial X}\right)_{X=1} = 0.0 \tag{12}$$

$$O^* - \frac{1}{P}\left(\frac{\partial O^*}{\partial X}\right)_{X=0} = O_0^*, \left(\frac{\partial O^*}{\partial X}\right)_{X=1} = 0.0 \tag{13}$$

Several assumptions are employed in the derivation and application of these equations to the experiments conducted using benzoate and salicylate as model compounds. The substrates are assumed to be readily available to the biomass with uptake from the solution phase only. These assumptions are valid given that benzoate and salicylate are not subject to sorption by the porous media. Substrate and oxygen concentrations are assumed to be the only constraints for biomass growth. For the simpler version of the model, biomass is considered immobile. Thus, cell transport is assumed to be negligible. As will be shown below, this assumption is invalid and is therefore relaxed for the more complete version of the model. The impact of cell transport is accounted for using a "cell loss" term, as seen in Eq. (2). The $M^*_{\text{cell loss}}$ term was obtained by converting the measured cell elution data into a "cell loss" function that is time dependent. This simplified approach for representing the effects of cell transport was deemed adequate for our well-controlled, homogeneous system.

For the simpler model, biodegradation is assumed to proceed immediately upon uptake (no lag phase exists). This assumption is valid for the benzoate system because the experiments were conducted using porous media preconditioned to the substrate. Conversely, biodegradation for the salicylate system was preceded by a measurable lag

phase, due to induction of the genes responsible for biodegradation of salicylate (Sandrin, 2001). This lag phase was described to follow a normal distribution.

$$M_t = \int_{t-\Delta t}^{t} \frac{1}{\sqrt{2\pi}\,\sigma} e^{-\frac{(t_l - t_{\mathrm{lag}})^2}{2\sigma^2}} \mathrm{d}t + M_{t-\Delta t} \tag{14}$$

and is incorporated in the mathematical model, where, M_t is the metabolizing biomass concentration at time t [ML^{-3}]; M_{t-1} is the metabolizing biomass concentration at time step $t-1$ [ML^{-3}]; t_l is the time since $C > C_{\mathrm{ind}}$ where C_{ind} is the induction concentration for the microbial population [ML^{-3}]; t_{lag} is the average lag time [T]; σ is the variance of lag time. Cell growth rates, or generation rates, were reported to be normally distributed by Kubitschek (1966), and bacterial growth was successfully modeled by Buchanan et al. (1997) assuming exponential growth preceded by a normal distribution of lag times.

2.2. Laboratory experiments

The results of laboratory experiments conducted in our laboratory (Brusseau et al., 1999b; Yolcubal, 2001), served as the data to be analyzed herein. Benzoate and salicylate, intermediaries in the biodegradation of aromatic hydrocarbons such as toluene and naphthalene, respectively, were used as the biodegradable solutes. Pentafluoro-benzoic acid (PFBA) was used as a non-reactive tracer to characterize the hydrodynamic properties of the packed columns.

2.2.1. Benzoate miscible-displacement experiments

An aquifer material collected from a site contaminated by petroleum hydrocarbons was used for the benzoate experiments. This material is primarily sand (> 96%), with a very low organic carbon content. A stainless steel column with dimensions of 2.1 cm ID by 7.0 cm in length was used for these experiments. The columns were sterilized

Table 1
Simulation results for growth-related parameters (benzoate)
μ_{m} value fitted, with $K_{\mathrm{c}} = 20.4$ mg/l and $K_{\mathrm{o}} = 0.18$ mg/l.

Substrate concentration (mg/l)	Velocity (cm/h)	Residence time (h)	Maximum specific growth rate (95%) (h^{-1})	R^2
$C_0 = 1$	2.54	2.76	0.32 (0.12, 0.53)	0.98
	25.5	0.27	1.43 (0.23, 2.63)	0.96
	125.3	0.06	14.41 (11.97, 16.84)	0.97
$C_0 = 10$	2.42	2.9	0.16 (0.14, 0.17)	0.97
	23.2	0.3	1.01 (0.80, 1.23)	0.95
	123.3	0.06	1.23 (0.69, 1.77)	0.99
$C_0 = 30$	2.27	3.08	N/A	N/A
	22.7	0.31	0.33 (0.20, 0.47)	0.98
	123.9	0.06	0.65 (−0.67, 1.98)	0.95
$C_0 = 100$	2.61	2.68	0.02 (0.0, 0.05)	0.99
	22.4	0.31	0.22 (−0.03, 0.48)	0.98
	114.7	0.06	0.75 (−0.18, 1.51)	0.99

Table 2
Simulation results for maximum specific growth rate (salicylate, $C = 20$ mg/l)

Velocity (cm/h)	Maximum specific growth rate (95%) (h^{-1})	R^2
2.0	0.60 (0.3, 0.9)	0.91
8.9	0.32 (0.3, 0.4)	0.97
82.0	0.45 (0.4, 0.5)	0.90

(autoclaved) and then incrementally packed with air-dried porous media to obtain a uniform bulk density (1.77 g/cm^3). The packed columns were slowly wetted from the bottom to establish saturation ($\theta = 0.37$) and approximately 100 pore volumes of electrolyte solution were pumped through the column prior to use. Experiments were conducted using various influent solute concentrations and pore-water velocities, as shown in Table 1.

