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Effect of Inorganic Nutrients on the Acclimation Period Preceding Mineralization of Organic Chemicals in Lake Water

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The addition of phosphate, nitrate, or sulfate (each at 10 mM) decreased the acclimation period for the mineralization of low concentrations of *p*-nitrophenol (PNP) in lake water. Added phosphate shortened the acclimation period for biodegradation of 2 ng to 2 μg of PNP per ml in various lake water samples and of 2,4-dichlorophenoxyacetate at 100 ng/ml. Added P enhanced the rate of growth of PNP-mineralizing microorganisms in waters containing 200 ng or 2 μg of PNP per ml. We suggest that the effect of P on the acclimation period results from an increase in the growth rate of the initially small population of microorganisms able to mineralize the synthetic chemicals.

The biodegradation of many synthetic organic chemicals does not appear to proceed immediately after the first introduction of the compounds into surface waters, groundwater, soils, or sediments, and loss of the chemical is evident only after an acclimation period. The acclimation period may reflect the time for a small population of mineralizing microorganisms to increase to a size that is adequate to cause detectable mineralization (10). According to this hypothesis, the length of the acclimation period depends on the rate of growth of the microorganisms that are responsible for degrading the chemical.

Several inorganic nutrients have been shown to limit the rates of biodegradation of organic chemicals (3, 8, 10), but little attention has been given to the influence of such nutrients on the destruction of the low levels of synthetic organic chemicals that contaminate many environments. Hence, a study was initiated to determine the effects of the availability of inorganic nutrients on the acclimation phase prior to the mineralization of low concentrations of organic compounds.

Lake water samples were collected at different times of the year from the top 10 cm of Beebe Lake or Cayuga Lake, Ithaca, N.Y., and used within 2 h of collection. Organic chemicals were added to 100 ml of the test solutions to give final concentrations of 2 to 2,000 ng, 10 ng, and 100 ng of *p*-nitrophenol (PNP), caprolactam, and 2,4-dichlorophenoxyacetate (2,4-D) per ml, respectively. ^{14}C-labeled compounds were added to lake water to give 500 to 1,500 dpm/ml. Caprolactam and PNP at the lower concentration were added only as labeled compounds. Unlabeled PNP and 2,4-D were added to solutions to give the correct final substrate concentration. In some instances in which 2 μg of PNP per ml was tested, only the unlabeled chemical was added to the solution. The solutions were placed in 250-ml Erlenmeyer flasks, which were incubated without shaking at 23°C in the dark. For P-amended samples of water, a solution of KH_2PO_4 and K_2HPO_4 was added at the pH of the unamended lake water, unless otherwise stated. Little or no change in pH was observed during the test periods.

At intervals, samples from each flask were acidified and bubbled with air to drive off radioactive CO_2, and the liquid was mixed with Liquiscint scintillation cocktail (National Diagnostics, Highland Park, N.J.). The amount of radioactivity remaining in the solution was determined with a liquid scintillation counter. Details of the method have been described (7). The disappearance of PNP from some lake water samples containing initial concentrations of 2 μg of unlabeled compound per ml was determined spectrophotometrically by measuring changes in A_{410}. No disappearance of ^{14}C from solution was detected when the test chemicals were incubated in water containing 500 μg of $HgCl_2$ per ml under the conditions of the various experiments.

The numbers of microorganisms that could use 2 μg of PNP per ml were estimated by the most-probable-number method previously described (10). Total counts of viable bacteria were determined in triplicate by plating the test samples on 10-fold diluted Trypticase soy broth amended with 1.5% agar. Colony counts were made after 7 days of incubation.

[^{14}C]PNP (30 mCi/mmol) was obtained from ICN Pharmaceuticals, Inc., Irvine, Calif., labeled 2,4-D (28 mCi/mmol) was obtained from Amersham Corp., Arlington Heights, Ill., and caprolactam was provided by Allied Corp., Syracuse, N.Y. PNP was labeled in carbons 2 and 6, caprolactam was labeled in the carbonyl carbon, and 2,4-D was labeled in the methylene carbon.

