APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 1993, p. 4266–4273 Vol. 59, No. 12
0099-2240/93/124266-08$02.00/0

Biodegradation, Sorption, and Transport of 2,4-Dichlorophenoxyacetic Acid in Saturated and Unsaturated Soils

M. ROCIO ESTRELLA,[1] MARK L. BRUSSEAU,[1] ROBERT S. MAIER,[2] IAN L. PEPPER,[1] PETER J. WIERENGA,[1] AND RAINA M. MILLER[1]*

Department of Soil and Water Science, University of Arizona, Tucson, Arizona 85721[1] and Army High Performance Computing Research Center, University of Minnesota, Minneapolis, Minnesota 55415[2]

Received 29 March 1993/Accepted 15 September 1993

The fate of an organic contaminant in soil depends on many factors, including sorption, biodegradation, and transport. The herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) was used as a model compound to illustrate the impact of these interacting factors on the fate of an organic contaminant. Batch and column experiments performed with a sandy loam soil mixture under saturated and unsaturated conditions were used to determine the effects of sorption and biodegradation on the fate and transport of 2,4-D. Sorption of 2,4-D was found to have a slight but significant effect on transport of 2,4-D under saturated conditions (retardation factor, 1.8) and unsaturated conditions (retardation factor, 3.4). Biodegradation of 2,4-D was extensive under both batch and column conditions and was found to have a significant impact on 2,4-D transport in column experiments. In batch experiments, complete mineralization of 2,4-D (100 mg kg^{-1}) occurred over a 4-day period following a 3-day lag phase under both saturated and unsaturated conditions. The biodegradation rate parameters calculated for batch experiments were found to be significantly different from those estimated for column experiments.

The potential transport of soil-applied pesticides to groundwater is a major environmental concern. The development of strategies to prevent pesticide contamination of groundwater depends on accurate estimates of the kinetic parameters which govern the fate of pesticides in the subsurface. For instance, pesticide sorption can decrease or eliminate pesticide availability for biodegradation or transport, although it does not affect the total amount of pesticide in the soil system (8). Biodegradation of the pesticide can partially reduce or totally eliminate the pesticide available for transport in the soil system (7). Thus, both sorption and biodegradation can significantly affect transport. Concurrent investigations of sorption, degradation, and transport have been rare since researchers have traditionally viewed these as separate processes, and only recently has it been realized that these processes are dependent on each other. If estimated sorption and biodegradation coefficients are obtained in isolation, the use of these coefficients to predict transport can lead to erroneous results. Another concern is that the values obtained for sorption and biodegradation coefficients can depend on the methods used for measurement.

In this study, a model pesticide, 2,4-dichlorophenoxyacetic acid (2,4-D), was used to examine both the effects of sorption and biodegradation on transport through soil and the effects of the method used on experimentally determined rate coefficients. The herbicide 2,4-D was chosen since this compound is an aromatic chlorinated molecule which is structurally similar to numerous other compounds of current interest. Batch and column experiments in which a sandy loam porous medium was used were performed to determine the relative contributions of sorption and degradation to transport of 2,4-D under both water-saturated and unsaturated conditions. Values for biodegradation rate parameters obtained with different techniques (batch and column) and under different conditions were compared to evaluate the influence of experimental approach on parameter values.

MATERIALS AND METHODS

Chemicals. Analytical grade 2,4-D was obtained from Sigma Chemical Co., St. Louis, Mo. Uniformly ring-labeled ^{14}C-2,4-D with a measured specific activity of 12.8 mCi $mmol^{-1}$ was also purchased from Sigma. Tritiated water (^3H_2O) with a specific activity of 1 mCi ml^{-1} was purchased from DuPont, Burbank, Calif. The levels of radiochemical purity were >98%.

Soil. A sandy loam soil was collected from the top 10 cm at an agricultural site in the Yaqui Valley, State of Sonora, Mexico, that had a history of repeated 2,4-D applications. This soil was sieved (pore size, <2 mm) and stored at 4°C to reduce changes in the indigenous microbial population. Preliminary 2,4-D sorption experiments performed with this soil revealed that only very limited sorption occurred; thus, to increase sorption, this soil was amended with a sandy loam soil that had an organic carbon content of 12.6% and was collected from Mount Lemmon, Ariz. An 80:20 (wt/wt) mixture of the Mexican sandy loam soil and Mount Lemmon soil was used for all of the experiments. Selected characteristics of this mixed soil, referred to below as soil, were as follows: particle size distribution, 77.7% sand, 18.1% silt, and 4.2% clay; pH 7.9, as determined after extraction with an equal volume of water; organic matter content, 2.7%; electrical conductivity, 0.2 dS m^{-1}, as determined after extraction with an equal volume of water. The following different soil conditions were used in this study: saturated soil (soil moisture tension, 0 Pa); unsaturated soil (tension, 30×10^5 Pa); sterile soil (autoclaved for 90 min at 120°C and

* Corresponding author.

21 lb/in^2); untreated soil; and spiked soil (soil spiked twice with 100 mg of 2,4-D per kg, once 2 weeks before the experiment and once 4 weeks before the experiment).

Batch sorption studies. Adsorption-desorption isotherms were obtained by the typical sequential desorption method and by a dilution desorption method, which was a modified batch equilibration method (4, 13). To obtain sorption isotherms, 10-g portions of sterile soil and 10-ml portions of a filter-sterilized 0.01 N $CaCl_2$ solution containing various concentrations of ^{14}C-2,4-D (500 mg liter^{-1}; 5 μCi liter^{-1}) were shaken for 72 h at 24°C. The amount of radioactivity in each supernatant was determined in triplicate with a model 1600TR Tri-Carb liquid scintillation counter (Packard, Meridian, Conn.). Sequential desorption isotherms were obtained by centrifuging samples and then removing the supernatants. An equal amount of 0.01 N $CaCl_2$ was added to each preparation, the samples were shaken for 24 h and centrifuged, and the amount of radioactivity in each supernatant was measured in triplicate. This desorption procedure was performed two more times; thus, a total of three resuspension-centrifugation steps were performed. To obtain dilution desorption isotherms, the supernatant was removed, and enough 0.01 N $CaCl_2$ was added to obtain a soil/solution ratio of 1:2.5. Samples were then shaken for 24 h, and the amount of radioactivity in each supernatant was measured.

