APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1986, p. 226–232
0099-2240/86/020226-07$02.00/0

Vol. 51, No.2

Anomalies in Mineralization of Low Concentrations of Organic Compounds in Lake Water and Sewage

DALLAS G. HOOVER,† GIORGIO E. BORGONOVI,‡ STEPHEN H. JONES, AND MARTIN ALEXANDER*

Laboratory of Soil Microbiology, Department of Agronomy, Cornell University, Ithaca, New York 14853

Received 5 July 1985/Accepted 28 October 1985

The rates of mineralization of nitrilotriacetic acid (NTA), 2,4-dichlorophenoxyacetic acid (2,4-D), *p*-nitrophenol, aniline, and isopropyl *N*-phenylcarbamate (IPC) at one or more concentrations ranging from 100 pg/ml to 1.0 μg/ml were proportional to chemical concentrations in samples of three lakes. The rates at 100 pg of NTA, 2,4-D, *p*-nitrophenol, and aniline per ml in samples of one or more lakes were less than predicted, assuming the rates were linearly related to the concentration. Neither NTA nor 2,4-dichlorophenol at 2.0 ng/ml was mineralized in some lake waters, but higher levels of the two chemicals were converted to CO_2 in samples of the same waters. In samples from two lakes, little or no mineralization of IPC or 2,4-D occurred at 1.0 μg/ml, but 10 ng/ml or lower levels of the herbicides were mineralized. The mineralization in sewage of 1.0 μg of NTA per ml was biphasic; about 20% of the substrate was mineralized in 20 h, and mineralization was only reinitiated after a period of 130 h. The biphasic transformation was not a result of the accumulation of organic products, and it was still evident if protozoan activity was inhibited. NTA also underwent a biphasic mineralization in lake waters, and the biphasic pattern was not altered by additions of growth factors and inorganic nutrients. From 40 to 60% of the carbon of aniline added to lake water at levels of 100 pg/ml to 1.0 μg/ml was mineralized, but more than 90% of the carbon of NTA, 2,4-D, or *p*-nitrophenol added to lake water at 10 ng/ml or 1.0 μg/ml was mineralized. The microbial communities of lake water acclimated to degrade IPC and *p*-nitrophenol even below the minimal concentration at which bacteria can use single carbon sources for growth. IPC at 400 pg/ml and 1.0 ng/ml and 2,4-D at 100 pg/ml, 10 ng/ml, and 1.0 μg/ml were not mineralized in waters from all lakes. We suggest that conclusions reached from tests of biodegradation at high concentrations of chemicals may not apply to the low levels found in many natural environments.

Many environments contain low concentrations of toxic chemicals. Soils that are treated with pesticides at rates up to a few kilograms per acre initially contain parts-per-million levels of the compound, but the concentration declines with time and distance from the site of application; moreover, breakdown products typically appear at levels much below that of the parent molecule. Fresh waters and marine waters receiving organic compounds from land runoff, inadequately treated wastes, or industrial effluents often have minute levels of these compounds; however, waters at a point remote from the point of original chemical introduction will only have trace amounts. Similarly, the levels of organic pollutants found in many groundwaters are extremely low. Moreover, the guidelines and standards for the quality of drinking water refer to acceptable levels that are frequently below 100 ng/ml.

Many of the organic pollutants present at low concentrations in soils, sediments, surface waters, and groundwaters are biodegradable at high levels, but few studies have been conducted of the microbial transformation of these chemicals at the concentrations characteristic of many natural ecosystems. The studies of low-level pollutants that have been carried out frequently give rise to findings that are unexpected based on investigations of higher levels of test substrates. For example, almost no mineralization of 2,4-dichlorophenoxyacetate (2,4-D) and 1-naphthyl-*N*-methylcarbamate occurs at concentrations below 1.0 ng/ml, although both pesticides are mineralized at higher concentrations (1). Likewise, marine bacteria fail to grow on low concentrations of substrates that support the proliferation of bacteria at higher levels (6). Similarly, trace amounts of phthalate esters are not destroyed when passed through soils, even though the esters are usually readily biodegradable (5). Conversely, 2,4-D is mineralized at 10 ng/ml but not at 200 ng/ml, although the herbicide is not known to be toxic to heterotrophs at these concentrations (10). The products of biodegradation may be different at low and high concentrations, and the biodegradation may shift from mineralization to cometabolism; thus, 400 pg of isopropyl *N*-phenylcarbamate (IPC) per ml is mineralized, but the chemical at 1.0 μg/ml is cometabolized in fresh waters (16). The kinetics of the process may change also, as illustrated in the shift from logarithmic to Monod to logistic kinetics in the degradation of 10, 1.0, and 0.10 μg of benzoate per ml of acclimated sewage (13).

During the course of our studies of the biodegradation of low concentrations of synthetic compounds in fresh water and sewage, we have observed patterns of mineralization that were frequently unexpected based on investigations of higher concentrations of the same or related chemicals. This paper presents these presumably anomalous findings and suggests that tests of biodegradation of organic compounds at concentrations not typical of natural ecosystems may lead to erroneous extrapolations about the transformations that occur in natural habitats.

MATERIALS AND METHODS

Water samples were collected from the top 5 cm of Beebe Lake (Ithaca, N.Y.), Cayuga Lake (Lansing, N.Y.), and White Lake (Old Forge, N.Y.). The pH values varied with the

* Corresponding author.
† Present address: Department of Food Science, University of Delaware, Newark, DE 19716.
‡ Permanent address: Istituto Guido Donegani, 28100 Novara, Italy.

