APPLIED AND ENVIRONMENTAL MICROBIOLOGY, June 2003, p. 3288–3298 Vol. 69, No. 6
0099-2240/03/$08.00+0 DOI: 10.1128/AEM.69.6.3288–3298.2003

Assessment of Bioavailability of Soil-Sorbed Atrazine

Jeong-Hun Park,[1,2] Yucheng Feng,[3]* Pingsheng Ji,[3] Thomas C. Voice,[1] and Stephen A. Boyd[2]

Department of Civil and Environmental Engineering[1] and Department of Crop and Soil Sciences,[2] Michigan State University, East Lansing, Michigan 48824, and Department of Agronomy and Soils, Auburn University, Auburn, Alabama 36849[3]

Received 16 October 2002/Accepted 4 March 2003

Bioavailability of pesticides sorbed to soils is an important determinant of their environmental fate and impact. Mineralization of sorbed atrazine was studied in soil and clay slurries, and a desorption-biodegradation-mineralization (DBM) model was developed to quantitatively evaluate the bioavailability of sorbed atrazine. Three atrazine-degrading bacteria that utilized atrazine as a sole N source (*Pseudomonas* sp. strain ADP, *Agrobacterium radiobacter* strain J14a, and *Ralstonia* sp. strain M91-3) were used in the bioavailability assays. Assays involved establishing sorption equilibrium in sterile soil slurries, inoculating the system with organisms, and measuring the CO_2 production over time. Sorption and desorption isotherm analyses were performed to evaluate distribution coefficients and desorption parameters, which consisted of three desorption site fractions and desorption rate coefficients. Atrazine sorption isotherms were linear for mineral and organic soils but displayed some nonlinearity for K-saturated montmorillonite. The desorption profiles were well described by the three-site desorption model. In many instances, the mineralization of atrazine was accurately predicted by the DBM model, which accounts for the extents and rates of sorption/desorption processes and assumes biodegradation of liquid-phase, but not sorbed, atrazine. However, for the Houghton muck soil, which manifested the highest sorbed atrazine concentrations, enhanced mineralization rates, i.e., greater than those expected on the basis of aqueous-phase atrazine concentration, were observed. Even the assumption of instantaneous desorption could not account for the elevated rates. A plausible explanation for enhanced bioavailability is that bacteria access the localized regions where atrazine is sorbed and that the concentrations found support higher mineralization rates than predicted on the basis of aqueous-phase concentrations. Characteristics of high sorbed-phase concentration, chemotaxis, and attachment of cells to soil particles seem to contribute to the bioavailability of soil-sorbed atrazine.

About 2 billion kilograms of chemicals are used as pesticides each year in the United States, with agricultural usage accounting for ~77% (4). Some pesticides accumulate in nature because release rates exceed rates of dissipation, including microbial and chemical degradation (39, 63). Low biodegradation rates can be attributed to a variety of factors, including limited bioavailability of pesticides to microorganisms. Limited bioavailability may lead to unexpected pesticide persistence in soils, thereby increasing the likelihood of ground- or surface-water contamination (J. J. Pignatello, B. L. Sawhney, and C. R. Frink, Letter, Science **236:**898, 1987). Bioavailability of pesticides and organic contaminants has been identified as a potential limitation to the complete bioremediation of contaminated soils (60, 62, 67).

Generally, soil-sorbed organic contaminants and pesticides have been considered unavailable for biodegradation without prior desorption (52, 62). However, some evidence suggests that sorbed contaminants can be degraded by microorganisms or at least that desorption into bulk solution is not a prerequisite for biodegradation. The apparent availability of soil-sorbed organic contaminants has been demonstrated by Guerin and Boyd (29), Calvillo and Alexander (10), Tang et al. (66), Guo et al. (30), Feng et al. (18), and Park et al. (56, 57). To degrade soil-sorbed pesticides, bacteria must either use sorbed molecules directly or facilitate desorption in some manner, for example, by producing surfactants. Calvillo and Alexander (10), however, showed that neither synthetic nor microbially produced surfactants resulted in utilization of sorbed biphenyl by bacteria. To establish the degradation of sorbed substrate requires that the effects of both rates and extents of desorption on bioavailability be fully accounted for (3, 10, 14, 24, 27–29, 45, 49, 53, 65, 66). Typically, sorption experiments are performed to evaluate solid/liquid distribution coefficients. Sorption experiments alone cannot predict desorption behavior, because hysteresis and irreversibility commonly occur to various degrees (11, 15, 33–35). Some investigators have conducted desorption experiments in an attempt to quantify the effects of desorption on mineralization of sorbed chemicals (10, 66). This approach is complicated by observations that sorbed material may reside in different compartments, each having a different desorption rate behavior (55, 56, 60a).

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) is the most commonly used herbicide in U.S. agricultural crop production, with over 3.2×10^7 kg being used each year (4). Both soil organic matter and clay minerals are effective sorbents for atrazine (40, 41, 51, 61). Several studies have shown that bioavailability of atrazine decreases as soil-atrazine contact time increases (12, 37, 60). However, it is unknown whether atrazine-degrading bacteria are able to access atrazine sorbed by soil.

To investigate the bioavailability of sorbed atrazine, integrated laboratory experiments were performed and analyzed

* Corresponding author. Mailing address: Department of Agronomy and Soils, Auburn University, Auburn, AL 36849. Phone: (334) 844-3967. Fax: (334) 844-3945. E-mail: yfeng@acesag.auburn.edu.

TABLE 1. Selected properties of sorbents used in this study

Soil	% O.C.[a]	% Sand	% Silt	% Clay	pH	CEC[b] (cmol/kg)
K-montmorillonite	ND[c]	0	0	100	6.8	81.6
Hartsells	1.29	59.1	32.1	8.78	5.3	7.10
Houston	1.55	4.80	31.9	63.3	5.4	50.3
Capac	3.28	54.6	24.0	21.4	6.8	24.4
Colwood	7.80	64.2	20.7	15.1	6.0	43.0
Houghton muck	38.3	ND	ND	ND	5.1	156

[a] O.C., organic carbon content.
[b] CEC, cation exchange capacity.
[c] ND, not determined.

using a mathematical model that links sorption with desorption, biodegradation, and mineralization (DBM). A three-site desorption model was employed in which atrazine can reside in equilibrium, rate-limited, and nondesorption sites.

MATERIALS AND METHODS

Organisms and growth conditions. Three bacterial isolates capable of utilizing atrazine as a sole nitrogen source were used in this study. *Pseudomonas* sp. strain ADP, which was isolated from a herbicide spill site (44), was provided by L. P. Wackett of the University of Minnesota. *Agrobacterium radiobacter* strain J14a, which was isolated from enrichment cultures of a surface soil collected from an atrazine-treated cornfield (64), was provided by T. B. Moorman of the USDA Agricultural Research Station, Ames, Iowa. *Ralstonia* sp. strain M91-3, which was isolated from a surface soil collected at a pesticide mixing area (59), was provided by M. Radosevich of the University of Delaware.

Pseudomonas sp. strain ADP was grown in minimal medium containing 0.1% (wt/vol) sodium citrate as the carbon source and atrazine as the sole nitrogen source at an atrazine concentration of 50 mg/liter for liquid cultures or 500 mg/liter for agar plates (44). *A. radiobacter* J14a was grown in minimal medium supplemented with sodium citrate (1 g/liter) and sucrose (1 g/liter) as carbon sources and 50 mg of atrazine/liter as the sole nitrogen source (64). *Ralstonia* sp. strain M91-3 was grown in a minimal medium containing atrazine (22 mg/liter) as the sole source of nitrogen and glucose (200 mg/liter) as the carbon source (59). Solid media contained 1.5% (wt/vol) Noble agar (Difco Laboratories, Detroit, Mich). Concentrated atrazine stock solutions (20 or 100 mg/ml in methanol) were used for making the above-mentioned minimal media. To prepare bacterial inocula, liquid cultures were incubated with shaking (200 rpm) at room temperature (24°C ± 1°C), and cell growth was monitored by measuring absorbance at 600 nm with a spectrophotometer. Bacterial cells in late log phase were pelleted by centrifugation, washed twice with sterile phosphate buffer (20 mM, pH 7), and resuspended in phosphate buffer. Nutrient agar plates containing 4 g of nutrient broth/liter were used for the numeration of bacterial cells.

Soils and soil extracts. The A horizon of agricultural soils from Michigan and Alabama was used in these studies (Table 1). Soils were air dried, ground, and passed through a 2-mm-pore-size sieve. A reference smectite clay, Na montmorillonite (SWy-2), was obtained from the Clay Minerals Society Source Clay Repository (Columbia, Mo.). The <2-μm-diameter clay particles were separated by wet sedimentation. The clay was then subjected to K^+ saturation. Briefly, clay (20 g) was dispersed in 1 liter of 0.1 M KCl solution. The clay suspensions were shaken for 24 h, and then fresh KCl solutions were used to displace the original solutions after centrifugation. This process was repeated four times to ensure complete K saturation. Distilled water (1 liter) was used to wash the clays to remove excess KCl. The clay was freeze dried and stored for later use. Cation exchange capacity and soil pH were determined according to methods described by Page et al. (54). Soil organic carbon contents and particle size distribution were determined at the Soil and Plant Nutrient Laboratory at Michigan State University. Table 1 summarizes the properties of the sorbents. Soil samples were sterilized by gamma irradiation (1.29 megarads/h; 5 megarads from a ^{60}Co source) and stored in sealed containers at room temperature. Before each experiment using soils, 0.1 g of each soil was placed on a half-strength nutrient agar plate and incubated at 30°C for 3 days to verify sterility. No colonies were observed.

To prepare extracts from all six sorbents, each sterile sorbent was suspended in sterile phosphate buffer (20 mM) at a sorbent/solution ratio of 1:40 for Houghton muck and potassium-saturated montmorillonite (K-montmorillonite) and 1:10 for the other soils. The suspensions were mixed for time periods corresponding to sorption and bioavailability experiments, and supernatants obtained after centrifugation were filtered through two layers of Whatman no. 2 filter paper.