Previous studies showed that the acclimation period preceding the mineralization of PNP decreased in lake water amended with $CaCl_2$, $MgSO_4$, KH_2PO_4, and K_2HPO_4 (10). To determine which nutrient was limiting, $CaCl_2$ (68 μM), $MgSO_4$ (0.41 mM), and two phosphates (11 mM KH_2PO_4 and 17 mM K_2HPO_4) were added separately to water samples collected from Beebe Lake on 12 December 1984 and amended with 200 ng of PNP per ml. KCl at a concentration (90 mM) equal to the ionic strength of the combined inorganic salts was also added to separate flasks. The pH values of all samples were 8.0 ± 0.5. The acclimation period was approximately 6 days in unamended samples and in lake water amended with $MgSO_4$ or $CaCl_2$, 5 days with KCl, and 1 day if the P salts were added (Fig. 1A).

Inorganic nutrients were added to samples from Beebe Lake collected on 6 May 1985. The pH of all water samples was 8.2 ± 0.2. The acclimation period was shortened somewhat by the addition of 10 mM KCl, and it was decreased

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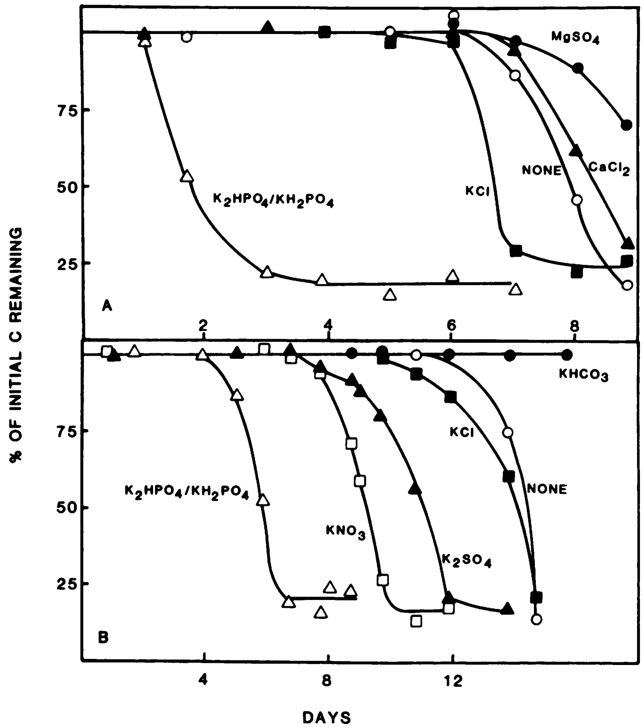

FIG. 1. Effect of addition of inorganic salts on mineralization of 200 ng of PNP per ml in lake water collected on 12 December 1984 (A) and 6 May 1985 (B).

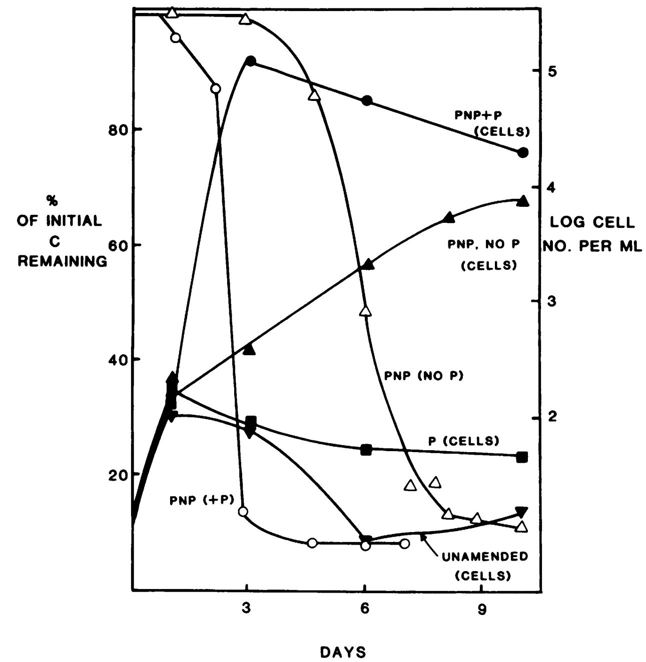

FIG. 2. Effect of 10 mM P on PNP mineralizers (closed symbols) and PNP mineralization (open symbols) in lake water receiving 200 ng of PNP per ml.