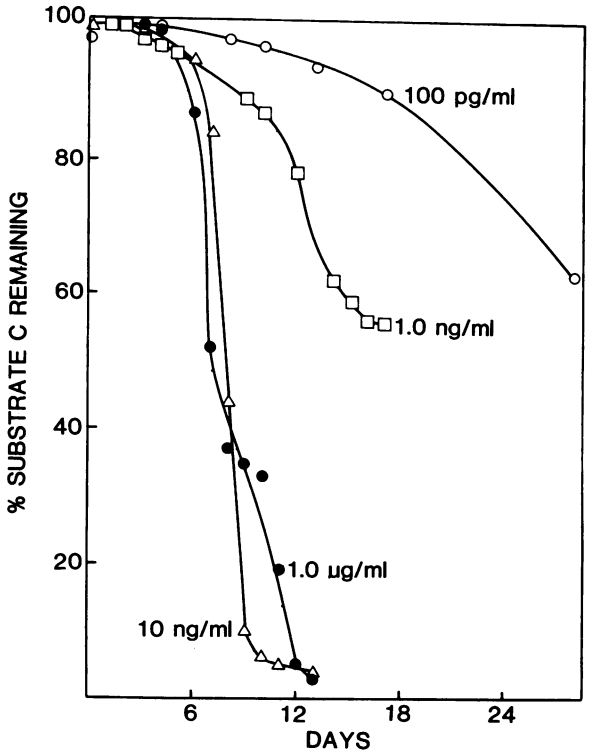

FIG. 1. Mineralization of *p*-nitrophenol added at four concentrations to Beebe Lake water.

sampling dates and ranged among the lakes from 7.5 to 8.2, 7.1 to 8.1, and 6.8 to 7.1, respectively. The samples from the first two lakes were processed within 2 h after collection, and water from White Lake was stored at 10°C for 16 h, after which the experiments were initiated.

Labeled organic compounds were added at various concentrations to the lake waters. Most of the chemicals at the higher concentrations were added in the unlabeled form, and only the labeled chemical was added at the lower concentrations. The labeled compound was added to give 250 to 400 dpm/ml or per vial. The volume of lake water added per flask varied from 100 to 400 ml, depending on the concentration and specific activity of the chemical. The flasks were covered with aluminum foil and incubated in the dark at 29°C without shaking.

At intervals, 1.5- to 10.5-ml samples were taken from each flask, and the liquid was acidified with concentrated H_2SO_4 to pH 2 and processed for measuring the radioactivity remaining in the liquid. The $^{14}CO_2$ generated by the microbial mineralization of the test compounds was removed from the solutions by bubbling air through the acidified samples for 5 to 15 min. A 1.0- to 10-ml portion of the sample was added to 6 to 10 ml of Liquiscint (National Diagnostics, Somerville, N.J.) contained in 20-ml polyethylene scintillation vials. The radioactivity of the samples was measured with an LS7500 scintillation system (Beckman Instruments, Fullerton, Calif.).

Mineralization is expressed as a percentage of the added radioactivity that remained in the solution, and the values are the averages of analyses from three flasks. The ^{14}C remained in solution when each of the test chemicals, except for 2,4-dichlorophenol, was incubated in waters containing 500 µg of $HgCl_2$ per ml under the test conditions; this indicated that volatilization, nonbiological mineralization, or sorption to the walls of the flasks had not occurred. Corrections were made for the loss of 2,4-dichlorophenol that took place in the presence of $HgCl_2$.

For studies of nitrilotriacetic acid (NTA) mineralization, freshly collected primary sewage from the sewage treatment plant of Ithaca, N.Y., was passed through coarse filter paper to eliminate the larger particulate matter; the sewage was used within 4 h after collection. Unlabeled NTA and radiolabeled NTA equivalent to 10^3 dpm/ml were added to 500 ml of sewage. The sewage was incubated at 23 ± 2°C with continuous bubbling of humidified air. At regular intervals, a 5.0-ml sample was withdrawn and passed through a 0.22-µm-pore membrane filter (Millipore Corp., Bedford, Mass.). The filters were boiled, washed, and stored in a solution of unlabeled NTA to avoid ^{14}C adsorption; after such treatment, less than 0.01% of the [^{14}C]NTA was retained by the filter. The sample was adjusted to pH 1.0 with H_2SO_4, and 8.0 ml of Liquiscint scintillation cocktail was added to 2 ml of acidified filtrate.

In tests in which products of NTA metabolism were sought by autoradiography, the labeled compound was added to give 10^4 dpm/ml; after filtration, 20-ml samples were evaporated under vacuum. The residue was dissolved in 2 ml of methanol by ultrasonic agitation. The recovery of the labeled material was about 95%. The methanol solution (100 µl) was spotted on a cellulose thin-layer chromatography plate (Eastman Kodak Co., Rochester, N.Y.) and developed by the method of Firestone and Tiedje (4). After evaporation of the solvents, the plates were placed in contact with X-ray-sensitive film (Eastman Kodak Co.). After 10 days in the dark, the films were developed according to the directions of the manufacturer.