2.2.2. Salicylate miscible-displacement experiments

The benzoate system was complicated by the presence of a consortium of indigenous microorganisms capable of degrading benzoate. To create a simpler, more well-defined

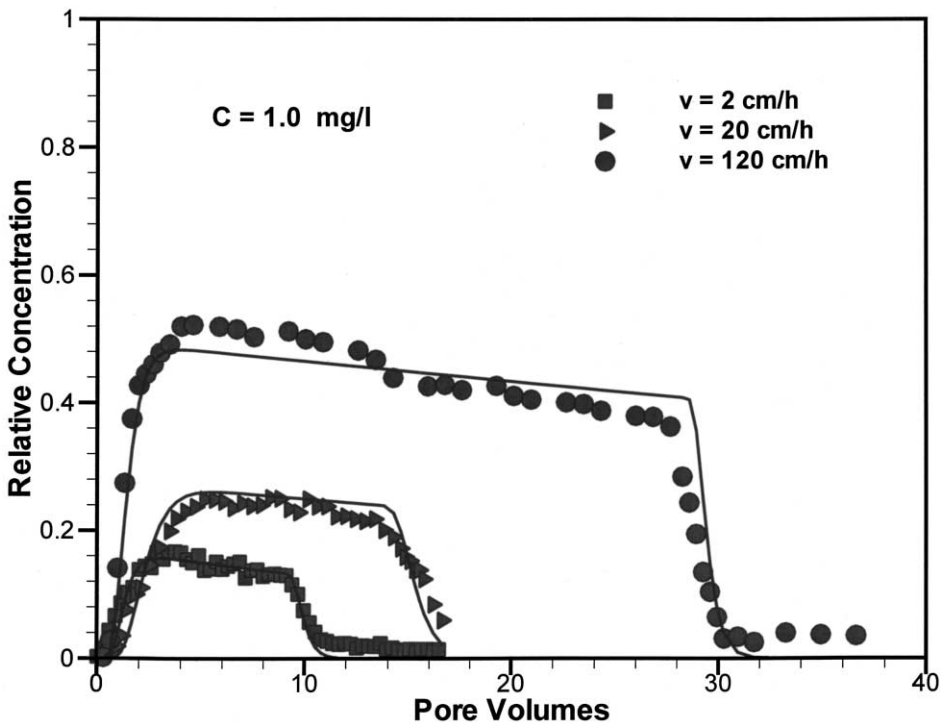


Fig. 1. Breakthrough curves for transport of benzoate in aquifer material for three pore-water velocities, with initial concentration 1 mg/l ($K_c = 20.4$ mg/l, $K_o = 0.1$ mg/l).

system, experiments were conducted using a sand inoculated with a single bacterial isolate. This isolate, *Pseudomonas putida* RB1353, contains the NAH7 plasmid, which enables degradation of naphthalene and its intermediaries (salicylate). A well-sorted (20/30 mesh), commercially available sand was used as the model porous medium (North Kato Supply, Mankato, MN). A stainless steel column (Modcol, St. Louis, MO) with dimensions of 5 cm ID $\times$ 10 cm in length was used for these experiments. For each salicylate column experiment, 420 g of sterile sand was inoculated to a cell density of about 1×10^7 cfu g^{-1} dry soil with the isolate. Subsamples were plated in duplicate for determination of initial cell density. The columns were then packed in incremental steps with the inoculated sand under sterile conditions to obtain uniform bulk density (1.76 g/cm^3), and saturated from the bottom for about 17 h ($\sim$ 15 pore volumes) with sterile nutrient/electrolyte solution at a flow rate of 1 ml min^{-1}. The solution reservoirs were continuously sparged with oxygen during both saturation and substrate injection to avoid oxygen limitation. Following saturation of the column ($\theta = 0.34$), salicylate solution was injected into the column at a concentration of 20 mg l^{-1} and at the flow rate of interest (see Table 2). Column effluent was monitored for salicylate, dissolved oxygen, and cell density. Effluent samples were periodically plated on LBK (Luria Broth with kanamycin) medium to monitor cell elution behavior of the *P. putida* RB1353. At the end of each

