APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 1988, p. 212–217
0099-2240/88/010212-06$02.00/0

Vol. 54, No. 1

Influence of Inorganic and Organic Nutrients on Aerobic Biodegradation and on the Adaptation Response of Subsurface Microbial Communities

C. MICHAEL SWINDOLL, C. MARJORIE AELION, AND FREDERIC K. PFAENDER*

Department of Environmental Sciences and Engineering, School of Public Health, University of North Carolina, Chapel Hill, North Carolina 27599-7400

Received 13 July 1987/Accepted 20 October 1987

The influence of inorganic and organic amendments on the mineralization of ethylene dibromide, *p*-nitrophenol, phenol, and toluene was examined in subsurface soil samples from a pristine aquifer near Lula, Okla. The responses indicate that the metabolic abilities and nutrient requirements of groundwater microorganisms vary substantially within an aquifer. In some samples, additions of inorganic nutrients resulted in a more rapid adaptation to the test substrate and a higher rate of metabolism, indicating that metabolism may have been limited by these nutrients. In other samples from the same aquifer layer, inorganic amendments had little or no influence on mineralization. In general, the addition of multiple inorganic nutrients resulted in a greater enhancement of degradation than did the addition of single substances. Additions of alternate carbon sources, such as glucose or amino acids, inhibited the mineralization of the xenobiotic substrates. This inhibition appears to be the result of the preferential utilization of the more easily degradable carbon amendments.

A considerable subsurface microbial community is known to exist (6, 12, 14) which has been shown to metabolize a number of xenobiotic compounds (1, 13; D. C. Dobbins and F. K. Pfaender, Microb. Ecol., in press). The rates of metabolism in subsurface communities are generally much slower than in aquatic or soil ecosystems (1, 13, 15; Dobbins and Pfaender, in press). It has also been shown in both laboratory (1) and field (15) studies that subsurface communities can adapt to the presence of pollutants. Once the communities in highly contaminated sites adapt, biodegradation rates may be controlled by hydrogeological characteristics, such as the rate of oxygen replenishment, rather than by the inherent abilities of the microbial community. At sites such as these a major question is how long an acclimation period is required for adaptation? In other instances, such as hazardous waste sites, where releases are slower and concentrations are lower, both the adaptation time and the rates achievable may be important in defining the fate of the pollutants.

Knowledge of the nutritional requirements of subsurface bacteria would add to our limited understanding of the physiology of these organisms and potentially reveal ways to successfully manipulate this physiology to enhance biodegradation. It has been noted that considerable variation exists among the nutritional requirements of organisms from different aquifers (7). Although not clearly defined, the biodegradation of organic compounds by groundwater bacteria is likely to be influenced by environmental factors such as dissolved oxygen, availability of nutrients, pH, E_h, temperature, and the concentration of the pollutant.

This investigation was undertaken to examine the factors which influence biodegradation of organic contaminants by indigenous groundwater microflora. Enhanced degradation due to the addition of a nutrient would indicate that the availability of this nutrient was limiting metabolism of the substrate. This information is important for adequate assessment of the long-term fate of organic compounds in aquifers and possibly for formulating bioreclamation techniques. The target substrates chosen for this study were ethylene dibromide (EDB), toluene, phenol, and *p*-nitrophenol (PNP). These compounds represent a variety of organic compounds that occur in aquifers, have a range of structures, and are available in radiolabeled form.

* Corresponding author.

MATERIALS AND METHODS

Subsurface samples. The aquifer solids used in this study were collected aseptically from an uncontaminated aquifer near Lula, Okla., in November 1985. Samples were stored at 5°C until used for experiments. Descriptions of the site and the aseptic sampling procedures are given elsewhere (3, 14). Briefly, the depth to the water table was 3.6 m below the surface, and the depth to bedrock was 7 m. Aquifer solids used in this study had a uniform fine sand texture and were collected from the layer 4.5 to 5.6 m below the surface in the saturated zone of the aquifer. Aquifer solids core samples used in this investigation were assigned the numbers 9JJ3, 9JJ4, 9KK5, 9LL4, 9NN7, and 9MM1 according to the Environmental Protection Agency, Ada, Okla., sample identification scheme. Although samples were collected from different corings (JJ, KK, LL, MM, and NN designate different cores), all were within the same 4.5- to 5.6-m-deep layer. Since there were no data to indicate any differences in the samples from this layer, we began with the assumption that they were replicate samples. In this layer of the aquifer the uniform fine sand contained approximately 0.22 ml of pore water per g, based on weight loss after drying. The total organic carbon content of the aquifer solids from this layer was 0.02% (J. T. Wilson, personal communication). Inorganic nutrients in well water from the Lula aquifer have been reported as follows (in milligrams per liter): P_i 0.12; NH_4^+ N, <0.05; and NO_2^- plus NO_3^- N, 0.8 (16).