[U-^{14}C]aniline hydrogen sulfate (98.8 mCi/mmol), ^{14}C-labeled 2,4-D (21.7 mCi/mmol), and [^{14}C]NTA (53 mCi/mmol) were from Amersham Corp., Arlington Heights, Ill.; isopropyl *N*-phenyl[U-^{14}C]carbamate (4.07 mCi/mmol) was obtained from New England Nuclear Corp., Boston, Mass. The 2,4-D was labeled in the methylene carbon; the NTA was labeled in the carboxyl carbon. *p*-[U-^{14}C]nitrophenol (24.1 mCi/mmol) was from California Bionuclear Corp., Sun Valley, Calif.

RESULTS

Rate different than expected. At substrate concentrations below K_s (the half-saturation constant for growth), the rate of mineralization is often directly proportional to concentration (10). In the present study, for example, the rate of mineralization was directly related to concentration in these instances: 10 ng and 1.0 µg of NTA per ml of Cayuga Lake water; 10 ng and 1.0 µg each of 2,4-D, *p*-nitrophenol, and aniline per ml of Beebe Lake water; 10 ng and 1.0 µg of aniline per ml of White Lake water; 400 pg and 10 ng of IPC per ml each of White Lake and Beebe Lake water; and 100 pg, 10 ng, and 1.0 µg of aniline per ml of Cayuga Lake water.

In contrast, the rate of mineralization of the following chemicals was less than would be predicted from tests of higher concentrations if it is assumed that the rates are directly proportional to the concentrations of the substrate: 1.0 ng of *p*-nitrophenol per ml of Beebe Lake water and 100-pg/ml concentrations each of 2,4-D in Beebe Lake water, NTA in Beebe Lake water, aniline in White Lake water, and *p*-nitrophenol in Beebe Lake, Cayuga Lake, and White Lake waters. This is illustrated for *p*-nitrophenol added to Beebe Lake water in Fig. 1. Thus, when plotted as the percentage of substrate C remaining, the curves for 10 ng and 1.0 µg of *p*-nitrophenol per ml of Beebe Lake water are

essentially superimposable, indicating that the rate is directly proportional to concentration. On the other hand, the plots for the percentages of substrate C left in lake water receiving 1.0 ng/ml and 100 pg/ml are displaced to the right and have lower slopes, showing that the actual rate is less than anticipated on the assumption that the rate is a linear function of initial *p*-nitrophenol level.

Extensive mineralization at only some concentrations. In some instances, mineralization was appreciable at the high but not at the low chemical concentrations. This was true, for example, of NTA added to water from Cayuga Lake. Thus, after approximately 1 week, extensive mineralization occurred if NTA was initially present at 10 ng/ml or 1.0 μg/ml (Fig. 2). At 100 pg of NTA per ml, however, some mineralization occurred within the first 2 weeks, but even after 61 days, more than 80% of the added carbon remained.

Mineralization sometimes occurred in water receiving moderate but not low chemical concentrations. For example, mineralization of NTA was evident in Beebe Lake water receiving 1.0 μg of NTA per ml, but radioactivity was not lost from the solution in 48 days if the NTA was added at 2.0 ng/ml. Similarly, after a 12-day acclimation period, 200 ng of 2,4-dichlorophenol per ml was mineralized in White Lake water, and the process was largely completed in 16 days. However, in lake water amended with 2.0 ng of 2,4-dichlorophenol per ml, no mineralization occurred in 47 days.

Evidence also exists for the lack of mineralization or unexpectedly slow rates at moderate concentrations. IPC at initial concentrations of 400 pg/ml and 10 ng/ml was mineralized in samples from White Lake (Fig. 3). However, even after 60 days, little or no loss of labeled carbon was evident

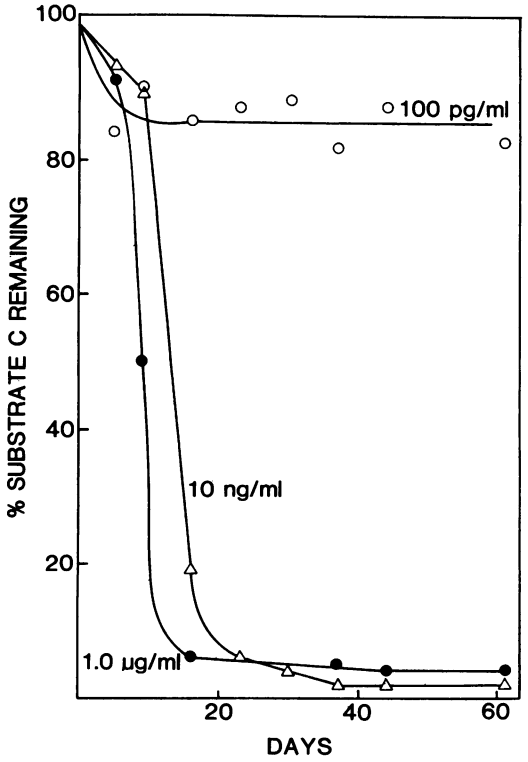

FIG. 2. Mineralization of NTA added at three concentrations to samples from Cayuga Lake.

