

# Quantitative Analysis of Experiments on Bacterial Chemotaxis to Naphthalene

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**Abstract:** A mathematical model was developed to quantify chemotaxis to naphthalene by *Pseudomonas putida* G7 (PpG7) and its influence on naphthalene degradation. The model was first used to estimate the three transport parameters (coefficients for naphthalene diffusion, random motility, and chemotactic sensitivity) by fitting it to experimental data on naphthalene removal from a discrete source in an aqueous system. The best-fit value of naphthalene diffusivity was close to the value estimated from molecular properties with the Wilke-Chang equation. Simulations applied to a non-chemotactic mutant strain only fit the experimental data well if random motility was negligible, suggesting that motility may be lost rapidly in the absence of substrate or that gravity may influence net random motion in a vertically oriented experimental system. For the chemotactic wild-type strain, random motility and gravity were predicted to have a negligible impact on naphthalene removal relative to the impact of chemotaxis. Based on simulations using the best-fit value of the chemotactic sensitivity coefficient, initial cell concentrations for a non-chemotactic strain would have to be several orders of magnitude higher than for a chemotactic strain to achieve similar rates of naphthalene removal under the experimental conditions we evaluated. The model was also applied to an experimental system representing an adaptation of the conventional capillary assay to evaluate chemotaxis in porous media. Our analysis suggests that it may be possible to quantify chemotaxis in porous media systems by simply adjusting the model's transport parameters to account for tortuosity, as has been suggested by others. © 2002 Wiley Periodicals, Inc. *Biotechnol Bioeng* 78: 626–634, 2002.

**Keywords:** chemotaxis; naphthalene; *Pseudomonas putida*; porous media; bioavailability; biodegradation

## INTRODUCTION

Most bacterial species are motile, and many are known to be chemotactic to various substances (Armitage, 1999). Although chemotaxis has long been believed to

provide a competitive advantage to bacteria in nutrient-limited environments, little attention has been paid to its corresponding influence on nutrient consumption. Such an influence may be important in the biodegradation of pollutants in contaminated environments, particularly in relatively quiescent and heterogeneous environments such as the subsurface. The subsurface environment is characterized by heterogeneity—over a range of scales—in both the nature of the solids and the distribution of contaminants (National Research Council Committee on Ground Water Cleanup Alternatives, 1994; Weber et al., 1998). Small-scale heterogeneity can lead to large differences in the local availability of a contaminant to the bacteria capable of degrading it, a condition in which chemotaxis may help cells locate substrate concentrations more favorable to growth and survival.

We recently demonstrated that chemotaxis to naphthalene by *Pseudomonas putida* G7 (PpG7) increased the rate of naphthalene degradation in an aqueous system in which a concentration gradient of naphthalene was imposed (Marx and Aitken, 2000a). The wild-type PpG7 degraded naphthalene at a much faster rate than either of two mutant strains, one deficient in chemotaxis to naphthalene and one that was immotile; naphthalene removal by the two mutant strains was constrained by the rate at which naphthalene diffused from its source (Marx and Aitken, 2000a). We have since developed a mathematical model of this experimental system, and have applied the model to a similar system in which the chemotaxis of PpG7 to naphthalene in a saturated porous medium was evaluated. The model was used both to fit parameters from the experimental data and to evaluate the sensitivity of simulated results to changes in selected parameter values. The influence of chemotaxis has been explicitly accounted for in a biodegradation model in only one previous study (Bosma et al., 1988), which did not provide corroborating experimental evidence of such an influence. Only limited research has been done to quantify bacterial motility and chemotaxis in porous media (Barton and Ford, 1995; 1997; Duffy et al., 1995; 1997; Dillon and Fauci, 2000).

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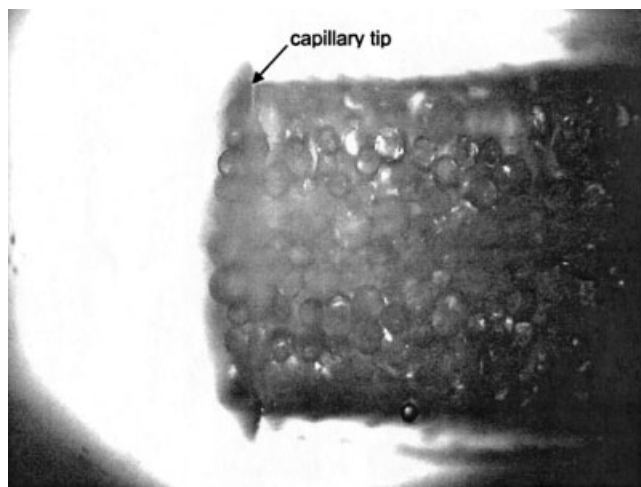
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## EXPERIMENTAL METHODS

The experimental system used to study naphthalene removal in free liquid is described elsewhere (Marx and Aitken, 2000a). Briefly, a capillary tube containing a buffered solution of naphthalene at its solubility limit in water was suspended into a reservoir containing a suspension of cells in the same buffer. Naphthalene that diffused out of the source capillary and was not degraded by the bacteria was trapped in a layer of heptamethylnonane (HMN) floating on top of the culture reservoir. The mass of naphthalene remaining in the source capillary and the combined mass remaining in the reservoir and trap were measured at discrete time intervals.

