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Effect of Adaptation to Phenol on Biodegradation of Monosubstituted Phenols by Aquatic Microbial Communities

ROBERT J. SHIMP[1]* AND FREDERICK K. PFAENDER[2]

Environmental Safety Department, The Procter and Gamble Company, Ivorydale Technical Center, Cincinnati, Ohio 45217,[1] and Department of Environmental Sciences and Engineering, University of North Carolina, Chapel Hill, North Carolina 27514[2]

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The adaptation of a mixed aquatic microbial community to phenol was examined in microcosms receiving phenol as a sole carbon source. Extended exposure (adaptation) to phenol resulted in adaptation of the microbial community to the structurally related aromatic compounds *m*-cresol, *m*-aminophenol, and *p*-chlorophenol. The increased biodegradation potential of the phenol-adapted microbial community was accompanied by a concurrent increase in the number of microorganisms able to degrade the three test compounds. Thus, adaptation to the three test chemicals was likely a growth-related result of extended exposure to phenol. The results indicate that adaptation to a single chemical may increase the assimilative capacity of an aquatic environment for other related chemicals even in the absence of adaptation-inducing levels of those materials.

The rate of biodegradation of pollutants in natural environments may be affected by a variety of factors, including adaptation of the microbial community to a particular chemical. Adaptation may be operationally defined as an increase in the ability of a microbial community to degrade a chemical after prolonged exposure to the material. Adaptation may be the result of several alterations in the structure and function of microbial communities. These include induction or derepression of enzymes, genetic change, and growth of specific degrader organisms (21).

Adaptation has been observed for a variety of chemicals in several environmental settings (14, 21–23). This research has primarily focused on direct adaptation to a single chemical. Relatively few papers have addressed the issue of coadaptation or the impact of exposure to a particular chemical on the biodegradation of other materials (9, 12, 17).

In previous publications (18, 19), we described the effect of readily biodegradable substrates (such as amino acids) and natural aquatic humic acids on the biodegradation of monosubstituted phenols. In this study, the influence of a structurally related material (phenol) on the biodegradation of *m*-creso, *m*-aminophenol, and *p*-chlorophenol is described. Studies were conducted on microbial communities from aquatic microcosms which were exposed to phenol for an extended period.

MATERIALS AND METHODS

Details of the adaptation and biodegradation methods employed in the study are described elsewhere (18). The following description provides an overview.

Adaptation to phenol was performed in continuous-flow, completely mixed microcosms containing water from Lake Michie, a relatively unpolluted mesotrophic reservoir near Durham, N.C. The microcosms had a relatively low dilution rate with a hydraulic residence time of approximately 3 days and were fed a solution of filter-sterilized (0.2-μm-pore-size filter), distilled, deionized water, inorganic nutrients at levels approximating those in Lake Michie, and the test chemical.

* Corresponding author.

Three microcosms were fed a solution containing phenol (1 mg/liter) as the sole carbon source. Two control microcosms, neither receiving phenol, were also used. One received filter-sterilized Lake Michie water (Lake Michie control), and the other received sterile feed solution containing no added organic substrate (nutrient control).

After an adaptation period of approximately 5 to 7 residence times (2 to 3 weeks), the water was removed from the microcosms and the microbial community of each microcosm was tested for its ability to biodegrade the radiolabeled monosubstituted phenols *m*-cresol, *m*-aminophenol, and *p*-chlorophenol. These radiolabeled substrates were obtained from Amersham Corp., Arlington Heights, Ill., and were more than 97% pure as determined by the supplier by gas chromatography.

A modification of the heterotrophic potential method described by Pfaender and Bartholomew (13) was used to measure biodegradation. Both respiration and net uptake of material into the cell biomass were measured over the following concentration ranges of the added radiolabeled test chemical: *m*-cresol and *p*-chlorophenol, 1 to 110 μg/liter; and *m*-aminophenol, 1 to 1,200 μg/liter. Total uptake was the sum of respiration and net uptake. In addition, the following microbiological parameters were assessed in the microbial community taken from the microcosms: the total size of the microbial community, measured by acridine orange direct counts (7); the general metabolic activity of the community, measured by ^{3}H-amino acid turnover time (3); and the number of specific degraders of the three test chemicals, counted in sequential fourfold dilutions of samples from the microcosms (11). Similar measurements were also performed on the original Lake Michie community placed in the microcosms.