Sorption of atrazine by soils. Batch sorption isotherms for all six sorbents were obtained by equilibrating U-^{14}C-labeled atrazine (Sigma, St. Louis, Mo.) (radiochemical purity, >95%; specific activity, 16.6 mCi/mmol) and nonradioactive atrazine with sorbents at a solid-to-solution ratio of 1:10 for Capac, Colwood, Houston, and Hartsells and 1:40 for Houghton muck and K-montmorillonite. An aliquot of [^{14}C]atrazine stock (in methanol; 2.4 g/liter) was spiked into 4.5-ml screw-cap glass vials containing 4.2 ml of sterile phosphate buffer (20 mM) and sterile soils to obtain the appropriate initial aqueous concentrations (0 to 2 mg/liter). The vials were capped with Teflon-lined polypropylene screw caps. The headspace was <1 ml in volume. Control vials without soil were prepared in triplicate. Vials were tumbled at 9 rpm for 2 days in the dark. After mixing, each vial was centrifuged for 10 min at 1,200 × *g* to separate soil and 1 ml of the supernatant was sampled in duplicate. The concentration of atrazine in the supernatant was determined by liquid scintillation counting (LSC) and verified by high-pressure liquid chromatography (HPLC) for some samples. Sorbed-atrazine concentrations were calculated by determining the difference between the initial and final liquid-phase concentrations. The use of difference calculations was validated by direct analysis of sorbed-phase atrazine concentrations in selected samples for each soil. To accomplish this, the soil was separated from the supernatant at the end of the sorption period and extracted with methanol. The concentrations of atrazine in the extracts were determined by LSC and verified by HPLC. All experiments were performed at room temperature (24 ± 1°C).

Desorption of sorbed atrazine. Using a similar procedure as in the sorption experiments, the desorption assay utilized batch soil slurries that had been equilibrated with an initial aqueous atrazine concentration of 2 mg/liter. An aliquot of [^{14}C]atrazine stock (in methanol; 2.4 g/liter) was spiked into 50-ml centrifuge tubes containing 45 ml of sterile phosphate buffer (20 mM) and sterile soils. The tubes were capped with Teflon-lined Mininert valves and screw-sealed with polypropylene caps. A control tube without soil was prepared in the same fashion. Tubes were tumbled at 9 rpm for 2 days in the dark, and then each tube was centrifuged for 20 min at 1,200 × *g* to separate soil and the supernatant was sampled. The final concentration of atrazine in the liquid phase was determined by LSC, and the amount of sorbed atrazine was calculated. The supernatant was then decanted to the extent possible, and the amount of residual water was determined gravimetrically. Desorption was initiated by adding fresh atrazine-free soil extract to make up the original volume. The tubes were tumbled again at 9 rpm and then removed periodically, and the liquid phase was sampled for analysis by LSC. The concentration of atrazine in the final desorption samples was determined by LSC and verified by HPLC. After the final desorption samples were analyzed, the soil was separated from the supernatant and extracted with methanol. The concentration of atrazine in the extracts was determined by LSC and verified by HPLC.

Atrazine degradation and mineralization in aqueous phase. Using an experimental setup similar to the bioavailability assays described below, degradation and mineralization kinetics of atrazine in phosphate buffer (20 mM) were determined with three bacterial strains. Appropriate amounts of phosphate buffer were placed in 160-ml serum bottles sealed with Teflon-coated butyl stoppers. Nonradioactive atrazine stock solution (20 mg/liter) and ^{14}C-labeled atrazine were added to each bottle to obtain an aqueous atrazine concentration of approximately 2 mg/liter and a radioactivity level of ~3.7 kBq. After mixing on a platform shaker, the solution (75 ml) in each serum bottle was inoculated with 0.75 ml of bacterial cell suspension to obtain a final cell density of ~10^8 CFU/ml. At precise time intervals, 1 ml of suspension and 1 ml of headspace gas were withdrawn from each bottle and injected into sealed tubes containing 0.5 ml of H_3PO_4 (0.3%). Radioactive CO_2 produced was detected using the procedures described below. To monitor biodegradation of atrazine, the acidified sample in each tube was centrifuged and the atrazine concentration in the supernatant was analyzed by HPLC as described in the chemical analysis section below.

Bioavailability assays. To evaluate the availability of soil-sorbed atrazine to bacteria, a mineralization assay adapted from the method described by Guerin and Boyd (29) and Feng et al. (18) was used. Soil extract controls and soil slurries were set up in 160-ml serum bottles sealed with Teflon-coated butyl stoppers. Sterile sorbents were placed into each bottle at a sorbent/solution ratio of 1:40 for Houghton muck and K-montmorillonite and 1:10 for other sorbents. Soil extracts or phosphate buffer solutions were used as soil-free controls for the soils or K-montmorillonite, respectively. Variable volumes of sterile phosphate buffer (20 mM) and nonradioactive atrazine stock solution were added to serum bottles to obtain the appropriate atrazine concentrations and a final volume of 75 ml. ^{14}C-labeled pesticide was then added to each serum bottle at a level of ~3.7 kBq.

Initial aqueous atrazine concentrations in both soil-free controls and soil slurries were 2 mg/liter. After a 24-h incubation on a platform shaker, soil slurries and soil-free controls were inoculated with cells harvested at early stationary phase to obtain a final cell density of $\sim 10^8$ CFU/ml. At precise time intervals, 1 ml of suspension and 1 ml of headspace gas were withdrawn from each bottle and injected into sealed tubes containing 1 ml of 2 M HCl. The radioactive CO_2 evolved was trapped on NaOH (2 M)-saturated filter paper strips in a center well hanging from a sleeve stopper. After overnight equilibration, the filter paper strips were transferred to a scintillation vial containing 10 ml of scintillation fluid and the center wells were washed with 2 ml of 50% (vol/vol) ethanol that was then combined with scintillation fluid. Samples were left in the dark for at least 24 h before LSC.

Characterization of atrazine-degrading bacteria. Several characteristics of the organisms that may potentially affect degradation of soil-sorbed atrazine were evaluated. Surface tension measurements were carried out using a DuNoüy tensiometer to determine whether biosurfactants were produced during bacterial growth in the culture media. Using a capillary assay, bacterial chemotactic response was tested at atrazine concentrations of 2 and 20 mg/liter (1). Briefly, bacterial cells harvested in late log phase were washed twice with 20 mM phosphate buffer, resuspended in chemotaxis buffer (20 mM phosphate buffer containing 10 μM EDTA), and placed in a chamber formed by placing a U-shaped glass tube between a microscope slide and a coverslip. Capillaries (1 μl) containing atrazine solution or phosphate buffer amended with 10 μM of EDTA were immersed in chambers containing bacterial suspensions of approximately 2×10^7 CFU/ml. The number of bacteria entering the capillaries after 60 min of incubation was determined by plate counts. The relative chemotaxis response was calculated as the ratio of the number of bacteria that enter the capillary containing atrazine to that with buffer only. A ratio of 2 or greater is considered a significant chemotactic response (1). To determine the number of cells attached to soil particles, sterile soil suspensions and soil-free controls were prepared in 25-ml Corex tubes in a manner consistent with that used for the bioavailability assay. The tubes were equilibrated on a rotating mixer for 24 h before inoculation with cells to obtain a final concentration of approximately 10^8 CFU/ml. The tubes were then equilibrated on a rotating mixer for 1 h to allow for attachment and centrifuged at $20 \times g$ for 3 min to settle the soil particles. The cell numbers in the supernatants and soil-free controls were determined by plate counts. The proportion of added bacteria that attached to soils was calculated as follows: percentage of cell attaching to soil = 100 − [(cell numbers of soil-free control − cell numbers of supernatant)/cell numbers in the soil-free controls) × 100]. Cell attachment to K-montmorillonite was not determined due to the difficulty of separating clay particles from bacterial cells by centrifugation.

DBM model. The DBM model adds sequential biodegradation and mineralization to the three-site desorption model (56, 57, 60a). The assumptions used in the developed DBM model are that (i) the solid is composed of equilibrium, nonequilibrium, and nondesorption sites; (ii) sorption equilibrium can be described by a nonlinear isotherm; (iii) the rate of release from nonequilibrium sites is proportional to the concentration gradient between these sites and the liquid phase; (iv) there is no time lag between biodegradation and mineralization; (v) desorption parameters developed in abiotic experiments are valid in the bioavailability assays; (vi) biodegradation and mineralization rates for attached cells to sorbents are the same as those of suspended cells; (vii) biodegradation and mineralization rate parameters evaluated from soil-free solutions are the same as those in soil slurry solutions; and (viii) both suspended and attached cells can utilize only liquid-phase contaminant. The nondesorption sites have been defined in this work and others (56, 57, 60a) as those containing sorbate that cannot be released to aqueous solution during the experimental desorption period.

Mathematically, equilibrium and nondesorption partitioning are described by

$$S_{eq} = f_{eq} \cdot K_F \cdot C^n \tag{1}$$

and

$$S_{nd} = f_{nd} \cdot K_F \cdot C_e^{\,n} \tag{2}$$

while the release from nonequilibrium sites follows the first-order expression

$$\frac{dS_{neq}}{dt} = \alpha \cdot (f_{neq} \cdot K_F \cdot C^n - S_{neq}) \tag{3}$$

where K_F is the Freundlich sorption coefficient, n is a constant characterizing isotherm curvature, C is the liquid-phase concentration in milligrams per liter, C_e is the liquid-phase concentration (in milligrams per liter) in sorption equilibrium, t is the desorption time in minutes, α is the first-order desorption rate coefficient (per minute) for nonequilibrium sites, f_{eq} is the equilibrium site fraction, f_{neq} is the nonequilibrium site fraction, f_{nd} is the nondesorption site fraction, and S_{eq}, S_{neq}, and S_{nd} are the sorbed concentrations (in milligrams per kilogram) in the solid equilibrium, nonequilibrium, and nondesorption sites, respectively. These three terms are related to the total sorbed-phase concentration (S) as follows:

$$S_{eq} + S_{neq} + S_{nd} = S \tag{4}$$

K_F and n were derived from the sorption isotherms and f_{nd} corresponds to the plateau of the desorption rate profile, while f_{eq}, f_{neq}, and α were estimated by nonlinear regression analysis of desorption data.