Experiments with porous media were conducted in a similar system except both the capillary tube and the vial used to hold the buffer reservoir were packed with glass beads (median dia 50  $\mu\text{m}$  and uniformity coefficient,  $d_{60}/d_{10}$ , 1.9; Blast-It-All, Salisbury, NC). The beads were washed successively with methanol, 0.01 N KOH, and 0.01 N  $\text{H}_2\text{SO}_4$ , with rinses by reagent water after each step. The beads were then dried at 120°C and cooled to room temperature. The measured porosity,  $\epsilon$ , of a packed bed of beads was 0.41.

Capillaries (10  $\mu\text{L}$  capacity, Drummond Scientific, Broomall, PA) were prepared by flame-sealing one end, placing them on a glass dish and heating the dish on a hot plate for at least 15 min. The open end of each capillary was immersed into a slurry of glass beads (10 g of beads added to 3 mL of buffer alone or buffer saturated with naphthalene) and allowed to cool for at least 5 min. Microscopic examination verified that the entire diameter (0.56 mm) of the capillary was packed tightly with the beads (Fig. 1). A small layer of free liquid ( $\approx 2$  mm) existed above the plug of beads ( $\approx 6$  mm) in each capillary.



**Figure 1.** Digital photomicrograph of a capillary tip filled with glass beads. The diameter of the capillary is 0.56 mm.

The reservoir consisted of a glass vial (8 mm dia  $\times$  43 mm) containing a suspension of PpG7 ( $10^5$  cfu/mL) in 0.5 mL of buffer, which was mixed with 1.2 g of glass beads. A capillary packed with the glass beads was placed into the reservoir and incubated for a predetermined time interval. The capillary was removed and the exterior rinsed with reagent water, then was broken and emptied into an aliquot of mineral salts buffer. The resulting suspensions from replicate incubations were diluted, if necessary, and plated on R2A agar (Difco, Detroit, MI). Plates were counted after 20–30 h of incubation at 30°C.

Parallel incubations with free liquid were conducted by omitting the glass beads from the capillaries and the culture reservoirs. Each capillary was held in place by inserting it into a teflon-lined septum that rested on top of the vial.

The potential adhesion of PpG7 to the glass beads was evaluated by incubating a 0.5-mL suspension of the culture in buffer ( $10^5$  cfu/mL) mixed with 1.2 g of glass beads in a test tube. Triplicate tubes were prepared with the glass beads and triplicate control tubes were prepared with the culture suspension but no glass beads. After time intervals of up to 2 h, 10 mL of buffer was added to each tube, which was then mixed gently. Each suspension was then diluted and plated as described above. After 2 h of incubation there was no significant difference between the incubations with the glass beads and the controls.

Naphthalene removal was not quantified in the porous-medium experiments; only cell accumulation data were obtained. To preclude confounding effects of bacterial growth on subsequent interpretation and analysis of the data, chloramphenicol (10  $\mu\text{g}/\text{mL}$ ) was added to the buffer in all experimental systems.

## Mathematical Model

### Approach

Our approach followed that originally proposed by Keller and Segel (1971), who assumed that the net motion of a population of cells was the sum of random and chemotactic motility. This approach treats the random motility of cells as analogous to molecular diffusion, and chemotaxis as a mechanism which biases the direction of cell motion as a function of the concentration gradient of the chemoeffector (an attractant or repellent). We further used the approach developed by Rivero et al. (1989) to define a chemotactic velocity term, which was later modified by Ford and co-workers (Chen et al., 1998; Ford and Cummings, 1992) for use in 3-D systems. The chemotactic velocity is given by (Chen et al., 1998):

$$v = \frac{2}{3}s \tanh\left(\frac{\chi_0}{2s} \frac{K_d}{(K_d + C)^2} \frac{\partial C}{\partial x}\right) \quad (1)$$

where  $s$  is the cell swimming speed,  $\chi_0$  is the chemotactic sensitivity coefficient,  $K_d$  is the dissociation constant for a chemoreceptor and its effector,  $C$  is the effector concentration, and  $x$  is a spatial coordinate. In this case the chemoeffector is an attractant, naphthalene, which is also consumed by the bacteria.

The chemotactic velocity expression shown in Equation (1) incorporates several important assumptions, including a constant cell swimming speed (independent of effector concentration) and a mechanism of chemoreceptor binding that can be defined by a single dissociation constant. We also assumed that the random motility coefficient,  $\mu$ , was constant, which is justifiable in most situations of interest (Ford and Lauffenburger, 1991a; 1991b; Widman et al., 1997). Although these assumptions may not hold for all chemotactic bacteria and experimental systems (Ford and Lauffenburger, 1991b), the expression in general captures the important features of chemotactic behavior.

### Model Development

An earlier model we developed to analyze the traditional capillary assay system (Marx and Aitken, 2000b) was based on a one-dimensional finite-difference analysis of both the capillary and the culture reservoir. The current model is based on a 2-D finite-element analysis of both domains, with much finer spatial discretization. The solution method was improved by incorporating adaptive time steps with formal methods to estimate and control time discretization errors, which is important for the larger physical dimensions and longer time scales of the experimental system simulated in this work. The model also includes parameter estimation capability.

The model consists of coupled partial differential equations representing material balances on the attractant and the bacterial cells. It accounts for diffusion and bacterial consumption (by Monod kinetics) of the attractant as well as bacterial transport by random motility and chemotaxis. The model also includes bacterial growth and decay, but the growth term was zero for the cases considered in this work. Cylindrical coordinates were used, consistent with the shapes of the capillary and reservoir. Using the center of the capillary tube as the longitudinal axis, the angle term in the model formulation was ignored because of symmetry. For the porous-medium experiment, the free-liquid volume above the glass bead packing inside the capillary was accounted for.

