Influence of Easily Degradable Naturally Occurring Carbon Substrates on Biodegradation of Monosubstituted Phenols by Aquatic Bacteria

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The influence of readily degradable, naturally occurring carbon substrates on the biodegradation of several monosubstitued phenols (*m*-cresol, *m*-aminophenol, *p*-chlorophenol) was examined. The natural substrate classes used were amino acids, carbohydrates, and fatty acids. Samples of the microbial community from Lake Michie, a mesotrophic reservoir, were adapted to different levels of representatives from each natural substrate class in chemostats. After an extended adaptation period, the ability of the microbial community to degrade the monosubstituted phenols was determined by using a radiolabeled substrate uptake and mineralization method. Several microbiological characteristics of the communities were also measured. Adaptation to increasing concentrations of amino acids, carbohydrates, or fatty acids enhanced the ability of the microbial community to degrade all three phenols. The stimulation was largest for *m*-cresol and *m*-aminophenol. The mechanism responsible for the enhancement of monosubstituted phenol metabolism was not clearly identified, but the observation that adaptation to amino acids also increased the biodegradation of glucose and, to a lesser extent, naphthalene suggests a general stimulation of microbial metabolism. This study demonstrates that prior exposure to labile, natural substrates can significantly enhance the ability of aquatic microbial communities to respond to xenobiotics.

A wide variety of factors can influence the biodegradation rates of organic pollutants in aquatic environments. These include the structure of the pollutant, temperature, salinity, pH, and the availability of inorganic nutrients and oxygen (2). Another important factor may be the organic materials to which the microbial community in a given environment has been exposed.

The types of organic materials a microbial community has encountered in the past can play an important role in determining the response of the community to new compounds. For example, previous exposure to an organic compound will often increase the rate at which it can be degraded by a microbial community. This has been demonstrated with both pollutants (10, 24) and natural substrates (13, 26). However, very little research has been conducted which has characterized the effects of exposure to organic substrates other than the compound of interest on the rates of degradation of that compound.

Except in highly polluted environments, the organic exposure history of most aquatic microbial communities is dominated by naturally occurring substrates. These natural materials are all ultimately derived from living organisms and come from three major sources: phytoplankton, higher plants, such as epiphytes and macrophytes, and allochthonous runoff from surrounding terrestrial ecosystems (19, 28). They consist primarily of the "building blocks" of all organisms, namely, amino acids, carbohydrates, and fatty acids. These three classes of materials are quite labile and are rapidly assimilated by aquatic microbial communities (23).

Most aquatic environments also contain significant quantities of humic materials, which are believed to result from

the complexation of the "leftovers" of microbial activities on lignin and other compounds (7). In general, these materials are biologically quite stable (1).

We have examined how the adaptation of samples of a natural aquatic microbial community to representatives of the easily degradable natural substrate classes (amino acids, carbohydrates, and fatty acids) affects the ability of the adapted bacteria to degrade three monosubstituted phenols, *m*-cresol, *m*-aminophenol, and *p*-chlorophenol. For the purpose of this work, the term "adaptation" refers to the process of long-term exposure of a microbial community to carbon substrates which causes the community to respond in such a way that it becomes acclimated to the new conditions. This definition does not imply that adaptation to a new substrate will necessarily enhance any metabolic function within the community, such as biodegradation of phenols. It simply refers to the process of exposure and subsequent response.

Samples of the microbial community were adapted to the selected natural substrates in completely mixed, long-residence-time chemostats. After an extended period, the adapted communities were removed from the chemostats and tested for their ability to degrade *m*-cresol, *m*-aminophenol, and *p*-chlorophenol. Several microbiological parameters were also measured on the adapted microbial communities.

MATERIALS AND METHODS

Study environment and sampling procedure. Lake Michie, a mesotrophic reservoir near Durham N.C., was chosen as the sample site because it is relatively unpolluted. There are no significant industrial sites near the lake and small amounts of agricultural runoff. Thus, it is unlikely that its microbial community has been exposed to significant levels of the three monosubstituted phenols.