Compounds used. [U-^{14}C]phenol, [U-^{14}C]PNP, [U-^{14}C]EDB, and [U-^{14}C]glucose were purchased from Amersham Corp. (Arlington Heights, Ill.) and had specific activities of

100, 8.5, 114, and 270 mCi/mmol, respectively. [U-^{14}C]toluene was purchased from Pathfinder Laboratories Inc. (St. Louis, Mo.) and had a specific activity of 10.9 mCi/mmol. The ^{14}C-amino acid mixture (New England Nuclear Corp., Boston, Mass.) had a specific activity of 50 mCi/mmol. Barium [^{14}C]carbonate was purchased from New England Nuclear.

Inorganic nutrients used were NH_4NO_3, $NaNO_3$, NH_4Cl, KH_2PO_4, and K_2HPO_4. The mineral salts medium contained the following (in grams per liter): $NaNO_3$, 4.0; KH_2PO_4, 1.5; $NaHPO_4$, 0.5; $FeSO_4 \cdot 7H_2O$, 0.0011; $MgSO_4 \cdot 7H_2O$, 0.01; $CaCl_2 \cdot 2H_2O$, 0.01 (final pH 7.0). The vitamin mixture was obtained from KC Biological Inc. (Lenexa, Kans.) and contained the following (in milligrams per liter); D-calcium pantothenate, 100; choline chloride, 100; folic acid, 100; *i*-inositol, 200; nicotinamide, 100; pyridoxal hydrochloride, 100; riboflavin, 10; thiamine hydrochloride, 100. Organic nutrients were glucose and amino acids (Difco Laboratories, Detroit, Mich.). NaN_3 (Sigma Chemical Co., St. Louis, Mo.) was used as a biological inhibitor.

Mineralization measurements. Metabolism of the organic substrates was measured by a modification of a procedure described by Dobbins and Pfaender (in press). A sample of aquifer solids was mixed with sterilized, distilled water to form a slurry. Samples of the slurry containing approximately 1 g (dry weight) of aquifer solids were pipetted into 25-ml glass vials (Pierce Chemical Co., Rockford, Ill.). Radiolabeled substrate and nutrient amendments were then added. The target substrate was the only added source of carbon and energy, except in experiments with carbon nutrient amendments. The low carbon content of these samples (0.02%) suggests little natural organic content. The vials were filled with sterile, distilled water, leaving no headspace, and sealed with Teflon-lined septa and caps. The oxygen provided was that dissolved in the suspension water. Samples were inverted and incubated in the dark at 17°C, the ambient temperature of the Lula aquifer. Abiotic controls consisted of similarly treated samples amended with a final concentration of 0.5% sodium azide. After incubation, the samples were transferred by using connector caps (Wheaton Scientific Co., Millville, N.J.) to 40-ml vials. The samples were acidified, and the $^{14}CO_2$ produced from the mineralization of the substrate was collected in traps containing KOH. Respiration values were corrected for abiotic activity by means of NaN_3-treated samples. The $^{14}CO_2$ recovery efficiency was determined by means of $Ba^{14}CO_3$-treated samples processed simultaneously with the other samples. The percentage of the target substrate mineralized was calculated as the percentage of the initial substrate measured as $^{14}CO_2$ after correcting for abiotic activity (generally <1% of live activity) and recovery efficiency (50 to 80%). Each treatment consisted of three to five replicates for each time point.

Determination of $^{14}CO_2$ was done with a 1214 Rackbeta Excel liquid scintillation counter (LKB Instruments Inc., Gaithersburg, Md.). Counts were corrected for quench and background radiation. All analyses were performed with a microcomputer and the SYSTAT statistical package (L. Wilkinson, SYSTAT: the system for statistics; SYSTAT, Inc., Evanston, Ill.). Data were analyzed by nonlinear regression analysis. A significant difference was attributed to all tests producing a P of <0.05.