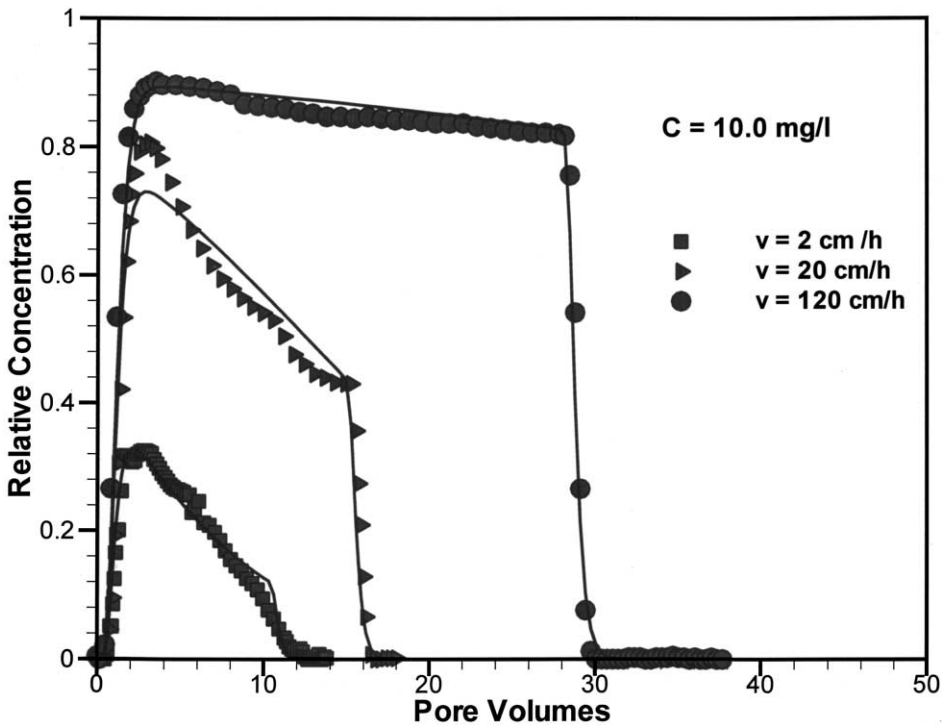


Fig. 2. Breakthrough curves for transport of benzoate in aquifer material for three pore-water velocities, with initial concentration 10 mg/l ($K_c = 20.4$ mg/l, $K_o = 0.1$ mg/l).

experiment, subsamples of the sand were analyzed to measure the spatial distribution of cell density.

2.2.3. Batch experiments

Batch experiments were conducted for both systems to measure growth-related parameters (μ_m, K_c, Y). Quantification of $^{14}CO_2$ evolution was used to evaluate the mineralization of benzoate by the indigenous bacterial populations. Values for K_c (20.4 mg/l), Y (0.65) and μ_m (2.3/h) were calculated from the experiments with six initial substrate concentrations (0.1, 1, 10, 30, 100 and 500 mg/l). For salicylate, loss of substrate with time was monitored for a series of aqueous concentrations (0.1, 0.5, 1, 5, 20, 50, 100, 250 and 500 mg/l) of salicylate. Values for K_c (55.0 mg/l), Y (0.22) and μ_m (0.3/h) were obtained (Sandrin, 2001).

3. Results and discussion

The results of the miscible-displacement experiments show that benzoate generally exhibited non-steady transport behavior (see Figs. 1–4), with the exception of the

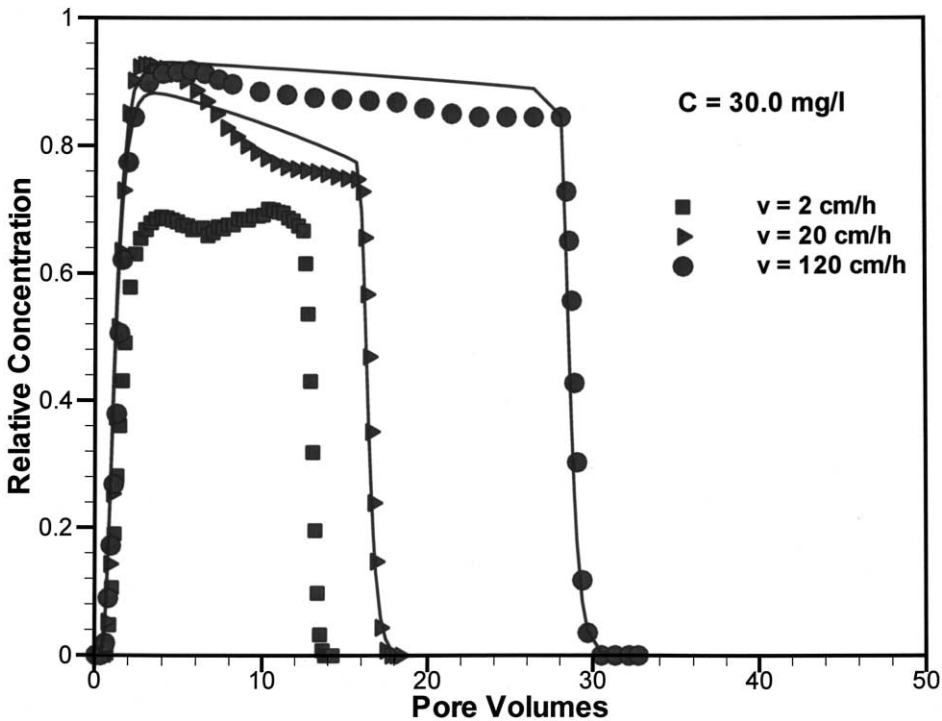


Fig. 3. Breakthrough curves for transport of benzoate in aquifer material for three pore-water velocities, with initial concentration 30 mg/l (K_c = 20.4 mg/l, K_o = 0.1 mg/l).