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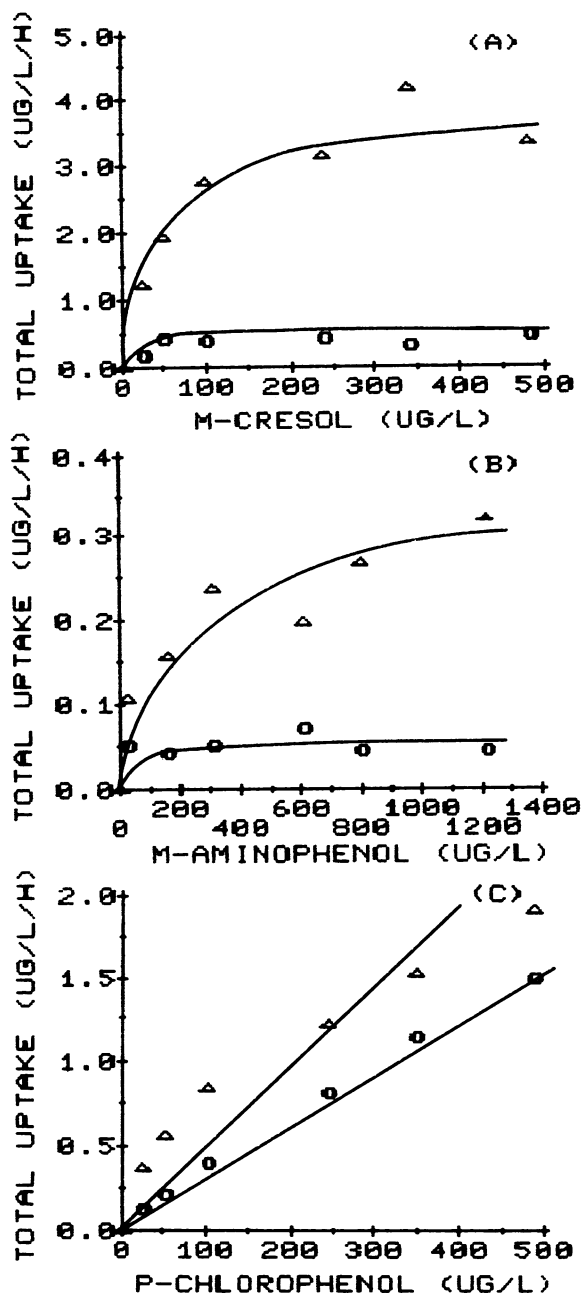
FIG. 1. Influence of adaption to amino acids on total uptake of (A) m-cresol, (B) m-aminophenol, and (C) p-chlorophenol from the natural substrate screening study. Symbols: O, 100 μg of amino acids per liter; △, 1,000 μg of amino acids per liter.

Grab samples of surface lake water were taken in 10-liter, acid-washed, polyethylene carboys. The water was filtered through a 5.0-μm Nuclepore filter (Nuclepore Corp., Pleasanton, Calif.) to remove phytoplankton and debris which could contribute carbon to the chemostats. This filtration was achieved by using a Pellicon tangential flow filtration system (Millipore Corp., Bedford, Mass.).

Chemostat system. The chemostats used in the study were continuous-flow, completely mixed (magnetic stirrer) devices. Each chemostat contained 1.5 liters of culture and was fed at a flow rate of 0.5 liter/day via a constant-head gravity feed system. This yielded a residence time of 3 days

($D = 0.014$ h^{-1}). The flow rate into each unit was regulated by gravity flow through a syringe needle. Chemostats were maintained at a constant temperature of 20 ± 1°C.

Composition of feed solutions. The solution fed to each chemostat was sterilized via filtration through a sterile rinsed 0.2-μm Gelman GA-8 filter (Gelman Sciences, Inc., Ann Arbor, Mich.). Filtration was used since it removes bacterial cells from the feed solution which could contribute additional carbon to the chemostats.

Each feed solution consisted of distilled, deionized, carbon-filtered water (Corning Megapure still and LD-5A demineralizer system; Corning Glass Co., Corning, N.Y.) which, after sterilization, was supplemented with inorganic nutrients and the desired natural carbon substrate.

The following inorganic ions were added to the feed: 0.024 mg of PO_4 P, 0.104 mg of NH_4 N, 0.254 mg of NO_3 N, 2.2 mg of calcium, 1.8 mg of potassium, 0.61 mg of iron, 5.13 mg of chloride and 1.05 mg of sulfate per liter and trace levels of magnesium, copper, and manganese. This mineral composition is based on an average of measured values for North Carolina Piedmont lakes (27).

The natural substrates selected for use in the adaptation studies were chosen on the basis of the structural composition and extracellular excretions of phytoplankton. We selected those compounds which appear repeatedly in studies of algal composition and extracellular products cited by Raymont (21). In our judgement, these compounds are amino acids, glutamic acid, alanine, lysine, and aspartic acid; carbohydrates; glucose, glycolate, mannose, and cellobiose; fatty acids; and butyric acid and acetate.

Adaptation and sampling. After Pellicon filtration, 1.5-liter samples of the lake water samples were placed in the chemostats and fed the desired natural substrate for a period of five to seven residence times. During this period, the chemostats were routinely sampled to monitor the stability of the microbial community by measuring its size and activity.

To assess the impact of a particular labile natural substrate class on the ability of the adapted microbial communities to degrade the pollutants, the concentration of the natural substrate fed to the chemostats was varied. Varying the concentration of the natural substrates fed to the chemostats represents the only method which can determine the impact of a particular substrate while simultaneously controlling for extraneous factors which result from the adaptation process itself. These include the general effects of containment in the culture vessel on the activity of the microbial community (8) and changes in the environment which are not related to the type of substrate fed to the chemostat, such as temperature.

