

Kinetics of Mineralization of Phenols in Lake Water

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The kinetics of mineralization of phenol and *p*-nitrophenol in lake water was determined at concentrations from 200 pg/ml to 5 µg/ml. The mineralization data were fit by nonlinear regression to equations for 14 kinetic models that describe patterns of biodegradation by nongrowing cells or by microorganisms growing on either the test chemical or other organic substrates. The kinetics of mineralization of phenol in water samples collected in July was best described by first-order models for 0.5 ng of phenol per ml; by Monod-without-growth, logistic, and logarithmic models for 1.0 and 2.0 ng/ml and 5.0 ng/ml to 1.0 µg/ml, respectively, if it is assumed that the mineralizing population uses phenol as the sole carbon source for growth; by models (for phenol at concentrations of 2.0 ng/ml to 1.0 µg/ml) that assume that the phenol-mineralizing populations do not grow or grow logarithmically or logistically on uncharacterized carbon compounds but metabolize the phenol when present at levels below and above K_m , respectively, for that compound; and by a logarithmic model at 5.0 µg/ml. Under the test conditions, usually less than 10% of the phenol C that was metabolized was incorporated into microbial cells or retained by other particulate material in the water at substrate concentrations of 10 ng/ml or less, and the percentage increased at higher substrate concentrations. The mineralization of 2.0 ng of phenol per ml in water samples collected at other times of year was best described by logistic or logarithmic models if the mineralizing bacteria were assumed to be growing on phenol, by a first-order model, or by a model assuming logarithmic growth of the phenol mineralizers on other organic compounds in the water. If the lake water was incubated for 12 h before phenol was added, mineralization was zero order. Removal of particles from lake water sampled after heavy runoff from land resulted in a change in kinetics of phenol mineralization. The patterns of mineralization of 0.5 ng to 1.0 µg of *p*-nitrophenol per ml were best fit by the Monod-with-growth model or the logistic model if it is assumed that *p*-nitrophenol was the carbon source for growth, or by models that assume logarithmic or logistic growth on uncharacterized organic matter in the water. Reasons for two models fitting the same data well are considered, as are bases for selecting between models.

Prediction of the persistence and concentration of toxic chemicals in natural environments and the possible exposure of humans and other species to such compounds requires information on the kinetics of biodegradation. Lakes receive many synthetic organic compounds from industrial effluents, rivers, and streams; runoff water from land areas; and eroding soil, and therefore many studies have been conducted of the biodegradation of such compounds in lake water or other surface freshwaters (3, 5, 11). Attention has also been given to the kinetics of biodegradation in aquatic environments (8, 14, 21), but little concern has been given to models for the kinetics of biodegradation of the low concentrations of chemicals that characterize most surface waters. These low levels may pose public health hazards (9) or may become toxic to the aquatic biota as a result of bioconcentration.

A number of kinetic models have been developed or applied to characterize biodegradation in natural environments (7, 10, 25). Recently, however, a number of new models have been formulated to characterize the kinetics of biodegradation by pure cultures of bacteria, and statistical means have been proposed for evaluating the fits of these models to data obtained in studies of microbial processes (12, 18). Some of these models are based on the assumption that the patterns of substrate disappearance can be modeled with information only on substrate concentration and population density together with the parameters of classical

Monod kinetics (18). In a more recent report, models were described for the kinetics of biodegradation of organic substrates by bacteria that grow logistically, logarithmically, or linearly while metabolizing (but not growing at the expense of) the organic substrate of interest (17).

In view of the availability of these new models, a study was initiated to determine their applicability to the kinetics of mineralization in lake water. For this purpose, two environmental pollutants, phenol and *p*-nitrophenol (PNP), were selected as test compounds. Because of their importance as pollutants, the biodegradation of these compounds has been extensively studied (2, 8, 19, 20). A wide range of concentrations was used, and samples of lake water were collected at several times of year.

MATERIALS AND METHODS

Water samples were collected at various times in 1984 from Beebe Lake in Ithaca, N.Y. The pH values of the samples ranged from 8.0 to 8.4. Glassware was soaked in a No-Chromix (Godax Labs Inc., New York, N.Y.) solution for at least 2 h and then rinsed with distilled water and water that had been passed through a Milli-Q reagent-grade water system (Millipore Corp., Bedford, Mass.). The water samples were dispensed into 500-ml Erlenmeyer flasks, and the samples were incubated at $23 \pm 2^\circ\text{C}$.