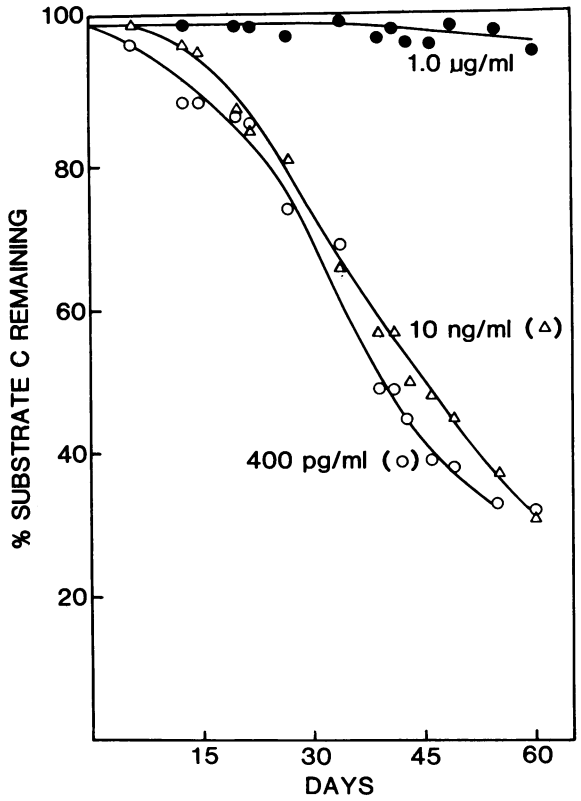

FIG. 3. Mineralization of three concentrations of IPC in water from White Lake.

if IPC was added at 1.0 μg/ml. The same observations were made for IPC added to water from Beebe Lake. Similarly, 47 and 16% of the C from 2,4-D added to Beebe Lake water were mineralized in 28 days when the amounts added were 100 pg/ml and 10 ng/ml, respectively, but only 5% was converted to CO_2 when added at 1.0 μg/ml.

Biphasic mineralization. The mineralization of NTA added to sewage at concentrations of 10 ng/ml or 1.0 μg/ml was biphasic (Fig. 4A). At the higher NTA level, about 20% of the C was converted in the first 20 h to volatile products, and then no mineralization was detected in the next 130 h. Thereafter, mineralization was reinitiated so that 96% of the C was mineralized in 8 days. At 10 ng/ml, mineralization never stopped, but two distinct phases of the degradation were still quite evident. The tests of the two concentrations were done with different samples of sewage collected at different times, so that the dissimilar patterns may be a function of the sewage sample and not the NTA level.

The biphasic pattern may result from protozoa reducing the size of the population initially active in destroying NTA, and the period during which mineralization was not detectable may reflect the time necessary for the survivors to multiply such that they could effect detectable mineralization. To test this hypothesis, sewage was amended with 2.0 μg of NTA per ml and 250 μg of cycloheximide per ml. Additional increments of the eucaryotic inhibitor (250 μg/ml) were added after 7 and 14 days. Microscopic examination of the cycloheximide-amended sewage samples failed to reveal the presence of viable protozoa. Under these circumstances, the biphasic mineralization still occurred (Fig. 4B). However, phase 2 of mineralization was slower in the antibiotic-supplemented samples. Autoradiography after thin-layer