The range of concentrations of natural substrates fed to the community in the chemostats was 100 to 1,000 μg/liter. This range was chosen to approximate the levels of labile substrates which occur in natural systems (29).

After the adaptation period, the communities were removed from the chemostats and tested for the following parameters: the total size of the community, the general metabolic activity of the community, the number of specific degraders of the three test pollutants (m-cresol, m-aminophenol, and p-clorophenol), and the biodegradation rates of the test pollutants. Each of these methods is described below.

Procedures. The total size of the bacterial community was determined with the acridine orange direct count microscopic method (11), using a Leitz Ortholux fluorescence microscope equipped with a Ploem illuminator. CFU were also measured for some studies to determine the number of

TABLE 1. Total uptake kinetic parameter estimates for the natural substrate screening study

Chemostat (μg/liter)	m-Cresol			m-Aminophenol			p-Chlorophenol	
	V_{max} (μg/liter per h)	K_m (μg/liter)	r^2	V_{max} (μg/liter per h)	K_m (μg/liter)	r^2	K_1 (h^{-1})	r^2
Amino acids								
100	0.42	23	0.94	0.05	7	0.90	0.0030	0.99
1,000	3.97	49	0.96	0.33	168	0.91	0.0041	0.94
Carbohydrates								
100	0.37	65	0.98	0.03	17	0.98	0.0025	0.99
1,000	1.20	38	0.93	0.18	168	0.83	0.0030	0.98
Fatty acids								
100	0.32	32	0.99	0.02	3	0.91	0.0029	0.99
1,000	2.73	51	0.92	0.46	148	0.98	0.0031	0.95

culturable bacteria in the community (5). The plating media contained 1/10-strength tryptic soy agar and Bacto-Agar (Difco Laboratories, Detroit, Mich.) dissolved in glass-fiber-filtered Lake Michie water.

All of the radioactivity measurements described below were performed on a Packard Tri-Carb 300CD liquid scintillation counter (Packard Instruments Co., Rockville, Md.). The sample/channels ratio standardization was used to convert from counts per minute to disintegrations per minute.

The general metabolic activity of the adapted microbial community was measured with the tritiated amino acids uptake and turnover time method (4), using a trace amount of ^{3}H-labeled amino acids formulated to mimic a phytoplankton hydrolysate (New England Nuclear Corp., Boston, Mass.).

The number of specific degraders of the test pollutants was determined with a most-probable-number (MPN) method (modified after reference 18). Five replicate 1-ml samples from each of a series of fourfold sample dilutions from each chemostat were spiked with approximately 10,000 dpm of ^{14}C-labeled test pollutant. The replicates were incubated in small shell vials placed in empty scintillation vials for 7 days in the dark. After incubation, the samples were acidified, and labeled CO_2 was trapped in 1 N KOH added to the scintillation vial. Tubes were scored as positive if the counts were twofold higher than control samples killed with NaN$_3$ (final concentration, 0.01%) (3). Calculations of MPN were made from tables in Fisher and Yates (9).

The method used to measure biodegradation rates has been previously described (20). Briefly, six different concentrations of ^{14}C-labeled test pollutants were added to 20-ml samples of water from the chemostats. Replicate samples were incubated at 20°C for 8 to 12 h. After incubation, two of the replicates at each concentration were filtered through 0.2-μm filters and rinsed with 50% ethanol to provide a measure of net uptake of the pollutant into cells. Two of the other replicates were acidified, and radiolabeled CO_2 was trapped in 1 N KOH to determine the amount of respiration of the compound. Background levels of abiotic uptake and respiration were determined by killing two samples at each concentration with NaN$_3$ and treating as described above.

Each of the radiolabeled pollutants used in the study was obtained from Amersham Corp. (Arlington Heights, Ill.). They were all >97% pure as determined by the supplier, using gas chromatography. For use in the study, the specific activity of each of the isotopes was reduced to workable levels by dilution with unlabeled compound (99+% gold label; Aldrich Chemical Co., Milwaukee, Wis.). The final concentration ranges of each pollutant used in the biodegradation assay were the following: m-cresol, 0 to 500 μg/liter;

m-aminophenol, 0 to 1,200 μg/liter; p-chlorophenol, 0 to 500 μg/liter.

Data analysis for the pollutant metabolism method was based on the Michaelis-Menten enzyme kinetics model. The Hanes-Wolff linearization of the model was used to estimate the kinetic parameters V_{max} (the maximum uptake rate) and K_m (the half-saturation constant) from the model:

$$\frac{t}{f} = \left(\frac{1}{V_{max}}\right)S + \frac{K_m}{V_{max}}$$

where t is the incubation time, f is the fraction of added label metabolized, and S is the concentration of added substrate.