Labeled phenol or PNP was added to water samples to give 350 to 2,000 or 190 to 750 dpm/ml, respectively. The unlabeled phenol or PNP was added to samples containing the labeled chemicals to attain higher substrate concentra-

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tions. [U-¹⁴C]phenol (87 mCi/mmol) was purchased from Amersham Corp., Arlington Heights, Ill., and [U-¹⁴]PNP (24.1 mCi/mmol) was obtained from A. W. Bourquin, U.S. Environmental Protection Agency, Gulf Breeze, Fla. The two labeled compounds were 98% or more pure as determined by the manufacturer.

In a study in which suspended particles were removed from lake water samples, duplicate 100-ml portions were passed through 3.0- μ m-pore polycarbonate filters (Nuclepore Corp., Pleasanton, Calif.), and the weight of the particles was determined by weighing the filters after drying for 2 h at 102°C.

Mineralization was measured by the procedure of Subba-Rao et al. (20). In this procedure, 2.5- or 5.1-ml samples were removed at regular intervals and acidified with 3 drops of concentrated H₂SO₄. Air was then passed through the samples for 5 to 10 min to drive ¹⁴CO₂ out of the solution. Subsamples (2.0 or 5.0 ml) were then placed in scintillation vials, 6.0 ml of Liquiscint scintillation cocktail (National Diagnostics, Inc., Somerville, N.J.) was added, and radioactivity was determined with a model LS 7500 liquid scintillation counter (Beckman Instruments, Inc., Irvine, Calif.). Approximations of the amount of phenol assimilated by microorganisms were made by filtering 5.0-ml subsamples through 0.2- μ m-pore polycarbonate filters (Nuclepore). No decrease in radioactivity was observed in lake water that was amended with HgCl₂ and either [¹⁴C]PNP or [¹⁴C]phenol and that was incubated under the same conditions of the various studies.

The results from tests of mineralization were analyzed by nonlinear regression by the procedures of Simkins and Alexander (18). An Apple II Plus microcomputer (Apple Computers, Inc., Cupertino, Calif.) was used to run the MARQFIT computer program (18) for minimizing the least squares of the differences between the data and the model curves. Attempts were made to fit the data to the models described by Simkins and Alexander (18) and Schmidt et al. (17). The six models of Simkins and Alexander (18) and all models except for model II of Schmidt et al. (17) were used for analyzing the data. Because the first-order, Michaelis-Menten or Monod-no-growth, and the zero order models are included in both sets of models, a total of 14 different models was used to analyze the data. Of the 14 models, 9 have been used in our previous studies (17, 18). Equations for the models and partial derivatives were incorporated into existing computer programs (18) for the five remaining models. We have been unsuccessful in analyzing the data with model II, which has six parameters and is more complex than are the other 14 models.

The model of best fit for each set of data was determined by comparing the residual sums of squares from fits for the 14 different models. The model fit with the lowest residual sum of squares was accepted as the model of best fit if the difference between it and models with fewer parameters was significant at the ≤ 0.1 level of probability by an *F* test (12). If there was no statistically significant better fit between two models having different numbers of parameters, the model with fewer parameters was selected. The *F* test was also used to determine the best model for the data from among models with equal numbers of parameters and between models from the two different families of models. If fits by models were not significantly different, models were rejected if the magnitude of any asymptotic standard deviation associated with estimated values for parameters was on the order of the parameter estimates themselves or if an extremely high correlation between parameters was indicated.

RESULTS

Lake water collected on 3 July 1984 was amended with 10 different concentrations of phenol. Mineralization was evident at the time the first sample was taken, namely 1.0 h, and the rate fell markedly by 24 h (Fig. 1). The curves for the two lowest concentrations were concave-up, whereas the pattern for 2.0 ng of phenol per ml was S-shaped. The curves at concentrations of 5.0 to 5,000 ng/ml were concave down, and the time for mineralization to be detected increased as the substrate concentration increased.