Dissolved oxygen (D.O.) was measured with an oxygen meter (Yellow Springs Instrument Co, Yellow Springs, Ohio), which was calibrated by using the azide modification of the Winkler technique (2). Samples remained aerobic during the incubation periods used with no D.O. value less than 1.0 mg per liter recorded at the end of the incubation period. The total number of bacteria in the soil samples was determined using an acridine orange direct count procedure (6).

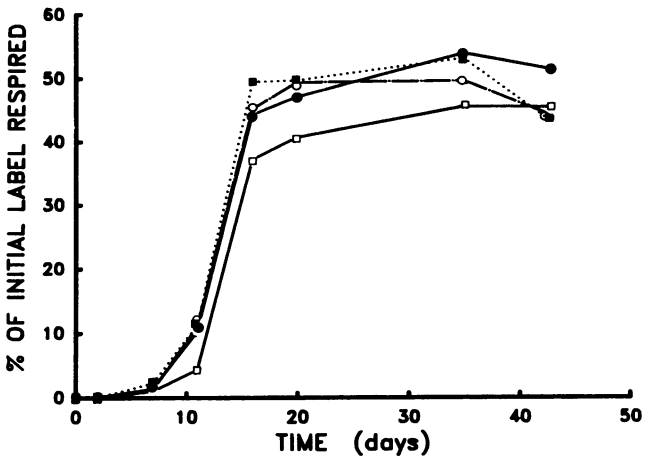

FIG. 1. Influence of nitrogen and phosphorus additions on the mineralization of PNP (113 ng/g) in Lula solids sample 9KK5. Symbols: ●, control; ■, nitrogen; ○, phosphorus; □, nitrogen and phosphorus.

RESULTS

Groundwater microorganisms from the Lula site were capable of mineralizing all four organic compounds examined. The rates of mineralization and the influence of nutrient amendments varied among the different compounds as well as among different samples of solids from the same horizon in the aquifer. Other studies have reported similar intrasite variability in the metabolic response of the microbial community collected in close proximity within the Lula aquifer (1, 15; Dobbins and Pfaender, in press). The mineralization of PNP is an example of this intrasite variability. In most samples, the bacteria displayed a distinct adaptive response to PNP, which was characterized by a period during which no detectable mineralization occurred followed by a rapid increase in mineralization. In experiments with sample 9KK5 (control), the length of the lag period was <10 days (Fig. 1), whereas sample 9JJ4 (control) required much longer periods before adapting, with lag periods of approximately 60 days (Fig. 2). Another solids sample (9NN7) (control) did not display an adaptive response after 130 days of exposure (Fig. 3).

In samples in which PNP adaptation occurred within 10 days (Fig. 1) (9KK5), approximately 50% of the initial substrate (113 ng/g) was converted to $^{14}CO_2$. The addition of N or P alone or together did not alter the length of the period before the adaptive response or the extent of mineralization (Fig. 1). In solid sample 9JJ4, which required 60 days of exposure before an adaptive response was discernible in controls, all nutrient amendments to PNP (106 ng/g)-treated samples significantly ($P < 0.05$) decreased the lag period before adaptation (Fig. 2). Nutrient amendments were N (as NH_4NO_3, 25.9 ng/g), P (as KH_2PO_4, 5.9 ng/g; as K_2HPO_4, 7.5 ng/g), vitamins (0.1 ml), amino acids (2.1 μg/g), mineral salts (0.5 ml), and glucose (26.6 ng/g). The addition of a combination of nutrients (N, P, vitamins, amino acids, and mineral salts) resulted in a significantly shorter lag period than all other treatments and a significantly greater amount of the substrate being mineralized during the 40-day exposure.

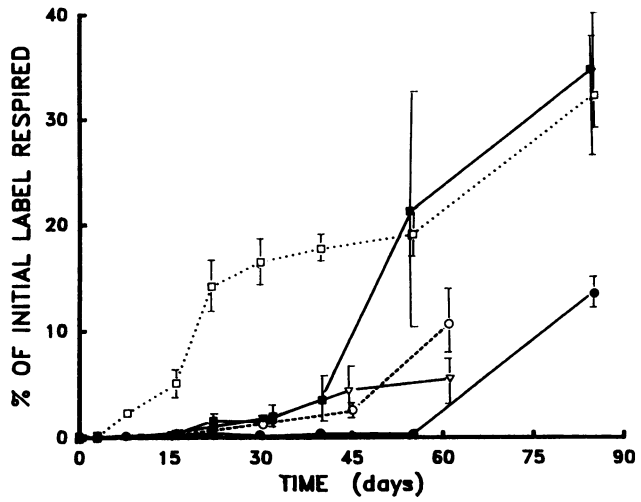

FIG. 2. Influence of nutrient additions on the mineralization of PNP (106 ng/g) in Lula aquifer solids sample 9JJ4. Symbols: ●, control; ■, vitamins; ▽, mineral salts; ○, glucose; □, vitamins, nitrogen, phosphorus, mineral salts, and amino acids combined.