If saturation kinetics were not obtained and the Hanes-Wolff line had an r^2 of <0.85, a first-order linear model was fitted to the data. K_1 represents the first-order rate constant for this model.

Estimates of the kinetic parameters from the chemostats were compared by using the small sample t-test for the difference between the slopes of two straight lines (15). When the Michaelis-Menten model was used, the slopes of two Hanes-Wolff lines ($1/V_{max}$) were compared. When the first-order model was used, values of K_1 were compared. There were never any cross comparisons between the two models.

RESULTS

Screening study. The first adaptation study conducted was a screening of all three of the easily degradable natural

TABLE 2. Microbiological parameters for the natural substrate screening study

Chemostat (μg/liter)	Total cells (× 10^6 cells per ml)	AA t/f (h)^a	MPNs (× 10^3 cells per ml)		
			−CH$_3$	−NH$_2$	−Cl
Amino acids					
100	2.17	0.57	2.64	0.07	5.28
1,000	2.04	1.01	3.48	0.04	10.5
Carbohydrates					
100	1.18	1.02	0.22	0.13	10.5
1,000	1.54	0.95	0.71	0.01	>16.5
Fatty acids					
100	1.47	1.24	0.40	0.01	10.5
1,000	1.52	1.03	0.30	0.46	0.16

^{a} AA t/f, Amino acids turnover times.
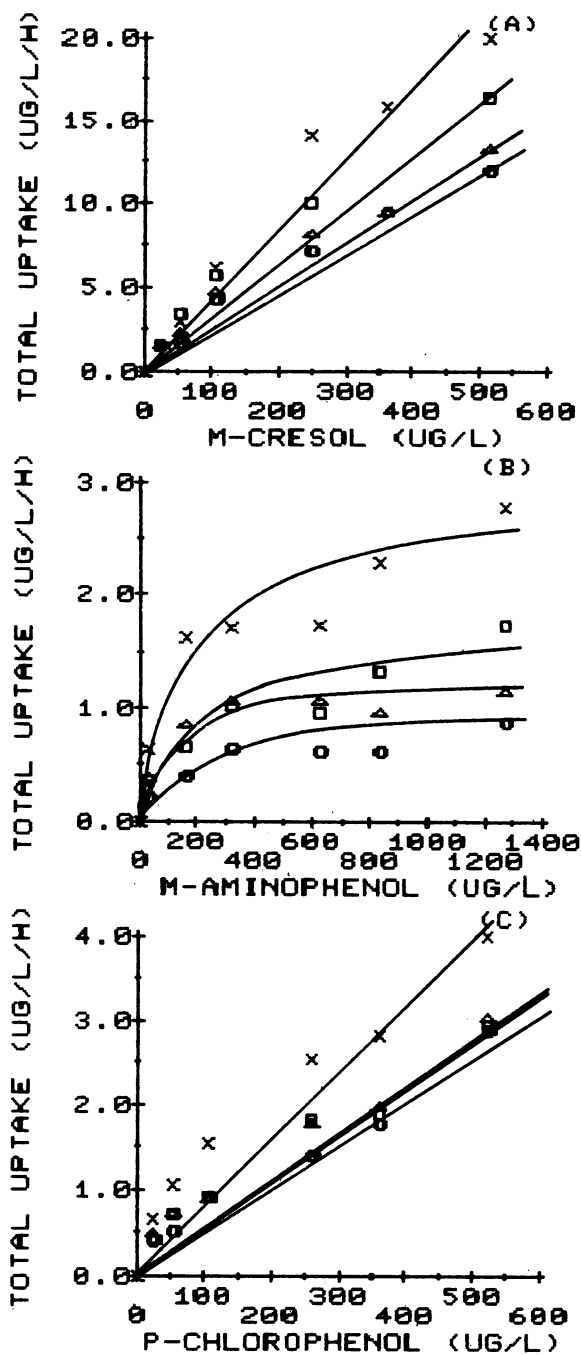
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FIG. 2. Total uptake of (A) m-cresol, (B) m-aminophenol, and (C) p-chlorophenol by microbial communities adapted to amino acids in the detailed concentration study. Symbols: amino acids at ○, 100 μg/liter; △, 300 μg/liter; □, 600 μg/liter; ×, 1,000 μg/liter.

TABLE 4. Microbiological parameters for the detailed concentration study of amino acids

Chemostat (amino acids, μg/ liter)	Total cells (× 10⁶ cells per ml)	AA t/f (h)[a]	MPNs (× 10³ cells per ml)			CFU (× 10⁵ cells per ml)
			CH₃	NH₂	Cl	
100	3.20	0.61	15.8	>4.1	6.37	2.53
300	1.60	1.09	7.42	>4.1	15.6	1.76
600	3.41	0.85	10.6	>4.1	42.3	1.84
1,000	3.45	0.67	>16.4	1.33	15.6	3.52

[a] AA t/f, Amino acids turnover times.

substrate classes. The chemostats were divided into three pairs, one set for each class of natural substrates. Each chemostat within a pair received either 100 or 1,000 μg of its respective natural substrate class per liter. Each of the constituents within each class were present in the feed solutions in equal amounts.

