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Biodegradation of 4-nitrophenol by indigenous microbial populations in Everglades soils

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Abstract

The Everglades in South Florida are a unique ecological system. As a result of the widespread use of pesticides and herbicides in agricultural areas upstream from these wetlands, there is a serious potential for pollution problems in the Everglades. The purpose of this study was to evaluate the ability of indigenous microbial populations to degrade xenobiotic organic compounds introduced by agricultural and other activities. Such biodegradation may facilitate the remediation of contaminated soils and water in the Everglades. The model compound selected in this study is 4-nitrophenol, a chemical commonly used in the manufacture of pesticides. The mineralization of 4-nitrophenol at various concentrations was studied in soils collected from the Everglades. At concentrations of 10 and 100 μg/g soil, considerable mineralization occurred within a week. At a higher concentration, i.e., 10 mg/g soil, however, no mineralization of 4-nitrophenol occurred over a 4-month period; such a high concentration apparently produced an inhibitory effect. The rate and extent of 4-nitrophenol mineralization was enhanced on inoculation with previously isolated nitrophenol-degrading microorganisms. The maximum mineralization extent measured, however, was less than 30% suggesting conversion to biomass and/or unidentified intermediate products. These results indicate the potential for natural mechanisms to mitigate the adverse effects of xenobiotic pollutants in a complex system such as the Everglades.

Introduction

The Everglades are a unique ecological system in South Florida, where the temperate zone meets the subtropics. They have been described as a River of Grass (Douglas 1947), a flat terrain with a maximum elevation of 6 meters extending over a hundred and fifty kilometers south from Lake Okeechobee to Florida Bay. Underlying the Everglades and much of South Florida are limestone formations. The limestone aquifer is covered by a thin layer of organic soil which is flooded under several inches of water in summer but is dry and exposed in winter. The porous limestone formation is an essential underground water reservoir known as the Biscayne Aquifer, that supplies the counties of Dade, Broward and Monroe their freshwater requirements.

Large parts of the Everglades have been drained for urban development along the coastal regions; extensive agricultural activities south of Lake Okeechobee, in the northern Everglades, have also had an adverse impact on the wetlands system. Increased water withdrawal from the Biscayne aquifer has caused considerable seawater intrusion in coastal areas, and the widespread use of fertilizers, pesticides, herbicides and insecticides has resulted in a deterioration of water quality for surface waters in the Everglades, potentially impacting the groundwater resources.

In an effort to understand the fate of xenobiotic chemicals in the Everglades, this study examines the ability of indigenous soil microorganisms to biodegrade 4-nitrophenol. Nitroaromatic compounds are ubiquitous contaminants which enter the environment as wastes, pesticides, explosives, and dyes, or are formed through photochemical (Lacorte & Barceto 1994; Grosjean 1985) or biological processes (Youngman et al. 1989; Sylvestre et al. 1982;

Giger et al. 1981). Several nitroaromatics, including 4-nitrophenol, are listed as priority pollutants, and nitrophenols have been widely studied as model compounds for examining the biodegradability of organic pollutants in both surface and subsurface environments. Nitrophenols in surface waters and surface soils are readily degraded by photochemical oxidation. However, in deeper soils and groundwater, nitrophenol degradation depends primarily on biodegradation. The biodegradation of nitroaromatics can be initiated by either reductive or oxidative mechanisms (Uberoi & Bhattacharya 1997; O'Connor & Young 1996; Jain et al. 1994; Aitken et al. 1994; Bradley et al. 1994; Blasco & Castillo 1992; Spain & Gibson 1991).

Numerous investigations into the microbial transformations of nitroaromatic compounds are reported in the literature (Folsom et al. 1994; Haigler et al. 1994; Nishino & Spain 1993; Hanne et al. 1993; Heitkamp et al. 1990; Zeyer & Kocher 1988; Bruhn et al. 1987; Schmidt et al. 1987). These studies often use microbial communities selected from engineered processes, e.g., activated sludge systems. Relatively little is known about the degradative capability of indigenous microbial communities, in particular in soils and aquifer materials. In this paper we report the potential for *in situ* biodegradation of 4-nitrophenol in Everglades soils, thereby providing some indication of the fate of xenobiotic chemicals released into this unique ecosystem. The effect of inoculating Everglades soil samples with previously isolated nitrophenol-degrading strains was also investigated. No attempt was made to determine intermediate products formed during microbial degradation or the biodegradation pathways involved. However, by monitoring the mineralization of 4-nitrophenol, only the complete degradation of 4-nitrophenol is reported.

