APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 1994, p. 4500–4508
0099-2240/94/$04.00+0

Vol. 60, No. 12

Effect of Mineral and Organic Soil Constituents on Microbial Mineralization of Organic Compounds in a Natural Soil†

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Received 16 May 1994/Accepted 11 September 1994

This research addressed the effect of mineral and organic soil constituents on the fate of organic compounds in soils. Specifically, it sought to determine how the associations between organic chemicals and different soil constituents affect their subsequent biodegradation in soil. Four ^{14}C-labeled surfactants were aseptically adsorbed to montmorillonite, kaolinite, illite, sand, and humic acids. These complexes were mixed with a woodlot soil, and $^{14}CO_2$ production was measured over time. The mineralization data were fitted to various production models by nonlinear regression, and a mixed (3/2)-order model was found to most accurately describe the mineralization patterns. Different mineralization patterns were observed as a function of the chemical and soil constituents. Surfactants that had been preadsorbed to sand or kaolinite usually showed similar mineralization kinetics to the control treatments, in which the surfactants were added to the soil as an aqueous solution. Surfactants that had been bound to illite or montmorillonite were typically degraded to lesser extents than the other forms, while surfactant-humic acid complexes were degraded more slowly than the other forms. The desorption coefficients (K_d) of the soil constituent-bound surfactants were negatively correlated with the initial rates of degradation (k_1) and estimates of $^{14}CO_2$ yield (P_0) as well as actual total yields of $^{14}CO_2$. However, there was no relationship between K_d and second-stage zero-order rates of mineralization (k_0). Microbial community characteristics (biomass and activity) were not correlated with any of the mineralization kinetic parameters. Overall, this study showed that environmental form had a profound effect on the ultimate fate of biodegradable chemicals in soil. This form is defined by the physicochemical characteristics of the chemical, the composition and mineralogy of the soil, and the mode of entry of the chemical into the soil environment.

Synthetic organic chemicals (xenobiotics) enter terrestrial environments as a result of intentional application, waste disposal practices, accidental spills, land use patterns, and atmospheric fallout. Their fate in soils is a function of both abiotic and biotic processes, which include transport and sorption, chemical catalysis, photodegradation, and biodegradation. Biodegradation of a chemical in soil is determined by the presence and size of degrader populations, as well as environmental conditions that affect the activity of these populations and the bioavailability of the chemical.

Several studies have shown that various soil constituents may alter the biodegradation of chemicals in solution. Benzylamine degradation was substantially inhibited when incubated in the presence of montmorillonite (31), whereas diquat degradation was severely inhibited in the presence of montmorillonite clays but not in the presence of kaolinite clays (48). In contrast, Scow and Alexander (41) found substantial decreases in the rates and extents of phenol and glutamate degradation in the presence of kaolinite. Other workers have shown that naphthalene or α-hexachlorocyclohexane must desorb from the soil matrix before biodegradation can occur (1, 36). Ogram et al. (33) likewise demonstrated that 2,4-dichlorophenoxyacetic acid had to be available in the soil solution before biodegradation could begin but found that both sorbed and free bacteria could degrade the 2,4-dichlorophenoxyacetic acid. In another study, the effect of the soil matrix on chemical biodegradation was microorganism specific, such that one bacterial species was capable of metabolizing sorbed naphthalene but another species required naphthalene to be in solution (13). In contrast to the aforementioned studies, other studies have shown that biodegradation of pentachlorophenol and toluene was not retarded by interactions with the soil matrix (3, 38).

Many of these previously mentioned studies were performed with dilute suspensions of the soil constituents, which are not representative of typical soil conditions. Only a few studies have assessed the effects of added soil constituents under environmentally realistic conditions. Bellin et al. (3) found that sorption of pentachlorophenol to alkaline and acidic soils increased with increasing additions of sludge but that biodegradation was not affected. O'Connor et al. (32) reported that sludge additions did not significantly affect either the adsorption or biodegradation of a related chemical, 2,4-dichlorophenol, in calcareous soils. In other studies, addition of various levels of different clays to soils generally resulted in decreased mineralization of a chemical to CO_2 (25, 44, 45). These studies did not differentiate the effects of the added clay on overall microbial activity and on the bioavailability of the test chemical. Furthermore, none of these studies of addition of sludge and clays to soils have specifically addressed the interaction between the chemicals and the added constituents.

The present study is unique in that organic chemicals were

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† Dr. Vestal died of brain cancer in August 1992 at the age of 49. We miss him greatly and valued him as a colleague, mentor, and friend. We hope that publication of his unfinished work will be a living memorial to his dedication to science.

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adsorbed to specific soil constituents before being introduced into a natural soil and the incubation conditions reflected in situ conditions of temperature and soil water activity (a_w). Experiments were designed to minimize changes in the soil chemistry and mineralogy while presenting the chemical to the natural microbiota in a defined environmental form. Four ^{14}C-labeled surfactants with different physicochemical properties were individually associated with purified soil constituents (sand, montmorillonite, kaolinite, illite, and humics), and small amounts of these complexes were introduced into a natural woodlot soil. The mineralization kinetics of the complexed surfactants were compared with one another and with those of controls, which were conventionally dosed with aqueous solutions or dispersions of the chemicals. In addition, the parameters describing mineralization were compared with the desorption coefficients (K_d) of the chemical from the soil constituent.

The four surfactants differed in their charge and hydrophobicity and therefore modeled many of the physicochemical interactions which can occur between organic chemicals and different soil constituents. In addition to serving as excellent model compounds, surfactants have a wide range of consumer and industrial uses (35) and are increasingly important in the remediation technology of in situ soil washing (10, 28). Most modern surfactants are usually quickly biodegraded in surface soils, with half-lives of 3 weeks or less (19, 47), but some nonionic surfactants may have longer residence times in some soils (46) and may affect microbial processes therein (22). In addition, the extent of mineralization of surfactants added to soils has been shown to vary greatly as a function of soil type (19).

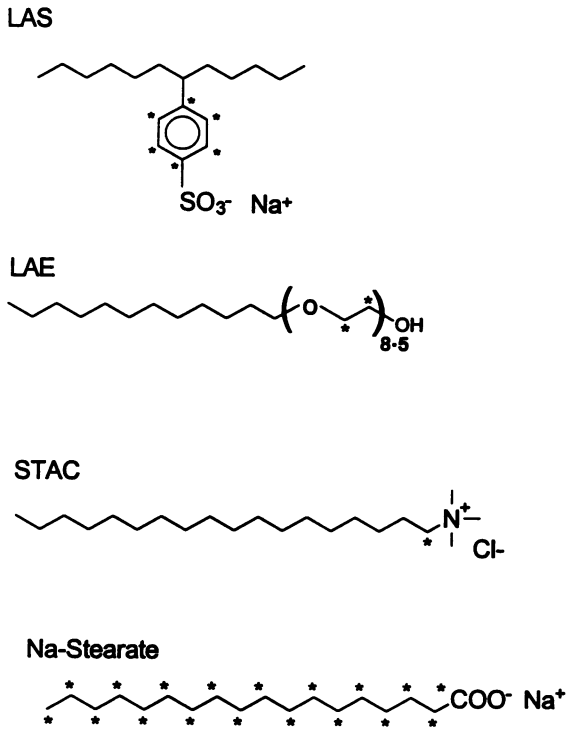

FIG. 1. Chemical structures of the compounds used in this study. The location of the ^{14}C is indicated by *.