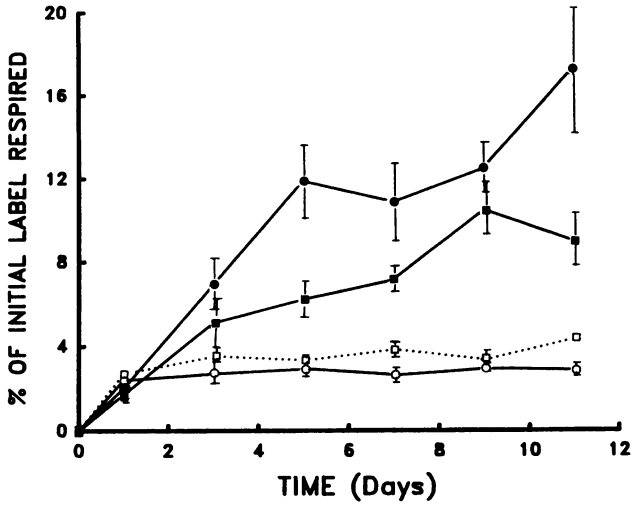

FIG. 4. Influence of nutrient additions on the mineralization of toluene (104 ng/g) in Lula aquifer solids sample 9JJ3. Symbols: ●, control; ■, mineral salts; ○, glucose; □, amino acids.

In sample 9NN7 treated with a high concentration of PNP (425 ng/g), there was not an adaptive response in the control (Fig. 3); mineralization was slow and increased linearly, with approximately 5% of the initial PNP mineralized to $^{14}CO_2$ after 130 days of exposure. Amending this sample with NH_4Cl resulted in an adaptive response with approximately 50% of the initial PNP being metabolized to $^{14}CO_2$ within 40 days. The length of the lag period was significantly shorter with the addition of 1.0 μg of NH_4Cl per g (approximately 20 days) than with the addition of 0.1 μg of NH_4Cl per g (approximately 30 days). In solid 9LL4, samples adapted to PNP (542 ng/g) with a lag period of 15 days (data not shown). Amending these PNP-treated samples with two concentrations of a mixture of $NaNO_3$ and NH_4Cl did not significantly shorten the length of the lag phase. However, at the higher of the two concentrations ($NaNO_3$, 1.5 μg/g; NH_4Cl, 1.0 μg/g) there was a significant increase in the total $^{14}CO_2$ production, with approximately 35% of the initial substrate mineralized by day 70. The amount of substrate mineralized at the lower concentration ($NaNO_3$, 0.15 μg/g; NH_4Cl, 0.10 μg/g) was approximately 10% of the initial amount and was not significantly different from that of the controls.

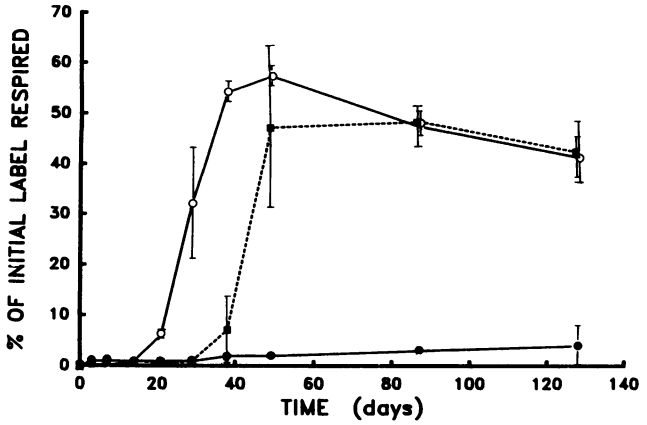

FIG. 3. Influence of two concentrations of ammonium chloride (0.1 and 1.0 μg/g) on PNP (425 ng/g) mineralization in Lula aquifer solids sample 9NN7. Symbols: ●, control; ■, 0.1 μg of NH_4Cl per g; ○, 1.0 μg of NH_4Cl per g.