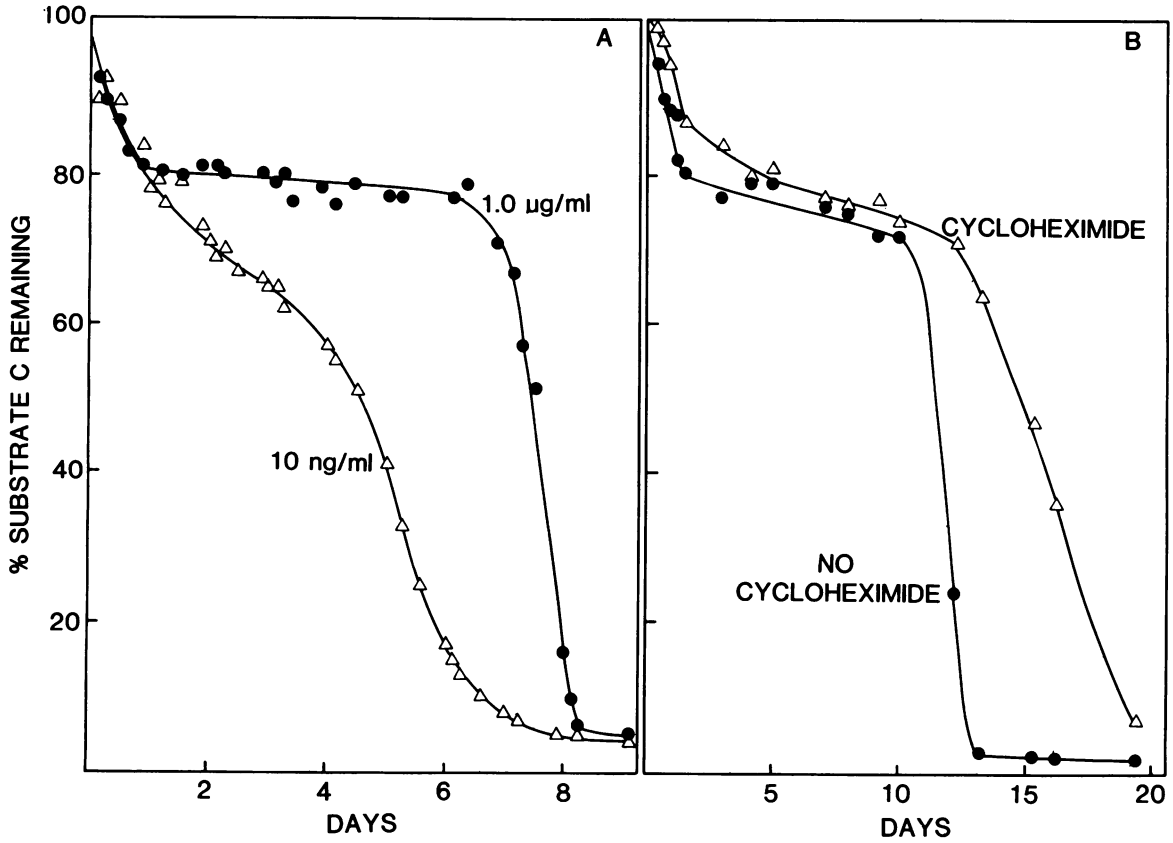

FIG. 4. Mineralization of NTA in sewage amended with (A) 10 ng or 1.0 μg of NTA per ml or with (B) 2.0 μg of NTA per ml with or without cycloheximide.

chromatography of the antibiotic-free samples revealed no organic products from NTA, suggesting that the accumulation of products does not explain the biphasic pattern of mineralization.

About 15% of the NTA C added to White Lake water at 1.0 μg/ml was mineralized in 1 day, but mineralization then ceased, only to begin again on day 7. The mineralization of 1.0 μg of NTA per ml was also biphasic in Beebe Lake water. In the first 2 to 3 days, about 15% of the C was mineralized, but then no mineralization was evident in the next 6 to 12 days. Thereafter, mineralization proceeded rapidly so that 90 to 95% of the carbon was mineralized within the next 4 days or so. The biphasic pattern of mineralization was not altered by the addition of 34 or 168 μg of yeast nitrogen base (Difco Laboratories, Detroit, Mich.) per ml, a mixture of 0.2 or 2.0 mM $CaCl_2$ and 0.8 or 8.0 mM $MgSO_4$, respectively, a mixture containing 0.1 mM $CaCl_2$ and 0.4 mM $MgSO_4$, 7.0 mM phosphate at pH 6.8, or a mixture of the phosphate with $CaCl_2$ and $MgSO_4$. These data suggest that the biphasic pattern is not related to the absence of growth factors. The pattern also was not altered if NTA was added 2 days after the incubation of Beebe Lake water began, when anomalies associated with placing the lake water in flasks may have occurred. Biphasic mineralization was unaltered if NTA was incubated for 2 days with 50 ml of filter-sterilized Beebe Lake water to allow for the possible formation of NTA-metal chelates before inoculating the flasks with 50 ml of nonsterile lake water. The pattern was likewise not affected if the lake water was passed through a 3.0-μm-pore polycarbonate filter (Nuclepore Corp., Pleasanton, Calif.) to remove most of the protozoa and particulate matter. Surprisingly, in Beebe Lake water that had been passed through the 3.0-μm-pore filter and had been amended with 250 μg of cycloheximide per ml, an initial phase of about 10% mineralization occurred in less than 5 days, but there was no further mineralization in a 28-day period.

At day 21, when more than 95% of the NTA that had been added to Beebe Lake water was mineralized, an addition of 1.0 μg of NTA per ml resulted in a mineralization that proceeded rapidly, without an acclimation period or biphasic pattern of destruction (Fig. 5). If, however, the additional increment of NTA was accompanied by 200 μg of chloramphenicol per ml and 100 μg of penicillin per ml, only about 20% of the carbon was mineralized in 7 days (Fig. 5).

Extent of mineralization. About 40 to 60% of the aniline C was mineralized when the chemical was added to waters from White Lake, Beebe Lake, and Cayuga Lake at levels of 100 pg/ml to 1.0 μg/ml. On the other hand, more than 90% of the C was mineralized when *p*-nitrophenol was added to Beebe Lake water (Fig. 1), when NTA was added to Cayuga Lake water (Fig. 2), or when 2,4-D was introduced into Beebe Lake water (data not shown) at 10 ng/ml or 1.0 μg/ml. In the last instance, the incubation period was 21 days. The extent of mineralization sometimes varied markedly with the substrate concentration, however. Thus, only about 15% of the C in NTA added at 100 pg/ml to Cayuga Lake water was mineralized, whereas the value was more than 90% when the NTA level was 10 ng/ml or 1.0 μg/ml (Fig. 2). In White Lake water, in contrast, 97% of the NTA was mineralized at 1.0 μg/ml in 15 days, but the values were 19 and 20% in samples incubated for 33 days with 100 pg/ml and 10 ng/ml, respec-

tively. This effect of concentration on the extent of mineralization was not restricted to NTA, moreover, because 59, 78, and 12% of the C of IPC added at levels of 400 pg/ml, 10 ng/ml, and 1.0 μg/ml to Beebe Lake water was converted to CO_2 in 49 days; 27, 27, and 81% of the C in carbaryl added at levels of 100 pg/ml, 10 ng/ml, and 1.0 μg/ml was mineralized in Beebe Lake water in 18 days. In each instance, the mineralization had essentially stopped by the last sampling date.

Acclimation. Concentrations of 1.0 μg/ml are generally not deemed to be toxic for heterotrophic microorganisms. The data for IPC reported above, however, suggest that such levels either are toxic or cause a shift from mineralization to reactions not resulting in CO_2 evolution. Similar concentrations may increase the acclimation period. In the mineralization of *p*-nitrophenol in White Lake water, for example, appreciable mineralization began after 6 days at levels of 1.0 and 10 ng/ml, but about 15 days elapsed before appreciable loss of carbon occurred at a concentration of 1.0 μg/ml (Fig. 6).