Rate data from the biodegradation assay were analyzed with the Hanes-Wolff linearization of the Michaelis-Menten kinetic model or with a simple first-order model (10). The Hanes-Wolff method estimates kinetic parameters V_{max} and K_m from the slope and y intercept of the Hanes-Wolff plot. At very low levels, the Michaelis-Menten model becomes essentially first order. Thus, from estimates of V_{max} and K_m, a first-order rate constant k_1, was calculated as V_{max}/K_m. If

saturation kinetics were not obtained over the range of tested concentrations and if the Hanes-Wolff line had an r^2 of less than 0.85, the simple first-order model was fitted to the rate data and k_1 was estimated as the slope of the first-order line.

Estimates of kinetic parameters were statistically compared by using the small-sample t test for the difference between the slopes of two straight lines (8). When the Michaelis-Menten model was appropriate, the slopes of the Hane-Wolff lines ($1/V_{max}$) were compared. When the first-order model was used, the values of k_1 were compared. No cross comparisons were made between the two models.

RESULTS

Adaptation of the Lake Michie microbial community to phenol as a sole carbon source led to an increase in the ability of the community to degrade *m*-cresol, *m*-aminophenol, and *p*-chlorophenol (Fig. 1). The response of the control microcosm communities to each test chemical remained near that of the initial Lake Michie community. In the phenol microcosms, monosubstituted phenol biodegradation was markedly increased relative to that of either the initial community or the control systems.

Both *m*-cresol and *p*-chlorophenol followed first-order kinetics over the range of concentrations tested. *m*-Aminophenol followed Michaelis-Menten kinetics in the phenol-adapted communities and first-order kinetics in the control and original samples. Estimates of the appropriate kinetic parameters are shown in Table 1. Statistical comparisons of the data demonstrated that the phenol-adapted microbial communities degraded each monosubstituted phenol at significantly higher rates than did communities which were not exposed to phenol. P values between phenol and control parameters were significant ($P < 0.05$) in all cases. Statistical analysis of the *m*-aminophenol data was not possible since the appropriate kinetic models differed in the test and control microcosms. However, Fig. 1C clearly shows a marked increase in the ability of the phenol-adapted community to degrade *m*-aminophenol relative to that of the controls.

The results of microbiological measurements on the microcosm communities are shown in Table 2. Exposure to phenol had little impact on the total size of the microbial community in the microcosms. Acridine orange direct counts were on the order of 10^6 cells per ml in all six microcosms, including the Lake Michie and nutrient controls. The turnover time of amino acid mixtures was more rapid in the six microcosms than in the initial community, but there was little difference in turnover times between microcosms. However, exposure increased the most probable number of microorganisms in the community capable of biodegrading *m*-cresol, *m*-aminophenol, and *p*-chlorophenol by at least a factor of 10 relative to that of the initial and control communities.

DISCUSSION

Extended exposure of the Lake Michie microbial community to phenol in the microcosms increased the ability of the community to biodegrade *m*-cresol, *m*-aminophenol, and *p*-chlorophenol. This increase was manifested by a corresponding increase in the number of specific degraders of the three test chemicals. Thus, the response to phenol was a chemical-specific phenomenon increasing the size and total activity of the community of organisms degrading the three