The system mass balance for biodegradation and mineralization, respectively, can be expressed as follows:

$$-\left(V_l \cdot \frac{dC}{dt} + m \cdot \frac{dS}{dt}\right) = V_l \cdot R_{bio} \tag{5}$$

and

$$\frac{dP_{co_2}}{dt} = Y_{co_2} \cdot R_{bio} \tag{6}$$

where m is the soil amount in kilograms, V_l is the total liquid volume in liters, P_{CO_2} is the CO_2 concentration (in milligrams per liter) produced, and Y_{CO_2} is the CO_2 yield coefficient (in milligrams of CO_2 as atrazine per milligram of substrate). R_{bio} is the liquid-phase biodegradation rate expression, which can be formulated for first-order kinetics and Michaelis-Menten kinetics, respectively, as follows:

$$R_{bio} = k_1 \cdot C \tag{7}$$

and

$$R_{bio} = \frac{k_m \cdot C}{K_s + C} \tag{8}$$

where k_1 and k_m are the first-order rate constant and maximum degradation rate for dissolved contaminant, respectively, and K_s is the half-saturation constant. In the system of equations presented above, k_1, k_m, and K_s were estimated by regression of the mineralization data in the soil extracts.

Mineralization data were predicted by the DBM model to evaluate atrazine bioavailability in soil slurries. If only dissolved atrazine in the bulk-aqueous phase can be degraded in soil slurries, the amount of CO_2 produced should be equal to the model prediction, which accounts for DBM. However, CO_2 production levels in soil slurries that exceed the predicted values indicate mineralization rates faster than would be expected on the basis of data for bulk-liquid-phase concentrations, i.e., enhanced bioavailability. Alternatively, CO_2 production below the predicted values indicates slower rates and may suggest that the slurry system somehow inhibits microbial activity.

For the observed enhanced bioavailability cases, mineralization data were further investigated by using the DBM model with the assumption of instantaneous desorption (DBM_{id}). DBM_{id} predicted line was calculated by using $f_{eq} = 1$, $f_{neq} = 0$, and $f_{nd} = 0$ in the DBM model (instead of the estimated parameters) and assuming bacteria were exposed to the theoretically maximized aqueous atrazine concentration.

Chemical analysis. Atrazine was analyzed by reverse-phase HPLC using an UV detector (220 nm). The mobile phase was 50% methanol and 50% water (vol/vol) at 1.0 ml/min. The retention time of atrazine was 11 min. Radioactivity was determined by LSC. The analytical detection limits of atrazine solution were 6 μg/liter and less than 1 μg/liter for HPLC and LSC, respectively.

RESULTS

Sorption of atrazine. The organic carbon content of sorbents used ranged from ~0% for K-montmorillonite to 38.3% for Houghton muck (Table 1). Sorption isotherms for atrazine on all soils were nearly linear over the concentration range evaluated, with n values ranging from 0.93 to 1.05. For K-montmorillonite, the sorption isotherm was less linear, with n = 0.82. Linear distribution coefficients (K_d) increased from 1.5 to 43 ml/g with increasing soil organic carbon contents, except for Houston soil and K-montmorillonite, which gave elevated values (K_d = 3.6 and 40 ml/g, respectively) with respect to organic carbon (Table 2 and Fig. 1). Clay mineralogy of the Houston

TABLE 2. Parameters for sorption of atrazine by the A horizons of selected soils and K-montmorillonite

Soil	Freundlich equation			Linear equation		
	K_F [(mg/kg)/(mg/liter)^n]	n	R^2	K_d (liters/kg)	R^2	K_{oc} (liters/kg)
K-montmorillonite	43.8	0.82	1.00	40.4	0.98	NA[a]
Hartsells	1.44	1.02	1.00	1.50	1.00	116
Houston	3.72	0.95	1.00	3.60	1.00	232
Capac	3.18	1.05	1.00	3.40	0.99	104
Colwood	12.4	0.93	0.99	11.9	0.99	153
Houghton muck	44.3	0.94	1.00	43.1	1.00	113

[a] NA, not available.

TABLE 3. Desorption parameters[a] evaluated by a three-site desorption model[b]

Soil	f_{eq}	f_{neq}	f_{nd}	α (min^{-1})	R^2
K-montmorillonite	0.20 (0.300)	0.74	0.06 (0.050)	0.0067 (0.0008)	0.89
Hartsells	0.35 (0.009)	0.14	0.51 (0.005)	0.0020 (0.0030)	0.97
Houston	0.45 (0.040)	0.46	0.09 (0.010)	0.0040 (0.0005)	0.98
Capac	0.63 (0.008)	0.14	0.23 (0.003)	0.0025 (0.0003)	0.98
Colwood	0.73 (0.020)	0.14	0.13 (0.010)	0.0025 (0.0095)	0.90
Houghton muck	0.72 (0.020)	0.17	0.11 (0.007)	0.0029 (0.0005)	0.95

[a] f_{eq}, f_{neq}, and f_{nd}, equilibrium, nonequilibrium, nondesorption site fractions, respectively; α, first-order desorption rate coefficient for nonequilibrium sites.
[b] Numbers in parentheses are standard deviations of the evaluated values.

soil is predominately smectite, whereas the other soils contain primarily illite and vermiculite (Colwood and Capac) or kaolinite and goethite (Hartsells). HPLC analysis indicated that atrazine was not transformed during the sorption period.

Desorption of atrazine. The three-site desorption model was used to fit desorption data and evaluate desorption parameters and site fractions (Table 3 and Fig. 2). The model fit the experimental data well, with R^2 values ranging from 0.89 to 0.98. The equilibrium site fraction tended to increase as soil organic carbon content increased, ranging from 0.20 to 0.73 except for Houston and K-montmorillonite, which gave values of 0.45 and 0.2, respectively. Nonequilibrium site fraction was similar among sorbents, ranging from 0.14 to 0.17 except for Houston and K-montmorillonite, which gave values of 0.46 and 0.74, respectively. For all soils, nondesorption sites were observed within the given desorption time (3 days) (Fig. 2). The nondesorption site fractions decreased as organic carbon content increased, ranging from 0.51 to 0.11 except for the smectitic Houston soil and K-montmorillonite, which gave values of 0.09 and 0.06, respectively. Desorption rate coefficients tended to increase as organic carbon content increased, ranging from 0.002 to 0.0029 min^{-1} except for the Houston and K-montmorillonite, which gave values of 0.004 and 0.0067 min^{-1}, respectively. HPLC analysis indicated that atrazine was not transformed during the sorption and desorption periods. The total recovery of atrazine was higher than 94% for all sorbents used.

Bioavailability of soil-sorbed atrazine. Biodegradation and mineralization of atrazine in phosphate buffer for *Pseudomonas* sp. strain ADP and *Ralstonia* sp. strain M91-3 were well described by first-order kinetics and for *A. radiobacter* J14a were well described by Michaelis-Menten kinetics (Fig. 3). No significant time lag between biodegradation and mineralization was observed, supporting the assumption used for model development. Since dissolved soil organic matter can potentially affect atrazine degradation and mineralization kinetics, soil extracts rather than phosphate buffer were used as soil-free controls in the bioavailability assays. The values for dissolved organic carbon content in soil extracts were 99.7, 252, 151, 319, and 554 mg/liter for Hartsells, Houston, Capac, Colwood, and Muck, respectively. The values for inorganic nitrogen concentration in soil extracts were 2.42, 2.69, 2.18, 3.08, and 33.5 mg/liter for Hartsells, Houston, Capac, Colwood, and Muck, respectively. For all three organisms, the rates of degradation and mineralization were lower in soil extracts than in phosphate buffer. Therefore, any enhanced bioavailability (greater than that predicted by the DBM model) observed here cannot be attributed to the stimulation of atrazine metabolism by dissolved substances such as organic matter, N, P, etc.

Bioavailability assays for *Pseudomonas* sp. strain ADP were performed using all six sorbents. The CO_2 yield coefficient for *Pseudomonas* sp. strain ADP ranged from 0.74 to 0.90 in the soil extracts (Table 4). To evaluate the bioavailability of sorbed atrazine, CO_2 production data from soil slurries were analyzed by the DBM model, which utilizes independently measured

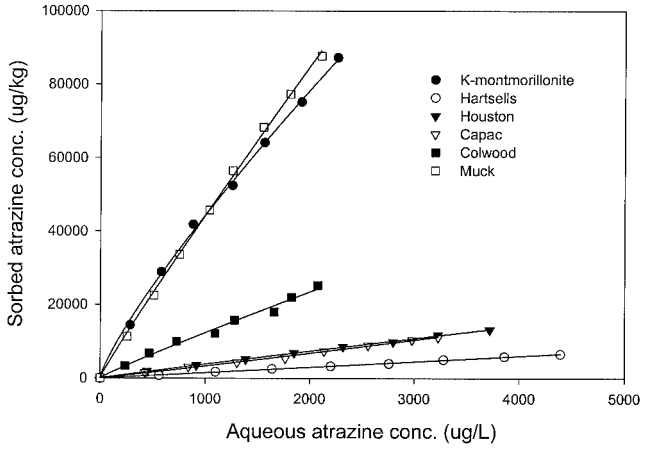

FIG. 1. Sorption isotherms of atrazine for different sorbents. Atrazine was equilibrated with sorbents for 2 days in the dark at 24 ± 1°C.