MATERIALS AND METHODS

Soil. Bonnell soil was obtained from a woodlot in Hamilton County, Ohio. The upper 5 to 8 cm was aseptically collected, partially dried under a stream of sterile air, and sieved. The ≤2-mm fraction was used for all experiments. The soil was a silty loam and was mapped as a fine mixed, Mesic, Typic Hapludalf (24). It had a pH of 7.13, an organic carbon content of 4.7%, a cation-exchange capacity of 27.5 meq 100 g^{-1} (2), a surface area of 49.6 m^2 g^{-1} (14), and a clay content of 6.3%. The clay fraction was predominantly mica, with a moderate level of vermiculite and a trace of smectite.

Soil constituents. Montmorillonite (SWy-1), kaolinite, and illite were purchased from the Clay Mineral Society, University of Missouri, Columbia. The illite was pulverized prior to use. The sand was collected near Daytona Beach, Fla., and combusted at 550°C for 4 h before use. Humic acid was purchased from Aldrich Chemical Co., Milwaukee, Wis.

Chemicals. [U-*ring*-^{14}C]dodecyl linear alkylbenzene sulfonate (LAS) with a specific activity of 67.8 μCi mg^{-1} was purchased from New England Nuclear, Wilmington, Del. [U-*ethoxy*-^{14}C]dodecyl linear alcohol ethoxylate (LAE) (average of 8.5 ethoxy residues per molecule) and [U-^{14}C]sodium stearate were purchased from Amersham, Arlington Heights, Ill. LAE had a specific activity of 5.4 μCi mg^{-1}, and stearate had a specific activity of 196.8 μCi mg^{-1}. [1-^{14}C]stearyl trimethylammonium chloride (STAC) was synthesized at Procter and Gamble and had a specific activity of 12.5 μCi mg^{-1}. [U-^{14}C]acetate was purchased from Amersham; its specific activity was 57 mCi $mmol^{-1}$. The radiochemical purity of the surfactants as determined by thin-layer chromatography was greater than 98%, except for sodium stearate, which had a purity greater than 96%. All other chemicals used were reagent grade. Structural formulas of the surfactants are shown in Fig. 1.

Microbiological analysis. Subsamples of the soil were analyzed for overall microbial biomass and activity at both the initiation and termination of the experiments. Microbial activity was determined by measuring the rate of [^{14}C]acetate incorporation into microbial lipids, using the method of McKinley et al. (29) with one modification. Instead of using a soil slurry, activity measurements were done at 100% water-holding capacity to represent more realistic a_w ranges in the soil. The concentration of [^{14}C]acetate was 42.6 μM. Soil microbial biomass was determined by measuring $CHCl_3$-extractable lipid phosphate as described by Findlay et al. (11).

Preparation of chemical-soil constituent complexes. All soil constituents were sterilized by autoclaving for 40 min at 121°C. A 4-g portion of each soil constituent was placed in a round-bottom (250 ml) flask with 4 to 12 ml of absolute methanol containing 14.5 μg of the ^{14}C-labeled chemicals. This ratio of surfactant and soil constituent resulted in a final concentration of approximately 50 ng of surfactant per g of soil in the mineralization experiments amended with 100 mg of the complex. The flasks were placed on a rotary evaporator, and the materials were mixed at 40°C. A vacuum was applied, and the solvent slowly evaporated as mixing continued. The complexes were further dried aseptically in a vacuum oven and stored over Drierite in sterile containers. Alcohols were used instead of water to avoid high temperatures during the drying process as well as to ensure sterility during the adsorption process.

Specific activity was determined by placing a known quantity of each complex in 0.5 ml of methanol or ethanol in a 20-ml scintillation vial (Kimble), vortexing for 5 min, and mixing with 15 ml of Scintiverse II (Fisher) and sufficient water to form a gel. Radioactivity determinations were made on a Packard

Tri-Carb 2200CA scintillation counter, using an efficiency tracing protocol for quench correction. Specific activities of the bound surfactants closely agreed with theoretical specific activities, based on the total ^{14}C activity added to each soil constituent.

Mineralization assays. Mineralization of the bound surfactants was measured in serum bottle radiorespirometers as described by Knaebel and Vestal (20). The surfactant-soil constituent complexes were added at a low level (1.25%, wt/wt) to avoid altering the biology and chemical and physical characteristics of the soil. A small amount (100 mg) of the surfactant-soil constituent complex was mixed with 8.0 (dry weight) of soil. Sterile deionized distilled water was then added to bring the mixture to 70% gravimetric water-holding capacity. Controls were treated similarly, except that the surfactant was dissolved or dispersed in the water used to adjust the water-holding capacity. Four replicates of each treatment were tested. Abiotic controls were autoclaved three times and amended with 4.7% formalin and 30 mg of thimerosol kg^{-1} as previously described (19). The respirometers were incubated in the dark at room temperature (ca. 22°C). Mineralization was measured over a period of 2 months.

Desorption assays. Desorption coefficients (log K_d) were determined by standard techniques (1a) adapted for desorption applications. An aliquot of each complex was placed into sterile 10 mM NaN_3 in acid-washed polypropylene or glass centrifuge tubes. The tubes were vortexed briefly and placed horizontally on an orbital shaker (120 rpm) at room temperature (22 to 24°C). At predetermined intervals, the tubes were centrifuged and a portion of the supernatant was removed for radioactivity determination. The complexes were resuspended, and the process was repeated until the level of radioactivity in the supernatant remained constant. Equilibria were assumed when the radioactivity in the supernatant was constant. This usually occurred within 2 to 4 h, but experiments were conducted for 24 to 48 h. The K_d was calculated from the ratio of radioactivity remaining bound to the soil constituents (dpm per milligram) to radioactivity in the supernatant (dpm per milliliter). The former was estimated by subtraction of the supernatant value from the total added radioactivity.

Data analysis. Mineralization results were fitted to first-order (23) and 3/2-order mineralization models (7). The two models are similar, but the 3/2-order model has additional terms for growth and a zero-order mineralization rate.

The first-order model has the following form: $P = P_0\,[1 - e^{(-k_1 t)}]$, where P is the percentage of compound mineralized at time t, P_0 is the asymptotic percentage of compound converted to $^{14}CO_2$, and k_1 is the first-order rate constant (day^{-1}). The 3/2 model (with linear growth term) has the following form: $P = P_0\,\{1 - e^{[-k_1 t - (k_2 t^2)/2]}\} + (k_0 t)$, where P is the percentage of compound mineralized at time t, P_0 is the percentage of compound converted to $^{14}CO_2$ during first-order metabolism, k_1 is a proportionality rate constant (day^{-1}), k_2 is a linear growth rate term, and k_0 is a zero-order rate constant (percent day^{-1}). On the basis of the small amounts of labile carbon (i.e., the surfactants) which were added to the soil (50 ng g^{-1}), no growth was assumed and k_2 was removed from the equation (7), to give the following: $P = P_0\,[1 - e^{(-k_1 t)}] + (k_0 t)$.

The mineralization data were fitted to the models by the Quasi-Newton minimization technique in the NONLIN module of Systat, Evanston, Ill. The model that best fit the mineralization data was determined by the F-test procedure outlined by Robinson (37). The GLM module of SAS Institute, Cary, N.C., and the MGLH module of Systat were used for statistical analyses. Comparisons within treatments (i.e., surfactants or soil constituents) were made by the Scheffé multiple-comparison test (9, 40, 43).