Materials and methods

Soil samples

The soil used was collected as grab samples from the top 10 to 15 cm at several locations in the Taylor Slough channel near the Royal Palm Visitor Center of the Everglades National Park (approximate coordinates: N 25° 30′, W 80° 28′). Because the soils were collected from locations within the boundaries of the National Park they are assumed to be relatively uncontaminated. Grab samples were mixed to yield composite soil samples that were sieved through U.S. Standard Sieve No. 12 (size 1.68 mm) without drying, and stored at 4 °C until used. The pH values measured for soil-water suspensions with distilled water was 8.2, and with tap water was between 7.2 and 7.9. The moisture content of the soil was gravimetrically determined to be approximately 63%. The moisture content of Everglades soils varies seasonally; although the samples were collected in February 1995, the moisture content was still relatively high because of unseasonal rainfall and flooding during the winter. The organic carbon content of the soil was determined by the Walkley-Black procedure to be 8.5% (ASA 1982).

Chemicals

Radiolabeled (^{14}C) 4-nitrophenol (uniformly labeled) was purchased from Sigma Chemical Co., St. Louis, MO. The specific activity was 6.6 mCi/mmol, and radiochemical purity exceeded 98%. Nonradiolabeled 4-nitrophenol (4-NP) was obtained from Aldrich Chemical Co., Milwaukee, WI (purity exceeded 98%) and was used as received. The inorganic reagents used were analytical grade.

Isolation of 4-NP-degrading bacteria

4-NP-degrading microorganisms were isolated from several soil sources by serial enrichment, and are identified as EG strains (from Everglades soils), BG strains (from Bulgarian soil samples), and KY strains (Kentucky soils). The Kentucky and Bulgarian soils used had been exposed to previous contamination; the Everglades soil samples were presumed to be uncontaminated since they were collected from various locations within the Everglades National Park boundaries. Microbial cultures used for inoculum were grown for about 24 hours in nutrient broth prepared in a mineral salts medium with 200 mg/L 4-NP. The inoculum provided between 10^5 and 10^7 cells/g soil. Growth of microorganisms was monitored spectrophotometrically.

Biomineralization tests

The rate and extent of 4-NP degradation were examined in soil-water systems for unamended soils as well as soils inoculated with previously isolated NP-degrading bacteria. Triplicate 125-mL flasks containing 5 g dry weight of soil and 10 mL of 4-NP solution were used for each variant. 4-NP solutions for the mineralization tests were prepared using both labeled and unlabeled

compound in a minimal salts medium. Analysis of [^{14}C]4-NP was taken as representative of the behavior of the compound as a whole. Samples were counted for ^{14}C on a Beckman LS 3801 liquid scintillation counter, using the H# quench monitoring technique with automatic quench compensation. The counts were obtained in disintegrations per minute (DPM) which could be related to the proportion of total mass of 4-NP in the system.

Degradation of ^{14}C-labeled 4-NP was detected by trapping and analyzing liberated $^{14}CO_2$. Individual flasks were sealed with rubber stoppers fitted with center wells containing filter paper soaked in 0.4 mL of 2 *N* NaOH to serve as CO_2 traps. The flasks were incubated statically in the dark at 30 °C. Poisoned controls (receiving mercuric chloride) were set up to detect any potential volatilization of 4-NP. To monitor $^{14}CO_2$ evolution, the NaOH-soaked filter papers from individual flasks were periodically removed, placed in 20-mL vials containing 10 mL Ecoscint scintillation cocktail (National Diagnostics, Atlanta, GA), and counted for ^{14}C after being stored in the dark overnight to minimize chemiluminescence. Calculations on cumulative mineralization were corrected for background DPM and reported values represent average $^{14}CO_2$ evolution for triplicate reactors. Acidification of the soil mixture at the conclusion of the experiments indicated that $^{14}CO_2$ release after acidification was not significant, and hence it was not necessary to account for [^{14}C] carbonate in samples.