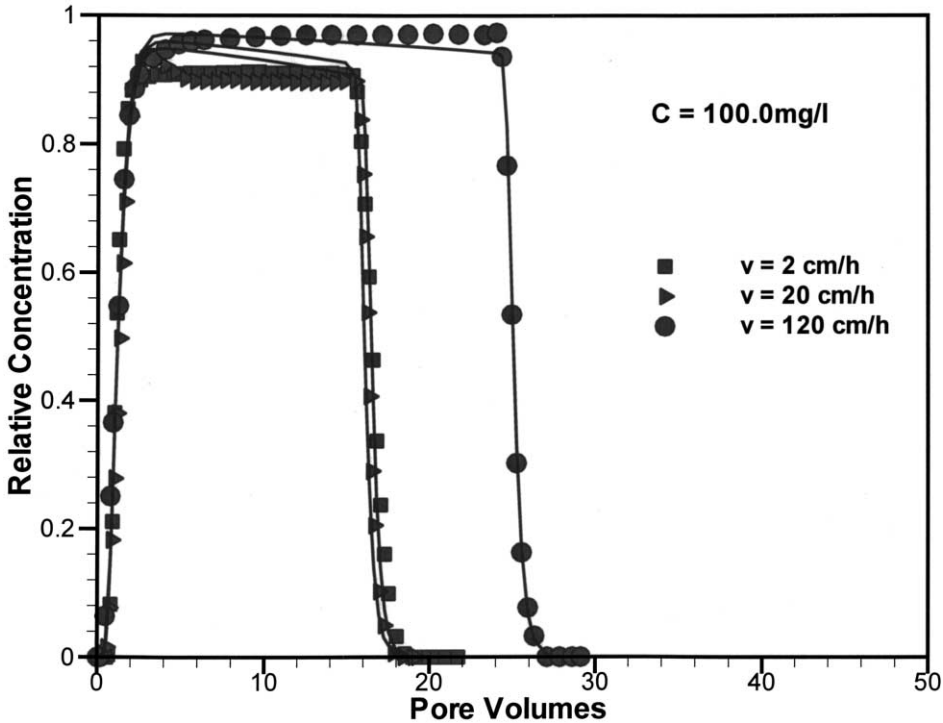
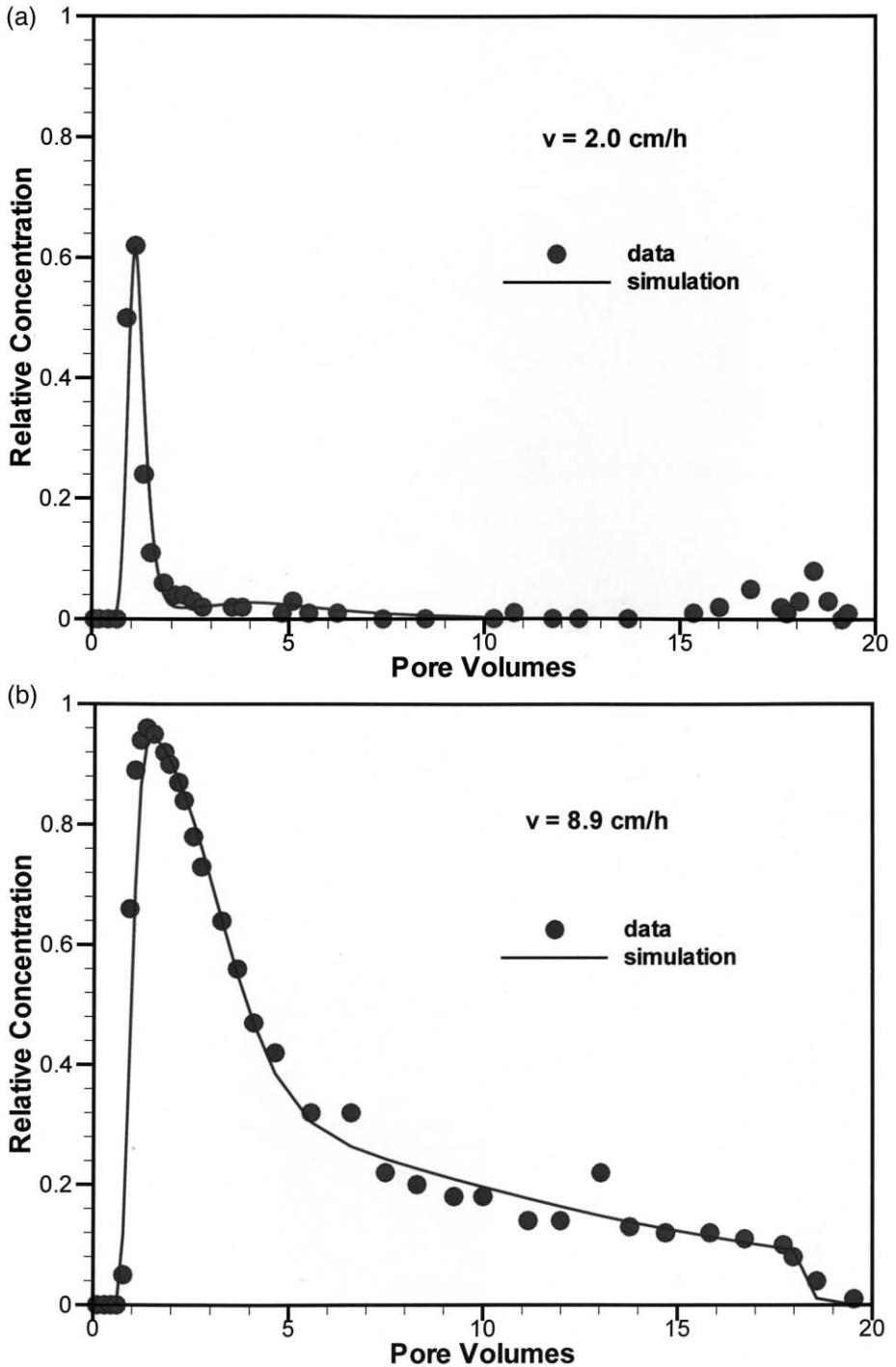