Batch biodegradation studies. For batch experiments, ^{14}C-2,4-D (100 mg kg^{-1}; 5 nCi g^{-1}) was added to 10-g portions of soil in 125-ml modified micro-Fernbach flasks (9). For unsaturated conditions, the soil volumetric water content was adjusted to 30% (soil water tension, 30×10^5 Pa) with 0.01 N $CaCl_2$. For saturated conditions, 10 ml of 0.01 N $CaCl_2$ was added, so that the soil/solution ratio was 1:1. Each experiment was performed in triplicate, and the results were compared with the results obtained with sterile (autoclaved) controls. Biodegradation of 2,4-D was determined by measuring the amounts of $^{14}CO_2$ and other volatile compounds, which were collected by periodically flushing the modified flasks with air through a series of six traps (9). In some cases, biodegradation was determined by the disappearance of organic solvent-extractable 2,4-D. To do this, 2,4-D was extracted from soil samples by adding 10 ml of acetone. The pH was then adjusted to <2.5 with HCl, and samples were shaken for 10 min. Chloroform (50 ml) and sodium sulfate (20 g) were added, and samples were shaken overnight and then filtered. Each filtered solution was concentrated with a rotoevaporator, and its volume was adjusted to 10 ml with acetonitrile-acidified water (50:50, vol/vol). The concentration of 2,4-D was determined by using a Waters Associates high-performance liquid chromatography (HPLC) system, which consisted of a model 600E delivery unit equipped with a Waters Associates model 715 Ultra Wisp sample processor and a Waters Associates model 490E multiwavelength detector operating at 235 nm. The column was a 5-μm Spherisorb ODS-2 column (250 by 4.6 mm) obtained from Alltech Associates, Inc., Deerfield, Ill. Elution was isocratic, and the mobile phase used was acetonitrile-acidified water (50:50, vol/vol); the flow rate was 1 ml min^{-1}. Acetic acid was used to acidify the water to pH 2.6.

Saturated-column displacement studies. Movement of 2,4-D through saturated soil columns was analyzed by using the miscible displacement technique (5). Chromatography columns (2.5 cm [inside diameter] by 5 cm; Kontes, Vineland, N.J.) were packed and saturated with a 0.01 N $CaCl_2$ solution until steady-state hydrodynamic conditions were established. To characterize the hydrodynamic properties of the columns, tritiated water (specific activity, 5 μCi liter^{-1}) was displaced through each column. Then, a ^{14}C-2,4-D solution (100 mg liter^{-1}; 5 μCi liter^{-1}) was introduced into the column at a constant flux until the effluent concentration of solute (C)/concentration of solute in the influent solution (C_0) was constant. Column effluents were collected in 1-ml aliquots with an automatic fraction collector. The level of radioactivity (^{14}C or ^{3}H) in the effluent was determined by liquid scintillation counting. In some cases the dissolved oxygen concentration in effluent samples was determined with a CHEMets dissolved oxygen kit (CHEMetrics, Inc., Calverton, Va.).

Unsaturated-column displacement studies. Transport experiments under unsaturated conditions (18) were performed by using a glass column (5 cm [inside diameter] by 30.5 cm) with a porous stainless steel plate (mean pore diameter, 0.5 μm) on the bottom. After the column was packed with soil (bed length, 27.5 cm), two tensiometers were installed, one 5 cm from the top of the column and one 5 cm from the bottom of the column. The column was then connected to a vacuum chamber containing an automatic fraction collector. The column was flushed with 0.01 N $CaCl_2$ at a flow rate of 100 ml day^{-1} until steady-state conditions were obtained. Steady state was defined as follows: (i) equal pressure heads on the two tensiometers ($-30 \times 10^5 \pm 2 \times 10^5$ Pa), (ii) equal influent and effluent flow rates, and, (iii) a constant column mass. A hand-held pressure transducer (Tensicorder; Soil Measurement Systems, Tucson, Ariz.) was used to measure suction on the two tensiometers.

Once steady state was reached, the influent solution was replaced with a 0.01 M $CaCl_2$ solution containing tritiated water (5 μCi liter^{-1}) and ^{14}C-2,4-D (100 mg liter^{-1}; 5 μCi liter^{-1}). Column effluent was collected in 7-ml aliquots, and the amount of radioactivity was measured by liquid scintillation counting.

Analysis of batch biodegradation kinetics. Initial cell mass concentration (X_0) values were estimated by performing nonlinear regression analyses of the simultaneous solutions to the Monod equations for growth of cell mass, substrate utilization, and CO_2 production as a function of time (10):

$$\frac{\partial X}{\partial t} = \mu_m \cdot \frac{XC}{K_s + C} - k_d X \quad (1)$$

$$\frac{\partial C}{\partial t} = -\frac{1}{Y} \cdot \mu_m \cdot \frac{XC}{K_s + C} \quad (2)$$

$$\frac{\partial CO_2}{\partial t} = Y_{CO_2\ \text{substrate}}\left(-\frac{\partial C}{\partial t}\right) + Y_{CO_2\ \text{endogenous}} k_d X \quad (3)$$

where X is the cell mass concentration, t is time, C is the solution phase concentration of solute (i.e., substrate), μ_m is the maximum specific growth rate, K_s is the half-saturation constant, k_d is the cell death rate coefficient, Y is the cell mass yield from substrate degradation, $Y_{CO_2\ \text{substrate}}$ is the CO_2 mass yield from substrate degradation, and $Y_{CO_2\ \text{endogenous}}$ is the CO_2 mass yield from endogenous decay of cells.

Analysis of saturated-column experiments. The results of saturated-column studies were analyzed by using a coupled process transport model (17) that included rate-limited sorption and first-order degradation, as well as the assumption that the increase in biomass was negligible during the experiment. The nondimensional governing equations for solute transport constrained by steady-state water flow, linear rate-limited sorption, and first-order degradation are:

$$\beta R \cdot \frac{\partial C^*}{\partial T} + (1 - \beta)R \cdot \frac{\partial S^*}{\partial T} = \frac{1}{P} \cdot \frac{\partial^2 C^*}{\partial X^2} - \frac{\partial C^*}{\partial X} - \xi C^* - \eta S^* \quad (4)$$

$$(1 - \beta)R \cdot \frac{\partial S^*}{\partial T} = \omega(C^* - S^*) - \eta S^* \quad (5)$$

where the nondimensional parameters are defined as follows:

$$C^* = C/C_0 \quad (6a)$$

$$S^* = S_2/[(1 - F)K_p C_0] \quad (6b)$$

$$X = x/l \quad (6c)$$

$$T = tv/l \quad (6d)$$

$$P = vl/D \quad (6e)$$

$$R = 1 + (\rho/\theta)K_p \quad (6f)$$

$$\beta = [1 + (\rho/\theta)FK_p]/R \quad (6g)$$

$$\omega = k_2(1 - \beta)Rl/v \quad (6h)$$

$$\xi = \mu_1 l/v + (\beta R - 1)\mu_{s1} l/v \quad (6i)$$

$$\eta = (1 - \beta)R\ \mu_{s2}\ l/v \quad (6j)$$

where S_2 is the sorbed-phase concentration for the rate-limited domain, t is time, x is distance, l is column length, ρ is the bulk density of the porous medium, θ is the fractional volumetric water content of the packed column, v is the average pore water velocity, D is the hydrodynamic dispersion coefficient, K_p is the equilibrium sorption constant, F is the fraction of sorbent for which sorption is instantaneous, k_2 is the reverse sorption rate constant, and μ_1, μ_{s1}, and μ_{s2} are the first-order degradation rate constants for the solution, equilibrium-sorbed, and rate-limited-sorbed phases, respectively.