The effect of 4-NP concentration on the rate and extent of mineralization was investigated by using three different 4-NP doses: 10 μg, 100 μg, and 10 mg 4-NP per g soil. The activity of [^{14}C]4-NP in individual microcosms was about 0.05 μCi ($\sim$ 100,000 DPM); this level of activity is expected to yield measurable $^{14}CO_2$ evolution (i.e., DPM levels significantly greater than background levels).

Extraction

Following completion of the mineralization tests, the soil-water residue in each flask was extracted by agitating overnight with 40 mL of 1 : 1 hexane-acetone mixture (Hickey et al. 1993; Brunner et al. 1985). The contents of the flasks were then allowed to settle, the solvent mixture decanted into graduated conical tip tubes, and the solvent allowed to evaporate to a final volume of 10 mL. The resulting solvent was sampled for ^{14}C.

Aqueous systems

In addition to growing liquid cultures for the purpose of inoculating soil-water systems, a number of reactors receiving ^{14}C-labeled 4-NP solutions and NP-degrading microbial strains were also set up. $^{14}CO_2$ evolution from these flasks was monitored to allow comparison with mineralization data from systems receiving soil.

Results and discussion

Three different 4-NP concentrations were used in the microbial mineralization tests: 10 μg, 100 μg, and 10 mg 4-NP per g soil. Each flask received 5 g dry weight of soil with a moisture content of approximately 63%, and 10 mL of 4-NP solution. The resulting soil-water ratio is, therefore, estimated to lie between 1 : 3 and 1 : 4 g soil per mL water. Assuming no partitioning of 4-NP onto soil, the three 4-NP dosages used correspond to aqueous concentrations of 5, 50, and 5,000 mg/L. The 5,000 mg/L concentration is an order of magnitude greater than the reported 4-NP concentration bacteria can tolerate.

Biological mineralization of 4-NP was monitored by the capture of $^{14}CO_2$ in caustic solution. Each biomineralization test was performed in triplicate. [^{14}C]4-NP losses through volatilization were assessed through the use of poisoned controls. Figures 1 and 2 show the microbial mineralization of 4-NP in soil-water systems receiving 10 μg 4-NP/g soil and 100 μg 4-NP/soil, respectively. Over the course of 75 days between 10 and 30% of the initial [^{14}C]4-NP was mineralized. The absence of ^{14}C in the NaOH-soaked filter paper for the control reactors indicates that abiotic processes do not contribute to the measurement of 4-NP mineralization. Most rapid rates of $^{14}CO_2$ evolution were observed within the first few days of initiating the biodegradation experiments; thereafter, mineralization continued at a much diminished rate.

In order to obtain estimates for the maximum rates and extent of mineralization in each microcosm, the observed mineralization data was modeled as a saturation type curve:

$$P = \frac{k_1 t}{(1 + k_2 t)} \tag{1}$$

where P is the percent mineralization, t is the time in days since initiation of mineralization, and k_1 and k_2 are constants derived from curve-fitting. This model is

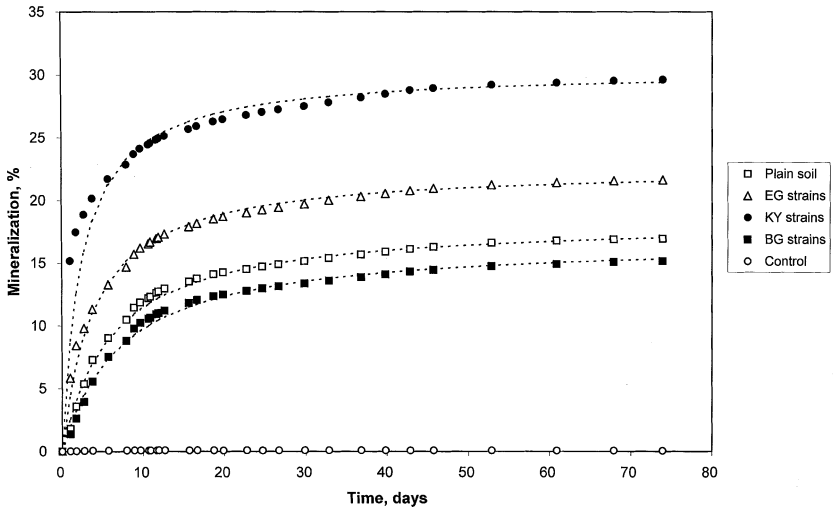

Figure 1. Microbial mineralization of 4-nitrophenol in soil-water systems with 10 microg 4-NP/g soil.