Representative results from the screening study are shown in Fig. 1. The data presented are for adaptation to amino acids. The response to carbohydrates or fatty acids was similar to the amino acids (data not shown). Estimates of the monosubstituted phenol degradation parameters for all three classes are shown in Table 1. Total uptake of m-cresol increased as the concentration of the amino acids was increased from 100 to 1,000 μg/liter (Fig. 1A). The differences between the estimates of m-cresol V_{max} (Table 1) for amino acids, carbohydrates, and fatty acids were highly significant ($P < 0.001$). In the cases of amino acids and fatty acids, the estimates of V_{max} from the 1,000-μg/liter chemostats were nearly ten times higher than those from the lower-concentration units. V_{max} estimates for carbohydrates were approximately five times greater at the high natural substrate concentration.

The results of the amino acid adaptation for m-aminophenol uptake were similar to those of m-cresol (Fig. 1B). The total uptake of m-aminophenol was substantially higher in communities from the chemostats receiving 1,000 μg of amino acids per liter compared with those receiving 100 μg/liter. Statistical comparisons of the estimates of m-aminophenol V_{max} from rate plots of all three substrates showed that the level of V_{max} increased significantly ($P < 0.001$) as the level of each of the labile substrates fed to the communities was increased.

p-Chlorophenol uptake by the communities adapted to amino acids was not as affected by the natural substrates as the uptake of the other two test pollutants, over the range of p-chlorophenol levels tested (Fig. 1C). Similar lack of effect was observed for carbohydrates and fatty acids. Saturation did not occur over the concentration range tested, so estimates of K_1 were made for the first-order model. Saturation probably failed to occur because the concentration range used for p-chlorophenol was not high enough. Estimates of the first-order rate constant did increase as the level of each

TABLE 3. Total uptake kinetic parameter estimates for the detailed concentration study of amino acids

Chemostat (amino acids, μg/liter)	m-Cresol			m-Aminophenol			p-Chlorophenol	
	K_1 (h⁻¹)	K_m (μg/liter)	r^2	V_{max} (μg/liter per h)	K_m (μg/liter)	r^2	K_1 (h⁻¹)	r^2
100	0.024	—[a]	0.98	0.88	185	0.92	0.0052	0.98
300	0.026	—	0.98	1.15	61	0.99	0.0056	0.97
600	0.033	—	0.97	1.86	287	0.87	0.0054	0.97
1,000	0.042	—	0.98	2.90	194	0.92	0.0076	0.96

[a] —, Not determined.

of the natural substrates was increased (Table 1). However, the differences between the estimates of K_1 were significant only for amino acids ($P = 0.015$).

Examination of the microbiological data (Table 2) shows that none of the microbiological parameters measured could account for the differences in test pollutant uptake observed. Both acridine orange direct counts and amino acids turnover times were relatively the same for each pair of chemostats. Estimates of the number of specific degraders (MPNs) varied widely. However, there were no consistent changes in MPNs which could account for the generally marked increase in pollutant uptake, particularly m-cresol and m-aminophenol, as the level of natural substrate fed to the chemostats was increased.

Detailed study of amino acids. Based on the results from the screening study, amino acids were selected for further work. In this experiment, four different levels of amino acids (100, 300, 600, and 1000 μg/liter) were fed to four chemostats. The response of the microbial communities adapted to the four different levels of amino acids (Fig. 2) was very similar to that in the screening study. As the level of amino acids was increased, the uptake rates of all three pollutants increased. Estimates of the kinetic parameters for the three pollutants are shown in Table 3. Note that both m-cresol and p-chlorophenol did not exhibit saturation kinetics at the levels tested, so first-order rate constants were estimated for that data. As the level of amino acids was increased, the estimates of K_1 or V_{max} for m-cresol or m-aminophenol, respectively, increased steadily. With the exception of the increase from 100 to 300 μg/liter, the increases in the estimates were significant ($P < 0.05$). Estimates of K_1 for p-chlorophenol uptake were essentially constant at the three lowest concentrations of amino acids. However, the value at 1,000 μg/liter was significantly higher than the values at the other three concentrations of amino acids (P ranged from <0.001 to 0.004).

The microbiological data from this experiment did not identify a community-level mechanism for the enhancement of pollutant uptake by amino acids (Table 4). Correlations of each of the microbiological parameters against either K_1 or V_{max} are shown in Table 5. There were no substantial correlations among acridine orange direct counts, amino acids turnover times, or MPNs. The correlations between plate counts (CFU) and the kinetic parameters were higher than those with the other parameters, but, with a data base of only four observations, these r values were not significant ($P > 0.20$).