The governing equations were solved with a finite-difference numerical approach under the following initial and flux type boundary conditions:

$$C^*(X,0) = S^*(X,0) = 0 \quad (7a)$$

$$C_0 = C^* - \frac{1}{P} \cdot \left.\frac{\partial C^*}{\partial X}\right|_{x=0} \quad (7b)$$

$$\frac{\partial C^*(1,T)}{\partial X} = 0 \quad (7c)$$

First-order biodegradation rate constants (μ_1) were estimated by using the following formula:

$$\mu_1 = \frac{v}{l}(-1n\ C^s) \quad (8)$$

where C^s is the steady-state relative effluent concentration (2).

Analysis of unsaturated-column experiments. The results from unsaturated column studies could not be simulated by a one-dimensional advective-dispersive solute transport model containing a first-order removal term such as that shown in equation 4. Therefore, the transport of 2,4-D and the accumulation of cell mass were modeled as a nonlinear parabolic system by using the following equations:

$$\beta R \cdot \frac{\partial C}{\partial t} = D \cdot \frac{\partial^2 C}{\partial x^2} - v \cdot \frac{\partial C}{\partial x} - \frac{\rho}{\theta} k_2[(1 - F)K_p C - S_2] - \frac{1}{Y}\left(\frac{\mu_m \cdot C \cdot X}{K_s + C}\right) \quad (9)$$

$$\frac{\partial S_2}{\partial t} = k_2[(1 - F)K_p C - S_2] \quad (10)$$

$$\frac{\partial X}{\partial t} = \frac{\mu_m \cdot C \cdot X}{(K_s + C)} \quad (11)$$

Note that the model described by these equations accounts for rate-limited sorption with a two-compartment approach and for transformation in the solution phase by using Monod kinetics. A transport model that accounted for rate-limited sorption (by diffusional constraints) and used a Monod-based description of biological activity was described by Ying and Weber (20). However, the model of Ying and Weber is of limited use for many soil systems because degradation was limited to the solid phase, whereas it appears that degradation actually occurs primarily in the solution phase. Equations 9 through 11 were solved under boundary and initial conditions similar to those shown in equation 7. The parameters fitted included μ_m and K_s, which have been shown to be far from linear in their estimation behavior (14). As a result, the estimates of these parameters were severely biased so that confidence intervals based on linearization were inaccurate. Therefore, no standard errors are reported below for fitted column parameters.

RESULTS

Batch sorption and biodegradation studies. A linear isotherm was obtained for sorption of 2,4-D by the soil (Fig. 1). A nonsingular isotherm was obtained with the sequential desorption method (Fig. 1A), as often reported previously. However, a singular isotherm was obtained with the dilution desorption method (Fig. 1B). This suggests that the nonsingularity observed in Fig. 1A is an artifact related to the successive periods of abrasion and centrifugation involved in the sequential desorption method. Similar results have been reported previously (4, 13). The K_p value was 0.25 ml g of soil^{-1}.

Biodegradation of 2,4-D under batch conditions was studied by conducting a series of experiments under both saturated and unsaturated conditions. As shown in Fig. 2, complete mineralization of 2,4-D occurred rapidly under both saturated and unsaturated conditions. Under saturated conditions 52% of the ^{14}C-2,4-D was recovered as $^{14}CO_2$ in 10 days, while only 36% of the 2,4-D was recovered as $^{14}CO_2$ under unsaturated conditions.

Under both saturated and unsaturated conditions, there was a lag phase of 2 to 3 days before significant biodegradation occurred (Fig. 2). The X_0 value in untreated soil was estimated to be 1.35 ± 0.25 mg liter^{-1} (mean ± standard error). To further examine the observed lag phase, a soil sample was spiked with 2,4-D (100 mg kg^{-1}) twice, once 2 weeks before biodegradation was measured and once 4 weeks before biodegradation was measured. In this case, the length of the lag phase decreased from 3 days to 1 day, and the X_0 value was estimated to be 5.25 ± 0.82 mg liter^{-1}

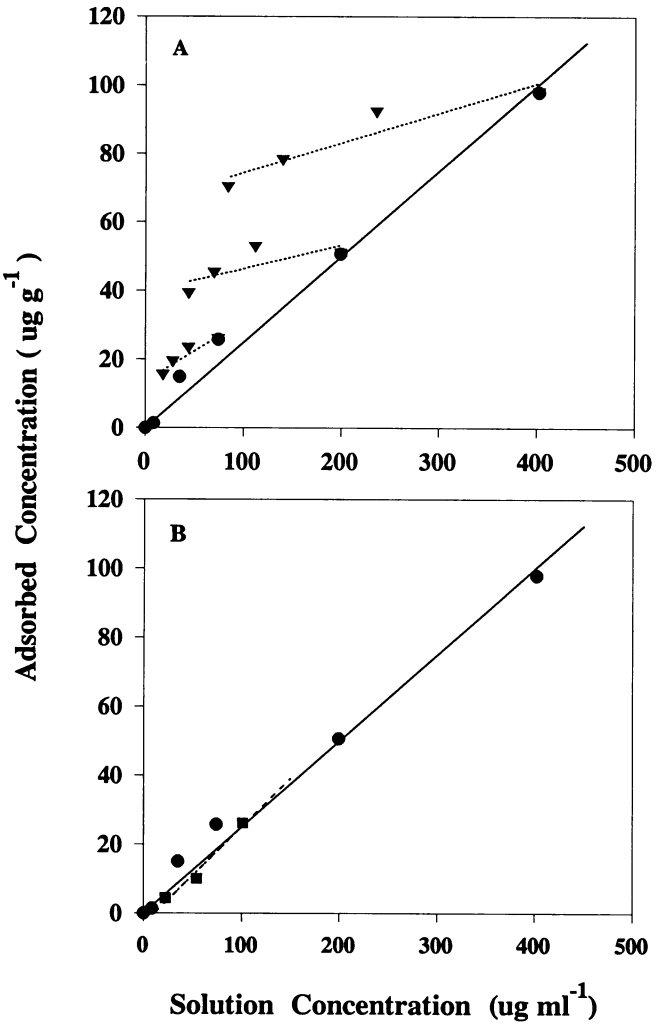

FIG. 1. Nonsingular (A) and singular (B) 2,4-D sorption-desorption isotherms when the K_p value was 0.2 ml g^{-1}. Symbols: ●, 2,4-D sorption; ▼, nonsingular 2,4-D desorption; ■, singular 2,4-D desorption. ——, regression of sorption data; ...., regression of desorption data.