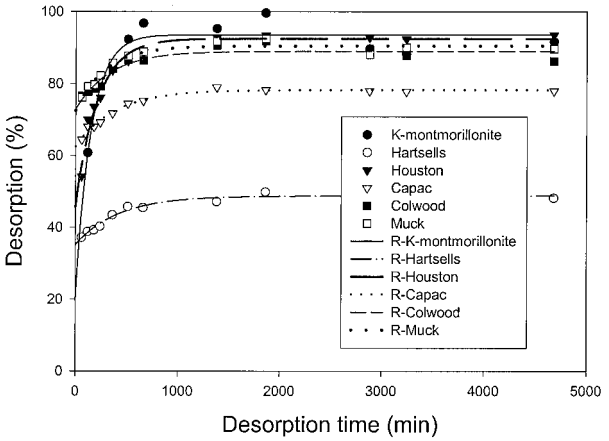

FIG. 2. Abiotic atrazine desorption data and regression (R-) lines from a three-site desorption model for sorbents.

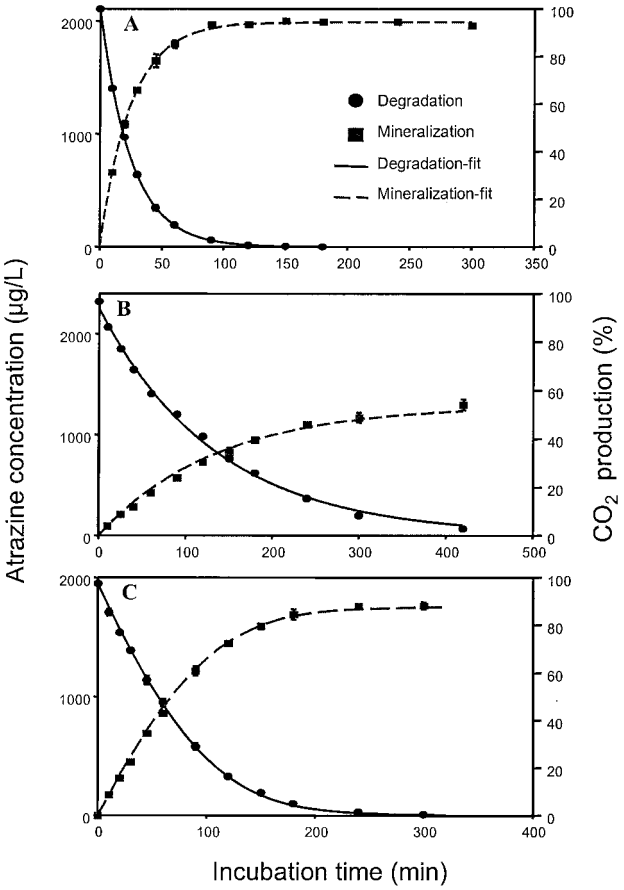

FIG. 3. Biodegradation and mineralization of atrazine by three bacterial strains in aqueous phase. (A) Degradation by *Pseudomonas* sp. strain ADP followed first-order kinetics ($k_1 = 0.0398\ \text{min}^{-1}$; $Y_{CO_2} = 0.944$; $R^2 = 1.0$); (B) degradation by *Ralstonia* sp. strain M91-3 followed first-order kinetics ($k_1 = 0.0074\ \text{min}^{-1}$; $Y_{CO_2} = 0.538$; $R^2 = 1.0$); (C) degradation by *A. radiobacter* J14a followed Michaelis-Menten kinetics ($k_m = 0.0340\ \text{min}^{-1}$; $K_s = 1.50$ mg/liter; $Y_{CO_2} = 0.877$; $R^2 = 1.0$).

desorption rates, liquid-phase biodegradation rates, and CO_2 yield coefficients (Fig. 4) to predict CO_2 production curves. Also plotted in Fig. 4 are the best-fit curves obtained by nonlinear regression for the soil extract controls. In cases in which data from the slurry systems fall on the DBM-predicted lines, the bioavailability of sorbed atrazine is well explained by the sequential processes of DBM. Data falling above the DBM-predicted line indicate enhanced bioavailability, i.e., that which cannot be described by the degradation rate of bulk liquid-phase atrazine alone. In such cases, mineralization was not controlled solely by the desorption rate of atrazine into bulk solution. Mineralization curves for atrazine in Houston and Capac soil slurries were well predicted by the DBM model. For Hartsells, Colwood, and Houghton muck soils, enhanced bioavailability was observed (Fig. 4). To further investigate, a DBM_{id}-predicted line was produced with an assumption of instantaneous desorption (Fig. 4). Mineralization of atrazine in Hartsells was well predicted by the DBM_{id} model, but rates in Colwood and Houghton muck soils were above the DBM_{id}-predicted line. The presence of K-montmorillonite inhibited mineralization of aqueous phase atrazine.

For *Ralstonia* sp. strain M91-3, the CO_2 yield coefficients ranged from 0.64 to 0.77 (Table 4). The measured CO_2 production curves and DBM predictions for soil extract controls and soil slurries are shown in Fig. 5. Enhanced bioavailability (albeit slight) was observed for Houghton muck soil. Mineralization data for other soils were well predicted by the DBM model. For *A. radiobacter* J14a, the CO_2 yield coefficients ranged from 0.73 to 0.91 (Table 4). The mineralization data were well predicted by the DBM model for the mineral soils. Enhanced bioavailability (>DBM or DBM_{id} predictions) was observed for Houghton muck soil (Fig. 6). As with *Pseudomonas* sp. strain ADP, K-montmorillonite inhibited mineralization of dissolved atrazine.

For all three atrazine-degrading bacteria, production of biosurfactants was not indicated by surface tension measurements. The differences in surface tensions between the uninoculated medium and corresponding bacterial culture were 6, −1.2, and −0.6 dynes/cm for *Pseudomonas* sp. strain ADP, *A. radiobacter* J14a, and *Ralstonia* sp. strain M91-3, respectively. The presence of biosurfactant has been reported to reduce the surface tensions of cell cultures by more than 20 dynes/cm (2). In the capillary assay, the numbers of bacteria accumulated in capillaries containing 2 mg of atrazine/liter were 420 ± 53, 367 ± 31, and 190 ± 17 CFU for *Pseudomonas* sp. strain ADP, *A. radiobacter* J14a, and *Ralstonia* sp. strain M91-3, respectively, and those in capillaries containing 20 mg of atrazine/liter were 733 ± 117, 560 ± 60, and 213 ± 90 CFU, respectively. The background accumulations in capillaries containing buffer only were 87 ± 12 CFU for *Pseudomonas* sp. strain ADP, 117 ± 29 CFU for *A. radiobacter* J14a, and 133 ± 35 CFU for *Ralstonia* sp. strain M91-3. Both *Pseudomonas* sp. strain ADP and *A. radiobacter* J14a exhibited a significant chemotactic response to atrazine, with *Pseudomonas* sp. strain ADP having

TABLE 4. Biokinetic parameters[a] evaluated for soil extract controls

Soil extracts	*Pseudomonas* sp. strain ADP		*Ralstonia* sp. strain M91-3		*A. radiobacter* strain J14a		
	k_1 (10^{-3}) (min^{-1})	Y_{CO_2}	k_1 (10^{-3}) (min^{-1})	Y_{CO_2}	k_m (10^{-3}) (mg liter^{-1} min^{-1})	K_s (mg liter^{-1})	Y_{CO_2}
K-montmorillonite	31.7	0.90	6.6	0.70	22.8	0.560	0.91
Hartsells	11.5	0.82	4.1	0.77	11.2	0.646	0.88
Houston	10.1	0.74	2.8	0.64	13.2	1.31	0.76
Capac	7.90	0.86	2.6	0.73	8.50	0.420	0.85
Colwood	5.80	0.78	3.6	0.68	16.9	1.10	0.83
Houghton muck	13.0	0.74	4.3	0.64	9.70	0.705	0.73

[a] k_1, first-order degradation rate constant; k_m, maximum degradation rate for dissolved atrazine; K_s, half-saturation constant; Y_{CO_2}, CO_2 yield coefficient (milligrams of CO_2 as atrazine/milligrams of atrazine).