The 14 models were fit to the data for the 10 concentrations of phenol. Residual sums of squares were calculated for the fits of each of these models at each substrate concentration. The models of best fit were then determined for each phenol concentration with the families of models of Simkins and Alexander (18) and Schmidt et al. (17). Except as noted below, the *F* test did not distinguish the model that gave the best representation of the pattern of mineralization of the various phenol concentrations because no significant difference was observed between the values for residual sums of squares for the models of best fit from each family. Therefore, one model of best fit was determined for each family of models. The models of best fit for the samples collected in July 1985 are given at the top of Table 1. Model IV represents logarithmic growth on a substrate other than the test compound and a concentration of the test chemical below K_m , and model III represents logistic growth on a compound other than the test substrate and a concentration of the test chemical above K_m . Of the models proposed by Simkins and Alexander (18), the best fits were obtained by the first-order model at 0.5 ng/ml, the Monod-no-growth model at 1.0 ng/ml, the logistic model at 2.0 ng/ml, and the logarithmic model at all higher concentrations. Of the models proposed by Schmidt et al. (17), the first-order model provided the best fit at 0.5 ng/ml, model IV and the Michaelis-Menten model provided the best fit at 1.0 ng/ml, and model IV provided the best fit to the data at all higher concentrations except 5.0 ng/ml. The models of Simkins and Alexander (18) were used to fit curves to the data in Fig. 1.

An *F* test was used to compare the residual sums of squares for the best models at each phenol concentration. By this statistical test, the logarithmic model was found to be the best model at a concentration of 5,000 ng of phenol per ml. The first-order model was the best representation for both sets of models at 0.5 ng/ml. The logarithmic model appears to be a more suitable choice than model III at 5.0 ng/ml is because the former has four parameters and is thus less complex than the latter is, which has five parameters. At each of the other seven concentrations, the *F* test revealed no significant differences between the models of best fit from the two families of models.

Determinations were made of the amount of ¹⁴C from phenol that was incorporated into particles greater than 0.2 μ m, that remained in solution, and that was mineralized after 48 h, when mineralization had ceased. The percentages of the phenol that was mineralized were similar at the various substrate concentrations, although the values declined somewhat as the concentration rose (Fig. 2). The ¹⁴C in the particulate material, which includes cells, was below 30%, and the percentages generally increased with increasing phenol concentrations; these particles may have included products sorbed by the small amount (15 μ g/ml) of suspended particles of diameters greater than 0.2 μ m. The amounts of ¹⁴C remaining in solution were reasonably constant at concentrations below 1 μ g/ml.