The governing equations for the attractant and bacteria are, respectively:

$$\frac{\partial C}{\partial t} = \frac{D}{\tau} \left[ \frac{\partial^2 C}{\partial z^2} + \frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{\partial C}{\partial r} \right) \right] - \frac{q_m BC}{K_s + C} \quad (2)$$

$$\frac{\partial B}{\partial t} = \frac{\mu}{\tau} \left[ \frac{\partial^2 B}{\partial z^2} + \frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{\partial B}{\partial r} \right) \right] - \left[ \frac{1}{\tau} \frac{\partial (v_z B)}{\partial z} \right] - \left[ \frac{1}{\tau} \frac{\partial (r v_r B)}{\partial r} \right] + \left( \frac{Y q_m BC}{K_s + C} \right) - k B \quad (3)$$

where  $r$  and  $z$  are the radial and longitudinal directions, respectively;  $t$  is time;  $D$  is the diffusion coefficient for the attractant;  $\tau$  is the tortuosity ( $=1.0$  for the free-liquid systems);  $B$  is the cell concentration;  $v_z$  and  $v_r$  are the chemotactic velocity components in the longitudinal and radial directions, respectively;  $q_m$  is the maximum specific rate of attractant utilization;  $K_s$  is the half-saturation coefficient;  $Y$  is the yield coefficient; and  $k$  is the microbial decay coefficient.

The chemotactic velocity expression shown in Equation (1) was used, with components in the  $z$  and  $r$  directions given by:

$$v_z = \frac{\frac{\partial C}{\partial z}}{\|\nabla C\|} \|v\| \quad (4)$$

$$v_r = \frac{\frac{\partial C}{\partial r}}{\|\nabla C\|} \|v\| \quad (5)$$

The relevant boundary conditions were:

$$\left. \frac{\partial C}{\partial n} \right|_{\Gamma} = 0 \quad (6)$$

$$\left. \frac{\partial B}{\partial n} \right|_{\Gamma} = 0 \quad (7)$$

where  $\Gamma$  represents a physical boundary (the inside and outside walls of the capillary, the inside wall of the vial, and the upper surface of the aqueous phase in the vial and the capillary) and  $n$  represents the direction normal to the boundary. For the systems containing an HMN trap on top of the culture reservoir, the boundary condition for naphthalene at the aqueous surface in the vial is approximate. However, the size of the reservoir (1000  $\mu\text{L}$ ) relative to the volume of the naphthalene source inside the capillary (2  $\mu\text{L}$ ), and the fact that naphthalene did not accumulate significantly in the reservoir in inoculated systems (Marx and Aitken, 2000a), suggests that this is a reasonable approximation. The initial attractant concentration was zero in the reservoir and at the aqueous saturation concentration ( $C_0$ ) in the capillary. The initial bacterial concentration was uniform in the reservoir ( $B_0$ ) and zero in the capillary. Initial concentrations of the attractant and bacteria at the nodes defining the interface between the capillary and the reservoir were  $0.5 C_0$  and  $0.5 B_0$ , respectively.

The governing system of equations was solved numerically. The method of lines (Schiesser, 1991) was used to reduce the system of partial differential equations to a system of ordinary differential equations. The

spatial derivatives were approximated by the Galerkin finite element method, with bilinear basis and test functions. Because the steepest gradients were confined to a relatively small but known part of the domain, we used variable spacing (fixed in time) with finer discretization in the region near the capillary mouth expected to be characterized by steep gradients. The resulting system of ordinary differential equations was integrated in time by the backward difference formula method (Schiesser, 1991) as implemented in DDASPK (Brown et al., 1994). The Levenburg-Marquardt optimization algorithm (Dennis and Schnabel, 1983), as implemented in the computer program LMDIF (Argonne National Laboratory, Minpack Project, March 1980), was used to estimate model parameters from the experimental data by minimizing the sum of the squared errors between the data and model simulations. The standard error of the parameter estimates was approximated from the sum of the squared errors, the degrees of freedom, and the optimization algorithm's estimate of the Jacobian (Draper and Smith, 1981).

Parameter values used in applications of the model are summarized in Table I; values used in specific simulations are identified in the text. Material balances on both naphthalene and cells were conducted with simulations that accounted for diffusion only or diffusion plus chemotaxis in the absence of naphthalene consumption and microbial decay. In all cases, the final naphthalene mass or cell number was within a factor of  $10^{-9}$  of the initial naphthalene mass or cell number.

Since the experimental system imposed a concentration gradient of naphthalene in a vertical direction, we considered the potential effect of gravity on the simulation results. A terminal settling velocity for a cell,  $v_s$ , was estimated using Stokes' law with a correction factor for an ellipsoid moving randomly (Berg, 1993). As a first approximation, this velocity was then assumed to rep-

resent a downward "drift velocity" (Berg, 1993) and was added to the vertical component of the chemotactic velocity expression [Eq. (4)]; this approach was also used by Shonnard et al. (1992). Based on a scaled electron micrograph of *P. putida* (Harwood et al., 1989), we assumed a cylindrical cell 0.9  $\mu\text{m}$  in diameter by 1.9  $\mu\text{m}$  long. The density difference between a cell and the buffer was assumed to be in the range of 0.04–0.05  $\text{g}/\text{cm}^3$ , which corresponds to a range of buoyant densities measured for groundwater bacteria cultured in the laboratory (Harvey et al., 1997). A drift velocity of 0.02  $\mu\text{m}/\text{s}$  was estimated on the basis of these assumptions.