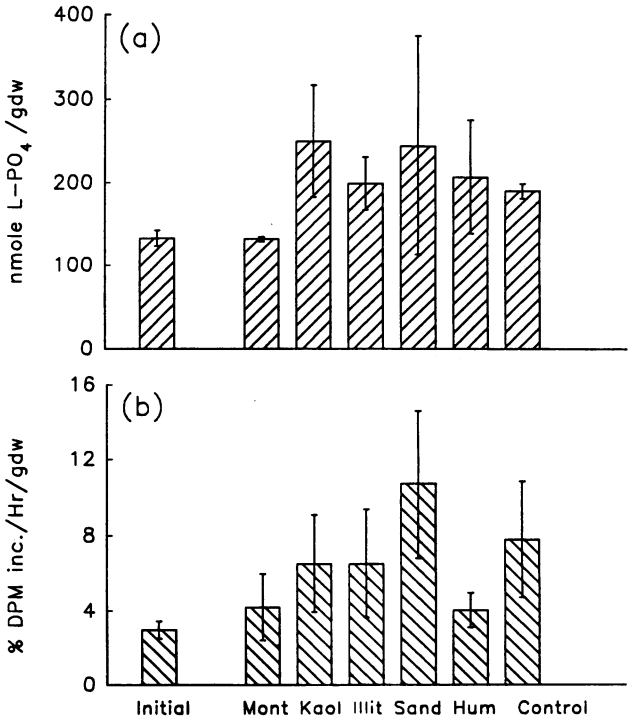

FIG. 2. Microbial biomass and activity in the Bonnell soil before and after the mineralization experiments. Data are shown as mean ± 1 standard deviation. (a) Microbial biomass, measured as nanomoles of lipid phosphate per gram (dry weight) (gdw) of soil (n = 3). (b) Microbial activity of the soil before and after the mineralization experiments, expressed in terms of soil mass (n = 4). Initial, initial biomass of the soil prior to mineralization experiments; Mont, montmorillonite; Kaol, kaolinite; Illit, illite; hum, humics.

RESULTS

The Bonnell soil had an initial microbial biomass of 141 nmol of lipid phosphate g (dry weight)$^{-1}$, which represents approximately 2.8 mg of biomass carbon g (dry weight)$^{-1}$ (49), and an initial activity of 2.97% [^{14}C]acetate incorporated into microbial lipids h^{-1} g (dry weight)$^{-1}$ (Fig. 2). The biomass and physiological activity of this soil were similar to those of other soils in this geographical region (19). After the mineralization experiments were terminated, mean microbial biomass and activity were elevated compared with the initial values in most treatments, including the control (Fig. 2). This increase in mean value was also accompanied by increased variability within each treatment. Hence, the biomass in treatments amended with surfactant-soil constituent complexes was not significantly different from that in the control treatments ($P \geq 0.05$) (Fig. 2a). Similarly, microbial activity, whether expressed in terms of soil mass (Fig. 2b) or microbial biomass (data not shown), did not exhibit any significant treatment effects ($P \geq 0.05$).

In 92% of the cases, the 3/2-order model showed significantly better fit to the mineralization data than the first-order model on the basis of F-tests that control for the extra terms in the model (40). Two examples of this better fit are shown in Fig. 3. This model estimates an initial exponential rate constant (k_1), a yield parameter (P_0), a linear (or exponential)

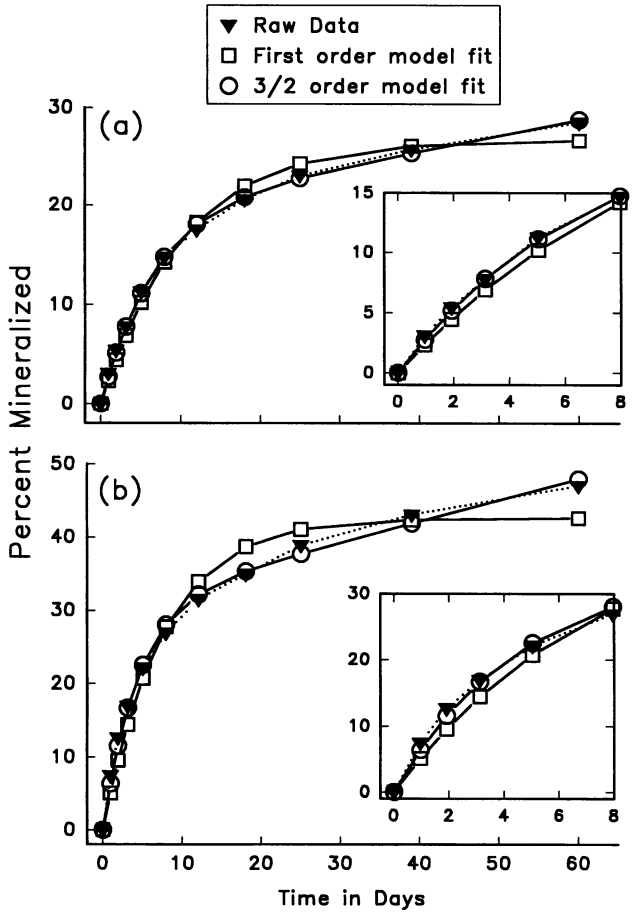

FIG. 3. Comparison of the fit of 3/2-order and first-order models to mineralization data. The mineralization data were from the stearate complexed with humics (a) and sand (b). The inset shows the details of the first 8 days of mineralization.

growth parameter (k_2), and a linear mineralization rate (k_0). The k_2 estimates were usually zero in this study (data not shown), so no growth was assumed. Therefore, the growth term was removed from the model (7). This was consistent with the minimal changes in biomass during the mineralization experiments (Fig. 2a) and the low level of added surfactants (50 ng g^{-1}).

Because of the error minimization calculations used by the nonlinear regression algorithm, the estimates of equivalent parameters of the 3/2-order model differed in a consistent fashion from those of the first-order model. The general trends were that the initial rate estimates (k_1) calculated by the 3/2-order model were slightly higher than the estimates of the first-order rate constant and that the yield estimates (P_0) tended to be lower than the asymptotic yield estimates of the first-order model. However, the P_0 values for the 3/2-order model are unique in that they indicate the point at which the mineralization rate changes from a pseudo-first-order reaction to a zero-order reaction. They do not indicate the asymptotic yield of CO_2. In most circumstances, P_0 represented 70% (for stearate and STAC) to 80% (LAE and LAS) of the total final percentage recovered as $^{14}CO_2$ (Table 1). For this reason, the total amounts of $^{14}CO_2$ evolved after 60 days are included in Table 1.