(mean ± standard error). Figure 3 shows the experimental growth curves and optimized growth curves which were generated by using the estimated X_0 values.

Column displacement studies: saturated conditions. Simultaneous interactions of sorption and biodegradation that occurred during 2,4-D transport were studied by performing a series of column experiments under saturated and unsaturated conditions. Typical 2,4-D breakthrough curves generated under saturated conditions are shown in Fig. 4. The breakthrough curves for the sterile columns slowly approached a C/C_0 value of 1 (i.e., tailing). This tailing indicates the impact of rate-limited sorption on transport (6).

In contrast to the breakthrough curves for the sterile columns, which eventually plateaued at a C/C_0 value of ≈1, the breakthrough curves obtained with the untreated and spiked soils, in which biodegradation occurred, exhibited plateaus at C/C_0 values of 0.96 and 0.89, respectively. This behavior is consistent with the impact of degradation on solute transport (2). To ensure that the effluent radioactivity was due to 2,4-D, effluent samples were analyzed by HPLC

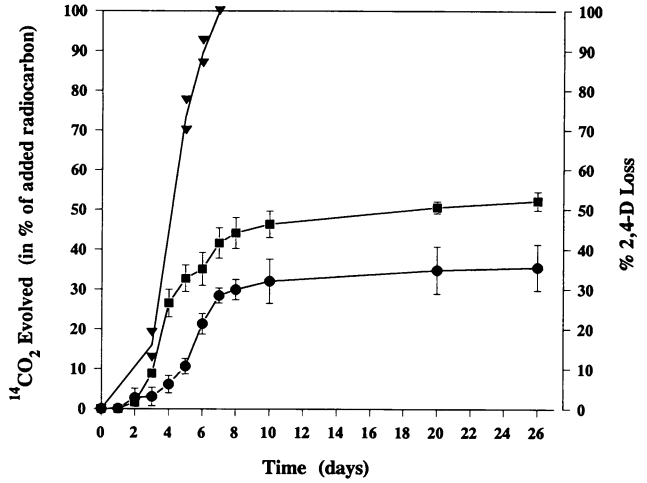

FIG. 2. Mineralization of 2,4-D under saturated and unsaturated conditions. $^{14}CO_2$ evolution and loss of 2,4-D were measured as described in Materials and Methods. Symbols: ■, $^{14}CO_2$ evolution under saturated conditions; ●, $^{14}CO_2$ evolution under unsaturated conditions; ▼, percentage of 2,4-D lost as determined by measuring extractable 2,4-D residues under unsaturated conditions.

in some experiments. The results of these analyses were identical to the results shown in Fig. 4. Some column effluents were also tested for dissolved oxygen concentrations. For both untreated and spiked columns, the level of dissolved oxygen was found to be ≤1 mg liter^{-1}. Equilibrium K_p values were estimated for each column treatment. K_p values were found to vary by a maximum of 30% between

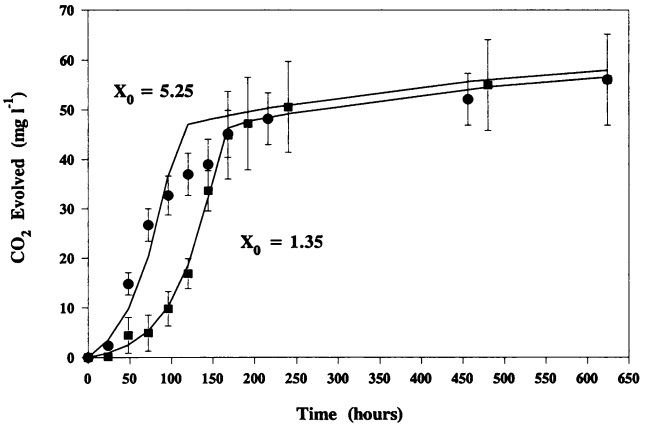

FIG. 3. Experimental and simulated mineralization of 2,4-D in untreated and spiked soils. The amount of $^{14}CO_2$ evolved was measured as described in Materials and Methods and was converted to concentration by using a carbon mass balance which assumed complete biodegradation of 2,4-D by 168 h (see Fig. 2). A modified Monod model was used to simulate 2,4-D biodegradation in untreated and spiked soils. The untreated soil optimized parameter values (means ± standard errors) were as follows: X_0, 1.35 ± 0.25 mg liter^{-1}; K_s, 7.2 ± 4.48 mg liter^{-1}; k_d, 0.06 ± 0.04 day^{-1}; Y_{CO_2} endogenous, 0.27 ± 0.11. The untreated soil fixed parameter values were as follows: C_0, 100 mg liter^{-1}; $CO_{2\ initial}$, 0 mg liter^{-1}; μ_m, 0.7 day^{-1}; Y, 0.58; Y_{CO_2} substrate, 0.45. The spiked soil optimized X_0 value was 5.25 ± 0.82 mg liter^{-1}; all other parameter values were the values used in the untreated soil simulation. Symbols: ■, untreated soil; ●, spiked soil; ——, simulations.