## RESULTS AND DISCUSSION

A number of models of bacterial chemotaxis have accounted for the simultaneous consumption of the chemoattractant and its influence on the motion of individual cells or cell populations (Barton and Ford, 1997; Blackburn et al., 1997; Bosma et al., 1988; Dillon and Fauci, 2000; Dillon et al., 1995; Ford and Lauffenburger, 1991a; 1992; Lauffenburger et al., 1982; Marx and Aitken, 2000b; Rivero-Hudec and Lauffenburger, 1986; Widman et al., 1997). However, only the model developed by Bosma et al. (1988) was used to analyze the potential influence of chemotaxis on degradation of the attractant. In that work, the corresponding experimental systems were not designed to distinguish between chemotactic and random motility, and in one case the fits to experimental data were unable to distinguish between models that included or excluded chemotaxis.

The model developed for the present study was first used to estimate coefficients for naphthalene diffusion, random motility, and chemotactic sensitivity by fitting it to naphthalene removal data from the free-liquid experimental system reported previously (Marx and Aitken, 2000a). The best-fit value of the naphthalene diffusion coefficient obtained from the uninoculated control (Fig. 2),  $7.5 \times 10^{-6} \text{ cm}^2/\text{s}$ , was used in all subsequent simulations and fits. The diffusion coefficient estimated from the Wilke-Chang equation,  $8.5 \times 10^{-6} \text{ cm}^2/\text{s}$  (Marx and Aitken, 1999), is well within the 95% confidence interval (CI) of the fitted value. Figure 2 also illustrates the excellent agreement between the numerical solution and an analytical solution that incorporated simplifying assumptions about the boundary condition at the mouth of the capillary (Marx and Aitken, 2000a). Results from this experiment demonstrate that naphthalene transport from the capillary to the reservoir was controlled by diffusion, contrary to observations by others that transport can be dominated by convection resulting from differences in fluid properties between the capillary and the reservoir (Shonnard et al., 1992; Vicker, 1981).

Data from the capillary systems inoculated with either wild-type PpG7 or the non-chemotactic mutant are re-

**Table I.** Parameter values used in applications of the model.<sup>a</sup>

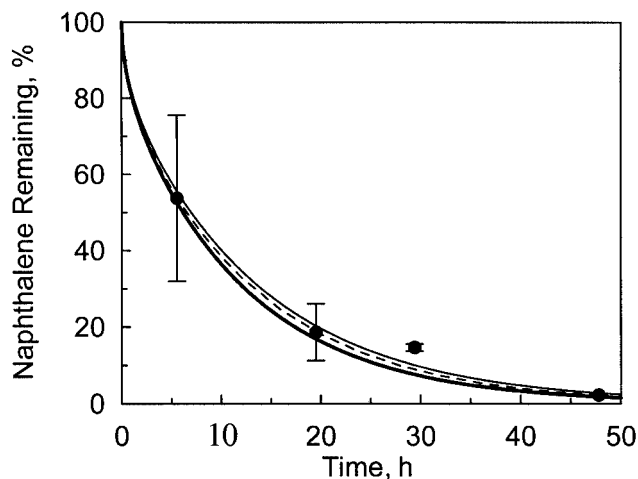
Parameter	Value
$B_0$ , wild-type ( $\text{cfu cm}^{-3}$ ) <sup>b</sup>	$4.04 \times 10^6$
$B_0$ , non-chemotactic ( $\text{cfu cm}^{-3}$ )	$3.49 \times 10^6$
$C_0$ ( $\text{g cm}^{-3}$ )	$2.83 \times 10^{-5}$
$D$ ( $\text{cm}^2 \text{s}^{-1}$ )	$7.5 \times 10^{-6}$
$\varepsilon$ (–)	0.41
$k$ ( $\text{s}^{-1}$ )	$1.22 \times 10^{-6}$
$K_d$ ( $\text{g cm}^{-3}$ ) <sup>c</sup>	$2.1 \times 10^{-6}$
$K_s$ ( $\text{g cm}^{-3}$ ) <sup>d</sup>	$1.3 \times 10^{-7}$
$\mu$ ( $\text{cm}^2 \text{s}^{-1}$ ) <sup>c</sup>	$3.2 \times 10^{-7}$
$q_m$ ( $\text{g cfu}^{-1} \text{s}^{-1}$ ) <sup>d</sup>	$7.9 \times 10^{-16}$
$s$ ( $\text{cm s}^{-1}$ ) <sup>c</sup>	$4.8 \times 10^{-3}$
$Y$ ( $\text{cfu g}^{-1}$ )	0

<sup>a</sup>Exceptions are indicated in the text.

<sup>b</sup>In the free-liquid system for which naphthalene removal was measured.  $B_0$  was  $1.36 \times 10^6 \text{ cfu cm}^{-3}$  in the porous-medium experiment and the corresponding free-liquid control.

<sup>c</sup>From Marx and Aitken (1999).