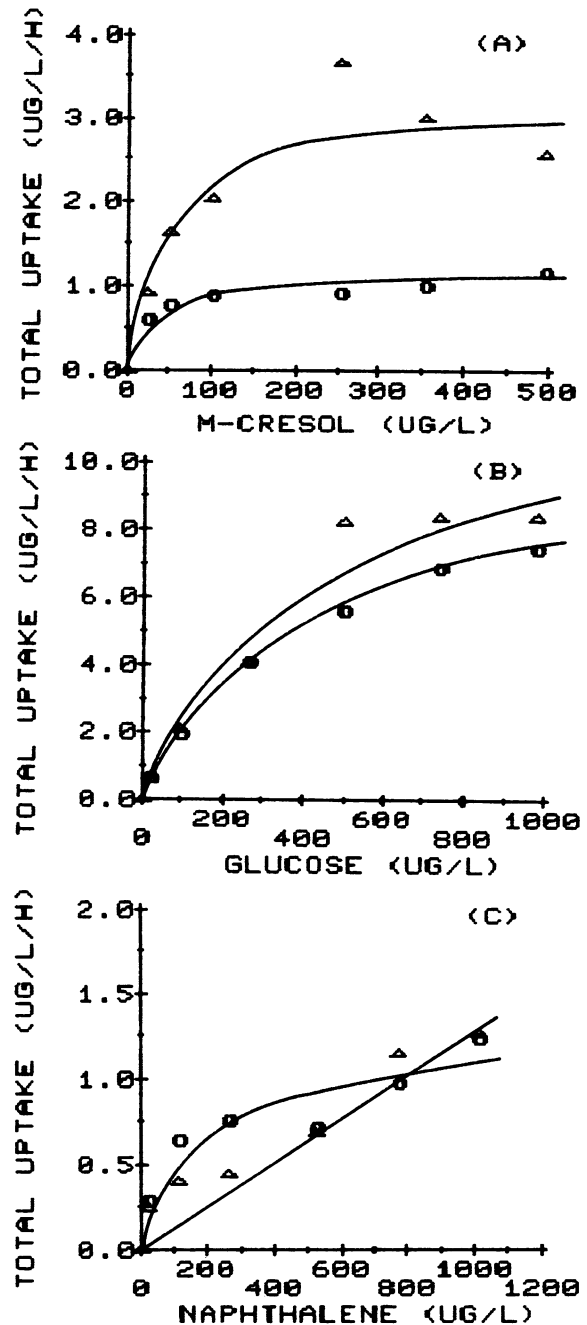**Effect of adaptation to amino acids on metabolism of other compounds.** In an additional study, the effect of adaptation

FIG. 3. Total uptake of (A) m-cresol, (B) glucose, and (C) naphthalene by communities adapted to amino acids, experiment 1. Symbols: O, 100 μg of amino acids per liter; △, 1,000 μg of amino acids per liter.

to amino acids on the metabolism of other, structurally diverse, organic compounds was examined. This experiment was performed to determine if the enhancing effect of labile natural substrates was the result of enhancements of the overall metabolic activity of the adapted community rather than the specific enhancement of monosubstituted phenol metabolism.

Samples of the Lake Michie microbial community were adapted to two levels of amino acids, 100 and 1,000 μg/liter. After adaptation, the communities were tested for their

TABLE 5. Correlation coefficients (r) of kinetic estimates with microbiological parameters from the detailed concentration study

Parameter[a]	M-Cresol, K_1 (h^{-1})	m-Aminophenol, V_{max} (μg/liter per h)	p-Chlorophenol K_1 (h^{-1})
AODC	0.531	0.506	0.291
AA t/f	−0.276	−0.26	−0.279
MPN			
−CH$_3$	0.394		
−NH$_2$		—[b]	
−Cl			−0.15
CFU	0.677	0.68	0.839

[a] AODC, Acridine orange direct count; AA t/f, amino acids turnover times.
[b] —, Not determined.

TABLE 6. Total uptake kinetic parameter estimates for the study of the effect of amino acids adaptation on microbial metabolism of several nonphenolic compounds

Chemostat (amino acids, μg/liter)	m-Cresol			Glucose			Naphthalene		
	V_{max} (μg/liter per h)	K_m (μg/liter)	r_2	V_{max} (μg/liter per h)	K_m (μg/liter)	r_2	V_{max} (μg/liter per h)	K_m (μg/liter)	r_2
Expt 1									
100	1.15	39	0.99	10.41	407	0.99	1.22	158	0.90
1,000	3.03	36	0.94	13.69	525	0.92	0.001[a]		0.94
Expt 2									
100	1.29	53	0.99	5.27	351	0.98	0.92	176	0.94
1,000	2.95	19	0.99	9.18	159	0.94	1.18	142	0.98

[a] Value reported is first-order rate constant, K_1 (hour^{-1}).

ability to degrade glucose and naphthalene, as well as m-cresol. Two identical experiments were performed.