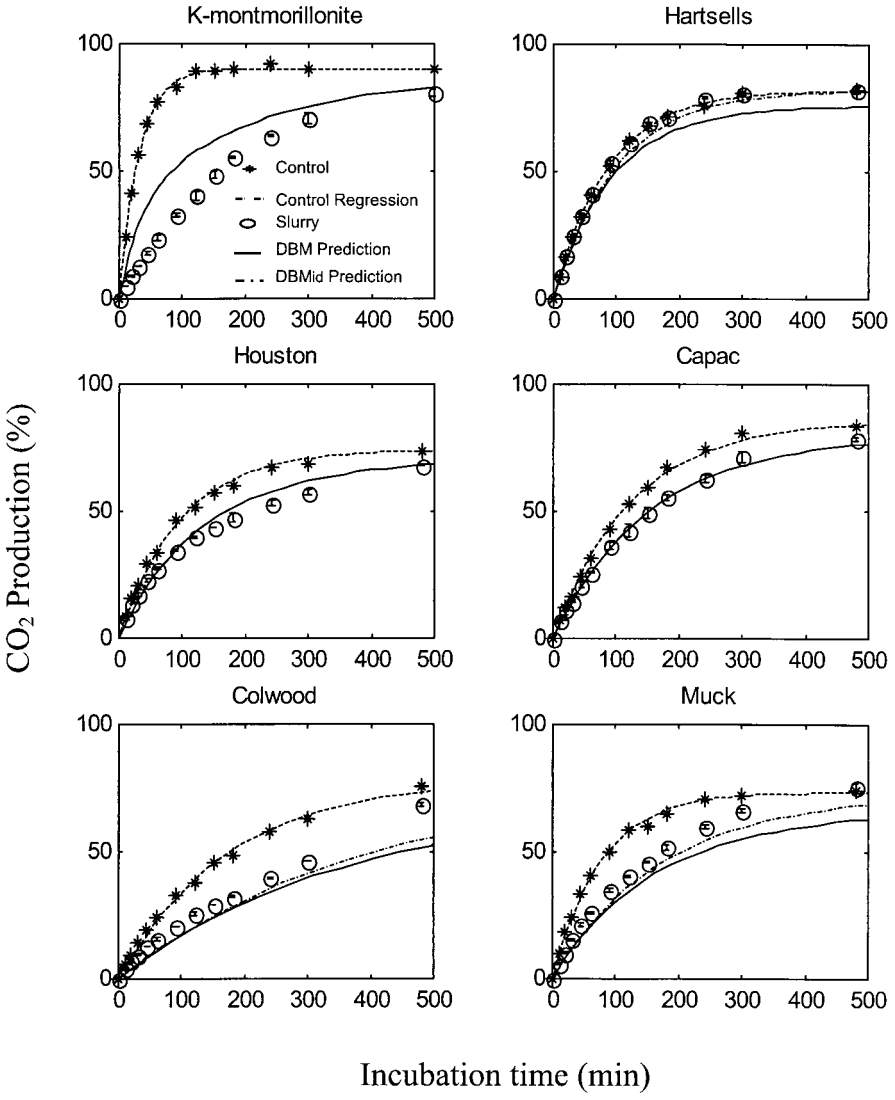

FIG. 4. Mineralization of atrazine by *Pseudomonas* sp. strain ADP in soil-free controls and soil slurries. Broken (control regression) lines represent the nonlinear regression line for soil-free control, solid (DBM prediction) lines represent the DBM-predicted line for soil slurry, and mixed dash and dot (DBM_{id}) lines represent the DBM_{id}-predicted line for soil slurry.

a higher relative response (Fig. 7A). *Ralstonia* sp. strain M91-3, however, did not show a significant chemotactic response. The relative response was less than 2 for both atrazine concentrations tested (Fig. 7A).

The percentages of added bacteria attaching to different soils varied from 8.5 to 17.2, 19.9 to 73.3, and 12.3 to 62.2% for *Pseudomonas* sp. strain ADP, *Ralstonia* sp. strain M91-3, and *A. radiobacter* J14a, respectively. Because the soil-to-solution ratio for muck soil was different from that used for the mineral soils, the number of attached cells is expressed in Fig. 7B in CFU per gram of soil rather than as a percentage. Judged on the basis of unit weight of soil, attachment of *Pseudomonas* sp. strain ADP and *A. radiobacter* J14a to muck soil was significantly higher than attachment to mineral soils. For *Ralstonia* sp. strain M91-3, cell attachment to muck soil was higher than that to Hartsells, Capac, and Colwood soils but similar to that to Houston soil.

DISCUSSION

Linear correlation between K_d and the organic carbon content in soils was observed for the Hartsells, Capac, Colwood, and Houghton muck soils. The K_{oc} values ranged from 104 to 153 (Table 2), in agreement with literature values for atrazine, suggesting that atrazine partitioning into soil organic matter was the predominant sorption mechanism for these soils (19, 32, 60). The high K_{oc} value for Houston (K_{oc} = 232) may be explained by atrazine sorption to the clay component of the soil as well as soil organic matter (61). Houston is composed of 63% clay which is predominantly smectite. The measured K_d value for sorption of atrazine by K-montmorillonite was 40.4, similar to that by muck soil (Table 2 and Fig. 1). This suggests that smectite clays in soils may be effective sorbents for atrazine. Sheng et al. (61) reported the high affinity of K-smectite for atrazine. High sorption of organics by K-montmorillonite

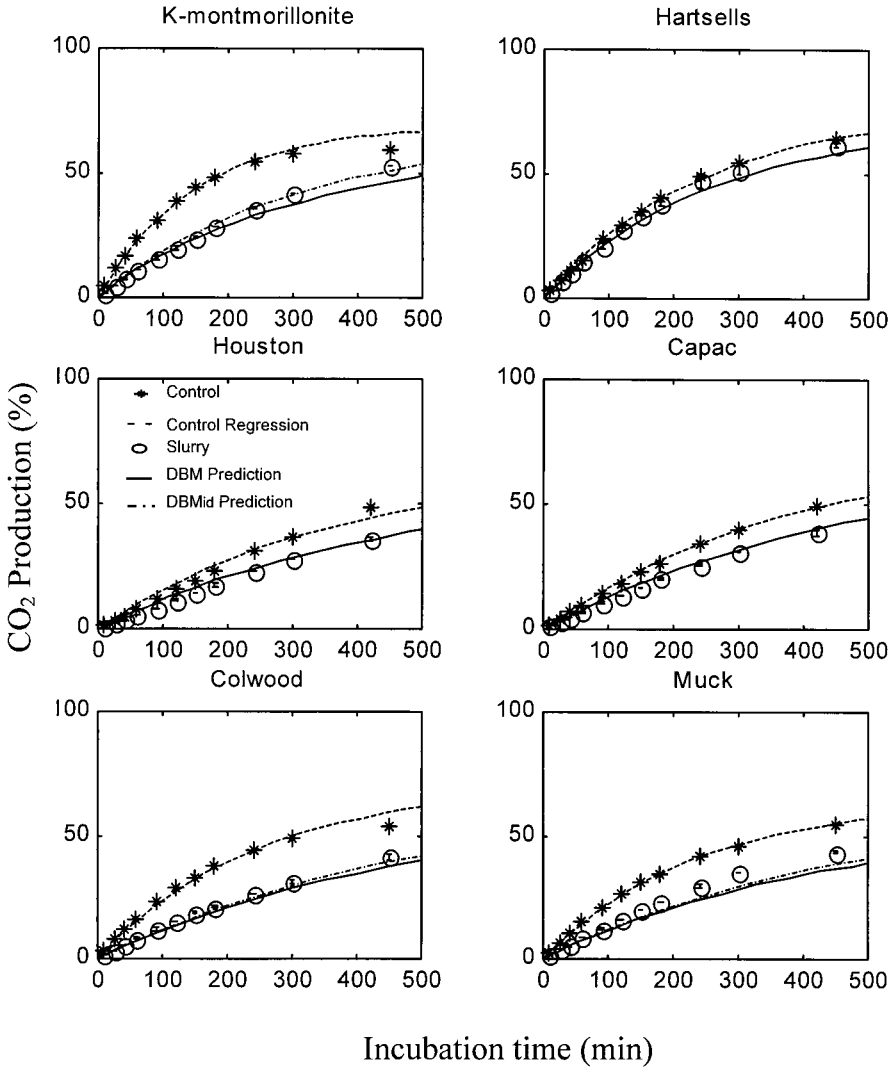

FIG. 5. Mineralization of atrazine by *Ralstonia* sp. strain M91-3 in soil-free controls and soil slurries. Broken (control regression) lines represent the nonlinear regression line for soil-free control, solid (DBM prediction) lines represent the DBM-predicted line for soil slurry, and mixed dash and dot (DBM_{id}) lines represent the DBM_{id}-predicted line for soil slurry.

has been explained by interstitial adsorption between expandable layers (38).

The desorption profiles show that three types of desorption occurred (Fig. 2). Equilibrium release was evidenced by high solution concentrations at the first time point. For all sorbents used except K-montmorillonite and Houston, f_{eq} increased in accordance with soil organic carbon content and hence with K_d (Table 1 and 3). This contradicts the two-compartment model of Karickhoff (36), which predicts that as sorption coefficients (K_d) increase, distribution of sorbate in the labile sorbed phase (comparable to f_{eq}) decreases. For all sorbents, rate-limited release was shown by the continued increase in aqueous phase concentrations over the next several time points. The presence of a nondesorbable fraction was indicated by failure to reach complete reversible desorption (Fig. 2 and Table 3). Irreversible sorption of atrazine in soils has been reported previously (13, 32, 43, 60a, 69). Several possible causes of apparent sorption/desorption hysteresis of atrazine in soils have been suggested, including loss of herbicide due to volatilization and degradation, changes in solution composition during desorption, nonattainment of equilibrium during sorption and desorption, and irreversible binding (13). In this study, loss of atrazine by volatilization in controls was not observed. In HPLC analysis of sorption and desorption samples, no discernible atrazine degradation was indicated. Soil extract solutions were used as the desorption medium to minimize changes in solution composition between sorption and desorption. To establish each equilibrium state, 2-day sorption and 3-day desorption times were used. No significant changes in sorption occurred from day 1 through day 7, and the desorption plateau was reached after 1 day (Fig. 2). According to Xie et al. (69), irreversible sorption of pesticides occurred in the humin fraction of soil. In their experiments, most irreversibly sorbed atrazine was found in the bound-humic acid and mineral fractions.