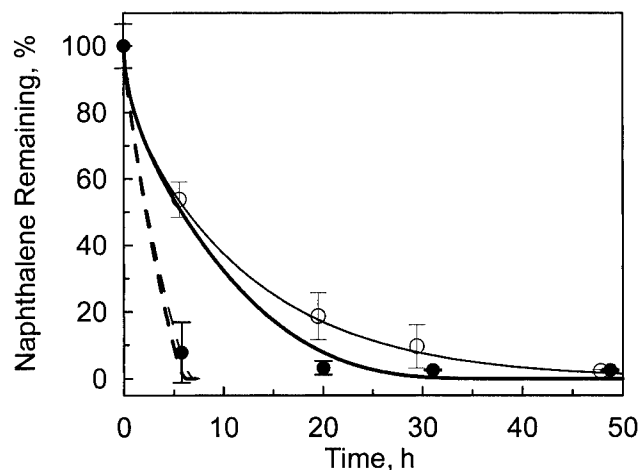
<sup>d</sup>Marx and Aitken (2000b).



**Figure 2.** Measured naphthalene diffusion from a capillary tube (mean and standard deviation of triplicates) compared to simulations using an analytical solution (dashed line; Marx and Aitken, 2000a) or the numerical solution (thick solid line) using a diffusion coefficient for naphthalene estimated by the Wilke-Chang equation (Marx and Aitken, 1999). The numerical solution with the best fit of the diffusion coefficient is also shown (thin solid line).

produced in Figure 3. When the previously measured value of  $\mu$  ( $3.2 \times 10^{-7}$  cm<sup>2</sup>/s; Marx and Aitken, 1999) was used in a simulation of the system with the non-chemotactic strain, significantly faster naphthalene removal was predicted than was observed experimentally (Fig. 3). The data for the non-chemotactic strain could only be fit with a random motility coefficient of essentially zero. This finding could be attributable to a rapid loss of motility caused by lack of access to substrate over the long experimental period, to a downward drift in net bacterial motion caused by gravity, or both. The rate of loss of motility by *P. putida* strain PRS2000 under nutrient-limited conditions has been measured at  $0.14 \text{ h}^{-1}$  (Barton and Ford, 1995), which corresponds to nearly two orders of magnitude decay within 24 h. Similar losses in motility under starvation conditions have been observed for three different strains of *Rhizobium meliloti* (Wei and Bauer, 1998). If a downward drift velocity of  $0.02 \text{ } \mu\text{m/s}$  is incorporated into the cell balance equation, the rate of naphthalene removal by the non-chemotactic strain is predicted to decrease (not shown); however, the decrease would only partially account for the difference between the predicted naphthalene removal in the absence of an influence of gravity and the experimentally observed naphthalene removal.

The model was also used to fit a value of  $\chi_0$  to the data for the wild-type strain (Fig. 3). The best-fit value was  $1.8 \times 10^{-5}$  cm<sup>2</sup>/s (95% CI  $1.6 \times 10^{-5}$  to  $2.0 \times 10^{-5}$  cm<sup>2</sup>/s), about four times lower than the value previously determined for this organism in capillary assays with naphthalene as the chemoattractant (Marx and Aitken, 1999). We do not yet understand the reason(s) for this difference, which could include batch-to-batch variability of the culture, differences in experimental systems



**Figure 3.** Naphthalene loss from a capillary in systems inoculated with wild-type PpG7 (●) or a mutant deficient in chemotaxis to naphthalene (○). The data for the non-chemotactic mutant strain essentially overlapped with that from an uninoculated control (shown in Fig. 2). Predicted naphthalene removal by the wild-type strain with the best-fit value of the chemotactic sensitivity coefficient,  $\chi_0$ , is illustrated with the thin dashed line. A simulation with the best-fit value of  $\chi_0$  but with random motility neglected is shown as the thick dashed line. The fit for the non-chemotactic mutant ( $\chi_0 = 0$ ) with the random motility coefficient,  $\mu$ , as the fitting parameter is shown as the thin solid line. The thick solid line represents a simulation for the mutant strain with the value of  $\mu$  measured previously ( $3.2 \times 10^{-7}$  cm<sup>2</sup>/s; Marx and Aitken, 1999).

used to quantify the sensitivity coefficient, or significant decreases in swimming speed or chemotactic response over the course of the experimental period. The capillary assays used to estimate  $\chi_0$  previously were conducted over a 1-h period, while the fitted value of  $\chi_0$  in this experiment was from an experimental period greater than 6 h. The capillary tubes used in this study also were substantially larger than those used in the capillary assay (0.56 mm vs. 0.2 mm diameter). A simulation with the previously measured value of  $\chi_0$  indicated that the naphthalene mass would have approached zero in less than half the time observed experimentally (not shown).