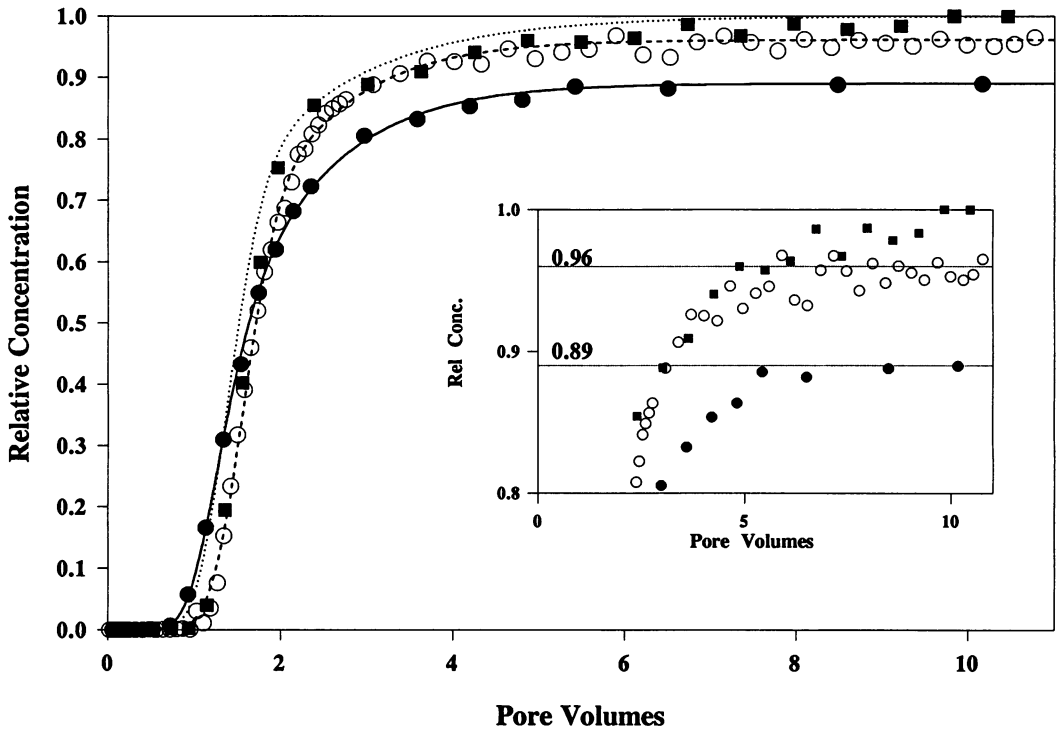

FIG. 4. Experimental and simulated breakthrough curves for ^{14}C-2,4-D from the saturated sterile soil (v, 6.2 cm h^{-1}; ρ, 1.59 g cm^{-1}), untreated soil (v, 4.7 cm h^{-1}; ρ, 1.37 g cm^{-1}), and spiked soil (v, 6.1 cm h^{-1}; ρ, 1.55 g cm^{-1}) experiments. (Inset) Expanded view of the graph for relative concentration values ranging from 0.8 to 1.0. Symbols: ■, sterile soil; ○, untreated soil; ●, spiked soil. Simulations of breakthrough curves for 2,4-D from the soil experiments were obtained with a coupled-process transport model that included first-order biodegradation (16). ...., μ_1 value of 0; – – – –, μ_1 value of 0.9 day^{-1}; ——, μ_1 value of 3.4 day^{-1}.

sterile columns (K_p, 0.2 ml g^{-1}) and nonsterile columns (K_p, 0.3 ml g^{-1} for untreated soil and spiked soil) (Table 1).

Finally, first-order biodegradation rate constants were calculated from the column data and, as expected, were found to be smaller for untreated soil (μ_1, 0.9 day^{-1}) than for spiked soil (μ_1, 3.4 day^{-1}). In order to compare these rate constants with batch and unsaturated column μ_m values, the first-order rate equation was equated with the Monod equation for substrate disappearance.

$$\mu_1 = \frac{\mu_m C_0 X_0}{Y(K_s + C_0)} \quad (12)$$

Equation 12 reveals that μ_1 reflects several of the parameters contained in the Monod equation, such as K_s, Y, μ_m, and X_0. Assuming that the magnitudes of the first three parameters remain constant, changes in X_0 would be expected to cause corresponding changes in μ_1. A comparison of μ_1 values for 2,4-D degradation in saturated soil columns containing either untreated or spiked soil (Table 1) revealed a 3.7-fold difference, which is very similar to the difference observed in X_0 values in untreated and spiked batch experiments (i.e., a 3.9-fold difference). In contrast, the μ_m values, which were independent of X_0, were similar for the column experiments performed with untreated and spiked soils (Table 1).

TABLE 1. Sorption and first-order biodegradation constants

Expt	Soil	K_p (ml g^{-1})	μ_1 (day^{-1})	μ_m (day^{-1})
Batch	Saturated, sterile	0.2[a]		
	Saturated, untreated			0.9[b]
	Unsaturated, untreated			0.7[b]
	Unsaturated, spiked			0.7[b]
Column	Saturated, sterile	0.2[c]		
	Saturated, untreated	0.3[d]	0.9	2.3[e]
	Saturated, spiked	0.3[d]	3.4	2.2[e]
	Unsaturated, untreated	0.6[d]		9.4[f]

[a] K_p was calculated from the batch sorption isotherm (Fig. 1).
[b] The experimental μ_m value was obtained from batch experiments.
[c] K_p was calculated from a mass balance analysis of the 2,4-D breakthrough curve.
[d] K_p was calculated from the optimized R value obtained by fitting the 2,4-D breakthrough curve with the appropriate model.
[e] μ_m was calculated from experimental μ_1 values by using the following assumption: $\mu_1 = \mu_m X_0 C_0 [Y(K_s + C_0)]^{-1}$. The following parameter values were used: X_0, 1.35 and 5.25 mg liter^{-1} (batch); C_0, 100 mg liter^{-1}; Y, 0.58 (batch); K_s, 472 mg liter^{-1} (unsaturated column).
[f] The optimized μ_m value was obtained from simulation of the unsaturated breakthrough curve.

Column displacement studies: unsaturated conditions. Breakthrough curves for ^{14}C-2,4-D and ^3H_2O transport in sterile and untreated soil under unsaturated conditions are shown in Fig. 5. The 2,4-D breakthrough curve obtained for transport in the sterile column plateaued at a C/C_0 value of ≈1 (Fig. 5A). This breakthrough curve is noticeably asymmetrical, reflecting the effect of rate-limited sorption on transport. Like the saturated column curves, the 2,4-D breakthrough curve for untreated soil was shifted to the right of the curve for the conservative tracer (^3H_2O) (Fig. 5). As shown in Fig. 5B, the concentration of 2,4-D in effluent samples increased for the first 3 pore volumes and then began to decrease. Surprisingly, after 5 pore volumes, the