No acclimation period was required for phenol (108 ng/g) mineralization in aquifer solids sample 9KK5 (data not shown). Metabolism was rapid and increased linearly to a maximum of 23% of the initial substrate within 10 days of exposure. Amending samples with nitrogen or phosphorus alone or together did not significantly alter the initial rate of mineralization or the amount of phenol mineralized.

The biodegradation of toluene (112 ng/g) with solids sample 9JJ4 did not require an acclimation period; mineralization increased linearly to a maximum of 1.3% within 10 days of exposure (data not shown). Amending the toluene-treated samples with N (as NH_4NO_3, 48.3 ng/g) or P (as KH_2PO_4, 11.1 ng/g; as K_2HPO_4, 14.2 ng/g) or both did not significantly alter the amount of the substrate mineralized after 60 days. However, the rate of mineralization during the initial 10 days of exposure varied among the treatments, with P alone or N and P together increasing the mineralization rate. These experiments were repeated with sample 9JJ3 and more frequent measurements early in the adaptation period (Fig. 4). The mineralization of toluene (104 ng/g) in control samples increased linearly throughout the exposure period, with 5% of the substrate recovered as $^{14}CO_2$ at day 10. The mineralization rates of samples amended with either N, P, or vitamins were not significantly different than those of the controls (data not shown). However, samples amended with either mineral salts (0.5 ml), $(NH_4)_2SO_4$ (136 ng/g; data not shown), or glucose (104 ng/g) had significantly lower rates of mineralization that the controls, with approximately 3% of the initial substrate recovered as $^{14}CO_2$ after 10 days of exposure. Amending the soil with amino acids (2.0 μg/g) or a combination of all of these nutrients (data not shown) resulted in significantly less metabolism than seen with the other treatments, with less than 1% of the toluene recovered as $^{14}CO_2$.

EDB-treated aquifer solids samples (9MM1, 108 ng/g) showed mineralization without an acclimation period (Fig. 5). The EDB mineralized increased linearly to 10 to 12% of the initial concentration by day 5 and more slowly to 14 to 17% by day 11. Amending EDB-treated samples with either

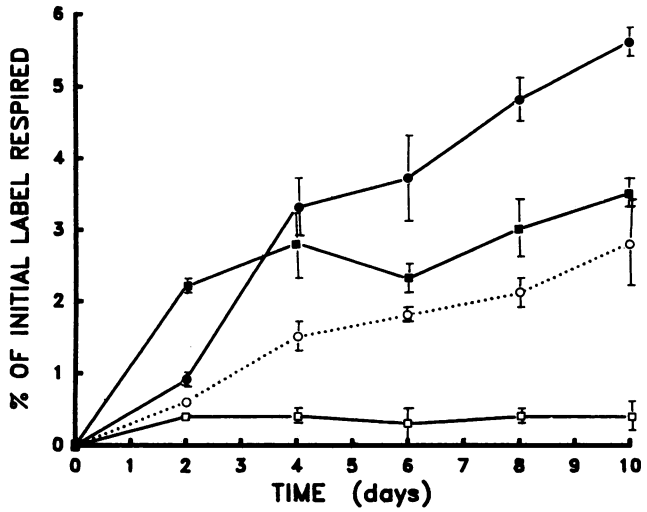

FIG. 5. Influence of nutrient additions on the mineralization of ethylene dibromide (108 ng/g) in Lula aquifer solids sample 9JJ3. Symbols: ●, control; ■, glucose; ○, amino acids; □, all nutrients combined (see text).

N (as NH_4NO_3, 6.2 ng/g), P (as KH_2PO_4, 2.8 ng/g; as K_2HPO_4, 3.6 ng/g) or both N and P together did not produce a response significantly different from that of the control after 55 days of exposure (data not shown). The initial rate of EDB mineralization was significantly increased, however, by the addition of P alone or N and P together. Experiments were repeated with material from aquifer solids sample 9JJ3 (Fig. 5). The rate of $^{14}CO_2$ production during the 11-day exposure was not significantly different between the controls and samples amended with either N (as NH_4NO_3, 132 ng/g), P (as KH_2PO_4, 29.7 ng/g; as K_2HPO_4, 37.6 ng/g), or vitamins (0.1 ml; data not shown), with 13 to 18% of the substrate recovered as $^{14}CO_2$ after 11 days. Amending the samples with either glucose (108 ng/g), $(NH_4)_2SO_4$ (141 ng/g; data not shown), amino acids (2.0 μg/g), or a combination of all of these nutrients significantly reduced EDB mineralization relative to that in the controls. After 11 days approximately 10% of the EDB was converted to $^{14}CO_2$ with $(NH_4)_2SO_4$- or glucose-amended samples, whereas 3% was recovered with samples amended with the amino acids or combined nutrients.