Fig. 4. Breakthrough curves for transport of benzoate in aquifer material for three pore-water velocities, with initial concentration 100 mg/l ($K_c = 20.4$ mg/l, $K_o = 0.1$ mg/l).

$C_0 = 100$ mg/l experiments which were influenced by oxygen constraints. The maximum relative concentration is different for each C_0 experiment, with larger maximum concentrations corresponding to larger influent concentrations. In addition, the magnitude of biodegradation was influenced by pore-water velocity, which results from the dependency of the magnitude of biodegradation on the mean contact time (i.e., residence time) between the substrate and the bacteria, as discussed by Angley et al. (1992) and Langner et al. (1998).

Salicylate shows the same residence-time-dependent behavior after the lag-phase effects have become minimal. The degree of salicylate degradation was greatest for the slowest pore-water velocity, as shown in Fig. 5. Cell elution data were collected by monitoring cell concentrations in the effluent. Cell elution was observed throughout the entire length of each experiment. Cell elution was greatest for the lowest velocity experiment and smallest for the largest velocity experiment.

For the benzoate data, the suite of growth-related parameters obtained from the batch experiment were initially used to simulate the column data sets. However, these simulations provided good results for only 2 of the 12 data sets. Thus, the experiment data were then calibrated with either K_o, K_c, or μ_m as the unknown fitting parameter. The most consistent simulation results were obtained by optimizing the maximum


(a)
v = 2.0 cm/h
data
simulation
Relative Concentration
Pore Volumes
(b)
v = 8.9 cm/h
data
simulation
Relative Concentration
Pore Volumes

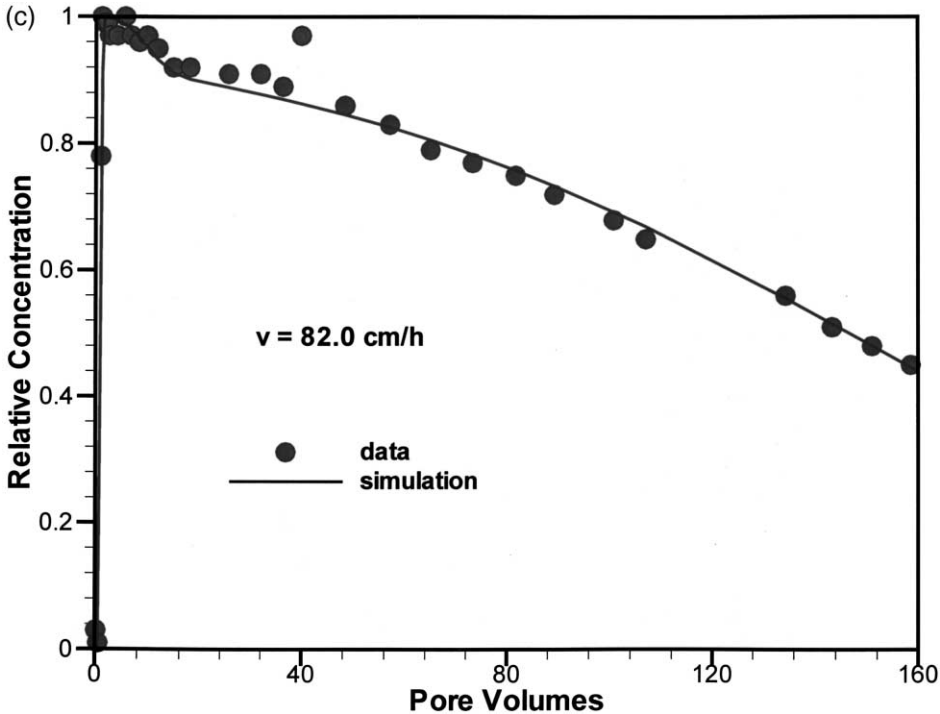


Fig. 5. Breakthrough curves for transport of salicylate in sand ($C_0 = 20$ mg/l) at (a) $v = 2.0$ cm/h; (b) $v = 8.9$ cm/h; (c) $v = 82.0$ cm/h.