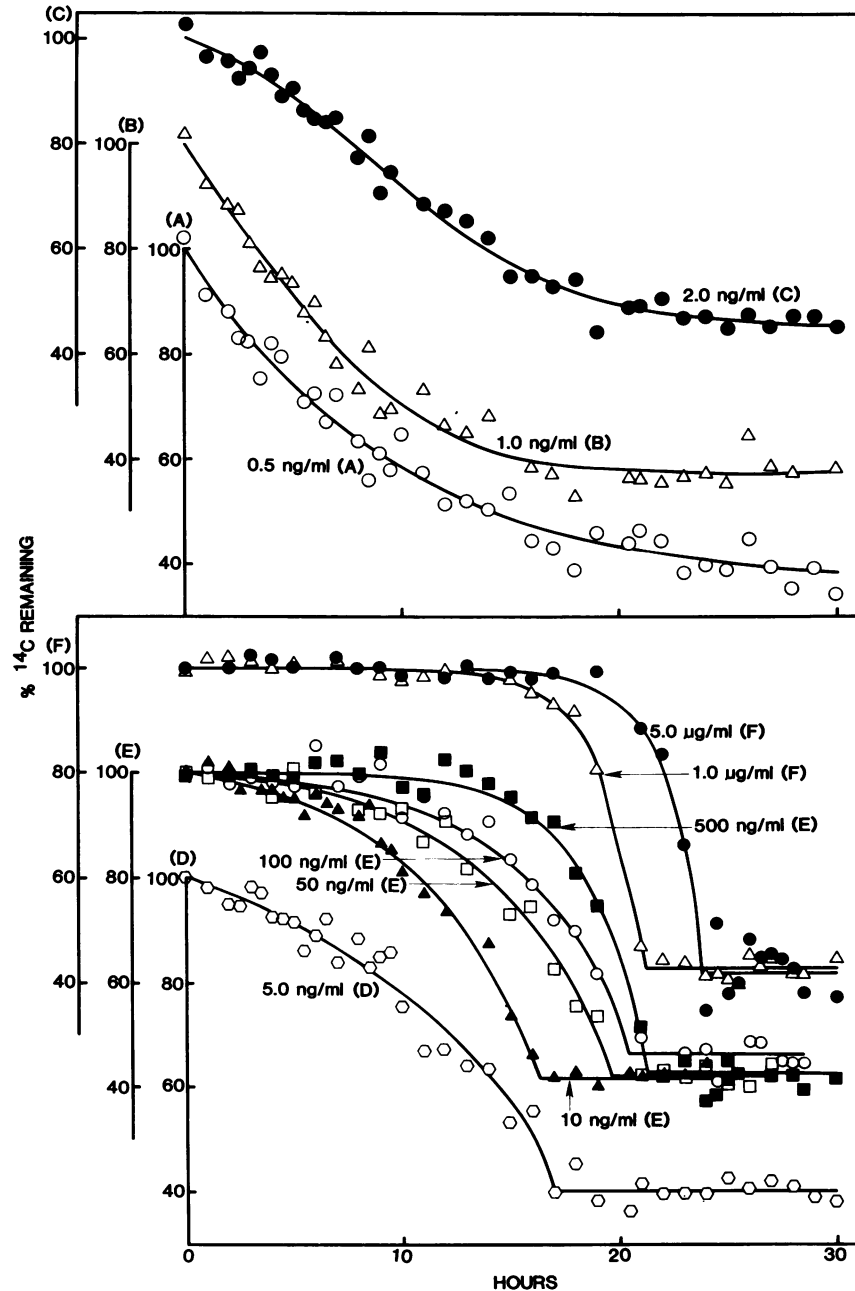


FIG. 1. Mineralization of phenol added to lake water at 10 concentrations. The letters adjacent to the concentration correspond to the letters on the ordinates.

Because nutrient availability, pH, and other chemical and biological characteristics of lake waters vary with time of year, the kinetics of phenol mineralization may also vary. A study was thus conducted to determine the temporal variability in kinetics of phenol mineralization, although the tests were conducted at a single temperature. This investigation and those that follow were done before the study reported above, but the study containing the most substrate concentrations was presented first to demonstrate the number of possible models. Samples were collected on 20 March 1984, and three substrate concentrations were tested. After 12 to 15 h with little detectable mineralization, the loss of ¹⁴C from the solution occurred at an accelerating rate (Fig. 3A).

Mineralization had ended by 24 h at all three concentrations. Analysis of the kinetics of mineralization by the family of models of Schmidt et al. (17) revealed that model IV gave the best fit for the data at all three concentrations. Analysis by the family of models of Simkins and Alexander (18) indicated that the best fits were obtained with (i) the logarithmic model for one replicate flask and the logistic model for the second replicate, with phenol at a level of 0.2 ng/ml; (ii) the logistic model at 2.0 ng/ml; and (iii) the logistic model for one replicate flask and the Monod-with-growth model for the second replicate, with phenol at 20 ng/ml (Table 1). The curves in Fig. 3A present the fits of model IV to the data. No significant differences between the models from each family

TABLE 1. Models of best fit describing kinetics of phenol mineralization in Beebe Lake water

Sample date ^a	Phenol concn ^b (ng/ml)	Models of best fit from:	
		Simkins and Alexander (17)	Schmidt et al. (16)
3 July	0.5	First order	X (first order)
	1.0	Monod-no-growth	IV, XI (Michaelis-Menten)
	2.0	Logistic	IV
	5.0	Logarithmic	III
	10	Logarithmic	IV
	50	Logarithmic	IV
	100	Logarithmic	IV
	500	Logarithmic	IV
20 March	1,000	Logarithmic	IV
	5,000	Logarithmic ^c	IV
	0.2	Logistic or logarithmic	IV
22 May	2.0	Logarithmic	IV
	2.0 ^d	Zero order	XII (zero order)
29 February	2.0	First order	X (first order)
	2.0 ^e	Logarithmic	IV

^a Date of sample collection and beginning of experiment.

^b Duplicate flasks in each study except individual flasks for samples collected on 3 July.

^c Model fit the data significantly ($P \leq 0.1$) better than model IV did.

^d Addition of phenol to lakewater was delayed 12 h.

^e Lakewater filtered through a 3.0- μm -pore filter before the test.

were evident by the F test. Thus, best-fit models were the same at 2.0 ng/ml of lake water collected in winter and in summer, but different models gave the best description of mineralization in the different water samples at the other concentrations.