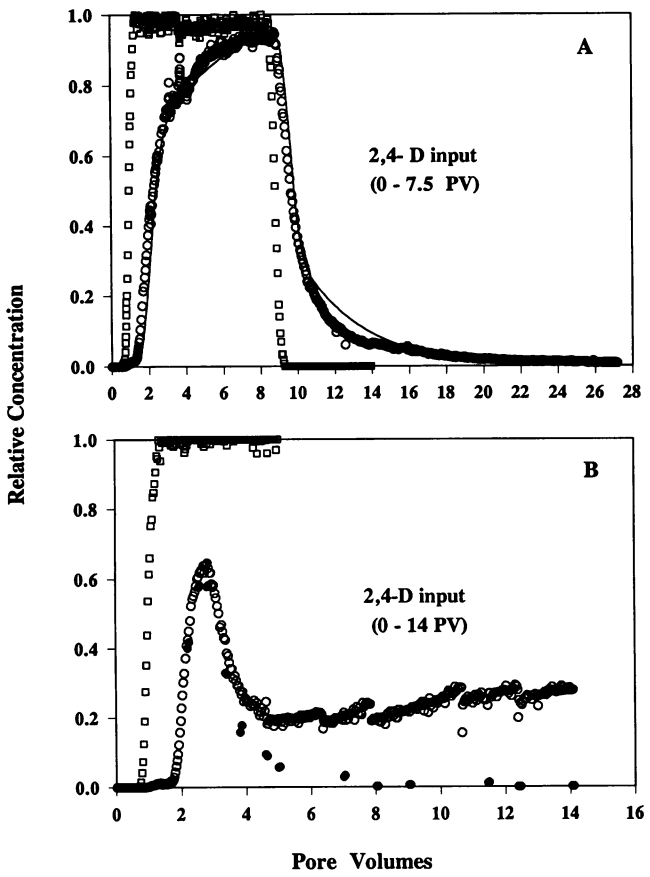

FIG. 5. Breakthrough curves for ^3H_2O and ^{14}C-2,4-D from the unsaturated untreated soil experiment (v, 0.7 cm h^{-1}; ρ, 1.25 g cm^3; volumetric water content, 0.29). (A) Sterile soil experiment. (B) Nonsterile soil experiment. Symbols: □, ^3H_2O; ○, ^{14}C radioactivity in the column effluent; ●, ^{14}C radioactivity in the column effluent minus $^{14}CO_2$ in the effluent; PV, pore volume.

2,4-D concentration in effluent samples began to slowly increase again. To ensure that the radioactivity measured in the effluent samples was actually 2,4-D radioactivity, the samples were placed in modified micro-Fernbach flasks, acidified to a pH of <2 to decrease the solubility of CO_2, and then flushed through a series of six traps designed to collect $^{14}CO_2$ and volatile compounds (9). Significant amounts of effluent radioactivity were found to be due to $^{14}CO_2$, and the values were used to correct the 2,4-D data shown in Fig. 5B.

The corrected data show that for the first 3 pore volumes the concentration of 2,4-D in the effluent steadily decreased until there was no 2,4-D at approximately 8 pore volumes, indicating that complete biodegradation of 2,4-D took place within the column. It is important to recognize that the decline and disappearance of 2,4-D in the effluent occurred while the 2,4-D solution was still being introduced into the column. This behavior indicates that temporally nonuniform biological activity occurred.

DISCUSSION

Sorption. Similar K_p values were obtained for untreated and spiked soils, suggesting that there was negligible sorption of 2,4-D by the microbial biomass. In addition, the K_p value obtained from the batch isotherm experiment (performed with sterile soil) was nearly identical to the value obtained from the sterile saturated-column experiments. Interestingly, the K_p value obtained for the unsaturated column was twice as large as the K_p values obtained from saturated column experiments. This may have been due to the time scale effect of the slower pore water velocity in the unsaturated column experiment (6). The overall consistency between the results of different experimental techniques suggests that the K_p values measured in batch systems are representative of sorption that occurs under dynamic transport conditions. The values obtained for F and k_2 (for example, 0.49 and 0.01 h^{-1} for the unsaturated column) are consistent with values reported previously (6).

Biodegradation. The μ_m values varied considerably depending on the experimental conditions used. The μ_m values obtained from the batch experiments were three times smaller than those obtained from the saturated-column experiments and 14 times smaller than those obtained from the unsaturated-column experiments (Table 1). Similar differences between the results of batch and column experiments (comparing first-order biodegradation rate constants) have been described for other organic compounds (2). This difference may be caused by differences in aeration and mixing in the batch and column systems or by the decreasing substrate concentrations in batch experiments compared with constant influent substrate concentrations in column experiments.

In addition to the effect of experimental technique (batch versus column), the influence of degree of water saturation on μ_m was investigated. While the μ_m values obtained from batch experiments under saturated and unsaturated conditions were similar, the μ_m value obtained in saturated-column experiments (2.3 day^{-1}) was much smaller than the μ_m value obtained in unsaturated-column experiments (9.4 day^{-1}). This is explained in part by the fact that there was insufficient time for the biodegrading population to increase because of the short duration (<12 h) of the saturated-column experiments. This difference may also have been due to oxygen limitations that slowed the biodegradation of 2,4-D during the saturated-column experiments. This hypothesis is supported by the fact that the dissolved oxygen concentration in saturated-column effluents was consistently ≤1 mg $liter^{-1}$.

Previous research has suggested that both efficiency and rate of pesticide degradation are different under aerobic and anaerobic conditions (12, 19). This finding results from the fact that there is a higher free energy yield (ΔF) when molecular oxygen is used as the terminal electron acceptor (aerobic respiration). It has been estimated that the ΔF is at least 2 times greater under aerobic conditions than under anaerobic conditions (11). While our results revealed a difference in efficiency of 2,4-D degradation under batch saturated conditions (52% CO_2) and unsaturated conditions (36% CO_2), the batch μ_m values were similar (0.7 day^{-1}). In contrast, for column experiments, the μ_m value for unsaturated conditions (9.4 day^{-1}) was higher than the μ_m value for saturated conditions (2.3 day^{-1}). The low μ_m value obtained in the batch experiments suggests that low availability of 2,4-D due to poor mixing may be more limiting to the rate of biodegradation under batch conditions than the amount of oxygen available. The dependence of estimated values for the parameter μ_m on the type of study (batch versus column, degree of saturation) indicates that predictions of biodegradation rates must reflect the experimental conditions used.