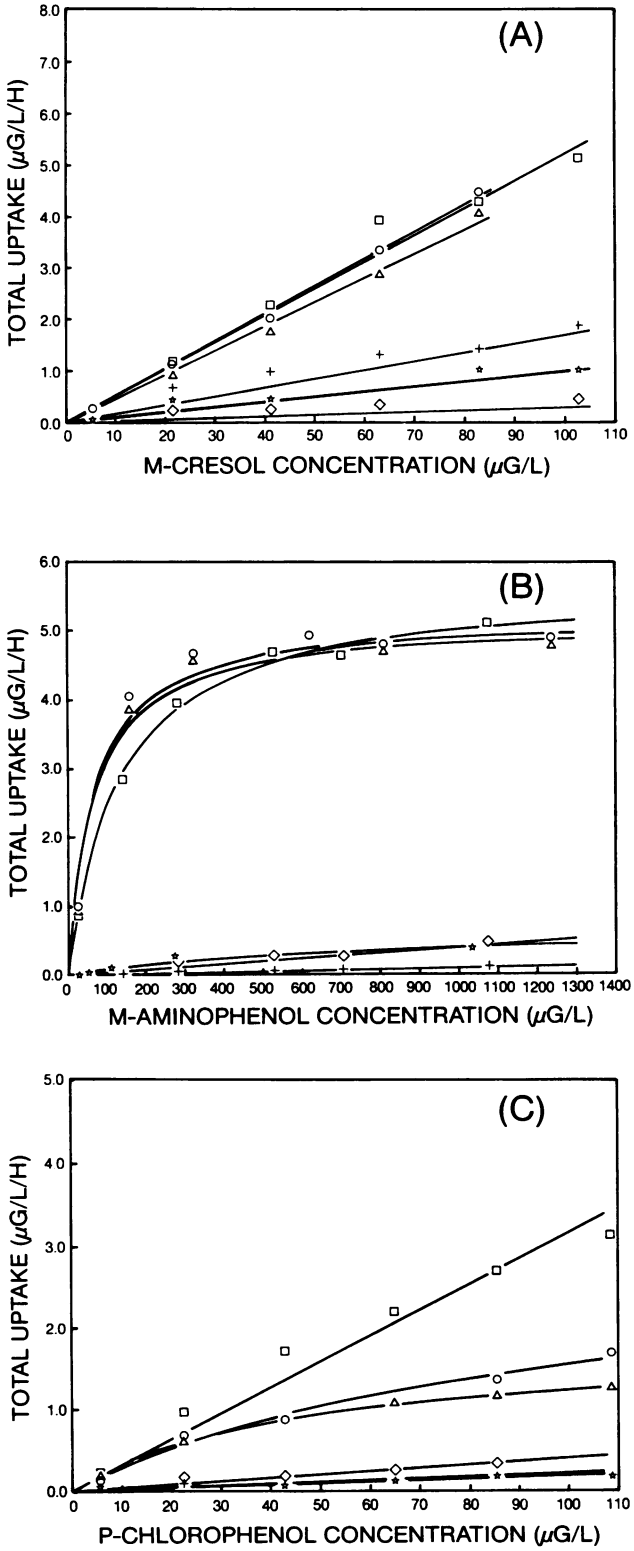

FIG. 1. Total uptake of (A) *m*-cresol, (B) *m*-aminophenol, and (C) *p*-chlorophenol by Lake Michie microbial communities adapted to 1,000 μg of phenol per liter in microcosms. Microcosm symbols: ○, phenol 1; △, phenol 2; □, phenol 3; ☆, initial community; +, Lake Michie control, receiving only sterile Lake Michie water; ◇, nutrient control, receiving only sterile unsupplemented inorganic-nutrient solution.

TABLE 1. Total-uptake kinetic-model estimates for the biodegradation of monosubstituted phenols in phenol-adapted and control microcosms

Test chemical and microcosm	V_{max} (μg/liter per h)	K_m (μg/liter)	k_1 (per h)[a]
m-Cresol			
Phenol 1			0.053
Phenol 2			0.047
Phenol 3			0.052
Initial community			0.010
Lake Michie control			0.017
Nutrient control			0.003
m-Aminophenol			
Phenol 1	5.21	64	0.081
Phenol 2	5.13	66	0.078
Phenol 3	5.68	133	0.042
Initial community	0.75	860	0.0009
Lake Michie control			0.0001
Nutrient control			0.0004
p-Chlorophenol			
Phenol 1	3.06	96	0.031
Phenol 2	1.78	44	0.040
Phenol 3			0.031
Initial community			0.002
Lake Michie control			0.002
Nutrient control			0.004

[a] When the Michaelis-Menton model was used to fit the biodegradation data, k_1 was estimated as V_{max}/K_m.

monosubstituted phenols. There was relatively little effect on either the total size of the microbial community or its general metabolic activity. Changes observed in the turnover of amino acids in the microcosms relative to that in the initial community were likely due to confinement effects (6). The presence of a stable and active microbial community in the control systems was probably due to confinement and the presence of background organic carbon in feed solutions even in the unsupplemented nutrient solution. A variety of microbial species have been shown to survive for extended periods on very low (microgram-per-liter) levels of organic carbon in both marine and fresh waters (2, 16, 20).