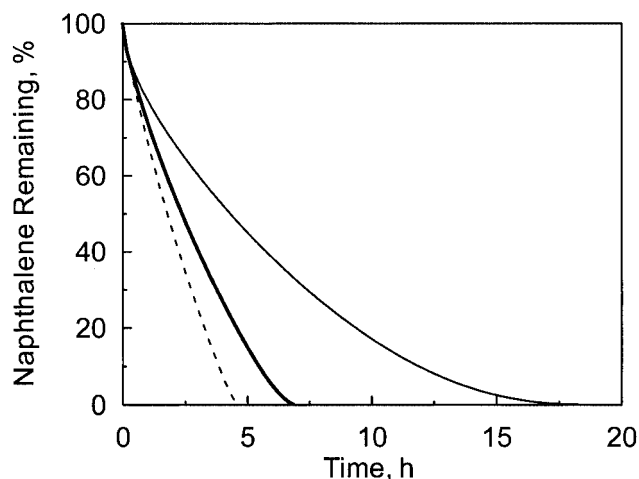
In simulations with the chemotactic wild-type strain we assumed a constant  $\mu$  of  $3.2 \times 10^{-7}$  cm<sup>2</sup>/s, on the assumption that any loss of motility that may have occurred in the absence of substrate would be much lower during active metabolism (Wei and Bauer, 1998). For this strain, the predicted naphthalene removal rate was slightly faster if random motility was neglected and the fitted  $\chi_0$  was used (Fig. 3). This result is consistent with the expected tendency of random motility to spread a concentrated band of cells moving towards the source of attractant (Marx and Aitken, 2000b). Gravity was predicted to have a negligible effect on naphthalene removal by the chemotactic strain under the experimental conditions examined in this study (not shown). We therefore ignored any influence of gravity in subsequent simulations for this strain so that we could isolate the effects of other parameters on chemotaxis.

The simulation results indicated that both the bacterial and naphthalene concentrations were uniform across the radial dimension inside the capillary. Therefore, a 1-D analysis in this domain would be a reasonable approach for similar physical systems.

### Sensitivity Analyses

Inspection of Equation (1) suggests that the value of the chemoreceptor dissociation constant,  $K_d$ , can have a significant influence on the chemotactic velocity term. We therefore examined the sensitivity of the simulated results to  $K_d$  in two ways. First, simulations were run using the best-fit value of  $\chi_0$  and  $K_d$  values either 10 times or 0.25 times the measured value of  $2.1 \text{ g/m}^3$  (simulations at  $K_d$  values lower than 0.25 times the measured value led to instability in the numerical solution). As illustrated in Figure 4, increasing  $K_d$  by an order of magnitude decreased the naphthalene removal rate, such that it would take about twice as long for essentially complete removal to occur relative to the experimentally observed removal. Decreasing the dissociation constant by a factor of four led to a modest increase in the predicted naphthalene removal rate.

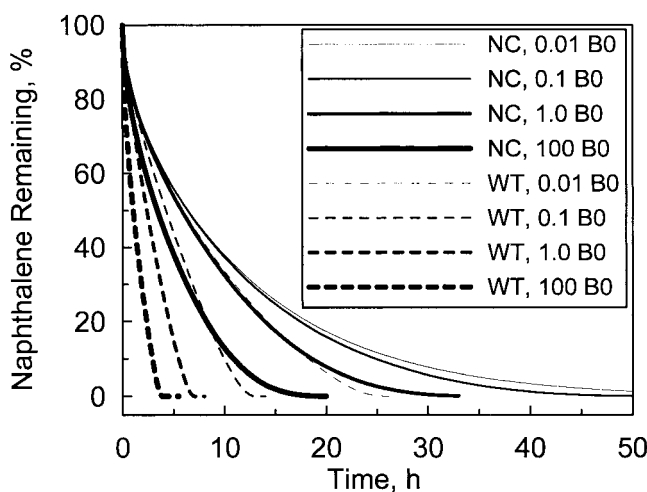
The sensitivity of the model to  $K_d$  was also examined by fitting the model with  $K_d$  as the fitting parameter, using the value of  $\chi_0$  ( $7.2 \times 10^{-5} \text{ cm}^2/\text{s}$ ) measured in our earlier work (Marx and Aitken, 1999). The resulting  $K_d$  of  $26 \text{ g/m}^3$  (95% CI 22 to  $30 \text{ g/m}^3$ ) was more than 12 times higher than the measured value, much greater than the relative change in  $\chi_0$  required to fit the model to the data. The fitted  $K_d$  is unrealistic, both because the method used to measure it is not likely to be wrong by



**Figure 4.** Predicted naphthalene loss from a capillary as a function of the  $K_d$  value used in the simulations: the measured value of  $2.1 \text{ g/m}^3$  (thick solid line), 10 times the measured value (thin solid line), and 0.25 times the measured value (dashed line). The  $\chi_0$  value used in the simulations was the best-fit value for the corresponding experiment ( $1.8 \times 10^{-5} \text{ cm}^2/\text{s}$ ).

an order of magnitude, and because it is similar to the saturation concentration of naphthalene in water; it does not seem likely that a chemoreceptor would evolve with a dissociation constant near the highest possible concentration of a chemoeffector that a cell could encounter. From the combined simulation and fitting results, the predicted naphthalene removal rate appears to be more sensitive to changes in the chemotactic sensitivity coefficient than to changes in the chemoreceptor dissociation constant.

For a given organism and chemoattractant, another important variable in determining the rate of attractant consumption is the initial biomass concentration. Simulations with the best-fit  $\chi_0$  and the previously measured value of  $\mu$  illustrate the extent to which the initial cell concentration influences the rate of naphthalene removal (Fig. 5). Of particular interest is that a two- to three-order of magnitude greater concentration of biomass is predicted to be required for the non-chemotactic strain to achieve similar rates of removal as the wild-type strain (compare, for example, the  $0.1 B_0$  curve for the wild-type strain with the  $100 B_0$  curve for the non-chemotactic strain). Note that simulations of the non-chemotactic strain would result in even slower naphthalene removal if random motility were insignificant, as was observed experimentally; this condition is approximated by the line representing the lowest initial cell concentration for the non-chemotactic strain. Also of interest is that a 100-fold increase in initial biomass concentration for the wild-type is predicted to result in slower removal of naphthalene than would a fourfold increase in the chemotactic sen-



**Figure 5.** Predicted naphthalene loss from a capillary as a function of the initial cell concentration in the reservoir,  $B_0$ . The legend indicates multiples of the initial concentration used in the experimental systems ( $4 \times 10^6 \text{ cfu/mL}$ ). The best-fit  $\chi_0$  value ( $1.8 \times 10^{-5} \text{ cm}^2/\text{s}$ ) was used together with the random motility coefficient measured previously ( $3.2 \times 10^{-7} \text{ cm}^2/\text{s}$ ), even for the non-chemotactic strain. Simulations for the non-chemotactic strain (NC) and the wild-type strain (WT) are shown with increasing line thicknesses corresponding to increasing cell concentration.