Mineralization was also measured in water samples collected on 22 May 1984 and supplemented with 2.0 ng of phenol per ml at either 0 or 12 h. The substrate was added at 12 h to determine whether the kinetics was affected by possible microbial growth in the samples before adding the test chemical. Mineralization was initially slow in the water that received the chemical at 0 h, but it was rapid from the time of phenol addition if the water was incubated for 12 h before adding the substrate (Fig. 3B). At the end of the incubation period, about 62% of the carbon was mineralized. The patterns of mineralization were analyzed with the 14 models. The logarithmic model and model IV gave the best fits to the data when phenol was added at 0 h (Table 1). The F test did not show a significant difference between the two models. When phenol was added at 12 h, the zero order model fit well when the data were fit by both families of models, and more complex models did not give statistically significantly better fits to the data than the zero-order model did.

Mineralization of 2.0 ng of phenol per ml also was measured in lake water collected on 29 February 1984 during a snow melt, which caused a high level of particulate matter to be washed into the lake. The waters contained abundant suspended particles (100 $\mu\text{g}/\text{ml}$) of size greater than 3.0 μm . Portions of the water were passed through a 3.0- μm -pore filter to determine the effect of particles on the kinetics of

phenol mineralization. The curve of phenol disappearance was concave-up for the unfiltered water and concave-down for the filtered samples (Fig. 3C). Analyses of the fits of these data to the 14 models showed that the best fits were obtained by the first-order model for the unfiltered water and by the logarithmic model and model IV for the filtered water (Table 1). For the latter, the difference between the residual sums of squares for the two models was not statistically significant.

Water samples collected on 7 September 1984 were amended with 12 concentrations of PNP, one flask per concentration (Fig. 4). The curves for 5.0, 7.0, 20, 50, 200, and 500 ng of PNP per ml (not shown) fell between the plots of 10 and 1,000 ng/ml. Mineralization was not detected in the first 200 h, and then the substrate was readily metabolized. Because the curves for percent substrate mineralized versus time were essentially the same at 5.0, 7.0, 10, 20, 50, 100, 200, 500, and 1,000 ng/ml, the actual rates were proportional to PNP concentration. In contrast, the rates at 0.5, 1.0, and 2.0 ng of PNP per ml were less than would be expected, assuming the rates were linearly related to concentration. The duration of the acclimation phase was similar at the various concentrations, as was the extent of mineralization of those concentrations for which the study was conducted long enough to determine the time at which mineralization ended.

The fits of the 14 models to the data were determined by nonlinear regression analysis. The patterns of mineralization of 5.0, 10, 20, 50, 100, 200, and 1,000 ng of phenol per ml were best fit by either the Monod-with-growth model (18) or model IV (17) (Table 2). The latter model was developed for processes involving logarithmic growth on one substrate but simultaneous metabolism of the test compound when the latter is at a concentration below K_m . Statistical analysis revealed no better fit of the data by the Monod-with-growth model than by model IV. Of the models of Simkins and Alexander (18), the best fits for the patterns of mineralization

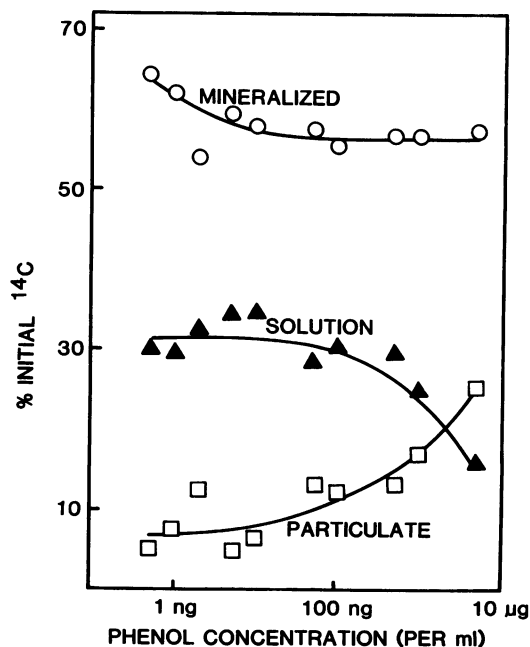


FIG. 2. Effect of initial phenol concentration on percent radioactivity that was mineralized, that remained in solution, or that was particulate in Beebe Lake water after 48 h.