specific growth rate μ_m, as shown in Table 1, while using K_c from the batch experiment, and a K_o value as suggested by Fritzsche (1994). For this case, the model provided reasonably close matches for a majority of the 12 data sets (see Figs. 1–4). A fewer number of successful matches were obtained using either K_c or K_o as the calibration parameter (data not shown). The inability to obtain good matches for all of the data sets is most likely due to the use of the simpler model, which may not account for all pertinent processes as discussed further below.

As discussed previously, substrate and oxygen concentrations, along with residence time, are the only growth-limiting factors considered in this application of the model. If the model incorporates all important processes that influence biodegradation, a single μ_m value should be applicable for all data sets, regardless of pore-water velocity or C_0. However, inspection of Table 1 shows that the magnitude of μ_m varies with pore-water velocity and initial substrate concentration. This indicates that one or more assumptions associated with the development or application of the simpler model may be invalid.

For the same pore-water velocity, the maximum specific growth rate coefficient generally increases as the initial substrate concentration decreases (Table 1). Thus, while the absolute amount of substrate degraded is smaller for smaller C_0 values as is predicted by the Monod equation, the relative efficiency of biodegradation (as denoted

by the magnitude of μ_m) appears to be greater. This phenomenon may be caused by toxicity or cell-elution effects, neither of which are incorporated into the simpler version of the mathematical model.

Aerobic transformation of benzoate, an aromatic compound, generates intermediates such as catechol, protocatechuate, and gentisate (Kiemer et al., 1996). Catechol, for example, has been shown to have an inhibitory effect on some microorganisms (Murray et al., 1972; Lu, 1993; Love and Leslie Grady, 1995). Such inhibitory effects may be expected to increase with increased intracellular concentrations of inhibitory intermediates, which likely correspond to higher substrate concentrations. If such inhibitory effects were present in our system, the efficiency of biodegradation would be reduced. However, because inhibition is not incorporated into the simpler version of the mathematical model, the impact of inhibition on reduced efficiency would be accounted for by a concentration-dependent value for μ_m (the calibrated parameter), with the magnitude of the "apparent" μ_m decreasing with increasing C_0.

It has been established that microbial cells can transfer to the solution phase and undergo aqueous-phase transport, especially under growth conditions (e.g., Reynolds et al., 1989; Harvey et al., 1984; Harvey and Barber, 1992; Sharma et al., 1993; Murphy et al., 1997; Shaw and Burns, 1998; Sandrin, 2001; Yolcubal, 2001). Thus, cell transport and elution may be a potentially significant factor for miscible-displacements experiments. For a given pore-water velocity, higher substrate concentrations correspond to higher biomass growth rates. It is possible that cell loss as a proportion of the total biomass is more significant at higher substrate concentrations, especially if newly produced cells comprise the majority of the eluting cells. In the simpler model, biomass is assumed to be immobile. Thus, any unaccounted for loss of cells from the system would be incorporated into the calibrated μ_m term, with greater cell loss corresponding to greater reductions in "efficiency" (reduction in global growth rate) and thereby resulting in lower apparent μ_m values.

For the same initial substrate concentration, the maximum specific growth rate increases with increasing pore-water velocity (decreasing residence time), as shown in Table 1. The change in μ_m as a function of velocity is consistent with both of the factors discussed above. The magnitude of biodegradation is greater at lower velocities due to the residence time effect. Thus, higher concentrations of inhibitory byproducts may be available at lower velocities, which would result in lower μ_m values at lower velocities. The biomass growth rate increases due to the longer contact time between bacteria and substrate at lower pore-water velocities. As discussed above, cell elution may be more significant when the growth rate is higher, which is consistent with smaller calibrated values of μ_m at lower velocities.

In order to further investigate the importance of factors that are not incorporated in the simpler model, such as cell elution, a set of simulations was conducted using the salicylate data. Because the system employed for these experiments was well characterized, it was possible to explicitly consider the observed cell elution behavior, as well as growth and lag effects, in the model. The simulation results are presented in Fig. 5, wherein it is observed that the measured breakthrough curves are very well described by the model simulations. The magnitude of the calibrated μ_m is statistically similar at the 95% confidence level for all three pore-water velocities (Table 2). Furthermore, for all

Table 3
Simulation results for maximum specific growth rate without considering lag phase and cell elution (salicylate, $C = 20$ mg/l)

Velocity (cm/h)	Maximum specific growth rate (95%) (h^{-1})	R^2
2.0	0.17 (0.1, 0.2)	0.18
8.9	0.18 (0.1, 0.2)	0.53
82.0	0.45 (0.4, 0.5)	0.87

three cases, the values of μ_m are similar to the value obtained from the batch experiment (0.3 h^{-1}).