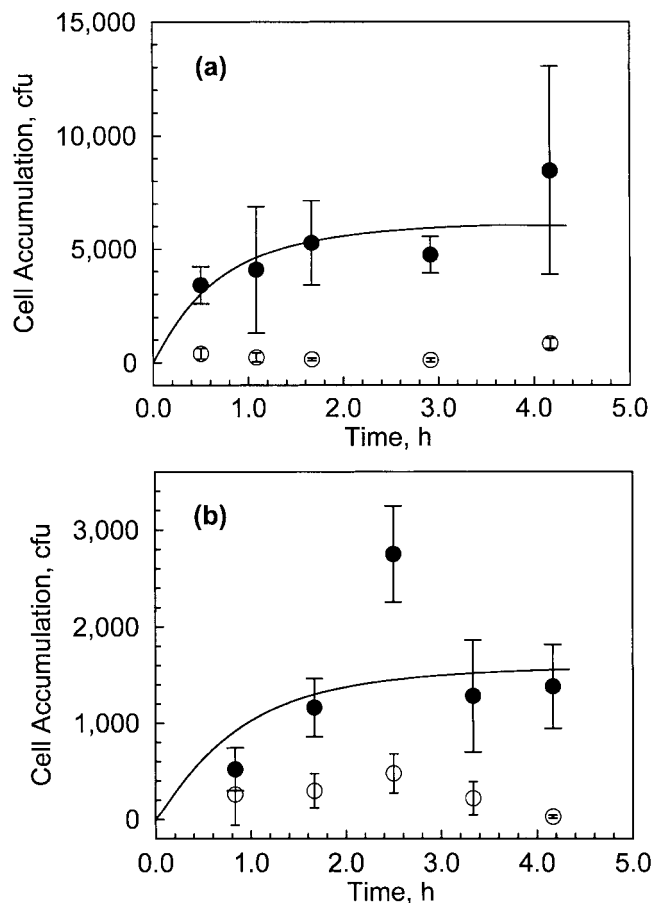
sitivity coefficient (not shown), again illustrating the sensitivity of the model to  $\chi_0$ .

Since the rate of naphthalene removal depends on the product of the cell concentration and the specific degradation rate, changes in the value of  $q_{max}$  for a given cell concentration should have the same effect as a corresponding change in cell concentration for a given  $q_{max}$ . We verified this by performing simulations at  $q_{max}$  values an order of magnitude higher and lower than the default value. The results were identical to a 10-fold increase or decrease, respectively, of the cell concentration (not shown).

### Porous-Medium Results

The conventional capillary assay used to quantify the chemotactic response of bacteria (Adler, 1973) was modified to include a model porous medium (glass beads) in both the capillary tube and the culture reservoir. An assay with a free-liquid system under otherwise identical conditions was conducted at the same time to determine a value for the chemotactic sensitivity coefficient (Fig. 6a). The resulting best-fit value of  $\chi_0$ ,  $2.9 \times 10^{-4} \text{ cm}^2/\text{s}$  (95% CI  $1.8$  to  $4.0 \times 10^{-4} \text{ cm}^2/\text{s}$ ), is about four times greater than the value measured in our earlier work (Marx and Aitken, 1999). Because maximum cell accumulations occurred over a time interval similar to that used in the earlier study, it is likely that differences in  $\chi_0$  between experiments with the same organism and chemoattractant are due to batch-to-batch variability in the culture or differences in the experimental apparatus. However, the fitted value of  $\chi_0$  in this experiment was considerably larger than the fitted value based on naphthalene degradation in the same experimental system but over a longer time scale. Thus, for a given experimental system, time may also influence the apparent value of  $\chi_0$ . As illustrated from the analysis of naphthalene removal data presented above, relatively small changes in  $\chi_0$  can lead to substantial differences in predicted responses.

The presence of porous media led, as expected, to lower cell accumulations in the capillary than in the comparable free-liquid system over the same time intervals (Fig. 6b). Nevertheless, significantly greater cell accumulations in capillaries initially containing naphthalene than in blank capillaries clearly demonstrate that chemotaxis can occur in porous media. Previous experiments in which evidence for chemotaxis in porous media was sought by following cell transport did not indicate an influence of chemotaxis (Barton and Ford, 1995; Reynolds et al., 1989). However, in those experiments either the distances separating the bacterial inocula and the chemoattractant were too long to result in significant concentration gradients (Barton and Ford, 1995; Barton and Ford, 1997), or the initial isotropic concentration of chemoattractant was so far above saturation of the chemoreceptor that a chemotactic re-



**Figure 6.** Cell accumulation data for parallel capillary assays in (a) free liquid and (b) saturated glass beads. Data represent means and standard deviations of triplicates at each time point for capillaries initially containing naphthalene (●) or blank capillaries (○). The line in panel (a) represents a fit of the model to the data, with  $\chi_0$  as the fitting parameter. The line in panel (b) is a fit of the model using the best-fit  $\chi_0$  from the free-liquid data ( $2.9 \times 10^{-4} \text{ cm}^2/\text{s}$ ) and  $\tau$  as the fitting parameter.

sponse could not occur (Barton and Ford, 1997). Compelling evidence for chemotaxis in soil has been obtained with rhizosphere bacteria (Bashan and Holguin, 1994; Soby and Bergman 1983). Indirect evidence also suggests that chemotaxis can occur in soil (van der Meer et al., 1987; Witt et al., 1999), although in these cases there was no experimental distinction between chemotactic and random motility.