of 0.5, 1.0, 2.0, 7.0, and 500 ng/ml were obtained with the logistic model. The Monod-with-growth model fit the data for these five concentrations somewhat better (i.e., lower residual sums of squares) than the other models did, but the differences in sums of squares were not statistically significant; nevertheless, the logistic model is listed in Table 2 because it contains fewer parameters and thus is simpler. The best models from among those of Schmidt et al. (17) were models IV at 0.5, 2.0, and 500 ng of PNP per ml and model I at 1.0 and 7.0 ng of PNP per ml. The differences between the best models from the two families of models were not statistically significant by an *F* test.

Maximum specific growth rates (μ_{\max} , a parameter of the logarithmic model) were estimated by nonlinear regression analysis of the data collected from the July water samples at phenol concentrations of 5 ng/ml to 5.0 $\mu\text{g/ml}$. A plot of these values of μ_{\max} against initial phenol concentration shows plateaus at μ_{\max} values of ca. 0.18 and 0.53 per h (Fig. 5). The phenol concentrations at which the μ values are half of these two values of μ_{\max} are ca. 5 and 400 ng/ml. A low K_m , a parameter of the Michaelis-Menten model, was estimated by nonlinear regression analysis of the data for the mineralization of 1.0 ng of phenol per ml in the water collected in July. The value thus obtained was 0.34 ng/ml.

DISCUSSION

The procedures used resulted in a rejection of most of the models as inappropriate to characterize the patterns of mineralization at the several concentrations of the two phenols. Nevertheless, statistically significant differences usually were not observed between the best fits from the two families of models considered. The analysis was thus, at individual concentrations, unable to distinguish between (i) model IV and logarithmic, logistic, Monod-no-growth, or Monod-with-growth models; (ii) model III and the logarithmic model; and (iii) model I and the logistic model. However, the theoretical curves for each of these models, which are given by Alexander (1) and Schmidt et al. (17), are quite similar, so that distinguishing among these models requires even more datum points and greater precision than are in the present study. It is not certain whether this additional effort is necessary to use kinetic analysis for predicting the fate of pollutants in natural environments, but it would be helpful in theoretical studies.

Choices among the models of best fit from the two families could also be made based on biological rather than statistical approaches, at least in those instances when the statistical approaches cannot distinguish among models. For example, theoretical considerations suggest that bacteria are unable to grow at the expense of single organic substrates when those compounds are at levels below several nanograms per milliliter (16), and these theoretical considerations have been confirmed in studies of *Salmonella typhimurium* (15). Thus, models based on considerations of microbial growth on individual substrates seem inappropriate for kinetics of biodegradation when those substrates are below the theoretical threshold levels for growth. Conversely, models based on considerations of the kinetics of biodegradation of organic compounds not supporting growth seem inappropriate to apply to the kinetics of biodegradation of higher concentrations of organic compounds known to support microbial proliferation. For example, the growth of phenol-mineralizing organisms was the same in Beebe Lake water with 1.0 ng of phenol per ml or with no added phenol, whereas the growth rate and final population size were greater in water receiving 1.0 μg of phenol per ml (13). Meaningful patterns