Experiments were conducted to further investigate why amending aquifer solids samples with alternate, degradable substrates such as amino acids or glucose resulted in a reduction in the amount of xenobiotic substrate mineralized relative to controls with substrate only. In Lula solids sample 9KK5 treated with PNP (113 ng/g), there was an 8-day lag period followed by an adaptive response, with 40 to 50% of the initial substrate recovered as $^{14}CO_2$ by 20 days (Fig. 6A). Amending PNP-treated samples with either amino acids (2.2 μg/g) or glucose (28 ng/g) significantly increased the length of the lag period and resulted in <15% of the substrate being mineralized after 60 days (Fig. 6A). Additional samples were processed in which solids (9KK5) were treated with nonlabeled PNP and either labeled amino acids or glucose (amounts given above). Mineralization of labeled amino acids added with nonlabeled PNP was rapid and immediate (Fig. 6B), with approximately 50% of the initial amino acids recovered as $^{14}CO_2$ after 1 day; however, no appreciable degradation of [^{14}C]PNP occurred in the presence of amino acids until after day 45 (Fig. 6A). Likewise, [^{14}C]glucose was readily metabolized in the presence of PNP, with approximately 20% of the initial [^{14}C]glucose recovered as $^{14}CO_2$ after 10 days (Fig. 6B). An adaptive response to PNP in the presence of glucose did not occur until approximately 30 days (Fig. 6A).

The number of cells in Lula solids sample 9JJ3, as determined by acridine orange direct count, was 2.5×10^7 cells per g. In samples treated with toluene (104 ng/g) or EDB (108 ng/g) cell numbers increased five- to sevenfold after 15 days (Table 1). Amending samples treated with toluene or EDB with either N (as NH_4NO_3, 130 ng/g), P (as KH_2PO_4, 30 ng/g; as K_2HPO_4, 35 ng/g), or NH_4SO_4 (140 ng/g) did not significantly alter cell numbers as compared with control samples. However, amending soils with either vitamins (5 μl), mineral salts (25 μl), glucose (100 ng/g), amino acids (2 μg/g), or a combination of all nutrients resulted in a 6- to 30-fold increase in cell numbers relative to nonamended samples.

DISCUSSION

Inorganic amendments substantially reduced the length of the acclimation period in samples requiring several months or longer to adapt to PNP (Fig. 2 and 3). Similar amendment effects have been reported for *p*-cresol and pond microorganisms (8). Differences in PNP mineralization among nonamended samples suggest spatial heterogeneity in the distribution of specific metabolic types. However, the influence of nutrient amendments indicates that nutrient availability,