In contrast, the lag phases for 2,4-D biodegradation were

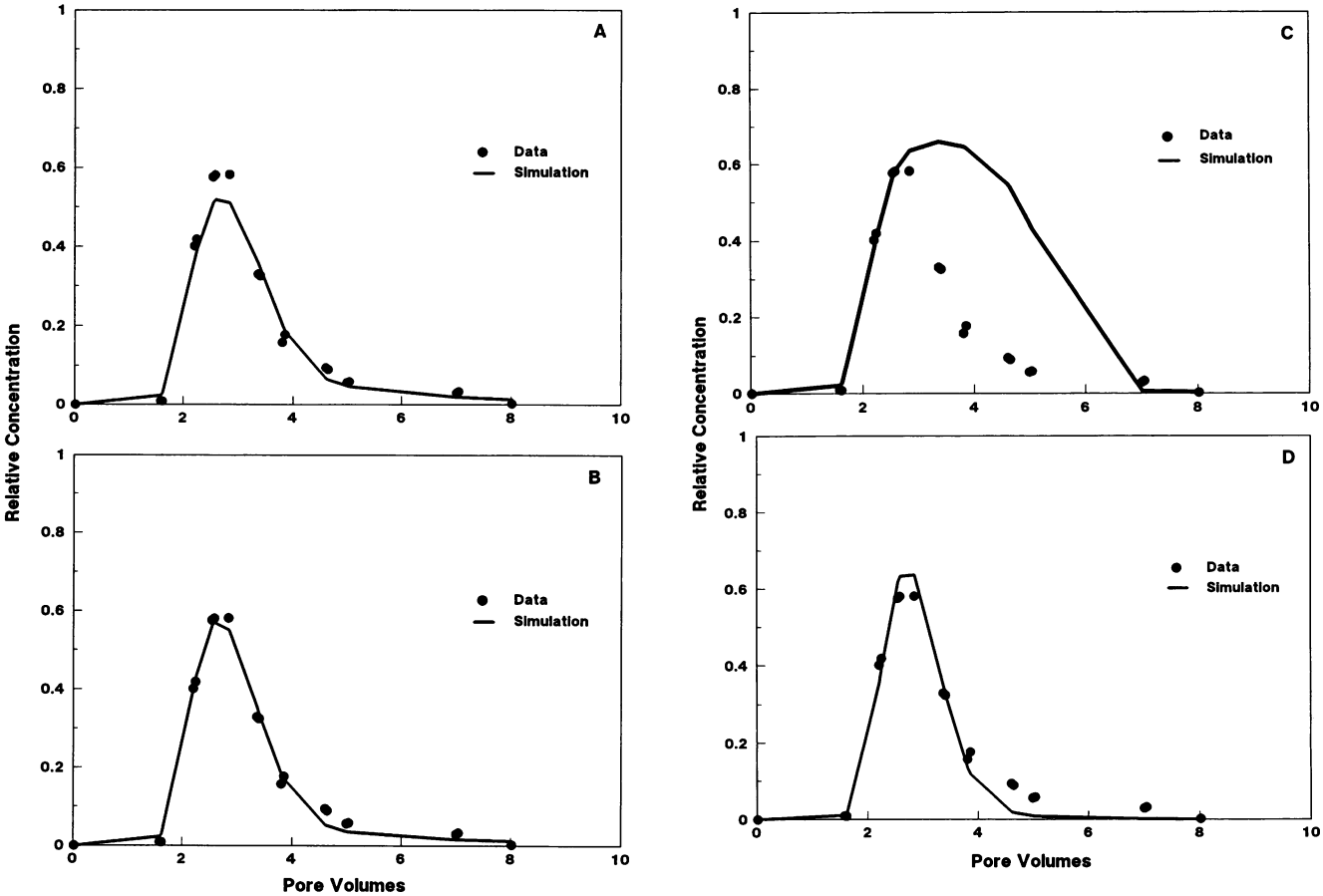

FIG. 6. Simulations of breakthrough curves for 2,4-D from the untreated unsaturated soil experiment (v, 0.7 cm h^{-1}; Y, 0.58; X_0, 1.4 mg $liter^{-1}$; D, 0.24 cm^2 h^{-1}). A coupled-process transport model with Monod biodegradation kinetics was used to analyze the data. (A) Optimized parameters: μ_m, 9.4 day^{-1}; K_s, 472 mg $liter^{-1}$. Fixed parameters: K_p, 0.6 ml g^{-1}; k_2, 0.2 day^{-1}. (B) Optimized parameters: μ_m, 176.6 day^{-1}; K_s, 1,000 mg $liter^{-1}$; K_p, 0.6 ml g^{-1}; k_2, 0.006 day^{-1}. (C) Fixed parameters: μ_m, 0.7 day^{-1}; K_s, 7.2 mg $liter^{-1}$; K_p, 0.6 ml g^{-1}; k_2, 0.2 day^{-1}. (D) Optimized parameters: μ_m, 196.8 day^{-1}; K_s, 9,996 mg $liter^{-1}$; K_p, 0.3 ml g^{-1}.

similar under batch and column conditions, suggesting that batch experiments may be predictive. Several groups of workers have reported that soils which have been exposed to 2,4-D exhibit a considerably shorter lag phase with subsequent additions of 2,4-D (3, 15, 16). Interestingly, even though the soil used in these experiments had recently been exposed to 2,4-D, there was still a 3-day lag period in both batch and unsaturated-column experiments before biodegradation began. However, when the soil was spiked with 2,4-D immediately prior to the experiment, the length of the lag phase was greatly reduced. In general, the lag phase can be attributed to (i) enzyme induction, (ii) random mutation, or (iii) an increase in the number of organisms capable of biodegradation (1). The soil used in these experiments had a history of 2,4-D treatment, suggesting that the lag phase was most likely due to an initial small biodegrading population rather than to enzyme induction or random mutation. The results of the modeling analyses performed to estimate X_0 values in untreated and spiked soils seem to confirm this, as shown in Fig. 3; the X_0 value was estimated to be approximately 3.9 times greater for the spiked soil than for the untreated soil in batch experiments. As stated above, the saturated-column μ_1 values for untreated and spiked soils exhibited a 3.7-fold difference, reflecting a similar difference in X_0 values.

Transport. A transport model based on rate-limited sorption and first-order biodegradation kinetics (equations 4 and 5) provided good simulations of the 2,4-D breakthrough curves obtained from saturated column experiments (Fig. 4, dashed and solid lines). The μ_1 value was calculated from the experimental data by using equation 8. Successful simulations of breakthrough curves influenced by simultaneous rate-limited sorption and biodegradation have been reported previously (2), suggesting that equations 4 and 5 provide an accurate description of transport under defined conditions. First-order biodegradation assumes that at constant biomass (insignificant growth) and limited substrate levels, the rate of degradation is directly proportional to the concentration of the substrate. Given the time frame of the saturated-column experiments (a maximum of 12 h), these assumptions seem to be valid.

For unsaturated conditions it was necessary to use a transport model which included a Monod-based representation of biodegradation kinetics and also accounted for rate-limited sorption. This model provided a relatively good simulation of the 2,4-D breakthrough curve for the unsaturated column (Fig. 6A). The parameters K_s (472 mg $liter^{-1}$) and μ_m (9.4 day^{-1}) were optimized to obtain this fit; the values for the other parameters (V, D, Y, K_p, k_2, and X_0) were obtained from experimental data. For comparison, Fig.

6B shows a curve in which K_p and k_2, as well as K_s and μ_m, were optimized. The optimized values for K_p and k_2 are very similar to the values that were determined in the sterile unsaturated-column experiment and were used to produce the simulation shown in Fig. 6A. This similarity suggests that both the equilibrium and kinetic components of sorption were not altered by biological activity.