We examined three strains of atrazine-degrading bacteria for their ability to degrade atrazine, as sorbed by six different sorbents. In 11 out of 18 cases, the experimental data con-

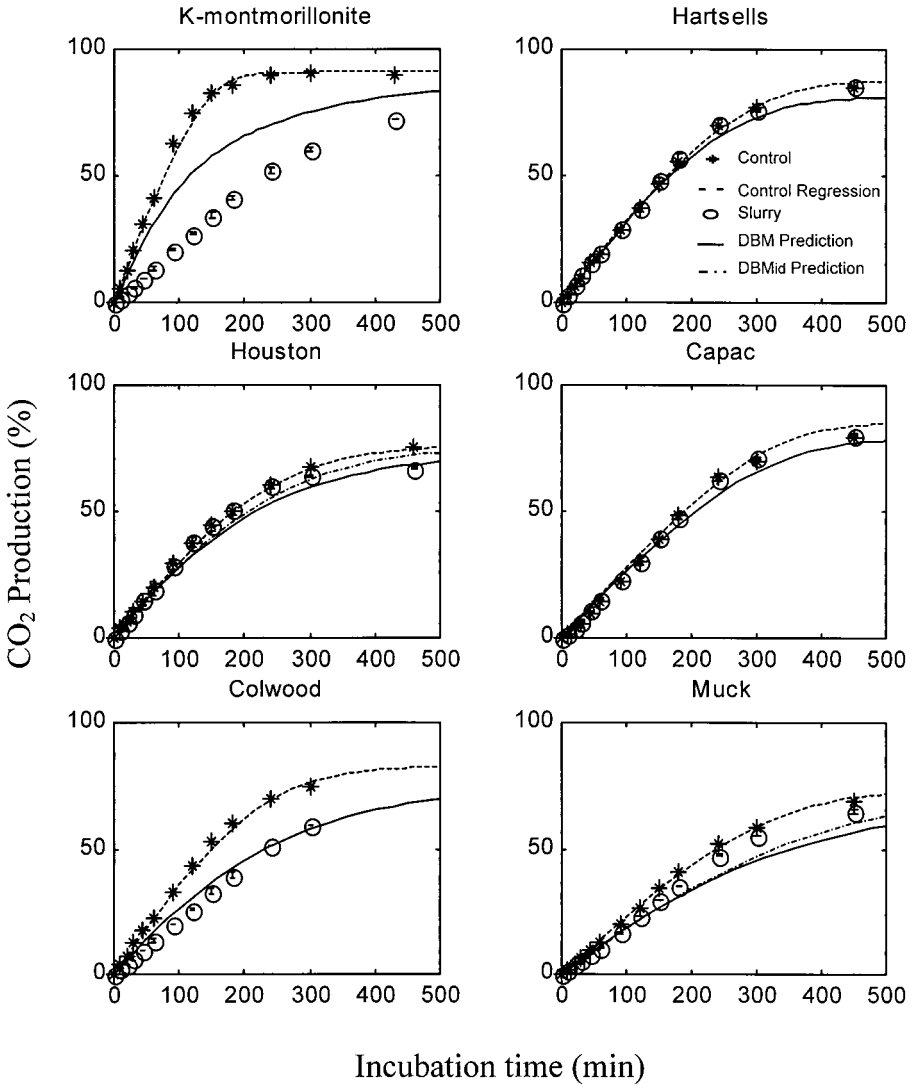

FIG. 6. Mineralization of atrazine by *A. radiobacter* J14a in soil-free controls and soil slurries. Broken (control regression) lines represent the nonlinear regression line for soil-free control, solid (DBM prediction) lines represent the DBM-predicted line for soil slurry, and mixed dash and dot (DBM_{id}) lines represent the DBM_{id}-predicted line for soil slurry.

formed to the DBM model, indicating that mineralization was limited to dissolved atrazine present initially plus that desorbing during the course of the experiment. In five cases, however, enhanced bioavailability was observed. Enhanced bioavailability occurred in all combinations of bacteria and the Houghton muck soil and additionally for *Pseudomonas* sp. strain ADP with the Colwood and Hartsells soils. These results are consistent with the enhanced bioavailability observed for hydrophobic compounds, i.e., biphenyl (10, 18), naphthalene (29, 56, 57), phenanthrene (58, 66), and fluoranthene (58), and extend such evidence to a more polar compound, atrazine. These results also indicate that the physiochemical properties of sorbents (e.g., organic carbon) as well as bacterial cell characteristics (e.g., chemotaxis and attachment ability) influence the bioavailability of sorbed atrazine.

For each organism, enhanced bioavailability was observed only in some soils; in many other soil-organism combinations, mineralization was well predicted by the DBM model. For all three organisms, enhanced bioavailability was observed for the Houghton muck, which possesses the highest organic carbon content among the sorbents used. This sorbent had the highest sorbed-phase concentrations, perhaps indicating a positive relationship between sorbed-phase concentrations and access to the pool of sorbed atrazine, i.e., that bacteria can access high-concentration surface-localized regions of sorbed atrazine. For the other sorbents, sorbed-phase atrazine concentrations were lower and, in general, mineralization was predicted adequately by the DBM model, except for the combinations of *Pseudomonas* sp. strain ADP with Colwood and Hartsells soils, where enhanced mineralization rates were observed.

The inhibition of atrazine mineralization by K-montmorillonite may result from clay particles coating (obscuring) the microbial cells. The clay particle size used was <2 μm; the diameters of fine clay particles were smaller than 0.2 μm, which is substantially smaller than the common dimensions of bacterial cells. Obscuration of the cell surface could easily lead

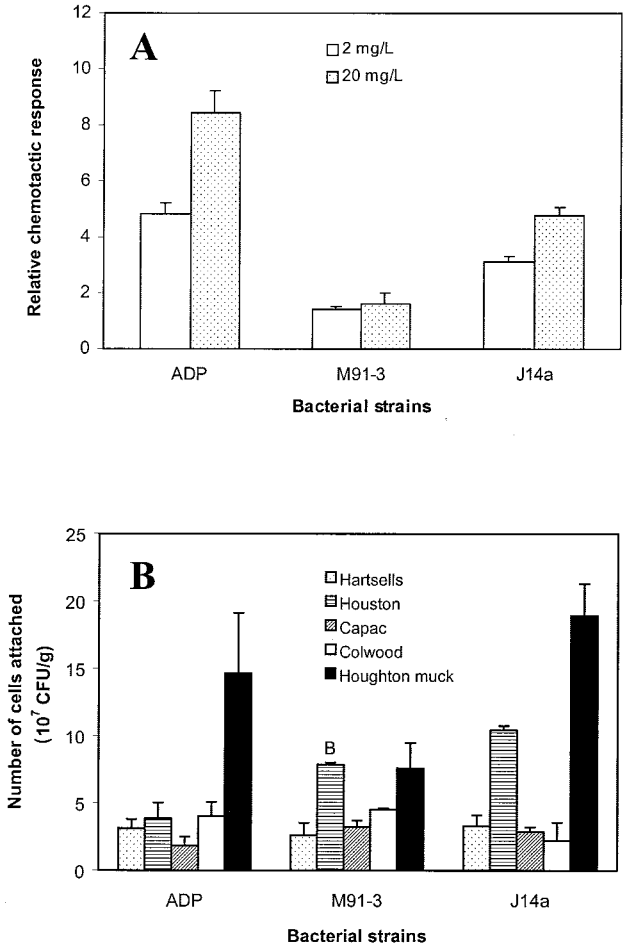

FIG. 7. Characteristics of atrazine-degrading bacteria. (A) Chemotactic responses of cells toward 2 and 20 mg of atrazine/liter after 60 min of incubation; (B) cell attachment to soils after 60 min of equilibration. Error bars represent the standard errors of triplicate measurements.

to diminished transport of atrazine into the cell. Access of bacteria to atrazine intercalated by the clay is not possible due to the small interlayer distances of <0.5 nm for K-montmorillonite (61); hence, enhanced bioavailability was not expected nor observed.

Mechanisms underlying the apparent availability of sorbed chemicals are complex due to the divergent properties of chemicals considered, the resultant sorption mechanisms, the metabolic diversity of microorganisms, and the heterogeneity of soils. Several microbially based mechanisms have been proposed for the apparent bioavailability of soil-sorbed organic chemicals: (i) production of biosurfactants (16, 50); (ii) production of extracellular enzymes to degrade target compounds; (iii) microorganisms with high substrate affinity, which efficiently reduce concentrations of the substrate close to the cell surface (5); (iv) reduction of the distance between cells and substrate by adhesion to sorbents (5, 26); and (v) reduction of the distance between cells and substrate by means of motility and chemotaxis (29).

The first three mechanisms do not seem to explain the bioavailability of soil-sorbed atrazine reported herein, since we did not detect the production of biosurfactants, extracellular enzyme activity on atrazine has not been observed, and no relationship is evident between K_s and bioavailability. Mechanisms four and five have been termed "direct uptake" but probably reflect a condition in which the substrate desorbs into the environment between the cell and the surface and then partitions to the cell surface, where it can be transported across the membrane. The critical issue here is that the substrate does not mix into the bulk solution, and thus, elevated concentrations can exist in this environment. Direct uptake of chemicals by bacteria at the aqueous phase/nonaqueous phase liquid interface has been demonstrated for hexadecane (8, 9, 17, 23), pyrene (7), naphthalene (6, 17, 68), and fluoranthene (7). In soils, sorbed pesticides are initially held predominately on organic matter surfaces of soil particles. We postulate that bacterial access to surface-localized pesticides at the soil-water interface has analogy to bacterial access to nonaqueous phase liquid molecules at its interface with water.

The influence of chemotaxis on substrate utilization has been studied extensively using hydrophilic chemicals (e.g., sugars, organic acids, and amino acids) not commonly considered pollutants. Recent studies showed that some bacteria were chemotactically attracted to organic contaminants such as benzene, toluene, trichloroethylene, biphenyl, and naphthalene (25, 47, 55). Chemotactic *P. putida* G7 can degrade naphthalene more rapidly than a mutant deficient in chemotaxis towards naphthalene (46). *P. putida* P106, which had nearly complete access to the pool of soil-sorbed biphenyl, showed a higher chemotactic response than *Rhodococcus erythropolis* NY05, an organism that degraded sorbed biphenyl to a lesser extent (18; G. Wu, Y. Feng, and S. A. Boyd, submitted for publication). Accumulations of atrazine-degrading bacteria in the capillaries were comparable to those reported for naphthalene- and biphenyl-degrading bacteria (46, 47; Wu et al., submitted) but were lower than those reported for bacteria chemotactic towards sugars or amino acids (1, 48). Two organisms used in this study, *Pseudomonas* sp. strain ADP and *A. radiobacter* J14a, were chemotactic towards atrazine and showed clear evidence of access to atrazine sorbed by the muck soil. *Ralstonia* sp. strain M91-3, however, was not chemotactic and also showed the least ability to access sorbed atrazine. Attachment of *Pseudomonas* sp. strain ADP and *A. radiobacter* J14a to the muck soil, where access to sorbed atrazine was indicated, was significantly higher than attachment to the mineral soils. Bacterial cell attachment to a sorbent has been shown to facilitate the degradation of sorbed chemicals (10, 31). Together, these results provide indications that access to sorbed atrazine may be favored by the bacterial characteristics of chemotaxis and cell attachment and by high sorbed-phase concentrations.