TABLE 1. Mineralization kinetic estimates and total yields of $^{14}CO_2$ from surfactant-soil constituent complexes in the Bonnell soil[a]

Soil constituent	LAE				LAS				Stearate				STAC			
	k_1	k_0	P_0	Total yield (%)	k_1	k_0	P_0	Total yield (%)	k_1	k_0	P_0	Total yield (%)	k_1	k_0	P_0	Total yield (%)
Sand	0.46 ± 0.04	0.19 ± 0.01	43.6 ± 3.9	54.0 ± 4.4	0.57 ± 0.04	0.26 ± 0.03	56.1 ± 6.9	70.3 ± 4.5	0.23 ± 0.02	0.29 ± 0.01	30.6 ± 1.9	47.1 ± 2.4	0.18 ± 0.01	0.12 ± 0.01	12.0 ± 0.9	18.6 ± 1.4
Kaolinite	0.43 ± 0.02	0.21 ± 0.03	50.0 ± 2.3	58.4 ± 3.2	0.49 ± 0.02	0.23 ± 0.02	62.8 ± 3.6	75.3 ± 7.9	0.25 ± 0.01	0.28 ± 0.03	27.3 ± 0.6	43.1 ± 1.5	0.13 ± 0.01	0.11 ± 0.01	14.2 ± 0.9	20.8 ± 1.0
Illite	0.49 ± 0.02	0.17 ± 0.01	33.7 ± 0.6	43.0 ± 0.5	0.47 ± 0.10	0.27 ± 0.02	52.9 ± 2.5	67.6 ± 2.8	0.20 ± 0.01	0.26 ± 0.02	25.1 ± 1.0	40.1 ± 1.8	0.18 ± 0.02	0.10 ± 0.01	10.5 ± 0.5	16.1 ± 0.9
Montmorillonite	0.45 ± 0.02	0.12 ± 0.02	27.8 ± 1.2	34.3 ± 2.2	0.60 ± 0.03	0.22 ± 0.02	47.5 ± 2.2	59.3 ± 2.7	0.24 ± 0.01	0.08 ± 0.09	25.6 ± 0.4	30.3 ± 5.0	0.24 ± 0.02	0.05 ± 0.01	4.5 ± 0.1	7.0 ± 0.3
Humic acids	0.23 ± 0.02	0.14 ± 0.02	46.0 ± 4.4	54.0 ± 3.5	0.19 ± 0.04	0.21 ± 0.02	37.5 ± 3.5	48.5 ± 4.5	0.15 ± 0.01	0.16 ± 0.01	19.1 ± 1.0	28.6 ± 1.1	0.10 ± 0.01	0.04 ± 0.01	6.8 ± 0.8	8.9 ± 1.3
Control	0.58 ± 0.03	0.18 ± 0.02	49.1 ± 1.1	58.8 ± 2.3	0.61 ± 0.09	0.21 ± 0.01	44.5 ± 3.5	55.8 ± 3.0	0.25 ± 0.01	0.24 ± 0.02	28.4 ± 0.6	42.0 ± 0.9	0.18 ± 0.02	0.10 ± 0.05	18.0 ± 7.7	20.1 ± 1.8
Mean	0.44 ± 0.12	0.17 ± 0.03	41.7 ± 9.0	50.4 ± 9.7	0.49 ± 0.16	0.23 ± 0.03	50.2 ± 9.0	62.8 ± 10.0	0.23 ± 0.02	0.24 ± 0.08	26.0 ± 3.9	35.5 ± 7.4	0.17 ± 0.05	0.09 ± 0.03	11.0 ± 4.9	15.2 ± 5.9

[a] Estimates shown are the mean values ± 1 standard deviation (n = 4). k_1 represents the initial rate of mineralization (day^{-1}), and k_0 represents the detachment-dependent mineralization rate, or the rate of mineralization of the chemicals as they become available from the environmental matrix (percent day^{-1}). P_0 represents the amount (percent) of $^{14}CO_2$ produced when the mineralization changes from pseudo-first-order to linear kinetics. The total yield represents the total percentage of $^{14}CO_2$ recovered after 60 days. The averages of the paraemeters for each surfactant are shown in the column averages.

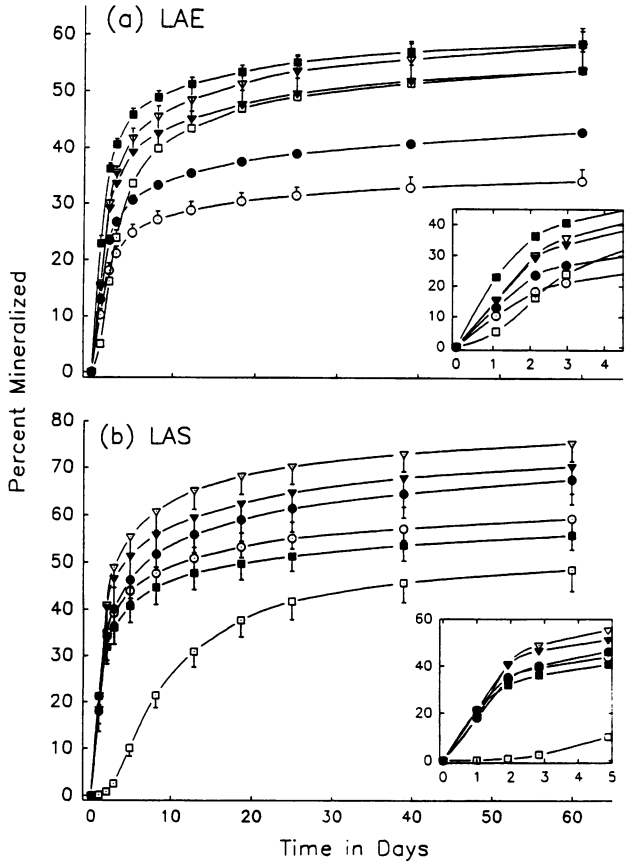

FIG. 4. Mineralization of bound or free LAE (a) and LAS (b) in the Bonnell soil, shown as the cumulative production of $^{14}CO_2$ over time. Each line represents four replicates. Error bars indicate 1 standard deviation. The initial 4 to 5 days of mineralization are shown in the insets. The soil constituents to which the surfactants were bound are shown in Fig. 3a. Symbols: ⊙, montmorillonite; ●, illite; ▽, kaolinite; ▼, sand; ⊡, humics; ■, control.

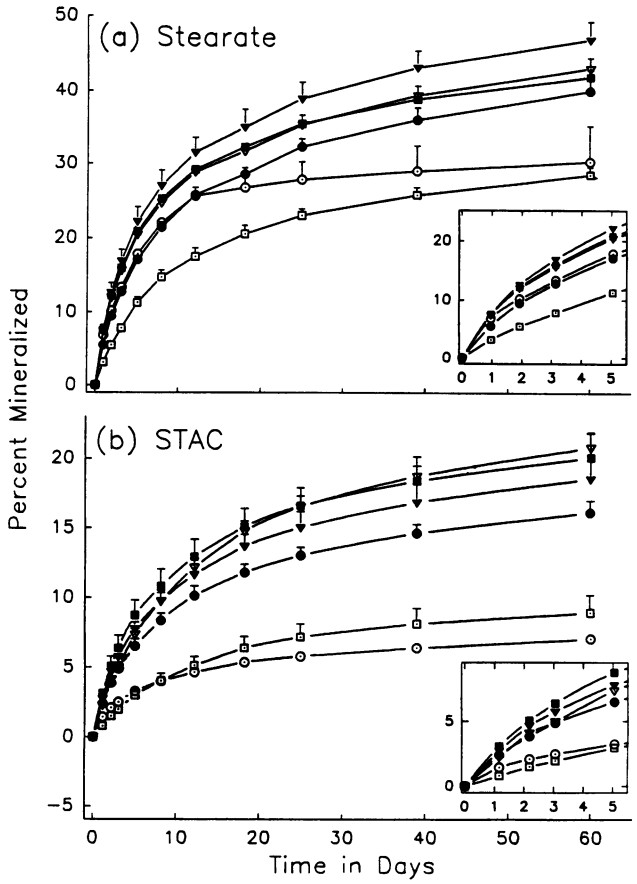

FIG. 5. Mineralization of bound or free stearate (a) and STAC (b) in the Bonnell soil, shown as the cumulative production of $^{14}CO_2$ over time. See the legend to Fig. 4 for explanation of symbols.