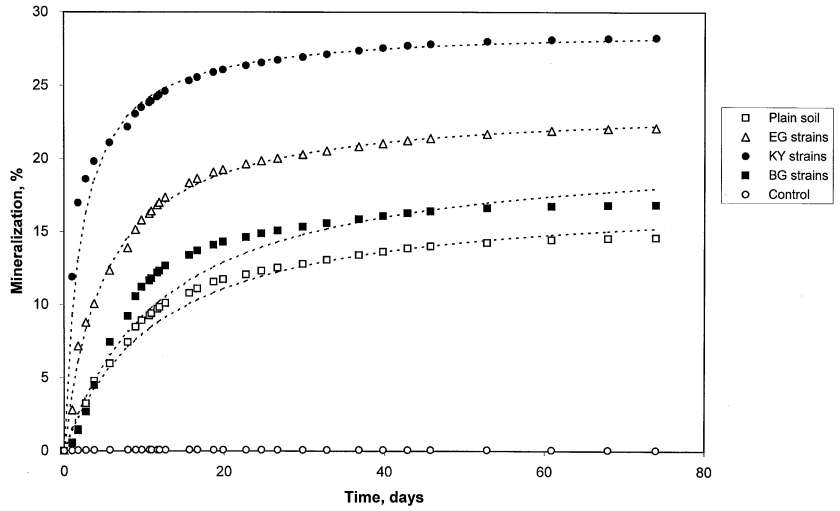

Figure 2. Microbial mineralization of 4-nitrophenol in soil-water systems with 100 microg 4-NP soil.

defined by a limiting or maximum extent of mineralization, $P_{max} = k_1/k_2$, and an initial linear mineralization rate given by $(dP/dt)_{max} = k_1$.

Equation (1) was linearized by taking the reciprocal of both sides and rearranging:

$$\frac{t}{P} = \frac{k_2}{k_1}t + \frac{1}{k_1} \qquad (2)$$

A plot of the ratio of time elapsed/percent mineralized versus time elapsed yields a straight line; parameters k_1 and k_2 were derived by linear regression. Table 1 presents a summary of the maximum rates and extent of mineralization of 4-NP observed in the soil-water microcosms; values for the squared correlation coefficient (R^2) for linear regression are also listed in Table 1. Model predictions are presented as dashed lines in Figures 1 and 2, along with experimental data for 4-NP mineralization in soil-water microcosms receiving 10 μg/g and 100 μg/g nitrophenol respectively.

The use of unamended Everglades soil samples resulted in maximum mineralization rates of 0.152 and 0.75 mg/L-day for systems receiving 10 and 100 μg 4-NP/g soil, respectively; the maximum extent of mineralization observed was about 18% in either case. The addition of microorganisms isolated from contaminated Kentucky soils (denoted as KY strains) was most

Table 1. Maximum rate and extent of mineralization of 4-nitrophenol in soil-water systems

System	4-NP added (μg/g of soil)	R^2	Maximum rate		Extent at conclusion	
			(%/day)	mg/L-day	(%)	μg
Unamended soil	10	0.9986	3.03	0.152	18.4	9.2
	100	0.9603	1.5	0.75	17.5	87.5
	10,000	–	–	–	–	–
Inoculated with KY strains	10	0.9993	12.4	0.62	30.4	15.2
	100	0.9998	13.8	6.9	29	144.8
	10,000	n/a	~ 0.5	~ 250	~ 11	~ 5500
Inoculated with EG strains	10	0.9996	5.74	0.287	22.7	11.3
	100	0.9992	4.77	2.3	23.7	118
	10,000	n/a	~ 0.3	~ 150	~ 11	~ 5500
Inoculated with BG strains	10	0.9973	2.31	0.115	16.8	8.4
	100	0.9098	1.68	0.84	20.9	104.4
	10,000	–	–	–	–	–