The model was fit to the data from the capillary assay in the presence of glass beads, using the estimated value of  $\chi_0$  from the parallel free-liquid experiment and the tortuosity,  $\tau$ , as the fitting parameter. As a first approximation, we assumed that the effective tortuosity for the bacterial cells was the same as for the attractant, naphthalene. In previous simulation studies, the tortuosity experienced by a cell was assumed to differ from that for a solute because the porous medium might affect important chemotactic properties in addition to impeding free swimming (Duffy et al., 1995; 1997). The best-fit value of  $\tau$  from the porous-medium experiment

was 2.2. This compares well with a value of 2.4 estimated from the empirical relationship  $\tau = 1/\varepsilon$  (Wakao and Smith, 1962).

## CONCLUSIONS

A mathematical model based on earlier approaches to quantifying chemotaxis and random motility (Chen et al., 1998; Ford and Cummings, 1992; Keller and Segel, 1971; Rivero et al., 1989) was able to reproduce experimental observations satisfactorily with fits based on a single parameter. Model simulations reinforced the experimentally observed impact of chemotaxis on naphthalene degradation in the heterogeneous aqueous system. For an immotile organism, cell concentrations would have to be several orders of magnitude greater than for a chemotactic organism to achieve similar rates of naphthalene degradation, assuming all other rate parameters were equal.

Although random motility was predicted to lead to higher rates of naphthalene removal than would occur in its absence, random motility appears to be insignificant over long experimental periods in the absence of substrate. For the conditions used in the experiments considered in this study, random motility also had a minor contribution to predicted results when chemotaxis was accounted for. Because random motility might be influenced significantly by time-dependent changes in cell swimming speed and by gravity, further experimental evaluation of these factors would be warranted.

We generally used the chemotactic sensitivity coefficient,  $\chi_0$ , as the fitting parameter for systems in which chemotaxis occurred because it is less directly quantifiable than the other model parameters. Fits of  $\chi_0$  to the naphthalene removal data and cell accumulation data for free-liquid systems were within a factor of 4 (in either direction) of the  $\chi_0$  value estimated in earlier work with a different capillary assay system (Marx and Aitken, 1999). Given the general lack of quantitative data on chemotactic sensitivity coefficients, we do not yet know whether these results are within an acceptable level of experimental reproducibility. Variability in apparent values of  $\chi_0$  may be due to differences in swimming behavior in different experimental systems and under different conditions (Liu and Papadopoulos, 1996; Phillips et al., 1994) or to changes in swimming over time. Further evaluation of the reproducibility and time-dependence of key parameters, such as the cell swimming speed, will help in establishing the extent to which models for chemotaxis and random motility can be extrapolated to different experimental systems and longer experimental time scales.

The standard capillary assay was adapted to demonstrate that chemotaxis can occur in porous media. Analysis of the data from the porous-medium experiment indicated that it may be possible to use models

applicable to free-liquid systems by adjusting the relevant transport terms (diffusivity, random motility coefficient and chemotactic velocity) to account for tortuosity, as suggested earlier by Barton and Ford (1997). Further work in porous media over a broader range of experimental conditions is required to verify this conclusion.

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## NOMENCLATURE

$\chi_0$	chemotactic sensitivity coefficient	( $L^2 t^{-1}$ )
$\varepsilon$	porosity	(—)
$\Gamma$	representation of a physical boundary	
$\mu$	random motility coefficient	( $L^2 t^{-1}$ )
$\tau$	tortuosity	(—)
$B$	cell concentration	(cfu $L^{-3}$ )
$B_0$	initial cell concentration in the culture reservoir	(cfu $L^{-3}$ )
$C$	attractant (naphthalene) concentration	(M $L^{-3}$ )
$C_0$	initial attractant concentration in the capillary	(M $L^{-3}$ )
$D$	attractant diffusion coefficient	( $L^2 t^{-1}$ )
$k$	biomass decay rate coefficient	( $t^{-1}$ )
$K_d$	chemoreceptor dissociation constant	(M $L^{-3}$ )
$K_s$	half-saturation coefficient for attractant consumption	(M $L^{-3}$ )
$n$	direction normal to a boundary	
$r$	radial coordinate	(L)
$s$	cell swimming speed	(L $t^{-1}$ )
$t$	time	
$v$	chemotactic velocity	(L $t^{-1}$ )
$v_s$	Stokes terminal settling velocity	(L $t^{-1}$ )
$q_m$	maximum specific rate of attractant consumption	(M cfu $^{-1}$ $t^{-1}$ )
$Y$	microbial yield coefficient	(M cfu $^{-1}$ )
$z$	longitudinal coordinate	(L)

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