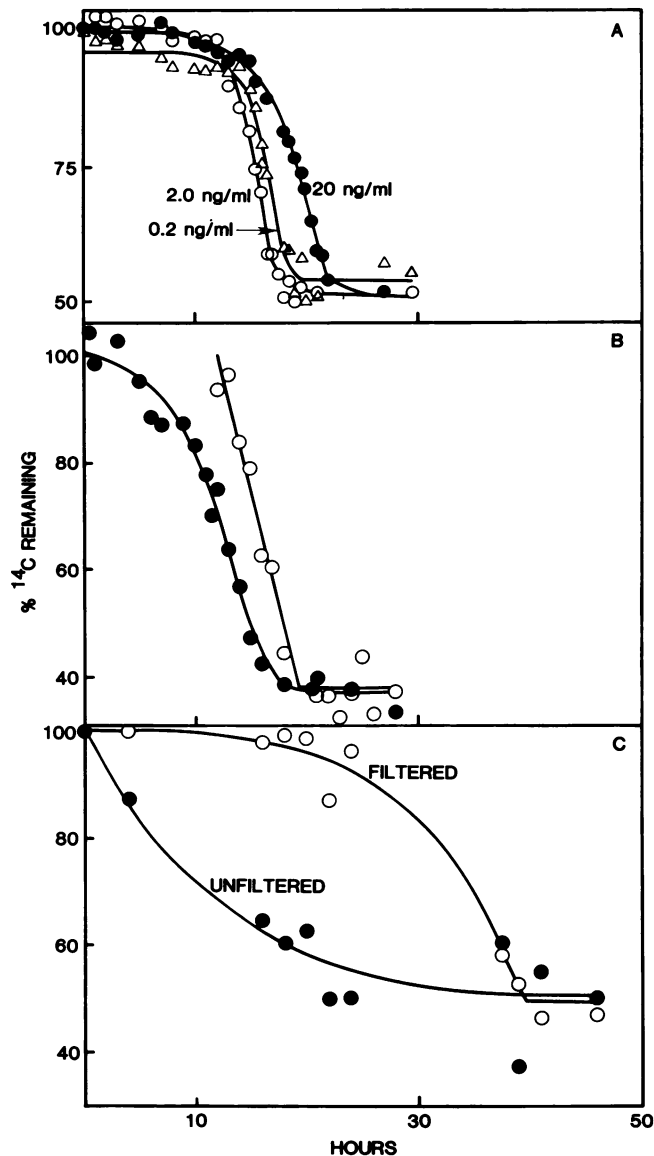


FIG. 3. Mineralization of phenol at several concentrations in lake water collected on 20 March (A), 22 May (B), and 29 February (C) 1984. The samples collected in May received 2.0 ng of phenol per ml at 0 or 12 h, and those collected on February 29 were or were not filtered before receiving 2.0 ng of phenol per ml.

emerge with this type of argument if the anomalies of the particle-rich water and the kinetics of mineralization when phenol was added after 12 h are ignored. For example, the only models that fit well at 2.0 ng/ml or lower are X (first order), XI (Michaelis-Menten), IV, or I if it is assumed (i) when the two phenols do not support growth at concentrations of 2.0 ng/ml or lower, only the family of models of Schmidt et al. (17) should be used, and (ii) when the phenols do sustain growth at higher levels, only the family of models of Simkins and Alexander (18) are appropriate. These models all are biologically meaningful at these concentrations because they reflect no growth or logarithmic or logistic growth on other organic nutrients in the lake water when the concentration of the test substrate is at or below K_m (17). In the same way, the relevant models for 5.0 ng/ml or higher are

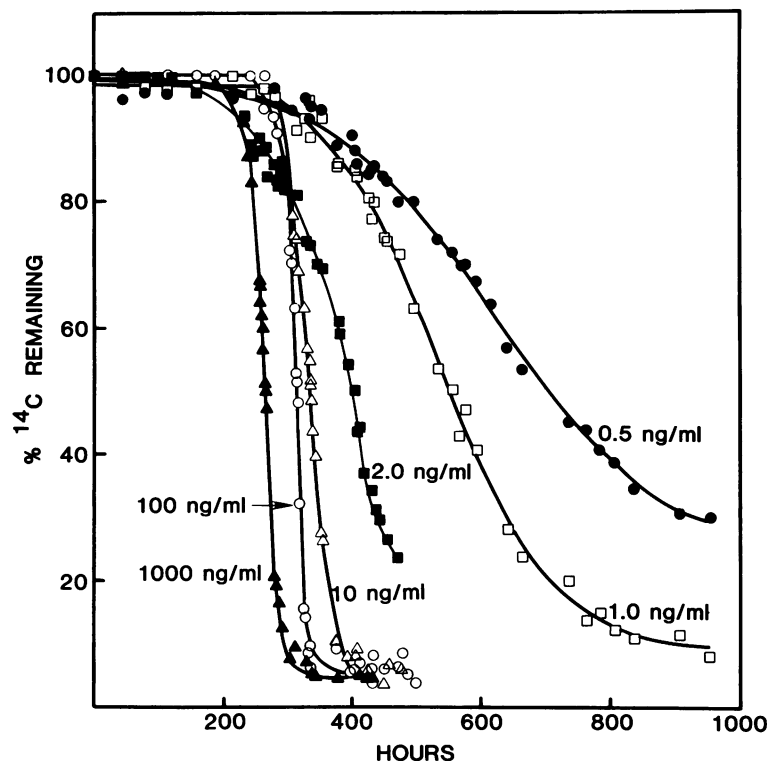


FIG. 4. Mineralization of various concentrations of PNP in lake water.