The results of the first experiment are shown in Fig. 3. Estimates of the kinetic parameters are shown in Table 6. Only the uptake of m-cresol was significantly enhanced by adaptation to increasing concentrations of amino acids. Glucose uptake was somewhat increased ($P = 0.07$, comparison of V_{max}), but naphthalene uptake was not affected by the adaptation to amino acids, at least over the range of naphthalene concentrations used in the biodegradation assay.

The results of the second experiment were somewhat different (Fig. 4, Table 6). The uptake of all of the test compounds increased as the level of amino acids was increased. However, the comparisons of V_{max} were not statistically significant for naphthalene ($P = 0.15$). Significant differences in the estimates of V_{max} were observed for glucose ($P = 0.002$) and m-cresol ($P < 0.001$).

DISCUSSION

The presence of labile, naturally occurring carbon sources can have a significant impact on the ability of microbial communities to degrade pollutants. In general, adaptation to increasing concentrations of amino acids, carbohydrates, or fatty acids enhanced the ability of the Lake Michie microbial community to degrade all three of the monosubstituted phenols (Fig. 1 and 2). The enhancement was largest for m-cresol and m-aminophenol. However, significant increases were also observed for p-chlorophenol.

The enhancement of the uptake of the test pollutants was qualitatively the same for each of three classes of natural substrates. Whereas the magnitude of the enhancements varied, the basic trends were very similar. Thus it seems likely that the results observed are due to the generally labile nature of the natural substrates used, and not to any particular biochemical relationship between a single class and the three monosubstituted phenols.

The ability of easily degradable substrates to enhance specific microbial activities has been observed by other researchers. Law and Button (17) found that the addition of amino acids to cultures of a marine coryneform, using glucose as their main carbon source, significantly reduced the steady-state levels of glucose present in the culture vessel. Their results indicate that there is a distinct interrelationship between the various metabolic pathways. Thus, the metabolism of one particular substrate might have a marked effect on the uptake of another apparently unrelated compound. Such an idea is consistent with our observation that labile natural substrates enhance monosubstituted phe-

nol uptake. It should be noted, however, that Law and Button's (17) work dealt with simultaneous exposure. Our work concerned the adaptation to one substrate followed by exposure to another; thus the mechanisms may not be directly comparable.

The process of cometabolism is an important example of the influence readily degradable secondary carbon substrates can have on the biodegradation of pollutants. In cometabolic processes, a compound which is normally stable and incapable of supporting bacterial growth can be partially degraded, but not used for carbon or energy, when a readily degradable secondary source of carbon is available (14). The secondary carbon source is thought to provide the carbon and energy required for the alteration of the parent compound.

Cometabolism does not include any mineralization of the parent compound, only an alteration in structure to some intermediate. Ecologically, however, the cometabolic products of one organism may be completely utilized by other bacteria which cannot use the intact parent molecule. Although this is only an hypothesis, it is possible that in our chemostats a readily utilizable carbon source, such as amino acids, could enhance the ability of a mixed aquatic community to degrade monosubstituted phenols by supplying the energy and carbon needed to permit degradation of the phenols by one group of organisms, while a second group mineralizes the products.

Easily degradable carbon sources do not necessarily enhance the metabolism of other substrates. Hollibaugh (12) found that the effect of secondary carbon sources on arginine metabolism was variable. The addition of glucose, citrate, or succinate would greatly shorten the lag period required for marine bacteria to degrade arginine. However, the presence of glutarate or glycolate had little effect. Rubin and Alexander (22) found that the addition of arginine and yeast extract would enhance the metabolism of phenol and p-nitrophenol in lake water whereas the addition of glucose, adenine, or propionate inhibited the metabolism of the pollutants. LaPat-Polasko et al. (16) found that methylene chloride metabolism at low concentrations (micrograms per liter) was greater when acetate was provided at higher concentrations (milligrams per liter); however, the situation was altered when the reverse experiment was conducted.