The estimated liquid diffusion film thickness under the conditions of this study (mixing at 200 rpm) was ~76 μm (42). It is possible that attached bacteria may have been exposed to higher atrazine concentrations within the inner boundary diffusion film compared to that of bulk solution, resulting in enhanced mineralization rates. Using an average desorption rate coefficient (α) of 0.003 min^{-1} (Table 3) and equation 3, the calculated half desorption time from sorbed phase to bulk aqueous phase is 13,800 s. Using Fick's second law, a liquid-phase diffusivity value for atrazine of 6.5×10^{-6} cm^2/s, and a liquid boundary layer thickness value of 76 μm, the calculated

half-diffusion time through the liquid boundary layer is 10 s. According to these calculations, the time required for diffusion through the liquid boundary layer is negligible compared to that for desorption of soil-sorbed atrazine. Another possible cause of enhanced mineralization rates is that depletion of atrazine within the diffusion film by attached cells can manifest enhanced desorption, and hence mineralization, by increasing the concentration gradient between the surface and interior of soil particles. The DBM_{id} model was used as a boundary condition to gain perspective on potential atrazine concentrations within the diffusion film as well as possible increased desorption rate. Because the DBM_{id} model assumes instantaneous equilibrium between sorbed and aqueous phase, it provides the theoretically maximum dissolved atrazine concentration and the maximum desorption rate possible.

Carbon dioxide production rates above those predicted by the DBM_{id} (Fig. 4 to 6) cannot be explained by the exposure of cells to elevated atrazine concentrations within the boundary layer or by improved desorption rates due to depletion of dissolved atrazine within the film. Rather, these results indicate bacterial access to the pool of sorbed atrazine. According to Gillette et al. (22) and Ghosh et al. (20, 21), sorbed concentrations of contaminants in soils differ from particle to particle and differ laterally and radially in the same particle, suggesting that sorption phenomena are heterogeneous at subparticle dimensions. In their study, polycyclic aromatic hydrocarbons accumulated more in organic carbon rich regions and on the exterior of soil particles. Based on these results, it seems plausible that surface-localized contaminant concentrations are higher than soil mass-normalized sorbed concentrations. In this study, the average atrazine concentration required to account for the observed enhanced mineralization (bioavailability) of atrazine was ca. 1.4 ($\pm$ 0.09) times the measured bulk liquid concentration. Because the calculated atrazine concentration is normalized by the total liquid volume in the system, the actual concentration that atrazine-degrading bacteria are exposed to may be much higher than the averaged value. It seems plausible that the properties of chemotaxis towards atrazine and/or attachment to soil surfaces facilitate access to surface-localized regions of elevated atrazine concentrations.

It is likely that the overall ability of an organism to access soil-sorbed compounds is influenced by more than one characteristic of bacteria and that these characteristics may be interrelated. Further studies are needed before definitive conclusions can be made regarding the effects of bacterial characteristics on their ability to access the pool of sorbed chemicals. However, there is an emerging body of evidence indicating the bioavailability of soil-sorbed pesticides and organic contaminants to bacteria.

ACKNOWLEDGMENT

This research was supported by the U.S. Department of Agriculture National Research Initiative Competitive Grants Program.

REFERENCES

1. **Adler, J.** 1973. A method for measuring chemotaxis and use of the method to determine optimum conditions for chemotaxis by *Escherichia coli*. J. Gen. Microbiol. **74:**77–91.
2. **Al-Tahhan, R. A., T. R. Sandrin, A. A. Bodour, and R. M. Maier.** 2000. Rhamnolipid-induced removal of lipopolysaccharide from *Pseudomonas aeruginosa*: effect on cell surface properties and interaction with hydrophobic substrates. Appl. Environ. Microbiol. **66:**3262–3268.
3. **Amador, J. A., and M. Alexander.** 1988. Effect of humic acids on the mineralization of low concentrations of organic compounds. Soil Biol. Biochem. **20:**185–191.
4. **Aspelin, A. L., and A. H. Grube.** 1999. Pesticides industry sales and usage: 1996 and 1997 market estimates. Office of Prevention, Pesticides & Toxic Substances, U.S. Environmental Protection Agency, Washington, D.C.
5. **Bastiaens, L., D. Springael, P. Wattiau, H. Harms, R. deWachter, H. Verachtert, and L. Diels.** 2000. Isolation of adherent polycyclic aromatic hydrocarbon (PAH)-degrading bacteria using PAH-sorbing carriers. Appl. Environ. Microbiol. **66:**1834–1843.
6. **Bouchez, M., D. Blanchet, B. Besnainou, J. Y. Leveau, and J. P. Vandecasteele.** 1997. Kinetic studies of biodegradation of insoluble compounds by continuous determination of oxygen consumption. J. Appl. Microbiol. **82:** 310–316.
7. **Bouchez, M., D. Blanchet, and J.-P. Vandecasteele.** 1997. An interfacial uptake mechanism for the degradation of pyrene by a *Rhodococcus* strain. Microbiology **143:**1087–1093.
8. **Bouchez-Naitali, M., D. Blanchet, V. Bardin, and J.-P. Vandecasteele.** 2001. Evidence for interfacial uptake in hexadecane degradation by *Rhodococcus equi*: the importance of cell flocculation. Microbiology **147:**2537–2543.
9. **Bouchez-Naitali, M., H. Rakatozafy, R. Marchal, J. Y. Leveau, and J. P. Vandecasteele.** 1999. Diversity of bacterial strains degrading hexadecane in relation to the mode of substrate uptake. J. Appl. Microbiol. **86:**421–428.
10. **Calvillo, Y. M., and M. Alexander.** 1996. Mechanism of microbial utilization of biphenyl sorbed to polyacrylic beads. Appl. Microbiol. Biotechnol. **45:** 383–390.
11. **Chen, W., A. T. Kan, G. Fu, L. C. Vignona, and M. B. Tomson.** 1999. Adsorption-desorption behaviors of hydrophobic organic compounds in sediments of Lake Charles, Louisiana, USA. Environ. Toxicol. Chem. **18:**1610–1616.
12. **Chung, N., and M. Alexander.** 1998. Differences in sequestration and bioavailability of organic compounds aged in dissimilar soils. Environ. Sci. Technol. **32:**855–860.
13. **Clay, S. A., R. R. Allmaras, W. C. Koskinen, and D. L. Wyse.** 1988. Desorption of atrazine and cyanazine from soil. J. Environ. Qual. **17:**719–723.
14. **Crocker, F. H., W. F. Guerin, and S. A. Boyd.** 1995. Bioavailability of naphthalene sorbed to cationic surfactant-modified smectite clay. Environ. Sci. Technol. **29:**2953–2958.
15. **Deitsch, J. J., and J. A. Smith.** 1999. Sorption and desorption rate comparisons for 1,2-dichlorobenzene to a peat soil. Environ. Toxicol. Chem. **18:** 1701–1709.
16. **Desai, J. D., and I. M. Banat.** 1997. Microbial production of surfactants and their commercial potential. Microbiol. Mol. Biol. Rev. **61:**47–64.
17. **Efroymson, R. A., and M. Alexander.** 1991. Biodegradation by an *Arthrobacter* species of hydrocarbons partitioned into an organic solvent. Appl. Environ. Microbiol. **57:**1441–1447.
18. **Feng, Y., J. H. Park, T. C. Voice, and S. A. Boyd.** 2000. Bioavailability of soil-sorbed biphenyl to bacteria. Environ. Sci. Technol. **34:**1977–1984.
19. **Garcia-Valcarcel, A. I., T. Matienzo, C. Sanchez-Brunete, and J. L. Tadeo.** 1998. Adsorption of triazines in soils with low organic matter content. Fresenius Environ. Bull. **7:**649–656.
20. **Ghosh, U., J. S. Gillette, R. G. Luthy, and R. N. Zare.** 2000. Microscale location, characterization, and association of polycyclic aromatic hydrocarbons on harbor sediment particles. Environ. Sci. Technol. **34:**1729–1736.
21. **Ghosh, U., J. W. Talley, and R. G. Luthy.** 2001. Particle-scale investigation of PAH desorption kinetics and thermodynamics from sediment. Environ. Sci. Technol. **35:**3468–3475.
22. **Gillette, J. S., R. G. Luthy, S. J. Clemett, and R. N. Zare.** 1999. Direct observation of polycyclic aromatic hydrocarbons on geosorbents at the subparticle scale. Environ. Sci. Technol. **33:**1185–1192.
23. **Goswami, P., and H. D. Singh.** 1991. Different modes of hydrocarbon uptake by 2 *Pseudomonas* species. Biotechnol. Bioeng. **37:**1–11.
24. **Griffith, P. C., and M. Fletcher.** 1991. Hydrolysis of protein and model dipeptide substrates by attached and nonattached marine *Pseudomonas* sp. strain NCIMB 2021. Appl. Environ. Microbiol. **57:**2186–2191.
25. **Grimm, A. C., and C. S. Harwood.** 1997. Chemotaxis of *Pseudomonas* spp. to the polyaromatic hydrocarbon naphthalene. Appl. Environ. Microbiol. **63:** 4111–4115.
26. **Grosser, R. J., M. Friedrich, D. M. Ward, and W. P. Inskeep.** 2000. Effect of model sorptive phases on phenanthrene biodegradation: different enrichment conditions influence bioavailability and selection of phenanthrene-degrading isolates. Appl. Environ. Microbiol. **66:**2695–2702.
27. **Guerin, W. F., and S. A. Boyd.** 1997. Bioavailability of naphthalene associated with natural and synthetic sorbents. Water Res. **32:**1504–1512.
28. **Guerin, W. F., and S. A. Boyd.** 1993. Bioavailability of sorbed naphthalene to bacteria: influence of contaminant aging and soil organic carbon content, p. 197–208. *In* D. M. Linn (ed.), Sorption and degradation of pesticides and organic chemicals in soil. Soil Science Society of America, Madison, Wis.
29. **Guerin, W. F., and S. A. Boyd.** 1992. Differential bioavailability of soil-sorbed naphthalene to two bacterial species. Appl. Environ. Microbiol. **58:**1142–1152.
30. **Guo, L., R. J. Wagenet, and W. A. Jury.** 1999. Adsorption effects on kinetics