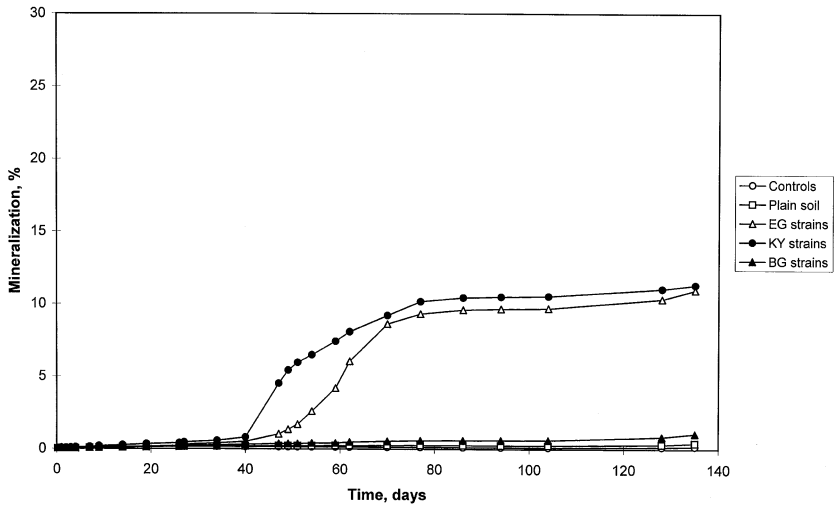

Figure 3. Microbial mineralization of 4-nitrophenol in soil-water systems with 10 mg 4-NP/g soil.

effective in increasing both the rate and extent of mineralization. For systems receiving 10 μg 4-NP/g soil and inoculated with KY strains, the maximum rate of mineralization measured was approximately 0.62 mg/L-day and the extent of 4-NP mineralized was ~ 30%; the corresponding values obtained for systems receiving 100 μg/g and KY strains are 6.9 mg/L-day and 29%, respectively. The addition of nitrophenol-degrading microorganisms previously isolated from Everglades soils (EG strains) also resulted in increased mineralization rates and extent. However, the addition of BG strains did not have a significant effect compared to uninoculated Everglades soil microcosms.

Figure 3 presents mineralization data for systems receiving 10 mg 4-NP/g soil. From this figure it is apparent that the increased nitrophenol concentration appears to have an inhibitory effect on microbial degradation. No mineralization was observed in any system until 40 days into the degradation studies; thereafter, soil-water systems inoculated with KY and EG strains exhibited some $^{14}CO_2$ evolution with approximately 10% mineralization observed four months after initiating the experiments. No $^{14}CO_2$ evolution was observed in unamended systems and those receiving the BG strains, suggesting that microbial activity in these systems continued to be inhibited due to the high 4-NP concentration.

A number of reactors was set up that received 25 mL of an aqueous solution of 200 mg/L 4-NP as well as ~ 11,000 DPM radiolabeled 4-NP. These reactors were

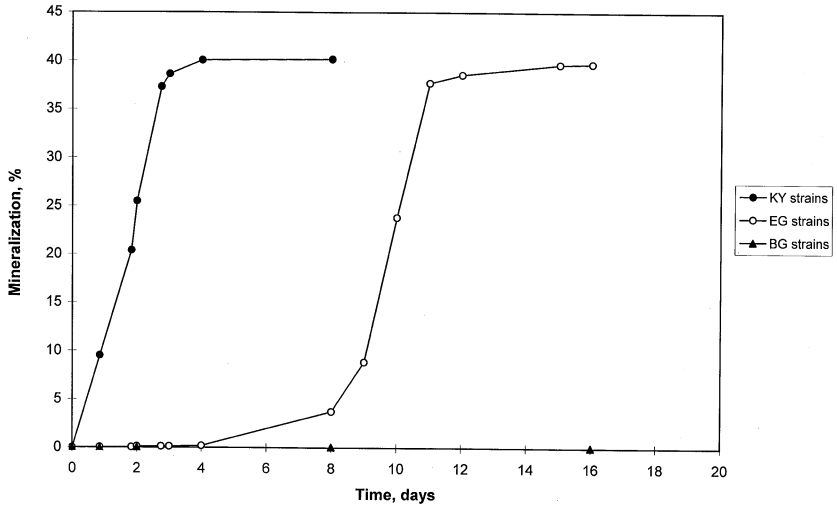

Figure 4. Microbial mineralization in aqueous systems.