Based on considerations of diffusion kinetics and demands for maintenance energy, it has been proposed that bacteria will not grow on organic substrates at concentrations of about or somewhat below 1.0 ng/ml (12). This theoretical argument is supported by tests showing that *Salmonella typhimurium* will grow at glucose levels of 5.0 but not 1.0 ng/ml (11). In instances in which acclimation in natural environments reflects the time for a small population to grow large enough to effect a detectable chemical change, such acclimation should not occur at concentrations of an organic nutrient below the threshold for growth if that compound is the sole carbon source for the organisms. Nevertheless, after the 9-day period during which no mineralization of 400 pg of IPC per ml was detected in White Lake water and after the 12-day period during which no destruction of 100 pg of *p*-nitrophenol per ml was noted in Cayuga Lake water, active breakdown was initiated (Fig. 7). Moreover, second additions of the chemicals at the same concentrations were destroyed with no apparent lag phase. Similar acclimation was noted in waters receiving 10 ng of each chemical per ml.

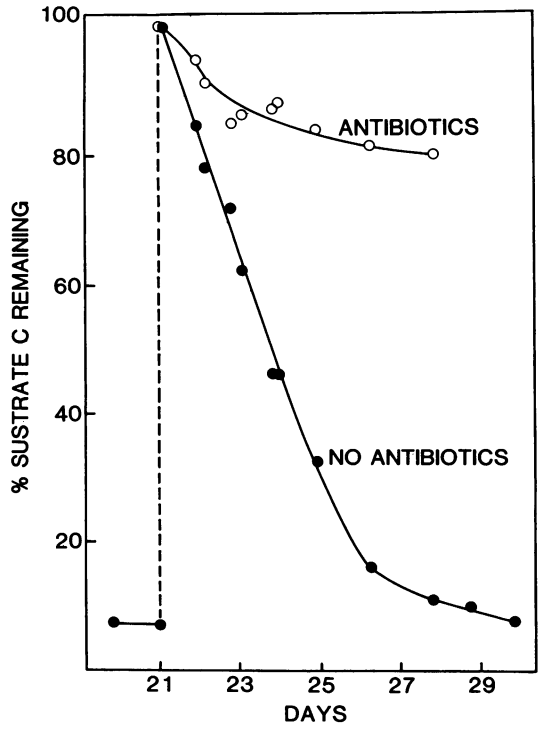

FIG. 5. Mineralization of 1.0 μg of NTA per ml in acclimated Beebe Lake water with or without penicillin and chloramphenicol.

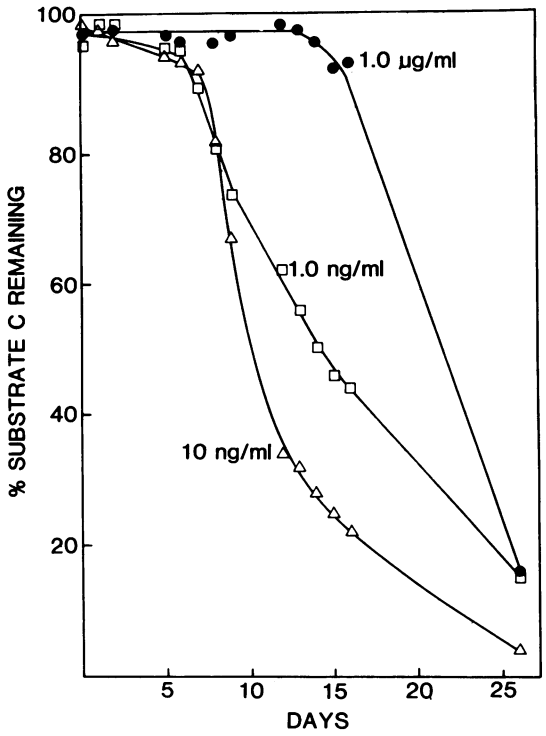

FIG. 6. Mineralization of three concentrations of *p*-nitrophenol in White Lake water.

In some instances, mineralization occurred in waters receiving the chemical initially but not upon another addition of the same compound. In White Lake water amended with 1.0 ng of *p*-nitrophenol per ml, for example, mineralization was evident at 5 days, yet addition of another 1.0 ng/ml was not followed by mineralization in an 11-day period after extensive breakdown of the first chemical amendment (Fig. 8). At these low substrate levels, toxicity of a product of decomposition would not be expected.

Mineralization in only some lake waters. IPC at 400 pg/ml and 10 ng/ml was mineralized in waters from White and Beebe lakes, and 2,4-D at 100 pg/ml, 10 ng/ml, and 1.0 μg/ml was mineralized in Beebe Lake water. Nevertheless, no degradation of 100 pg, 10 ng, or 1.0 μg of 2,4-D per ml on 31 May 1983 or of 400 pg, 10 ng, or 1.0 μg of IPC per ml on 9 June 1983 and 25 April 1984 was evident in Cayuga Lake water.