The environmental form of the surfactants had variable but significant effects on the rate and extent of mineralization of the radiolabeled chemicals. In some instances, different chemicals also exhibited distinct mineralization kinetics when associated with the same soil constituent. Figure 4 shows evolution of $^{14}CO_2$ from the various chemical-soil constituent complexes as a function of time. For LAE, the total yield of $^{14}CO_2$ during the experiment was similar (≈55%) when LAE was associated with kaolinite, sand, or humic acid or added to the soil in aqueous solution (Fig. 4a; Table 1). The yield was significantly lower when LAE was bound to illite (43%) or montmorillonite (34%). During the initial 4 days, LAE added as an aqueous solution was mineralized more rapidly (k_1 = 0.58 day^{-1}) than was LAE added in the bound form (Fig. 4a inset). The k_1 for LAE bound to montmorillonite, kaolinite, illite, or sand was approximately 0.45 day^{-1}, while that for LAE bound to humic acid was 0.23 day^{-1}. Notably, mineralization of the LAE-humic acid complex exhibited a sigmoidal mineralization pattern. The k_0 ranged from 0.12% day^{-1} for montmorillonite-bound LAE up to 0.21% day^{-1} for kaolinite-bound LAE.

The microbial mineralization of the various environmental forms of LAS was different from that observed for the analogous forms of LAE (Fig. 4b). The total yield of $^{14}CO_2$ for LAS after 60 days was greatest when LAS was associated with kaolinite (75%), sand (70%), or illite (68%). The yield was lower when LAS was added to the soil as an aqueous solution (56%) or when bound to montmorillonite (59%) or humic acids (48%). Notably, mineralization of the LAS-humic acid complex was preceded by a 3-day lag. The initial rate of LAS mineralization (k_1) was similar for most of the environmental forms ($k_1 \approx 0.55$ day^{-1}), except for the LAS-humic acid complex, which was mineralized much more slowly than the other forms ($k_1 \approx 0.19$ day^{-1}) (Fig. 4b inset; Table 1). The k_0 of all forms of LAS was relatively constant and averaged 0.23% day^{-1}.

Stearate mineralization in many ways mimicked that of LAS; however, the total yield of $^{14}CO_2$ from LAS was on average 1.8-fold higher than that for stearate (Fig. 5a; Table 1). The yield of $^{14}CO_2$ was highest when stearate was associated with kaolinite (43%), sand (47%), or illite (43%) or added as an aqueous solution (42%). The yield was lower when stearate was bound to montmorillonite (39%) or humic acid (29%). In all cases, mineralization of stearate was not preceded by a lag and did not exhibit a sigmoidal pattern. With the exception of the humic acid complex, the initial rates of mineralization of all forms of stearate were similar (k_1 = 0.20 to 0.25 day^{-1}). The k_1 of the humic acid complex was 0.15 day^{-1}. These initial rates were lower than those observed for LAE or LAS. The k_0 was again relatively constant across all treatments (0.24 to 0.29 day^{-1}), except for the montmorillonite (0.08 day^{-1}) and humic acid (0.16 day^{-1}) complexes.

TABLE 2. Desorption coefficients (K_d) for the surfactant-soil constituent complexes[a]

Soil constituent	K_d for:			
	LAE	LAS	Stearate	STAC
Sand	98	41	479	794
Kaolinite	162	155	1,413	2,399
Illite	141	66	676	1,258
Montmorillonite	98	22	33	275
Humic acid	135	1,698	2,884	2,239
Mean	123	110	832	1,071

[a] K_d is calculated as: $(G_s/B)/C_s$, where G_s is the total quantity of material desorbed (micrograms), B is the dry weight of soil (or soil constituent), and C_s is the concentration of chemical in solution at equilibrium (micrograms per milliliter).

The yield of $^{14}CO_2$ from STAC was the lowest for all the materials tested, regardless of environmental form (Fig. 5b). Like stearate, yields were greatest and comparable when STAC was added to the soil as an aqueous solution (20%) or as a kaolinite (21%), sand (19%), or illite (19%) complex (Table 1). They were substantially lower for humic acid (9%) or montmorillonite (7%) complexes, which also had the lowest initial mineralization rates ($k_1 \approx 0.12$ day^{-1}) compared with the other forms (≈ 0.20 day^{-1}). The k_0 values for STAC mineralization were also the lowest observed with any of the chemicals. Montmorillonite and humic complexes had k_0 values of approximately 0.05% day^{-1}, while those of the other forms averaged 0.11% day^{-1}.

Table 2 shows the desorption coefficients (K_d) for the various chemical-soil constituent complexes. The K_ds for LAE were similar for all soil constituents (98 to 162 liters/kg). In contrast, those for LAS were highly variable, ranging from 22 liters/kg for montmorillonite to 1,698 liters/kg for humic acid complexes. On average, the desorption coefficients for the stearate and STAC complexes were considerably greater than those of other two surfactants. The K_ds for the humic acid-bound surfactants were usually the largest for any of the soil constituent-surfactant complexes, and the K_ds for montmorillonite- and sand-bound surfactants were usually the lowest. The low values for montmorillonite were unexpected, given its large surface area. However, because of the use of alcohol rather than water during the adsorption process, this 2:1 clay was not fully expanded and many interstitial sites were not available.

Figure 6 shows the relationships between the desorption coefficient (log K_d) and the various mineralization parameters. For all of the surfactant-soil constituent complexes, log K_d was negatively correlated with k_1 ($r = -0.862$; $P \leq 0.01$), P_0 ($r = -0.663$; $P \leq 0.01$), and the total recovery of $^{14}CO_2$ ($r = -0.620$; $P \leq 0.01$) but was not significantly related to k_0 ($r = -0.182$; $P \geq 0.05$). For LAS and STAC, k_1 was inversely related to the desorption coefficient of the various soil constituent complexes. No such relationships, however, existed for the LAE and stearate complexes (Fig. 6a). More importantly, the average k_1 and log K_d for each compound was inversely correlated. Thus, the differences among chemicals as well as within the STAC and LAS treatments accounted for the observed correlation between k_1 and log K_d. In contrast, the negative correlation between P_0 and log K_d appears to be largely dependent on the differences between the surfactants and not to be due to the soil constituents to which they were bound.

The microbial characteristics of the soil (activity and biomass) were not correlated with any of the mineralization kinetic parameters or total yields of $^{14}CO_2$ ($P \geq 0.05$ in all cases).