Conversely, the values of μ_m are not statistically similar when the simpler model is used to calibrate the salicylate data (Table 3). In this case, wherein cell mobility and lag are ignored, the μ_m values obtained for the smaller pore-water velocities are smaller than the value obtained for the largest-velocity experiment, as was observed for the benzoate system. Inspection of Tables 2 and 3 reveals that the calibrated μ_m value for the fastest-velocity experiment is identical for both the simpler and more-complete model applications. However, the calibrated μ_m values obtained for the lower-velocity data are smaller for the simpler model than for the more complete model. This is consistent with the observed differences in the magnitude of cell elution observed for the three experiments. Cell elution was least significant for the highest-velocity experiment, and thus the use of the simpler model, wherein cell elution is ignored, would therefore be less constrained than for the other two experiments, wherein cell elution was greater. As a result, the calibrated μ_m values obtained with the simpler model would be expected to exhibit greater deviation from the true value for the lower-velocity experiments.

Comparison of Tables 1 and 3 shows that the range of μ_m values obtained for the salicylate data is not as great as was observed for the benzoate data. This is possibly related to the greater overall growth that occurred in the benzoate system. This difference in growth is consistent with the relative magnitudes of μ_m and K_c measured for benzoate and salicylate in the batch experiments. The results discussed herein illustrate the importance of accounting explicitly for all important processes when simulating the biodegradation and transport of contaminants in porous media.

4. Conclusions

The calibrated maximum specific growth rate coefficients obtained with use of the simpler model appear to be a function of pore-water velocity and initial substrate concentration for the experiments investigated herein. For the same pore-water velocity, the apparent maximum specific growth rate coefficient generally increases as the initial substrate concentration decreases. For the same initial substrate concentration, the apparent maximum specific growth rate increases with increasing pore-water velocity (decreasing residence time). These results suggest that one or more of the assumptions

employed in the application of the simpler model, which are assumptions common to most biodegradation/transport models, are invalid. It is likely that μ_m is a lumped parameter in this case, accounting for processes not explicitly incorporated into the simpler model. We postulate that cell mobility or the existence of inhibitory byproducts is the factor that could possibly be influencing the benzoate system, and whose potential impacts are consistent with the results of the simulations. The impact of cell mobility is illustrated by the results obtained from applying a model that does explicitly account for cell mobility to the salicylate data. In this case, the μ_m values obtained for three pore-water velocities were similar, both to each other and to the batch-measured value. These results demonstrate the difficulty in simulating the influence of microbial processes on contaminant transport, and point to the need for more comprehensive (and complex) mathematical models, along with the importance of characterizing the many factors potentially influencing biodegradation of organic contaminants in subsurface systems.

Acknowledgements

This research was supported by a grant provided by the US Environmental Protection Agency, under the auspices of the Joint Bioremediation Research Program, and the NIEHS superfund Basic Research program.

References

Alexander, M., Scow, K.M., 1989. Kinetics of biodegradation in soil. Reactions and Movement of Organic Chemicals in Soils, Special Pub. No. 22. Soil Science Society of America, Madison, WI, pp. 243–270.

Angley, J.T., Brusseau, M.L., Miller, W.L., Delfino, J.J., 1992. Nonequilibrium sorption and aerobic biodegradation of dissolved alkylbenzenes during transport in aquifer material: column experiments and evaluation of a coupled-process model. Environ. Sci. Technol. 26, 1404–1410.

Borden, R.C., Bedient, P.B., Lee, M.D., Ward, C.H., Wilson, J.T., 1986. Transport of dissolved hydrocarbons influenced by oxygen-limited biodegradation: 2. Field application. Water Resour. Res. 22, 1983–1990.

Brusseau, M.L., Xie, L.H., Li, L., 1999a. Biodegradation during contaminant transport in porous media: 1. Mathematical analysis of controlling factors. J. Contam. Hydrol. 37, 269–293.

Brusseau, M.L., Hu, M.Q., Wang, J., Maier, R.M., 1999b. Biodegradation during contaminant transport in porous media: 2. The influence of physicochemical factors. Environ. Sci. Technol. 33, 96–103.

Buchanan, R.L., Whiting, R.C., Damert, W.C., 1997. When is simple good enough: a comparison of the Gompertz, Baranyi, and three-phase linear models for fitting bacterial growth curves. Food Microbiol. 14, 313–326.

Chen, Y.M., Abriola, L.M., Alvarez, P.J.J., Anid, P.J., Vogel, T.M., 1992. Modeling transport and biodegradation of Benzene and Toluene in sandy aquifer material: comparisons with experimental measurements. Water Resour. Res. 28 (7), 1833–1847.

Estrella, M.R., Brusseau, M.L., Maier, R.S., Pepper, I.L., Wierenga, P.J., Miller, R.M., 1993. Biodegradation, sorption, and transport of 2,4-Dichlorophenoxyacetic acid in saturated and unsaturated soils. Appl. Environ. Microbiol. 59 (12), 4266–4273.