from 13 to 5 days by supplementation with 10 mM P as a K_2HPO_4-KH_2PO_4 mixture (Fig. 1B). The acclimation period was also shortened, from 13 to 8 days, in the presence of 10 mM KNO_3 or 10 mM K_2SO_4, but no mineralization was observed in 16 days in water receiving 10 mM $KHCO_3$. Adding N and P together as 10 mM $(NH_4)_2HPO_4$ gave no more of a decrease in the acclimation period than did 10 mM P alone, and the responses were the same upon the addition of P as Na_2HPO_4, K_2HPO_4, or $(NH_4)_2HPO_4$ to a final concentration of 10 mM (data not shown). Adding P, usually as K_2HPO_4 at 10 mM, to Beebe Lake water decreased the duration of the acclimation periods for PNP mineralization in 17 of 18 water samples collected in a period of 18 months, and mineralization was sometimes observed only in P-amended water.

The acclimation period for mineralization of 200 ng of PNP per ml in Cayuga Lake water collected on 22 May and 6 July 1985 decreased from 20 to 10 days and 11 to 9 days, respectively, following the addition of P to a final concentration of added P of 10 mM. The final pH in all samples was approximately 8.0. An acclimation period was not detected preceding the mineralization of 10 ng of caprolactam per ml of Beebe Lake water collected on 11 June 1985, although the rate of mineralization was faster in the presence of 10 mM P. The acclimation period for mineralization of 100 ng of 2,4-D per ml was 24 days with 10 mM P in Beebe Lake water collected on 20 November 1985, but no mineralization was detected in 29 days in lake water without added P. The addition of P (final concentration, 10 mM) to Beebe Lake water collected on 2 April 1985 shortened the acclimation periods for mineralization of PNP at concentrations of 2, 6, 20, and 60 ng/ml from greater than 20 days to 8, 7, 6, and 2 days, respectively, and it shortened the acclimation for 200 ng of PNP per ml from 10 to 2 days. No mineralization of PNP at concentrations of 2 to 60 ng/ml was observed in 20 days in samples not amended with P.

The effect of added P on the growth of microorganisms in lake water was also determined. The total counts of viable bacteria increased from an initial density of 3.2×10^5 per ml to 3.6×10^6 per ml after 2 days in Beebe Lake water collected on 7 May 1985 and amended with P (10 mM), but the counts did not increase in unamended lake water. The cell numbers decreased to 1.9×10^6 and 1.6×10^5 per ml in P-amended and unamended samples, respectively, after 6 days.

The influence of added P (10 mM) on the growth and activity of PNP-mineralizing microorganisms was also investigated. The initial density of PNP mineralizers was approximately 10 cells per ml, which is a small percentage of the total bacterial density (usually approximately 10^5 per ml). In the first day, these organisms multiplied in Beebe Lake water (collected on 17 March 1986) with or without additions of P or PNP (Fig. 2). After the first day, the growth rate of microorganisms able to use PNP was greater in the presence than in the absence of P. The addition of P (10 mM) also increased the rate of growth on PNP degraders in lake water amended with 2 μg of PNP per ml. Thus, added P appears to decrease the acclimation period by allowing more rapid growth of the organisms capable of mineralizing PNP.

Several explanations have been proposed for the acclimation period (2, 3, 6). Recent evidence indicates that the acclimation period in aquatic environments may reflect the time required for small populations of microorganisms capable of degrading some organic compounds to multiply to cell densities that are high enough to cause detectable mineralization (10). The same investigation showed that the acclimation period preceding PNP mineralization in lake water was markedly decreased when a mixture of several inorganic salts was added. The present study shows that the acclimation period for PNP mineralization was shortened by adding

P, N, or S. A stimulation of biodegradation of organic chemicals in lake water by added inorganic nutrients, including P, N, Fe, and Mg, has been reported previously (1a, 3–5, 9). It is noteworthy that the greatest effects noted in the present study were obtained at P concentrations much higher than those typically found in New York lakes, which usually range from 1 to 70 μg of P per liter (1).

P additions had a greater effect on the acclimation period than did additions of other inorganic nutrients. The concentration of available P thus may be an important factor in determining the persistence of polluting chemicals at low levels in fresh waters. The data show that the growth rate of PNP-mineralizing microorganisms was increased by added P, and it is thus plausible to suggest that the shortened acclimation period results from the shortened time for the initially small population to become sufficiently large to bring about detectable mineralization.

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