DISCUSSION

Seven presumed anomalies are evident in the present investigation of the biodegradation of low concentrations of organic compounds. (i) The rate of mineralization may be less than anticipated if it is assumed that the rates are linearly related to concentration. (ii) Chemicals mineralized at one concentration may not be converted to CO_2 at lower levels. (iii) Organic compounds may not be mineralized at low and presumably nontoxic levels in water, but they may be metabolized to CO_2 at still lower concentrations. (iv) Mineralization may not follow the commonly described

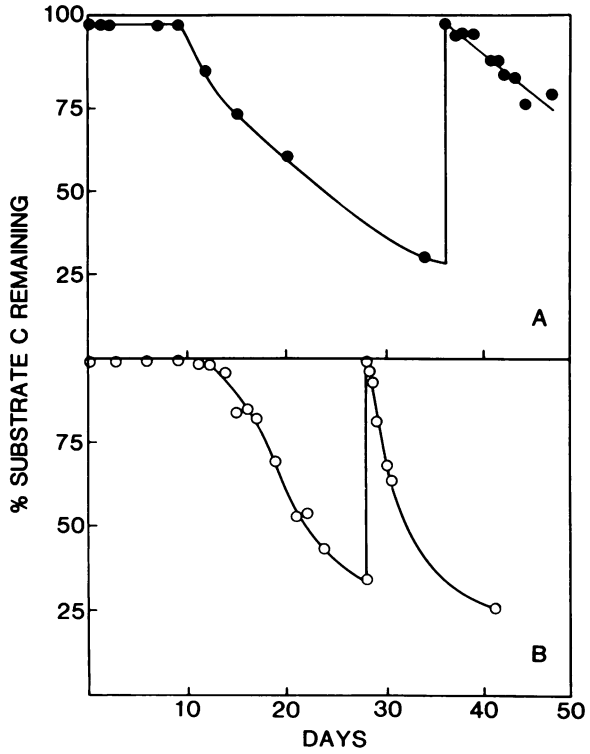

FIG. 7. Mineralization of (A) 400 pg of IPC per ml of White Lake water and of (B) 100 pg of *p*-nitrophenol per ml of Cayuga Lake water. An additional increment of chemical was added to each concentration at the times shown by the vertical curve segments.

kinetics (13) but may proceed in a biphasic manner. (v) The extent of mineralization in samples from a single body of water may vary markedly. (vi) Microbial communities may acclimate to mineralize a substrate even though the substrate concentration is below the threshold level to sustain growth. (vii) Compounds may be mineralized in some but not all waters. Given these putative anomalies, it seems likely that erroneous conclusions will be reached if knowledge from studies of chemicals at high concentrations is facilely extrapolated to environments in which the chemicals exist at low concentrations.

It is generally expected that the rate of biodegradation of substrates at low levels, e.g., below K_s, is directly related to the substrate concentration (8). Evidence exists for a linear relationship between rates of mineralization and concentration of organic substrates added to fresh waters (2, 10). However, the observations reported here (that low concentrations of organic compounds are mineralized at lower than predicted rates) are not unprecedented. Similarly, the failure to observe mineralization of 2.0 ng each of NTA and 2,4-dichlorophenol per ml of waters in which higher levels were mineralized is also not unprecedented. Thus, the rate of mineralization of 2,4-D and 1-naphthyl-*N*-methylcarbamate in stream water is less than predicted from a linear extrapolation from the rates at higher concentrations (1). Moreover, no growth of *Escherichia coli* occurred at 18 ng of glucose per ml (7) or of *S. typhimurium* at 1.0 ng of glucose per ml (11), although both bacterial species grow well at higher levels of the sugar.

Herbicides have not been described as being appreciably toxic to heterotrophic microorganisms at levels as low as 1.0 μg/ml. Nevertheless, neither IPC nor 2,4-D was mineralized at this concentration, yet both were destroyed at lower concentrations. The apparent suppression of the degradation of pesticides at levels of 1.0 μg/ml or lower has been noted heretofore (10, 16), and data have been presented that the IPC at such levels is cometabolized rather than mineralized (16).

The biphasic mineralization of NTA is surprising. A similar pattern has been reported for *m*-chlorobenzoate in sewage (3). For NTA, the biphasic conversion apparently did not result from the accumulation of organic products in phase 1 and their mineralization in phase 2, because none was found by chromatographic analysis. It also was not a result of an initial mineralization by bacteria before the increase of protozoa in sewage reduced bacterial activity, and a subsequent period of mineralization when grazing by the protozoa subsequently became less pronounced.

Several studies have dealt with the extent of mineralization in natural waters. For example, 98% of the aniline added to lake water at 5.7 pg/ml was mineralized (15), whereas 74 to 86% of the carbon from amino acids supplied at 10 to 50 nM was assimilated by marine bacteria (9). The present study shows that the extent of mineralization may vary appreciably even in samples from a single body of water.

Theoretical arguments have been advanced that bacteria will not multiply if the concentration of their carbon source is below about 1.0 ng/ml (12), and tests with bacteria in culture are in agreement with this view (6, 7, 11). Inasmuch as acclimation to synthetic compounds may often involve growth from a few cells to a population density large enough to cause a detectable loss in chemical, it is noteworthy that mineralization took place in this study after an apparent lag phase in lake water that was amended with only 100 pg of *p*-nitrophenol per ml, and that a subsequent increment of the test chemical was mineralized without an acclimation pe-