Fritzsche, C., 1994. Degradation of Pyrene at low define oxygen concentration by a mycobacterium sp. Appl. Environ. Microbiol. 60 (5), 1687–1689.

Harvey, R.W., Barber II, L.B., 1992. Associations of free-living bacteria and dissolved organic compounds in a plume of contaminated groundwater. J. Contam. Hydrol. 9, 91–103.

Harvey, R.W., Smith, R.L., George, L., 1984. Effect of organic contamination upon microbial distributions and heterotrophic uptake in a Cape Cod. Mass., Aquifer Appl. Environ. Microbiol. 48, 1197–1202.

Kelsey, J.W., Alexander, M., 1995. Effect of flow rate and path length on *p*-Nitrophenol biodegradation during transport in soil. Soil Sci. Soc. Am. J. 59, 113–117.

Kiemer, P., Tshisuaka, B., Fetzner, S., Lingens, F., 1996. Degradation of benzoate via benzoyl-coenzyme A and gentisate by *Bacillus stearothermophilus* PK1 and purification of gentisate 1,2-dioxygenase. Biol. Fertil. Soils 23, 307–313.

Kubitschek, H.E., 1966. Normal distribution of cell generation rates. Nature 209, 1039–1040.

Langner, H.W., Inskeep, W.P., Gaber, H.M., Jones, W.L., Das, B.S., Wraith, J.M., 1998. Pore water velocity and residence time effects on the degradation of 2,4-D during transport. Environ. Sci. Technol. 32, 1308–1315.

Love, N.G., Leslie Grady Jr., C.P., 1995. Impact of growth in benzoate and *m*-toluate liquid media on culturability of *Pseudomonas putida* on benzoate and *m*-toluate plates. Appl. Environ. Microbiol. 61 (8), 3142–3144.

Lu, Y.-T., 1993. Analysis of plasmid instability among populations carrying the TOL plasmid in a single substrate limited environment. PhD dissertation. Clemson University, Clemson, S.C.

MacQuarrie, K.T.P., Sudicky, E.A., 1990. Simulation of biodegradable organic contaminants in groundwater: 2. Plume behavior in uniform and random flow fields. Water Resour. Res. 26, 223–239.

Molz, F.J., Widdowson, M.A., Benefield, L.D., 1990. Simulation of microbial growth dynamics coupled to nutrient and oxygen transport in porous media. Water Resour. Res. 22 (8), 1207–1216.

Murphy, E.M., Ginn, T.R., Chilakapati, A., Resch, C.T., Phillips, J.L., Wietsma, T.W., Spadoni, C.M., 1997. The influence of physical heterogeneity on microbial degradation and distribution in porous media. Water Resour. Res. 33 (5), 1087–1103.

Murray, K., Duggleby, C.J., Sala-Trepat, J.M., Williams, P.A., 1972. The metabolism of benzoate and methylbenzoates via the meta-cleavage pathway by *Pseudomonas arvilla* mt-2. Eur. J. Biochem. 28, 301–310.

Reynolds, P.J., Sharma, P.K., Jenneman, G.E., McInerney, M.J., 1989. Mechanisms of microbial movement in subsurface materials. Appl. Environ. Microbiol. 55, 2280–2286.

Sandrin, S.K., 2001. The impact of bacterial population dynamics on the transport and biodegradation of organic compounds. PhD Dissertation, The University of Arizona, Tucson, AZ.

Sharma, P.K., McInerney, M.J., Knapp, R.M., 1993. In situ growth and activity and modes of penetration of *Escherichia coli* in unconsolidated porous materials. Appl. Environ. Microbiol. 59, 3686–3694.

Shaw, L.J., Burns, R.G., 1998. Biodegradation-transport interactions of pulse applied 2,4-D in repacked soil column microcosms. J. Environ. Qual. 27, 1472–1478.

Sykes, J.F., Soyupak, S., Farquhar, G.J., 1982. Modeling of leachate organic migration and attenuation in groundwater below sanitary landfills. Water Resour. Res. 18 (1), 135–145.

Widdowson, M.A., Molz, F.J., Benifield, L.D., 1988. A numerical transport model for oxygen- and nitrate-based respiration linked to substrate and nutrient availability in porous media. Water Resour. Res. 24 (9), 1553–1565.

Wood, B.D., Ginn, T.R., Dawson, C.N., 1995. Effects of microbial metabolic lag in contaminant transport and biodegradation modeling. Water Resour. Res. 31 (3), 553–563.

Wookeun, B., Odencrantz, J.E., Rittmann, B.E., Valocchi, A.J., 1990. Transformation kinetics of trace-level halogenated organic contaminants in a biologically active zone (BAZ) induced by nitrate injection. J. Contam. Hydrol. 6, 53–68.

Ying, W., Weber, W.J., 1979. Bio-physicochemical adsorption model systems for wastewater treatment. J. Water Pollut. Control Fed. 51, 2661–2677.

Yolcubal, I., 2001. In-situ monitoring of biodegradation and microbial activity during solute transport in porous media. PhD Dissertation, The University of Arizona, Tucson, AZ.