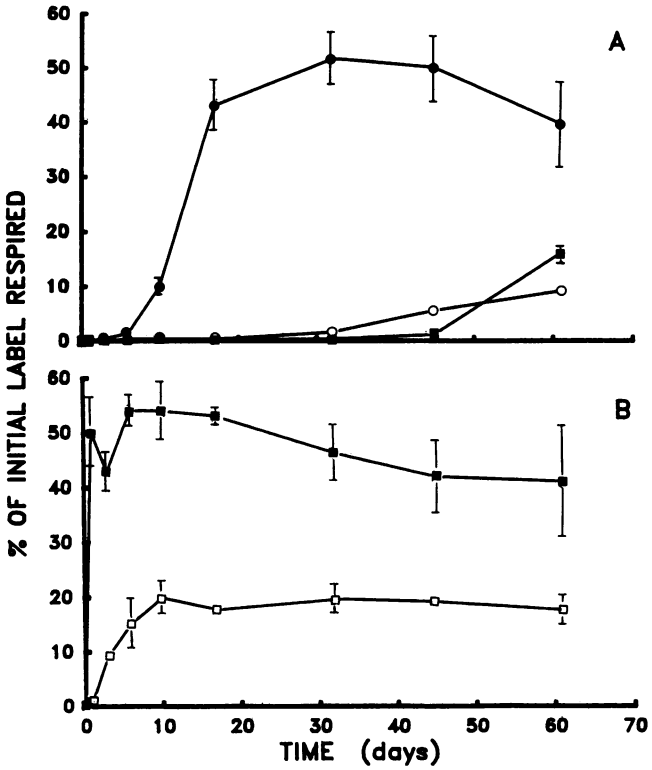

FIG. 6. Concurrent mineralization of two carbon sources. (A) Influence of amino acids (2.2 μg/g) or glucose (28 ng/g) on the mineralization of [^{14}C]PNP (113 ng/g); the control has [^{14}C]PNP only. Symbols: ■, amino acids; ○, glucose, ●, control. (B) Influence of PNP (113 ng/g) on the mineralization of ^{14}C-amino acids (2.2 μg/g) and [^{14}C]glucose (28 ng/g). Symbols: ■, ^{14}C-amino acids; □, [^{14}C]glucose.

TABLE 1. Changes in numbers of microorganisms with nutrient additions with xenobiotic substrates[a]

Treatment	No. of cells/g of solids (10^8) with added:	
	EDB	Toluene
Control	1.2	1.9
Nitrogen	1.7	1.5
Phosphorus	6.5	2.8
Mineral salts		29.0
Vitamins	8.3	16.9
Ammonium sulfate	2.0	2.2
Glucose	10.4	10.6
Amino acids	37.2	25.2
All nutrients	33.6	26.5

[a] Determined by acridine orange direct counting. The initial number was 2.5×10^7 cells per g of aquifer solids. The exposure period was 15 days. See the text for concentrations of amendments.

rather than the absence of degraders, may have contributed to the metabolic variability observed in Lula subsurface soils. Whatever the mechanisms of adaptation (i.e., growth, population changes, enzyme induction, etc.) (10), it is apparent that nutrient availability can play an important role in adaptation.

Amending samples with ammonium sulfate or mineral salts (toluene or EDB as the substrate) resulted in a decrease in mineralization relative to that in nonamended samples. These results are unexpected, since these nutrients are thought to be essential for microorganisms. In these particular samples the numbers of bacteria in the amended solids were equal to or greater than those in aquifer materials without nutrient amendments (Table 1). Since mineralization of the test substrate did not increase with an increase in cell numbers, this may indicate that ammonium sulfate and mineral salts were present in adequate amounts in the aquifer materials or that they stimulated the nondegraders rather than those microbes that utilize the xenobiotic substrates.

In general, adding combinations of inorganic nutrients to samples resulted in greater mineralization than did the addition of any single inorganic nutrient. Since several types of organisms may be required to sequentially degrade some xenobiotic compounds, and each species may have its own particular nutrient requirements, a number of nutrients may be influencing metabolism by a heterogeneous population at any given time. Therefore, the concept of a single limiting nutrient may not be applicable to heterogeneous groundwater populations. Organic nutrient amendments resulted in less mineralization of the target substrate than was observed in nonamended samples (Fig. 4 and 5) and increased the length of the lag period before mineralization began. Similar results have been reported by others (9, 11) and may be due to catabolite repression (7).

The results obtained from this investigation provide some insight into the biodegradation of organic compounds within aerobic aquifers and could have implications for the potential reclamation of contaminated sites. Although these studies were conducted with samples from a single layer in one aquifer, the responses indicate that the metabolic abilities and nutrient requirements of groundwater microorganisms can vary substantially within an aquifer. The cause of the variability within samples that are nominally from the same aquifer horizon is not clear but may be the result of a combination of factors, including (i) the existence of a variety of microhabitats within the aquifer, (ii) the patchy distribution of microbes in the aquifer (4), (iii) differences in microbial community structure over short distances, and (iv) differences in nutrient requirements and availability in different microhabitats within superficially uniform aquifer material. In addition, variability would be expected to increase as the aquifer material is experimentally diluted and therefore smaller amounts are represented in the incubations (17).

In relation to aquifer restoration, the addition of inorganic nutrients would appear to be either helpful or have no effect, at least in aquifers where aerobic conditions exist. When an aquifer is contaminated with more than one organic compound, a plethora of possible substrate interactions can occur, such as sequential or concurrent substrate utilization. The addition of organic nutrients with the goal of enhancing biodegradation could, however, inhibit or delay the metabolism of the contaminant both through substrate inhibition, as seen in this study, and through oxygen depletion.