The biological mechanism for the enhancing properties of the labile natural substrates cannot be completely determined by the experiments conducted. On the basis of the microbiological parameters measured, it appears that the mechanism is not evident at the community levels, but likely acts at the biochemical level of individual cells or enzymes.
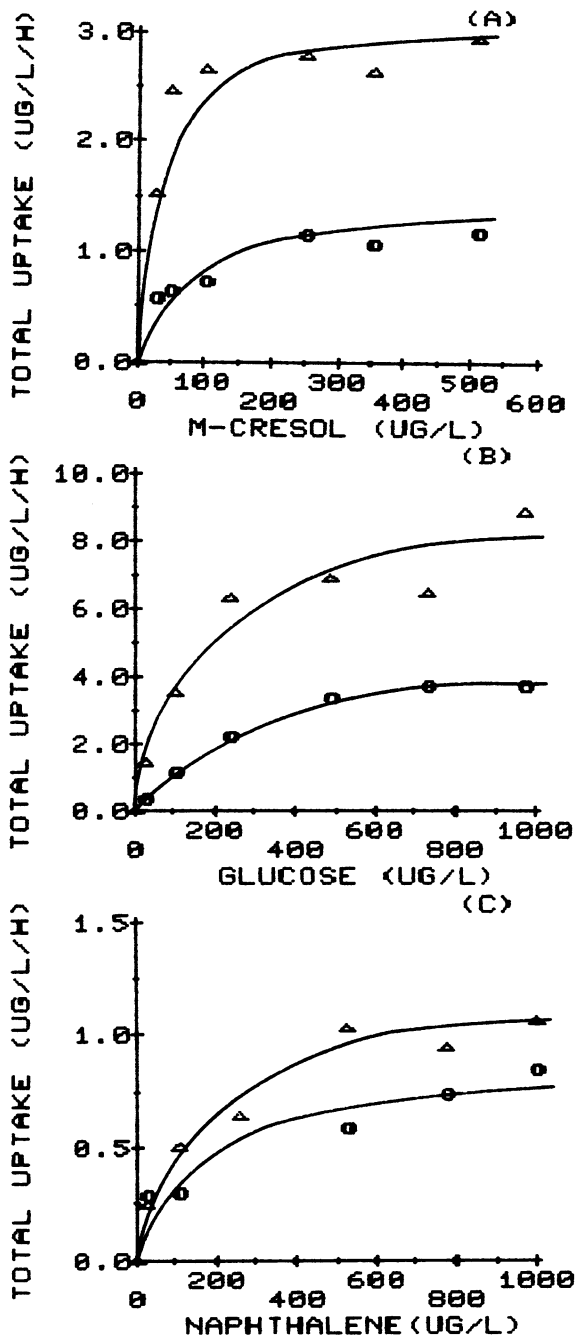
FIG. 4. Total uptake of (A) m-cresol, (B) glucose, and (C) naphthalene by communities adapted to amino acids, experiment 2. Symbols: O, 100 μg of amino acids per liter; Δ, 1,000 μg of amino acids per liter.

There was some evidence to support the idea that the enhancing properties of the amino acids was fairly general in nature. Adaptation to amino acids did enhance the biodegradation of both glucose and, to a lesser extent, naphthalene (Fig. 3 and 4). However, this enhancement was relatively small compared with the enhancement of m-cresol metabolism.

The pathways of phenolic metabolism have been extensively studied (6). The pathways for the metabolism of amino acids, carbohydrates, or fatty acids involve funda-

mental conversions such as deamination, glycolysis, or beta-oxidation. There is no obvious direct biochemical link between these fundamental pathways and the pathways for the degradation of the monosubstituted phenols. Thus, a specific enhancement of monosubstituted phenol metabolism by any of the natural substrate classes seems unlikely. The smaller enhancement of glucose and naphthalene metabolism may be due to a lack of responsiveness in the bacteria metabolizing these compounds rather than an actual preference for m-cresol, as the data suggest.

Although it cannot be directly determined from the data, it is possible that the bacteria degrading glucose and naphthalene were not as affected by the amino acids as the m-cresol degraders because the enzymes responsible for the degradation of glucose and naphthalene were not as sensitive to changes in their environment. The half-saturation constant, K_m, is generally regarded as a measure of the responsiveness of a microorganism to an organic substrate (25). Bacteria with low K_m values generally respond more effectively to low levels of a substrate than cells with high values do. After adaptation to amino acids, the K_m values were relatively high for both glucose and napthalene, compared with those for m-cresol, indicating relatively low sensitivity to glucose and naphthalene (Table 6). If glucose and naphthalene degraders were relatively insensitive to changes in the concentration of these materials, they may also have been relatively insensitive to other substrates, such as amino acids. Thus the levels of amino acids used in the study might not have been high enough to evoke a significant response from these bacteria.

Whereas the glucose and naphthalene data do not conclusively affirm the concept that amino acids were generally enhancing to the metabolic activities of the adapted microbial communities, the idea cannot be conclusively refuted either. More data are required describing the effects of adaptation to labile substrates on a wide variety of organic compounds before a conclusive determination can be made.

The results of the adaptation studies with amino acids, carbohydrates, and fatty acids indicate that these compounds can play an important role in determining the fate of pollutants in aquatic systems. The results of this research suggest that, whereas the "same substrate" adaptations described by other researchers (10, 24) may be important, the naturally occurring organic materials which enter the environment must also be considered in any assessment of factors which influence the fate of man-made materials. Both the types and the concentrations of natural materials in the environment can influence the response of the microbial community to new compounds, even though the new molecules may not be structurally or biochemically related to the natural substrates.

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