To evaluate the viability of batch-determined biodegradation parameters, a breakthrough curve was simulated by using the values for K_s (7.2 mg liter^{-1}) and μ_m (0.7 day^{-1}) obtained in the unsaturated batch experiment. It is evident that this curve does not provide a good description of the data (Fig. 6C). This suggests that the values determined for K_s and μ_m in the batch experiments are not appropriate for the column experiments. This may be a reflection of the different conditions (e.g., mixing, aeration, solid/water ratio) associated with the two systems and the resulting impact on biological activity.

A breakthrough curve produced with a model that was based on the assumption that there was instantaneous sorption is shown in Fig. 6D. This curve was obtained by optimizing K_p (0.3 ml g^{-1}), as well as K_s (9,996 mg liter^{-1}) and μ_m (196.8 day^{-1}). While the curve provides a fairly good match to the data, the values obtained for the fitted parameters are unrealistic. For example, since sorption was assumed to be instantaneous, the tailing exhibited by the data could not be accounted for by the true cause (rate-limited sorption). Thus, the tailing had to be accounted for by the biodegradation parameters. This was done by a greatly increased value for K_s, which resulted in a lower rate of degradation at small substrate concentrations. Note also that the fitted K_p value is less than the value measured in the sterile unsaturated-column experiment (0.6 ml g^{-1}). This artificially small K_p value does not account for all of the solute mass that was retained by the system. To maintain a mass balance, this "extra" mass had to be lumped into the mass degraded. Thus, the μ_m value was increased greatly to allow for greater mass lost per unit of time. This example illustrates the danger associated with using an inappropriate transport model to fit a data set; while the fitted curve may reproduce the data, the fitted parameter values may have no physical meaning.

The demand for accurate simulations of field scale behavior of organic contaminants is increasing so that contaminant impact on the environment can be accurately predicted. Our results demonstrate that a thorough understanding of the individual processes governing 2,4-D transport and the interactions among these processes is necessary when the impact of 2,4-D and/or its fate in the environment is considered.

ACKNOWLEDGMENTS

We thank W. J. Maier for scientific discussions.

This research was supported by National Institute of Environmental Health Science Superfund Project IP42ES4940 and by the U. S. Department of Agriculture Cooperative State Research Service Water Quality Program.

REFERENCES

1. **Aelion, C. M., C. M. Swindoll, and F. K. Pfaender.** 1987. Adaptation to and biodegradation of xenobiotic compounds by microbial communities from a pristine aquifer. Appl. Environ. Microbiol. **53**:2212–2217.
2. **Angley, T. A., M. L. Brusseau, W. L. Miller, and J. J. Delfino.** 1992. Nonequilibrium sorption and aerobic biodegradation of dissolved alkylbenzenes during transport in aquifer materials: column experiments and evaluation of coupled-process model. Environ. Sci. Technol. **26**:1404–1410.
3. **Audus, L. J.** 1960. Microbial breakdown of herbicides in soils, p. 1–19. *In* E. K. Woodford and G. R. Sagar (ed.), Herbicides and the soil. Blackwell Scientific Publications, Oxford.
4. **Bowman, B. T., and W. W. Sans.** 1985. Partitioning behavior of insecticides in soil-water systems. J. Environ. Qual. **14**:270–273.
5. **Brusseau, M. L., R. E. Jessup, and P. S. C. Rao.** 1990. Sorption kinetics of organic chemicals: evaluation of gas-purge and miscible-displacement techniques. Environ. Sci. Technol. **24**: 727–735.
6. **Brusseau, M. L., and P. S. C. Rao.** 1989. Sorption nonideality during organic contaminant transport in porous media. Crit. Rev. Environ. Control **19**:33–99.
7. **Cheng, H. H., and W. C. Koskinen.** 1986. Processes and factors affecting transport of pesticides to groundwater, p. 12–14. *In* W. Y. Garner, R. C. Honeycutt, and H. N. Nigg (ed.), Evaluation of pesticides in groundwater. American Chemical Society Symposium Series no. 315. American Chemical Society, Washington, D.C.
8. **Greer, L. E., and D. R. Shelton.** 1992. Effect of inoculant strain and organic matter content on kinetics of 2,4-dichlorophenoxyacetic acid degradation in soil. Appl. Environ. Microbiol. **58**: 1459–1465.
9. **Marinucci, A. C., and R. Bartha.** 1979. Apparatus for monitoring the mineralization of volatile ^{14}C-labeled compounds. Appl. Environ. Microbiol. **38**:1020–1022.
10. **Monod, J.** 1949. The growth of bacterial cultures. Annu. Rev. Microbiol. **3**:371–394.
11. **Que Hee, S. S., and R. G. Sutherland.** 1981. The phenoxyalkanoic herbicides, vol. 1. CRC Press, Inc., Boca Raton, Fla.
12. **Rao, P. S. C., and J. M. Davidson.** 1980. Estimation of pesticide retention and transformation parameters required in nonpoint source pollution models, p. 23–67. *In* M. R. Overcash and J. M. Davidson (ed.), Environmental impact of nonpoint source pollution. Ann Arbor Science Publishing Co., Ann Arbor, Mich.
13. **Rao, P. S. C., J. M. Davidson, and D. P. Kilcrease.** 1978. Examination of non-singularity of adsorption-desorption isotherms for soil-pesticide systems. Agron. Abstr., p. 34. American Society of Agronomy, Madison, Wis.
14. **Ratkowsky, D. A.** 1990. Handbook of nonlinear regression models. Marcel Dekker, New York.
15. **Sinton, G. L., L. T. Fan, L. E. Erickson, and S. M. Lee.** 1986. Biodegradation of 2,4-D and related xenobiotic compounds. Enzyme Microb. Technol. **8**:395–403.
16. **Torstensson, L.** 1988. Microbial decomposition of herbicides in the soil. Outlook Agric. **17**:120–124.
17. **van Genuchten, M. T., and R. J. Wagenet.** 1989. Two-site/two-region models for pesticide transport and degradation: theoretical development and analytical solutions. Soil Sci. Soc. Am. J. **53**:1303–1310.
18. **van Genuchten, M. T., and P. J. Wierenga.** 1986. Solute dispersion coefficients and retardation factors, p. 1025–1054. *In* A. Klute (ed.), Methods of soil analysis, part 1, 2nd ed. American Society of Agronomy and Soil Science Society of America, Madison, Wis.
19. **Yaron, B., Z. Gerstl, and W. F. Spencer.** 1985. Behavior of herbicides in irrigated soils, p. 121–217. *In* B. A. Stewart (ed.), Advances in soil science, vol. 3. Springer-Verlag, New York.
20. **Ying, W., and W. J. Weber.** 1979. Bio-physiochemical adsorption model systems for wastewater treatment. J. Water Pollut. Control Fed. **51**:2661–2677.