inoculated with the three previously-isolated microbial strains. Figure 4 presents the mineralization results obtained for the aqueous 4-NP solutions receiving KY, EG, and BG strains. No lag period was observed for the systems receiving KY strains, and approximately 40% of the [^{14}C]4-NP was mineralized within the first three days. For aqueous systems receiving EG strains a one-week lag period was observed, following which cumulative mineralization reached about 40% in another five days. The systems inoculated with BG strains indicated no measurable 4-NP mineralization during the course of the two-week study suggesting that these microorganisms may not be as effective at degrading nitrophenols. These results support observations in the soil microcosms, i.e., that inoculation with KY strains was most effective at enhancing the biomineralization of 4-NP and that BG strains were unable to promote 4-NP mineralization. 4-NP is a highly colored compound and its presence at concentrations > 1 mg/L in solution produces a bright yellow color. This provides a visual indication of the presence of 4-NP, although it is acknowledged that acid production may also cause the yellow color of 4-NP to disappear. The KY systems turned colorless within four days whereas the reactors receiving EG strains lost their yellow color after two weeks. The reactors were acidified prior to the last sampling to ensure no $^{14}CO_2$ remained in soluble carbonate form.

Mineralization accounted for less than 30% of parent 4-NP in the biodegradation experiments suggesting the possible formation of some unidentified intermediate products and/or biomass. The purpose of extracting soil residues with the hexane-acetone solvent mixture was to obtain reasonable mass balances for radiolabeled nitrophenol in our experiments. However, ^{14}C recovery efficiencies obtained from these extractions were very disappointing. For those systems receiving 10 or 100 μg 4-NP/g soil, almost 60% of the [^{14}C]4-NP added to the abiotic control was recovered in the solvent; for the remaining reactors less than 5% of the initial activity was recovered in the hexane-acetone solvent. This only accounted for between 17 and 60% of the radiolabeled 4-NP added to the systems, and was unacceptable for mass balance purposes. For systems receiving higher concentrations of 4-NP, i.e., 10 mg/g, percent recoveries from the hexane-acetone solvent were on average approximately 25% for the abiotic controls, 21% for the unamended soils, 13% for soils amended with BG strains and less than 5% for systems receiving EG and KY strains. This accounted for between 14 and 25% of the ^{14}C added to the soil microcosms, again rendering the data unacceptable for mass balance purposes.

Conclusion

In this study, the microbial mineralization of 4-NP was examined in soil samples collected from the Everglades. Experimental results indicate that at contamination levels up to 100 μg/g soil about 20 percent of 4-NP was mineralized in unamended soils within a month; no lag period was detected prior to the onset of mineralization. The rate and extent of mineralization

are almost doubled when the soil-water systems were inoculated with microbial strains isolated from contaminated Kentucky soils (KY strains). The addition of NP-degraders previously isolated from various Everglades soil samples also resulted in enhanced 4-NP mineralization. For soil microcosms receiving 10 mg 4-NP/g soil (or 5,000 mg/L 4-NP), inoculation with EG or KY strains resulted in significant mineralization (10%) following a forty-day acclimation period. No efforts were undertaken to confirm that the EG and KY microbes that were added to the soil survived the 40 day acclimation period. It is possible that an enrichment or genetic selection process may have occurred over the long acclimation period. However, in the absence of additional studies, it is currently hypothesized that the lag period observed with the 5,000 mg/L 4-nitrophenol dose was a result of the initial toxicity destroying the majority of the inoculum which then takes some time to recover to significant numbers.

The results obtained are significant in that microorganisms indigenous to the Everglades appear able to biodegrade 4-NP without any observed lag period up to concentrations of 100 μg/g, suggesting the potential for natural mechanisms to mitigate the adverse effects of xenobiotic pollutants in natural soil environments.

Such implications are supported by results reported by other researchers. In one study evaluating the viability of bacterial inoculation in enhancing the biodegradation of organic pollutants, 4-NP was observed to be mineralized extensively in lake water inoculated with 4-NP degraders whereas no mineralization was measured in uninoculated lake water over a one week incubation (Zaidi et al. 1989). Spain & van Veld (1983) report on the ability of microbial communities in various sediment-water samples collected from the Pensacola area, Florida, to degrade radiolabeled 4-NP. After a lag period of two to four days during which mineralization was insignificant, the rates of $^{14}CO_2$ release increased considerably in all freshwater communities. Preexposure of freshwater ecocores to 4-NP resulted in microbial adaptation marked by the disappearance of lag periods. The extent of mineralization observed in these sediment-water systems ranged from 20 to 60 percent. In another study on the aerobic biodegradation of phenolic hydrocarbons in laboratory microcosms using sediment and groundwater from an aerobic aquifer, 4-NP was rapidly degraded with lag periods of 5 days or less (Nielsen & Christensen 1994).