of aldicarb degradation: equilibrium model and application to incubation and transport experiments. Soil Sci. Soc. Am. J. **63:**1637–1644.
31. **Harms, H., and A. J. B. Zehnder.** 1995. Bioavailability of sorbed 3-chlorodibenzofuran. Appl. Environ. Microbiol. **61:**27–33.
32. **Jenks, B. M., F. W. Roeth, A. R. Martin, and D. L. McCallister.** 1998. Influence of surface and subsurface soil properties on atrazine sorption and degradation. Weed Sci. **46:**132–138.
33. **Kan, A. T., G. Fu, M. Hunter, W. Chen, C. H. Ward, and M. B. Tomson.** 1998. Irreversible sorption of neutral hydrocarbons to sediments: experimental observations and model predictions. Environ. Sci. Technol. **32:**892–902.
34. **Kan, A. T., G. Fu, M. A. Hunter, and M. B. Tomson.** 1997. Irreversible adsorption of naphthalene and tetrachlorobiphenyl to Lula and surrogate sediments. Environ. Sci. Technol. **31:**2176–2185.
35. **Kan, A. T., G. Fu, and M. B. Tomson.** 1994. Adsorption/desorption hysteresis in organic pollutant and soil/sediment interaction. Environ. Sci. Technol. **28:**859–867.
36. **Karickhoff, S. W.** 1980. Sorption kinetics of hydrophobic pollutants in natural sediments, p. 193–205. *In* R. A. Baker (ed.), Contaminants and sediments: analysis, chemistry, and biology, vol. 2. Ann Arbor Press, Ann Arbor, Mich.
37. **Kelsey, J. W., B. D. Kottler, and M. Alexander.** 1997. Selective chemical extractants to predict bioavailability of soil-aged organic chemicals. Environ. Sci. Technol. **31:**214–217.
38. **Knaebel, D. B., T. W. Federle, D. C. Mcavoy, and J. R. Vestal.** 1994. Effect of mineral and organic soil constituents on microbial mineralization of organic compounds in a natural soil. Appl. Environ. Microbiol. **60:**4500–4508.
39. **Kolpin, D. W., E. M. Thurman, and D. A. Goolsby.** 1996. Occurrence of selected pesticides and their metabolites in near-surface aquifers of the midwestern United States. Environ. Sci. Technol. **30:**335–340.
40. **Laird, D. A., E. Barriuso, R. H. Dowdy, and W. C. Koskinen.** 1992. Adsorption of atrazine on smectites. Soil Sci. Soc. Am. J. **56:**62–67.
41. **Laird, D. A., P. Y. Yen, W. C. Koskinen, T. R. Steinheimer, and R. H. Dowdy.** 1994. Sorption of atrazine on soil clay components. Environ. Sci. Technol. **28:**1054–1061.
42. **Levich, V. G.** 1962. Physicochemical hydrodynamics. Prentice-Hall, Inc., Englewood Cliffs, N.J.
43. **Ma, L., L. M. Southwick, G. H. Willis, and H. M. Selim.** 1993. Hysteretic characteristics of atrazine adsorption-desorption by a Sharkey soil. Weed Sci. **41:**627–633.
44. **Mandelbaum, R. T., D. L. Allan, and L. P. Wackett.** 1995. Isolation and characterization of a *Pseudomonas s*p. that mineralizes the *s*-triazine herbicide atrazine. Appl. Environ. Microbiol. **61:**1451–1457.
45. **Marshman, N. A., and K. C. Marshall.** 1981. Bacterial growth on proteins in the presence of clay minerals. Soil Biol. Biochem. **13:**127–134.
46. **Marx, R. B., and M. D. Aitken.** 2000. Bacterial chemotaxis enhances naphthalene degradation in a heterogeneous aqueous system. Environ. Sci. Technol. **34:**3379–3383.
47. **Marx, R. B., and M. D. Aitken.** 1999. Quantification of chemotaxis to naphthalene by *Pseudomonas putida* G7. Appl. Environ. Microbiol. **65:**2847–2852.
48. **Mazumder, R., T. J. Phelps, N. R. Krieg, and R. E. Benoit.** 1999. Determining chemotactic responses by two subsurface microaerophiles using a simplified capillary assay method. J. Microbiol. Methods **37:**255–263.
49. **McGhee, I., F. Sannino, L. Gianfreda, and R. G. Burns.** 1999. Bioavailability of 2,4-D sorbed to a chlorite-like complex. Chemosphere **39:**285–291.
50. **Noordman, W. H., W. Ji, M. L. Brusseau, and D. B. Janssen.** 1998. Effects of rhamnolipid biosurfactants on removal of phenanthrene from soil. Environ. Sci. Technol. **32:**1806–1812.
51. **Novak, J. M.** 1999. Soil factors influencing atrazine sorption: implications on fate. Environ. Toxicol. Chem. **18:**1663–1667.
52. **Ogram, A. V., R. E. Jessup, L. T. Ou, and P. S. C. Rao.** 1985. Effects of sorption on biological degradation rates of (2,4-dichlorophenoxy)acetic acid in soils. Appl. Environ. Microbiol. **49:**582–587.
53. **Ortega-Calvo, J.-J., and C. Saiz-Jimenez.** 1998. Effect of humic fractions and clay on biodegradation of phenanthrene by a *Pseudomonas fluorescens* strain isolated from soil. Appl. Environ. Microbiol. **64:**3123–3126.
54. **Page, A. L., R. H. Miller, and D. R. Keeney.** 1982. Methods of soil analysis (part 2). American Society of Agronomy, Inc., Madison, Wis.
55. **Parales, R. E., J. L. Ditty, and C. S. Harwood.** 2000. Toluene-degrading bacteria are chemotactic towards the environmental pollutants benzene, toluene, and trichloroethylene. Appl. Environ. Microbiol. **66:**4098–4104.
56. **Park, J.-H., X. Zhao, and T. C. Voice.** 2001. Biodegradation of non-desorbable naphthalene in soils. Environ. Sci. Technol. **35:**2734–2740.
57. **Park, J.-H., X. Zhao, and T. C. Voice.** 2002. Development of a kinetic basis for bioavailability of naphthalene in soil slurries. Water Res. **36:**1620–1628.
58. **Poeton, T. S., H. D. Stensel, and S. E. Strand.** 1999. Biodegradation of polyaromatic hydrocarbons by marine bacteria: effect of solid phase on degradation kinetics. Water Res. **33:**868–880.
59. **Radosevich, M., S. J. Traina, Y. L. Hao, and O. H. Tuovinen.** 1995. Degradation and mineralization of atrazine by a soil bacterial isolate. Appl. Environ. Microbiol. **61:**297–302.
60. **Radosevich, M., S. J. Traina, and O. H. Tuovinen.** 1997. Atrazine mineralization in laboratory-aged soil microcosms inoculated with s-triazine-degrading bacteria. J. Environ. Qual. **26:**206–214.
60a. **Sharer, M., J.-H. Park, T. C. Voice, and S. A. Boyd.** J. Environ. Qual. in press.
61. **Sheng, G. Y., C. T. Johnston, B. J. Teppen, and S. A. Boyd.** 2001. Potential contributions of smectite clays and organic matter to pesticide retention in soils. J. Agric. Food Chem. **49:**2899–2907.
62. **Smith, S. C., C. C. Ainsworth, S. J. Traina, and R. J. Hicks.** 1992. Effect of sorption on the biodegradation of quinoline. Soil Sci. Soc. Am. J. **56:**737–746.
63. **Squillace, P. J., and E. M. Thurman.** 1992. Herbicide transport in rivers: importance of hydrology and geochemistry in nonpoint-source contamination. Environ. Sci. Technol. **26:**538–545.
64. **Struthers, J. K., K. Jayachandran, and T. B. Moorman.** 1998. Biodegradation of atrazine by *Agrobacterium radiobacter* J14a and use of this strain in bioremediation of contaminated soil. Appl. Environ. Microbiol. **64:**3368–3375.
65. **Subba-Rao, R. V., and M. Alexander.** 1982. Effect of sorption on mineralization of low concentrations of aromatic compounds in lake water samples. Appl. Environ. Microbiol. **44:**659–668.
66. **Tang, W. C., J. C. White, and M. Alexander.** 1998. Utilization of sorbed compounds by microorganisms specifically isolated for that purpose. Appl. Microbiol. Biotechnol. **49:**117–121.
67. **U.S. Environmental Protection Agency.** 1991. Summary and report—high-priority research on bioremediation, Bioremediation Research Needs Workshop. U.S. Environmental Protection Agency, Washington, D.C.
68. **Wodzinski, R. S., and D. Larocca.** 1977. Bacterial-growth kinetics on diphenylmethane and naphthalene-heptamethylnonane mixtures. Appl. Environ. Microbiol. **33:**660–665.
69. **Xie, H., T. F. Guetzloff, and J. A. Rice.** 1997. Fractionation of pesticide residues bound to humin. Soil Sci. **162:**421–429.