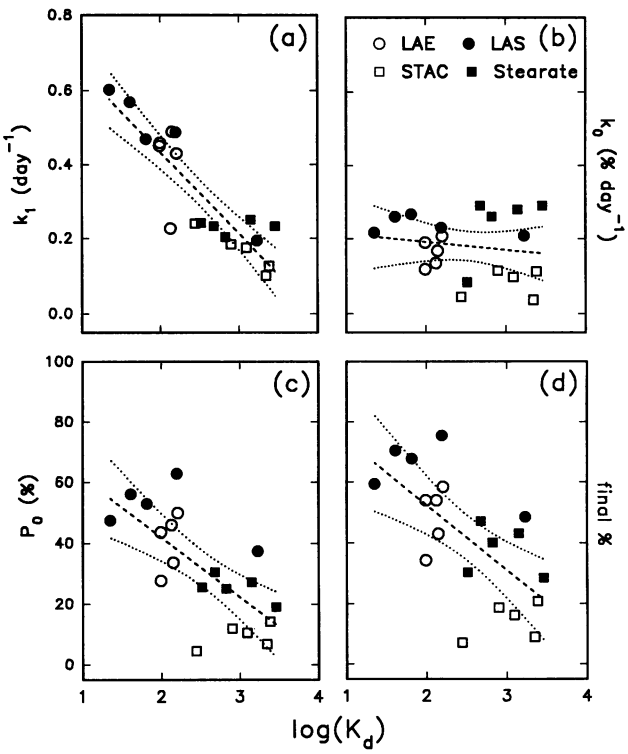

FIG. 6. Relationship between the mineralization kinetic parameters (k_1, k_0, P_0, and final yields of $^{14}CO_2$) and log K_d. (a) Log K_d versus the initial mineralization rate, k_1 (day^{-1}); (b) log K_d versus detachment-dependent rate, k_0 (% day^{-1}); (c) log K_d versus yield component, P_0 (%); (d) log K_d versus final yields of $^{14}CO_2$ (%). Confidence intervals (99% level) are shown. The different surfactants are identified in panel b.

DISCUSSION

These experiments demonstrated that under realistic soil conditions, the microbial mineralization of organic chemicals in soil is controlled in part by interactions between the chemicals and the soil matrix constituents. These interactions affected both the yields of $^{14}CO_2$ from radiolabeled chemicals introduced into soils and the kinetics of mineralization. While other studies have demonstrated that purified soil constituents can adsorb and potentially affect the subsequent degradation of chemicals, most of these studies have not been performed under typical soil solution conditions with intact soil communities. Commonly, the adsorption and desorption of chemicals is examined in dilute solutions of the soil constituent, which do not represent the typical soil solutions or soil water activity (a_w) (6, 12, 31, 38, 39, 41). The unique value of this study is that mineralization of chemicals in known environmental forms was measured under realistic a_w conditions, at which the biological, chemical, and physical characteristics of a natural soil were largely preserved.

Mineralization in this study exhibited multiphasic kinetics, consistent with multiple controlling factors. Typically, the mineralization data were better described by the 3/2-order model than the first-order model. The improved fits resulted

from the inclusion of the k_0 term in the 3/2-order model, which describes a zero-order process that follows the initial first-order phase of mineralization (7). Brunner and Focht (7) found that k_0 was constant for a variety of substrates in any one soil and suggested that it describes the indigenous rate of carbon release in a soil. Others have concluded that biphasic biodegradation kinetics are related to desorption, since models that have a desorption term typically provide better fits to soil biodegradation data (17, 21, 30, 42). Furthermore, biphasic desorption kinetics have been frequently observed for organic chemicals in soil and are characterized by a relatively quick initial release of the chemical from the soil or soil constituent, followed by a slower desorption phase (4, 18, 30, 34, 38). In the present study, it is likely that k_0 was indicative primarily of slow desorption of the ^{14}C substrates from the soil matrices. This conclusion is based on observations that the k_0 values varied as a function of the surfactant and the soil constituent to which it was bound. The k_0 was greatest when the surfactants had been preadsorbed to sand or kaolinite and lowest when they had been bound to humics or montmorillonite.

Association of the surfactants with soil minerals (sand and clays) led to variable effects on the mineralization kinetic parameters. Sand and kaolinite complexes exhibited some of the highest rates (k_1 and k_0) and extents (P_0 and total yields of $^{14}CO_2$) of mineralization, while illite and montmorillonite complexes generally exhibited lower P_0 and total yields of $^{14}CO_2$ but similar initial mineralization rates (k_1). P_0 values for the illite complexes were 9 to 34% lower than those for the corresponding kaolinite complexes, with LAE and STAC exhibiting the greatest treatment effect (Table 1). Montmorillonite had an even more dramatic effect on P_0, with 6 to 68% lower values for the montmorillonite complexes than for the corresponding kaolinite complexes. Notably, k_1 for all clay complexes were similar to each other and to those for sand complexes. In contrast, k_0 values for the montmorillonite complexes of LAE, stearate, and STAC were approximately half of what was observed for the corresponding illite, kaolinite, and sand complexes.

These observations are consistent with different degrees of bioavailability related to different types of association between the chemicals and the soil minerals. One fraction of the chemical appeared to be readily bioavailable and rapidly mineralized, and the rate at which this fraction was mineralized was described by the initial mineralization rate (k_1). Significantly, k_1 and the desorption coefficients (K_d) were significantly negatively correlated with each other. The relative size of this readily mineralized fraction was reflected by the magnitude of P_0, which also was inversely correlated with K_d. A second fraction was more slowly mineralized, and its mineralization appeared to be equilibrium dependent. This equilibrium dependence was reflected in the similarity of the k_0 values of the kaolinite, illite, and sand complexes to those of the treatments involving aqueous solutions. A third fraction, exclusive to montmorillonite, was much more slowly mineralized and not entirely equilibrium dependent. The predominance of this form was reflected in the much lower k_0 rates for the montmorillonite complexes of LAE, stearate, and STAC.

Attributing the observed mineralization kinetics to the desorption behavior of the chemicals is consistent with the different nature of the sand and clays involved. Sand has exclusively external binding sites. Kaolonite and illite are nonswelling 1:1 clays, whose binding surfaces are primarily external. Illite has 3 to 5 times the ion-exchange capacity and 5 to 10 times the surface area of kaolinite (5). In contrast, montmorillonite is an expandable 2:1 clay, with 2 to 7 times the ion-exchange capacity and 3 to 8 times the surface area of illite, but approximately 80% of the binding surfaces are interstitial (5). Differences in P_0 among the clay complexes probably reflect the relative abundance of binding sites, whereas the lower k_0 values exhibited by montmorillonite complexes of LAE, stearate, and STAC were probably due to much slower desorption and decreased bioavailability of chemicals which migrated into interstitial surfaces following their hydration in the soil. Notably, the k_0 of the LAS-montmorillonite complex was similar to that of all other LAS complexes. This unique behavior for LAS is probably associated with its size-related exclusion from the interstitial spaces of montmorillonite. While the linear surfactants (LAE, stearate, and STAC) have small enough diameters (<10 Å [<1 nm]) to migrate into the interlayers (10 to 20 Å [1 to 2 nm]), the benzene ring (diameter, 35 Å [3.5 nm]) makes this migration nearly impossible for LAS. A similar observation (exclusion of a chemical from within the interlayer spaces of montmorillonite) was made by Ogram et al. for 2,4-dichlorophenoxyacetic acid (33).

These results expand on and are generally consistent with experiments that demonstrate the ability of clays to inhibit biodegradation of organic compounds. They are consistent with the observations that benzylamine degradation by a *Pseudomonas* sp. was slower when montmorillonite (1 g liter^{-1}) was present (31) and that nucleic acids bound to montmorillonite were generally oxidized to lesser extents than those bound to kaolinite or illite (16). They were not, however, consistent with the observations of Scow and Alexander (41) that the degradation of phenol, glutamate, and *p*-nitrophenol was slower when they were incubated in aqueous solutions containing kaolinite. This incongruity demonstrates the different results that can be observed when testing biodegradation processes at realistic soil a_w in contrast to testing them in dilute solutions.