In the current study, the extent of mineralization measured in soil-water systems receiving up to 10 μg 4-NP/g soil was between 15 and 30%. Attempts at performing mass balances on the systems were unsuccessful so it is not possible to assess the extent of conversion of 4-NP to intermediate products. Acidification of soil-water systems at the conclusion of biodegradation tests did not produce an increase in $^{14}CO_2$ evolution indicating the absence of dissolved [^{14}C] carbonate. The extent of mineralization measured in liquid cultures containing 200 mg/L 4-NP was approximately 40% for the EG and KY strains; the EG strains displayed a one-week lag period. 4-NP is moderately soluble in water with an aqueous solubility reported as 0.08 M (Schwarzenbach et al. 1988), and no problems were encountered in preparing 200 mg/L 4-NP solutions. The compound is not very hydrophobic, with an octanol/water partition constant log K_{ow} value reported between 1.91 and 2.04 (Lyman et al. 1990; Schwarzenbach et al. 1988). This suggests that excessive 4-NP sorption onto soil organic carbon is unlikely, and probably does not completely account for the poor recovery efficiencies obtained in our mass balances.

Comparing the rate of mineralization observed in the current study with earlier investigations, it is noted that Heitkamp et al. (1990) observed between 45 to 70% 4-NP mineralization within four days in liquid culture, Herman & Costerton (1993) report almost 60% mineralization by a bacterial species capable of utilizing 4-NP as sole carbon and nitrogen source, and Schmidt & Gier (1989) measured almost 50% mineralization of 2,4-dinitrophenol (DNP) in soil-water systems receiving up to 200 μg/g DNP, and approximately 10% mineralization in systems receiving 500 μg/g DNP. The extent of mineralization measured in this investigation are somewhat less than this; however, observations are generally in agreement with earlier work. 4-NP is a readily biodegraded organic pollutant, so that the ability of indigenous microbial populations to mineralize 4-NP is not surprising, and does not necessarily imply the existence of nitrophenolic pollutants at the site. In order to determine the existence of such organic contamination, additional sampling and analysis of soil and water are required. Additional work is also planned to develop a better understanding of the process of acclimation: specifically to determine whether the nitrophenol degraders after a long acclimation period are the same as those originally added, or whether they are new strains or genetic variants of the original inoculum.

References

ASA (1982) Methods of Soil Analysis, Part 2: Chemical and Microbiological Processes, American Society of Agronomy, Inc., Madison, Wisconsin

Aitken MD, Massey IJ, Chen T & Heck PE (1994) Characterization of reaction products from the enzyme catalyzed oxidation of phenolic pollutants. Wat. Res. 28: 1879–1889

Blasco R & Castillo F (1992) Light-dependent degradation of nitrophenols by the phototrophic bacterium *Rhodobacter capsulatus* E1F1. Appl. Environ. Microbiol. 58: 690–695

Bradley PM, Chapelle FH, Landmeyer JE & Schumacher JG (1994) Microbial transformation of nitroaromatics in surface soils and aquifer materials. Appl. Environ. Microbiol. 60: 2170–2175

Bruhn C, Lenke H & Knackmuss HJ (1987) Nitrosubstituted aromatic compounds as nitrogen source for bacteria. Appl. Environ. Microbiol. 53: 208–210

Brunner W, Sutherland FH & Focht DD (1985) Enhanced biodegradation of polychlorinated biphenyls in soil by analog enrichment and bacterial inoculation. J. Environ. Qual. 14: 324–328

Douglas MS (1947) The Everglades: River of Grass. DBA/Mockingbird Books: Marietta, GA

Folsom BR, Stierli R, Schwarzenbach RP & Zeyer J (1994) Comparison of substituted 4-nitrophenol degradation by enzyme extracts and intact cells. Environ. Sci. Technol. 28: 306–311

Giger W, Schaffner CLH & Keith E (1981) Advances in the identification and analysis of organic pollutants in water. Vol. 1: 141–154; Ann Arbor Science Publishers, Inc.: Ann Arbor, MI