Further investigations of the interactions of nutrients and xenobiotic compounds are needed to describe the nature of the subsurface microbial community and what regulates its response to added materials. This should include additional sample areas with other characteristics and sites contaminated with xenobiotic compounds where anaerobic conditions exist.

ACKNOWLEDGMENTS

We thank John T. Wilson for providing the subsurface soil samples used in this study.

This work was supported under Cooperative Agreement CR 811828 by the Environmental Protection Agency.

LITERATURE CITED

1. **Aelion, C. M., C. M. Swindoll, and F. K. Pfaender.** 1987. Adaptation to and biodegradation of xenobiotic compounds by microbial communities from a pristine aquifer. Appl. Environ. Microbiol. **53**:2212–2217.
2. **American Public Health Association.** 1985. Standard methods for the examination of water and wastewater, 16th ed. American Public Health Association, American Water Works Association, Water Pollution Control Federation, Washington, D.C.
3. **Balkwill, D. L., and W. C. Ghiorse.** 1985. Characterization of subsurface bacteria associated with two shallow aquifers in Oklahoma. Appl. Environ. Microbiol. **50**:580–588.
4. **Federle, T. W., M. A. Hullar, R. J. Livingston, D. A. Meeter, and D. C. White.** 1983. Spatial distribution of biochemical parameters indicating biomass and community composition of microbial assemblies in estuarine mud flat sediments. Appl. Environ. Microbiol. **45**:58–63.
5. **Gaudy, A. F., Jr., and E. T. Gaudy.** 1980. Microbiology for environmental scientist and engineers. John Wiley & Sons, Inc., New York.
6. **Ghiorse, W. C., and D. L. Balkwill.** 1983. Enumeration and morphological characterization of bacteria indigenous to subsurface environments. Dev. Ind. Microbiol. **24**:213–224.
7. **Lee, M. D., and C. H. Ward.** 1985. Biological methods for the restoration of contaminated aquifers. Environ. Toxicol. Chem. **4**:743–750.
8. **Lewis, D. L., H. P. Kollig, and R. E. Hodson.** 1986. Nutrient limitation and adaptation of microbial populations to chemical transformations. Appl. Environ. Microbiol. **51**:598–603.
9. **Rozich, A. F., and R. J. Colvin.** 1986. Effects of glucose on phenol biodegradation by heterogeneous populations. Biotechnol. Bioeng. **28**:965–971.
10. **Spain, J. C., P. A. VanVeld, C. A. Monti, P. H. Pritchard, and C. R. Cripe.** 1984. Comparison of *p*-nitrophenol degradation in field and laboratory test systems. Appl. Environ. Microbiol. **45**:944–950.

11. **Stumm-Zollinger, E.** 1968. Substrate utilization in heterogeneous bacterial communities. J. Water Pollut. Control Fed. **40**:R212–R229.
12. **White, D. C., G. A. Smith, M. J. Gehron, J. H. Parker, R. H. Findlay, R. F. Martz, and H. L. Frederickson.** 1983. The groundwater aquifer microbiota: biomass, community structure, and nutritional status. Dev. Ind. Microbiol. **24**:201–211.
13. **Wilson, J. T., and J. F. McNabb.** 1983. Biological transformation of organic pollutants in groundwater. EOS **64**:505–506.
14. **Wilson, J. T., J. F. McNabb, D. L. Balkwill, and W. C. Ghiorse.** 1983. Enumeration and characterization of bacteria indigenous to a shallow water-table aquifer. Ground Water **21**:134–142.
15. **Wilson, J. T., J. F. McNabb, J. W. Cochran, T. H. Wang, M. B. Tomson, and P. B. Bedient.** 1985. Influence of microbial adaptation on the fate of organic pollutants in groundwater. Environ. Toxicol. Chem. **4**:721–726.
16. **Wilson, J. T., G. D. Miller, W. C. Ghiorse, and F. R. Leach.** 1986. Relationship between the ATP content of subsurface material and the rates of biodegradation of alkylbenzenes and chlorobenzene. J. Contam. Hydrol. **1**:163–170.
17. **Wilson, J. T., and M. J. Noonan.** 1984. Microbial activity in model aquifer systems, p. 117–133. *In* G. Bitton and C. P. Gerba (ed.), Ground water pollution microbiology. John Wiley & Sons, Inc., New York.