The mineralization of the surfactants was consistently inhibited when they had been preadsorbed to humics. Whereas most of interactions between the chemicals and clays (or sand) were probably ionic or involved hydrogen bonding, those with the humics were probably hydrophobic and in some instances may have involved covalent bonding (8, 27). The initial mineralization rates of all chemical-humic acid complexes were much lower than those of the other complexes. In addition, the onset of humic acid-LAS complex mineralization was preceded by a 3-day lag period, and mineralization of LAE exhibited a sigmoidal pattern. Furthermore, the desorption coefficients for humic complexes of LAS, stearate, and STAC were much greater than those of the other complexes. These complexes exhibited much lower P_0 values than the other complexes, which suggests a smaller pool of readily bioavailable chemical in the humic complexes. These results agree with other reports that showed that nonbiological binding events or enzymatic incorporation between chemicals and soil organic matter would lead to much longer persistence in the environment (8, 15, 26, 27).

The log K_d values of the compounds and soil constituents were negatively correlated with the kinetic parameters k_1, P_0, and total measured yield (Fig. 6). These correlations suggest that the greater the affinity of a chemical for the environmental matrix, the less it will be available to the indigenous degrading populations. These relationships, however, should be viewed with caution, because a short-term equilibration constant (determined over 24 to 48 h) is being compared with a longer-term (60 day) process. Furthermore, in the short-term K_d determinations, the surfactants partition between a large amount of solution and a small amount of soil constituent solids. In the biodegradation experiments, the surfactants partition into a much smaller volume of soil solution and are

available not only to the microorganisms but also to other soil processes. Since these K_d measurements did not take into consideration the full complexity of the interactions of a chemical with the various components of soil, it should not be taken for granted that sorptive chemicals with high measured K_d will not degrade as extensively or rapidly as less sorptive chemicals.

Laboratory studies that examine the biodegradation of a chemical in soil usually are conducted by adding the chemical to a soil as an aqueous solution or dispersion or by adding insoluble chemicals to the soil in an organic solvent. The results of this study indicate that the soil constituents with which the chemical becomes associated can have a profound effect on the observed results. As a consequence, to most accurately assess the biodegradation of a chemical in a soil, it is important that the chemical be added in the same manner that it normally enters the soil environment. Thus, chemicals that enter soil in association with sewage sludge, such as surfactants, are best studied preassociated with sludge. Similarly, the fate of other chemicals that enter soils (e.g., pesticides) should be studied in the same form in which they are customarily applied.

In summary, a chemical in a soil environment can be in solution, sorbed weakly or tightly to inorganic and organic soil constituents, or covalently incorporated into soil organic matter, such as humics. The bioavailability of the chemical for biodegradation will depend on which of these environmental forms is predominant. How the chemical is distributed among these forms is determined by its physical and chemical characteristics, its mode of entry, and the composition and mineralogy of the soil. The ultimate fate of a chemical in soil is the result of several competing processes: biodegradation, irreversible sorption, humification, and diffusion into interstitial spaces not accessible to microorganisms. The outcome of this competition is dependent on the kinetics of these various processes and the points from which they begin. These starting points are defined by the mode of entry of the chemical into the soil matrix and its form at the time of this entry.

ACKNOWLEDGMENTS

We thank Carl Johnston, Jack Pretty, and Joseph Caruso for assistance with the inductively coupled plasma emission spectroscopy analysis; B. Grosser, C. Johnston, and D. Moll for helpful discussions; and C. Bollinger for editorial assistance.

This work was supported by a grant from the Environmental Science Department of the Procter and Gamble Company.

REFERENCES

1. **Al Bashir, B., T. Cseh, R. Leduc, and R. Samson.** 1990. Effect of soil/contaminant interactions on the biodegradation of naphthalene in flooded soils under denitrifying conditions. Appl. Microbiol. Biotechnol. **34**:414–419.

1a. **American Society for Testing and Materials.** 1987. American Society for Testing and Materials method E1195-87, standard test method for determining a soption constant (K_{oc}) for an organic chemical in soil and sediments. American Society for Testing and Materials, Philadelphia.

2. **Bascomb, C. L.** 1964. Rapid method for the determination of cation exchange capacity of calcareous and non-calcareous soils. J. Sci. Food Agric. **15**:821–823.

3. **Bellin, C. A., G. A. O'Connor, and Y. Jin.** 1990. Sorption and degradation of pentachlorophenol in sludge amended soils. J. Environ. Qual. **19**:603–608.

4. **Boesten, J. J. T. I., and L. J. T. van der Pas.** 1988. Modeling adsorption/desorption kinetics of pesticides in a soil suspension. Soil Sci. **146**:221–231.

5. **Bohn, H. L., B. L. McNeal, and G. A. O'Connor.** 1979. Soil chemistry. John Wiley & Sons, Inc., New York.

6. **Bosetto, M., P. Arfaioli, and P. Fusi.** 1993. Interactions of alachlor with homoionic montmorillonites. Soil Sci. **155**:105–113.

7. **Brunner, W., and D. D. Focht.** 1984. Deterministic three half-order kinetic model for degradation of added carbon substrates in soil. Appl. Environ. Microbiol. **47**:167–172.

8. **Dec, J., and J.-M. Bollag.** 1988. Microbial release and degradation of catechol and chlorophenols bound to synthetic humic acid. Soil Sci. Soc. Am. J. **52**:1366–1371.

9. **Einot, I., and K. R. Gabriel.** 1975. A study of the powers of several methods of multiple comparisons. J. Am. Stat. Assoc. **70**:574–583.

10. **Ellis, W. D., J. R. Payne, and G. D. McNabb.** 1985. Treatment of contaminated soils with aqueous surfactants. United States EPA project summary. EPA/600/S2-85/129. U.S. Environmental Protection Agency, Washington, D.C.

11. **Findlay, R. H., G. M. King, and L. Watling.** 1989. Efficacy of phospholipid analysis in determining microbial biomass in sediments. Appl. Environ. Microbiol. **55**:2888–2893.

12. **Gu, B., and H. E. Doner.** 1992. The interaction of polysaccharides with silver hill illite. Clays Clay Miner. **40**:151–156.

13. **Guerin, W. F., and S. A. Boyd.** 1992. Differential bioavailability of soil-sorbed naphthalene to two bacterial species. Appl. Environ. Microbiol. **58**:1142–1152.

14. **Heilman, M. D., D. L. Carter, and C. L. Gonzalez.** 1965. The ethylene glycol monoethyl ether (EGME) technique for determining soil surface area. Soil Sci. **100**:409–413.

15. **Hsu, T.-S., and R. Bartha.** 1976. Hydrolyzable and nonhydrolyzable 3,4-dichloroaniline-humus complexes and their respective rates of biodegradation. J. Agric. Food Chem. **24**:118–122.

16. **Ivarson, K. C., M. Schnitzer, and J. Cortez.** 1982. The biodegradability of nucleic acid bases adsorbed on inorganic an organic soil components. Plant Soil **64**:343–353.

17. **Jacobsen, C. S., and J. C. Pedersen.** 1992. Mineralization of 2,4-dichlorophenoxyacetic acid (2,4-D) in soil inoculated with *Pseudomonas cepacia* DBO1 (pRO101), *Alcaligenes eutrophus* AEO106 (pRO101) and *Alcaligenes eutrophus* JMP134 (pJP4): effects of inoculation level and substrate concentration. Biodegradation **2**: 253–263.