Grosjean D (1985) Reactions of o-cresol and nitrocresol with NO_x in sunlight and with ozone-nitrogen dioxide mixtures in the dark. Environ. Sci. Technol. 19: 968–974

Haigler BE, Wallace WH & Spain JC (1994) Biodegradation of 2-nitrotoluene by *Pseudomonas* sp. strain JS42. Appl. Environ. Microbiol. 60: 3466–3469

Hanne LF, Kirk LL, Appel SM, Narayan AD & Bains KK (1993) Degradation and induction specificity in actinomycetes and degrade *p*-nitrophenol. Appl. Environ. Microbiol. 59: 3505–3508

Heitkamp MA, Camel V, Reuter TJ & Adams WJ (1990) Biodegradation of *p*-nitrophenol in an aqueous waste stream by immobilized bacteria. Appl. Environ. Microbiol. 56: 2967–2973

Herman DC & Costerton JW (1993) Starvation-survival of a *p*-nitrophenol-degrading bacterium. Appl. Environ. Microbiol. 59: 340–343

Hickey WJ, Searles DB & Focht DD (1993) Enhanced mineralization of polychlorinated biphenyls in soil inoculated with chlorobenzoate-degrading bacteria. Appl. Environ. Microbiol. 59: 1194–1200

Jain RK, Dreisbach JH & Spain JC (1994) Biodegradation of *p*-nitrophenol via 1,2,4-benzenetriol by an *Arthrobacter* sp. Appl. Environ. Microbiol. 60: 3030–3032

Lacorte S & Barceto D (1994) Rapid degradation of fenitrothion in estuarine water. Environ. Sci. Technol. 28: 1159–1163

Lyman WJ, Reehl WF & Rosenblatt DH (1990) Handbook of Chemical Property Estimation Methods. American Chemical Society: Washington, D.C.

Nielsen PH & Christensen TH (1994) Variability of biological degradation of phenolic hydrocarbons in an aerobic aquifer determined by laboratory batch experiments. J. Contam. Hydrol. 17: 55–67

Nishino SF & Spain JC (1993) Cell density-dependent adaptation of *Pseudomonas putida* to biodegradation of *p*-nitrophenol. Environ. Sci. Technol. 27: 489–494

O'Connor OA & Young LY (1996) Effects of six different functional groups and their positions on the bacterial metabolism of monosubstituted phenols under anaerobic conditions. Environ. Sci. Technol. 30: 1419–1428

Schmidt SK & Gier MJ (1989) Dynamics of microbial populations in soil: indigenous microorganisms degrading 2,4-dinitrophenol. Microb. Ecol. 18: 285–296

Schmidt SK, Scow KM & Alexander M (1987) Kinetics of *p*-nitrophenol mineralization by a *Pseudomonas* sp.: effects of second substrates. Appl. Environ. Microbiol. 53: 2617–2623

Schwarzenbach RP, Stierli R, Folson BR & Zeyer J (1988) Compound properties relevant for assessing the environmental partitioning of nitrophenols. Environ. Sci. Technol. 22: 83–92

Spain JC & Gibson DT (1991) Pathway for biodegradation of *p*-nitrophenol in a *Moxarella* sp. Appl. Environ. Microbiol. 57: 812–819

Spain JC & van Veld PA (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

Sylvestre M, Masse R, Messier F, Fauteux J, Bisaillon G & Beaudet R (1982) Bacterial nitration of 4-chlorobiphenyl. Appl. Environ. Microbiol. 44: 871–877

Uberoi V & Bhattacharya SK (1997) Toxicity and degradability of nitrophenols in anaerobic systems. Water Environ. Res. 69: 146–156

Youngman RR, Toscano NC & Gaston LK (1989) Degradation of methyl parathion to p-nitrophenol on cotton and lettuce leaves and its effects on plant growth. J. Econ. Entomol. 82: 1317–1322

Zaidi BR, Murakami Y & Alexander M (1989) Predation and inhibitors in lake water affect the success of inoculation to enhance biodegradation of organic chemicals. Environ. Sci. Technol. 23: 859–863

Zeyer J & Kocher HP (1988) Purification and characterization of a bacterial nitrophenol oxygenase which converts *ortho*-nitrophenol to catechol and nitrite. J. Bacteriol. 170: 1789–1794