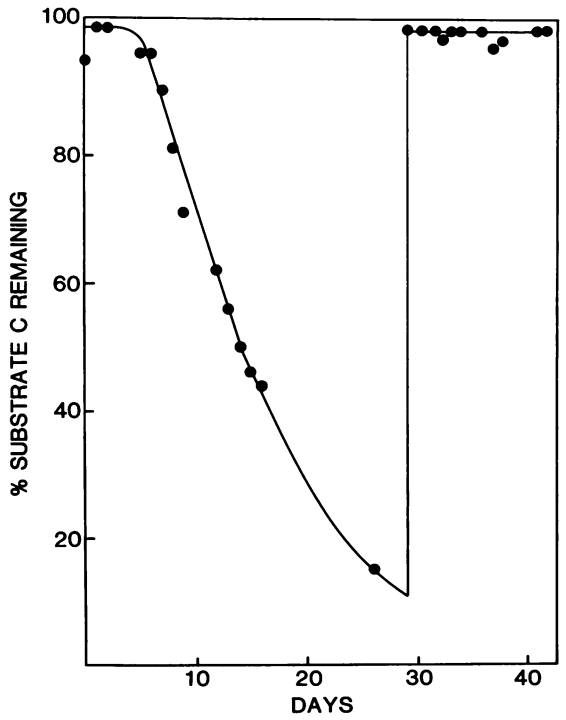

FIG. 8. Mineralization of two additions of 1.0 ng of *p*-nitrophenol per ml in samples from White Lake.

riod. These data suggest that growth had occurred, although the microorganisms could have grown at the expense of other organic materials in the water. In contrast, Spain and Van Veld (14) found that acclimation occurred in natural waters containing sediment only if the *p*-nitrophenol level was above 10 ng/ml. The latter threshold is in line with those proposed for growth.

ACKNOWLEDGMENTS

This research was supported by funds provided by the U.S. Army Research Office, U.S. Department of the Interior, and U.S. Department of Agriculture under agreement USDA-TPSU-CU-2057-261, and by Public Health Service Training Grant ES-07052 from the Division of Environmental Health Sciences, National Institutes of Health.

LITERATURE CITED

1. **Boethling, R. S., and M. Alexander.** 1979. Effect of concentration of organic chemicals on their biodegradation by natural microbial communities. Appl. Environ. Microbiol. **37**:1211–1216.
2. **Boethling, R. S., and M. Alexander.** 1979. Microbial degradation of organic compounds at trace levels. Environ. Sci. Technol. **13**:989–991.
3. **DiGeronimo, M. J., R. S. Boethling, and M. Alexander.** 1979. Effect of chemical structure and concentration on microbial degradation in aquatic ecosystems, p. 154–166. *In* A. W. Bourquin and P. H. Pritchard (ed.), Microbial degradation of pollutants in marine environments. U.S. Environmental Protection Agency, Gulf Breeze, Fla.
4. **Firestone, M. K., and J. M. Tiedje.** 1978. Pathway of degradation of nitrilotriacetate by a *Pseudomonas* species. Appl. Environ. Microbiol. **35**:955–961.
5. **Hutchins, S. R., M. B. Tomson, and C. H. Ward.** 1983. Trace organic contamination of ground water from a rapid infiltration site: a laboratory-field coordinated study. Environ. Toxicol. Chem. **2**:195–216.
6. **Jannasch, H. W.** 1967. Growth of marine bacteria at limiting concentrations of organic carbon in seawater. Limnol. Oceanogr. **12**:264–271.
7. **Koch, A. L., and C. H. Wang.** 1982. How close to the theoretical diffusion limit do bacterial uptake systems function? Arch. Microbiol. **131**:36–42.
8. **Larson, R. J.** 1980. Role of biodegradation kinetics in predicting environmental fate, p. 67–86. *In* A. W. Maki, K. L. Dickson, and J. Cairns, Jr. (ed.), Biotransformation and fate of chemicals in the aquatic environment. American Society for Microbiology, Washington, D.C.
9. **Palumbo, A. V., and R. L. Ferguson.** 1983. Efficient utilization of dissolved free amino acids by suspended marine bacteria. J. Exp. Mar. Biol. Ecol. **69**:257–266.
10. **Rubin, H. E., R. V. Subba-Rao, and M. Alexander.** 1982. Rates of mineralization of trace concentrations of aromatic compounds in lake water and sewage samples. Appl. Environ. Microbiol. **43**:1133–1138.
11. **Schmidt, S. K., and M. Alexander.** 1985. Effects of dissolved organic carbon and second substrates on the biodegradation of organic compounds at low concentrations. Appl. Environ. Microbiol. **49**:822–827.
12. **Schmidt, S. K., M. Alexander, and M. L. Shuler.** 1985. Predicting threshold concentrations of organic substrates for bacterial growth. J. Theor. Biol. **114**:1–8.
13. **Simkins, S., and M. Alexander.** 1984. Models for mineralization kinetics with the variables of substrate concentration and population density. Appl. Environ. Microbiol. **47**:1299–1306.
14. **Spain, J. C., and P. A. Van Veld.** 1983. Adaptation of natural microbial communities to degradation of xenobiotic compounds: effects of concentration, exposure time, inoculum, and chemical structure. Appl. Environ. Microbiol. **45**:428–435.
15. **Subba-Rao, R. V., H. E. Rubin, and M. Alexander.** 1982. Kinetics and extent of mineralization of organic chemicals at trace levels in freshwater and sewage. Appl. Environ. Microbiol. **43**:1139–1150.
16. **Wang, Y.-S., R. V. Subba-Rao, and M. Alexander.** 1984. Effect of substrate concentration and organic and inorganic compounds on the occurrence and rate of mineralization and cometabolism. Appl. Environ. Microbiol. **47**:1195–1200.