18. **Karickhoff, S. W.** 1980. Sorption kinetics of hydrophobic pollutants in natural sediments, p. 193–205. *In* R. H. Baker (ed.), Contaminants and sediments: analysis, chemistry, biology, vol. 2. Ann Arbor Science Publishing, Ann Arbor, Mich.

19. **Knaebel, D. B., T. W. Federle, and J. R. Vestal.** 1990. Mineralization of linear alkylbenzene sulfonate (LAS) and linear alcohol ethoxylate (LAE) in 11 contrasting soils. Environ. Toxicol. Chem. **9**:981–988.

20. **Knaebel, D. B., and J. R. Vestal.** 1988. Comparison of double-vial to serum bottle radiorespirometry to measure microbial mineralization in soils. J. Microbiol. Methods **7**:309–317.

21. **Knaebel, D. B., and J. R. Vestal.** 1992. Effects of intact rhizosphere microbial communities on the mineralization of surfactants in surface soils. Can. J. Microbiol. **38**:643–653.

22. **Laha, S., and R. G. Luthy.** 1991. Inhibition of phenanthrene mineralization by nonionic surfactants in soil-water systems. Environ. Sci. Technol. **25**:1920–1930.

23. **Larson, R. J.** 1984. Kinetic and ecological approaches for predicting biodegradation rates of xenobiotic organic chemicals in natural ecosystems, p. 677–686. *In* M. J. Klug and C. A. Reddy (ed.), Current perspectives in microbial ecology. American Society for Microbiology, Washington, D.C.

24. **Lerch, N. K., W. F. Hale, and D. D. Lemaster.** 1982. Soil survey of Hamilton County, Ohio. U.S. Department of Agriculture, Soil Conservation Service, Washington, D.C.

25. **Martin, J. P., and K. Haider.** 1986. Influence of mineral colloids on turnover rates of soil organic carbon, p. 283–304. *In* P. M. Huang and M. Schnitzer (ed.), Interactions of soil minerals with natural organics and microbes. Soil Science Society of America, Inc., Madison, Wis.

26. **Martin, J. P., K. Haider, and L. F. Linhares.** 1979. Decomposition and stabilization of ring-^{14}C-labeled catechol in soil. Soil Sci. Soc. Am. J. **43**:100–104.

27. **Martin, J. P., A. A. Parsa, and K. Haider.** 1978. Influence of intimate association with humic polymers on biodegradation of [^{14}C]labeled organic substrates in soil. Soil Biol. Biochem. **10**:483–486.

28. **McDermott, J. B., R. Unterman, M. J. Brennan, R. E. Brooks, D. P. Mobley, C. C. Schwartz, and D. K. Dietrich.** 1989. Two strategies for PCB soil remediation: biodegradation and surfactant extraction. Environ. Prog. **8**:46–51.
29. **McKinley, V. L., T. W. Federle, and J. R. Vestal.** 1982. Effects of petroleum hydrocarbons on plant litter microbiota in an arctic lake. Appl. Environ. Microbiol. **43**:129–135.
30. **Mihelcic, J. R., and R. G. Luthy.** 1991. Sorption and microbial degradation of naphthalene in soil-water suspensions under denitrifying conditions. Environ. Sci. Technol. **25**:169–177.
31. **Miller, M. E., and M. Alexander.** 1991. Kinetics of bacterial degradation of benzylamine in a montmorillonite suspension. Environ. Sci. Technol. **25**:240–245.
32. **O'Connor, G. A., J. R. Lujan, and Y. Jin.** 1990. Adsorption, degradation and plant availability of 2,4-dichlorophenol in sludge-amended calcareous soils. J. Environ. Qual. **19**:587–593.
33. **Ogram, A. V., R. E. Jessup, L. T. Ou, and P. S. C. Rao.** 1985. Effects of sorption on biological biodegradation rates of (2,4-dichlorophenoxy)acetic acid in soils. Appl. Environ. Microbiol. **49**: 582–587.
34. **Pavlostathis, S. G., and K. Jaglal.** 1991. Desorptive behavior of trichloroethylene in contaminated soil. Environ. Sci. Technol. **25**: 274–279.
35. **Richtler, H. J., and J. Knaut.** 1988. World prospects for surfactants, p. 3–58. *In* Proceedings of the 2nd World Surfactant Congress. European Committee for Surfactants and their Organic Intermediates (CESIO), Brussels.
36. **Rijnaarts, H. H. M., A. Bachmann, J. C. Jumelet, and A. J. B. Zehnder.** 1990. Effect of desorption and intraparticle mass transfer on the aerobic biomineralization of α-hexachlorocyclohexane in a contaminated calcareous soil. Environ. Sci. Technol. **24**:1349–1354.
37. **Robinson, J. A.** 1985. Determining microbial kinetic parameters using nonlinear regression analysis: advantages and limitations in microbial ecology. Adv. Microb. Ecol. **8**:61–114.
38. **Robinson, K. G., W. S. Farmer, and J. T. Novak.** 1990. Availability of sorbed toluene in soils for biodegradation of acclimated bacteria. Water Res. **24**:345–350.
39. **Rowlands, W. N., and R. J. Hunter.** 1992. Electroacoustic study of adsorption of cetylpyridiunium chloride on kaolinite. Clays Clay Miner. **40**:287–291.
40. **SAS Institute, Inc.** 1985. SAS user's guide: statistics, version 5 edition. SAS Institute, Inc., Cary, N.C.
41. **Scow, K. M., and M. Alexander.** 1992. Effect of diffusion on the kinetics of biodegradation: experimental results with synthetic aggregates. Soil Sci. Soc. Am. J. **56**:128–134.
42. **Scow, K. M., and J. Hutson.** 1992. Effect of diffusion and sorption on the kinetics of biodegradation: theoretical considerations. Soil Sci. Soc. Am. J. **56**:119–127.
43. **Sokal, R. R., and F. J. Rohlf.** 1981. Biometry, 2nd ed. W. H. Freeman & Co., New York.
44. **Sorensen, L. H.** 1972. Stabilization of newly formed amino acid metabolites in soil by clay minerals. Soil Sci. **114**:5–11.
45. **Sorensen, L. H.** 1975. The influence of clay on the rate of decay of amino acid metabolites synthesized in soil during the decomposition of cellulose. Soil Biol. Biochem. **7**:171–177.
46. **Valoras, N., J. Letely, J. P. Martin, and J. Osborn.** 1976. Degradation of a nonionic surfactant in soils and peat. Soil Sci. Soc. Am. J. **40**:60–63.
47. **Ward, T. E., and R. J. Larson.** 1989. Biodegradation kinetics of linear alkylbenzene sulfonate in sludge-amended agricultural soils. Ecotoxicol. Environ. Saf. **17**:119–130.
48. **Weber, J. B., and H. D. Coble.** 1968. Microbial decomposition of diquat adsorbed on montmorillonite and kaolinite clays. J. Agric. Food Chem. **16**:475–478.
49. **White, D. C., R. J. Bobbie, J. S. Herron, J. D. King, and S. J. Morrison.** 1979. Biochemical measurements of microbial mass and activity from environmental samples, p. 69–81. *In* J. W. Costerton and R. R. Colwell (ed.), Native aquatic bacteria: enumeration, activity and ecology. ASTM STP 695. American Society for Testing and Materials, Philadelphia.