The ability of phenol to affect the biodegradation of related phenolic compounds might be surmised from the structural similarity of the materials and common elements within their biodegradation pathways. Aromatic biodegradation may follow several metabolic pathways which ultimately result in *ortho* or *meta* fission of an intermediate metabolite (e.g., catechol) (5). However, structurally similar aromatic chemicals could have similar cell membrane transport systems or similar catabolic pathways after ring fission. Thus, it is not unreasonable to expect prolonged exposure to phenol to increase the ability of mixed microbial communities to biodegrade *m*-cresol, *m*-aminophenol, and *p*-chlorophenol. This phenomenon has not, however, been previously demonstrated for such materials.

TABLE 2. Microbiological parameters measured in phenol-adapted and control microcosms

Microcosm	Total no. of cells (10^6/ml)	Amino acid turnover time (h)	Most probable number (10^3 cells/ml)[a]		
			CH_3	NH_2	Cl
Phenol 1	8.14	1.16	0.72	0.33	5.31
Phenol 2	2.29	0.89	1.32	0.66	15.9
Phenol 3	5.29	0.82			
Initial community	4.98	16.27	0.05	0.008	0.99
L. Michie control	1.45	3.11	0.008	0.002	0.09
Nutrient control	7.19	0.65	0.09	0.003	0.29

[a] CH_3, *m*-Cresol; NH_2, *m*-aminophenol; Cl, *p*-chlorophenol.

Adaptation to phenol not only increased the biodegradation of the three monosubstituted phenols relative to unadapted rates, but it also resulted in relatively similar adapted biodegradation rates for the three chemicals. In soil microbial communities (1) and activated sludge (15), biodegradation of the three monosubstituted phenols has been shown to have the following relationship: *m*-cresol > *p*-chlorophenol > *m*-aminophenol. Similar results were obtained for the initial Lake Michie community (Table 1). The initial first-order rate constant for *m*-cresol was 5-fold greater than that of *p*-chlorophenol and 12-fold greater than that of *m*-aminophenol. However, after adaptation to phenol, the Lake Michie microbial community was able to degrade *m*-cresol and *m*-aminophenol at roughly comparable rates and *p*-chlorophenol at rates approximately two times lower (Table 1).

Characterization of the phenol-adapted Lake Michie microbial community demonstrated that adaptation to phenol increased the proportion of *m*-cresol, *m*-aminophenol, and *p*-chlorophenol degraders in the microbial community. Adaptation to phenol was probably a growth-related phenomenon. Most-probable-number estimates generally increased more than 15-fold relative to levels in the initial community and the two control microcosms. The absolute numbers of degraders for each of the three test chemicals were similar, varying from a low of 330 (most probable number) per ml for *m*-aminophenol to 1,590 (most probable number) per ml for *p*-chlorophenol. As judged by the published intrinsic accuracy of fourfold-dilution data from the most-probable-number method (4), these variations are not different. Thus the most-probable-number data are consistent with the approximately equal biodegradation rate constants for each of the three phenols in the adapted Lake Michie communities.

The results of this research illustrate the versatility of mixed microbial communities during their response to chemicals. On exposure to phenol, the Lake Michie microbial community shifted its distribution of organisms towards those species with the ability to degrade a variety of phenolic materials. Adaptation to phenol induced a general increase in the ability of the community to degrade related aromatic chemicals. Such a response may have important implications for assessing the fate of complex mixtures containing a variety of structurally related chemicals. The ability of the community to adapt to a single substrate (e.g., phenol) may in turn increase the capacity of an environment to assimilate other structurally related compounds even in the absence of adaptation-inducing levels of those materials.

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