logarithmic for phenol or Monod-with-growth or, at low concentrations, logistic for PNP; the choices of these models is consistent with the basis for their formulation (18). In the waters that were rich in particles or that received phenol after 12 h, it is likely the initial populations of phenol degraders were sufficiently large that significant growth did not occur during the time of mineralization, and models (first order and zero order) that assume that no growth occurred fit these data better than did model IV, which was the best model for phenol at 2.0 ng/ml in other water samples. Nevertheless, further study is necessary to determine whether such reasoning is appropriate in making choices between models fitting well in conditions under which the goodness of fit cannot be distinguished statistically.

A variety of factors may confound the facile application of these kinetics to natural waters. For example, if the dominant species responsible for mineralization are different at different times of year or at different substrate concentrations, or if they are in the water phase as contrasted with the particulates in the water, apparent anomalies will be evident in the models of best fit should these species have dissimilar K_s values for growth (18) or K_m values for metabolism without growth (17) on the test compounds. Such apparent anomalies may explain the different models that best fit the data when water samples were collected at different times of year or when particulate matter was present or removed. The difficulty is not presently resolvable because the organisms carrying out the transformations or the relevant physiological properties of these populations are not known.

Zero order kinetics at low substrate concentrations, as observed in the degradation of phenol in water incubated for 12 h before the chemical was added, is more difficult to explain by these two families of models. Linear rates of biodegradation at low concentrations of organic compounds

have been noted previously, however (20, 25). Nevertheless, if bacteria grew during the 12 h by using nutrients in the lake water, zero order kinetics would apply in one family of models if the added phenol concentration was above K_s and the cell density that appeared was high (18). Alternatively, if the 12 h were associated with the depletion of organic matter in the water on which the bacteria might have grown, zero order kinetics would apply in the other family of models if the concentration of added phenol available to the nongrowing cells was greater than K_m (17). The explanations suffer from the fact that they require that K_s or K_m be below 2.0 ng/ml.

The data suggest two K_s values (ca. 0.053 and 4.25 μM)

TABLE 2. Models of best fit describing kinetics of PNP mineralization in Beebe Lake water

PNP concn (ng/ml)	Models of best fit from:	
	Simkins and Alexander (18)	Schmidt et al. (17)
0.5	Logistic ^a	IV
1.0	Logistic ^a	I
2.0	Logistic ^a	IV
5.0	Monod-with-growth	IV
7.0	Logistic	I
10	Monod-with-growth	IV
20	Monod-with-growth	IV
50	Monod-with-growth	IV
100	Monod-with-growth	IV
200	Monod-with-growth	IV
500	Logistic ^a	IV
1,000	Monod-with-growth	IV

^a Data were also well fit by Monod-with-growth model.

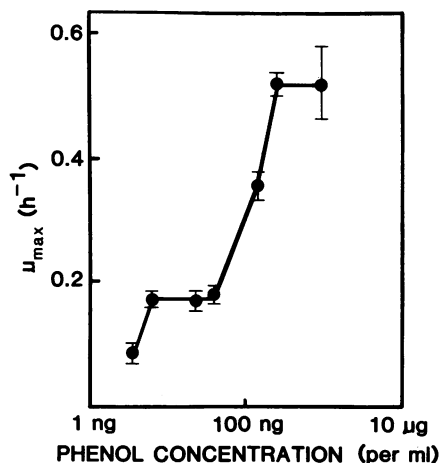


FIG. 5. Effect of phenol concentration on the μ_{max} of indigenous phenol-degrading microorganisms. The error bars represent the asymptotic standard deviations.

and two concentration ranges at which the phenol concentration is saturating for the growth of the organisms bringing about phenol mineralization. Low K_s and low K_m have been reported for some bacteria: e.g., K_s values of 0.17 μM for growth of *Aeromonas hydrophila* on glucose (23), 0.04 μM for *Pseudomonas aeruginosa* on arginine (24), and ≤ 0.03 μM for *Flavobacterium* sp. on maltoselike compounds (22); and a K_m of 0.074 μM for glutamate assimilation by *Aeromonas* sp. (23). The presence of several K_s (4) and K_m values (6) in some bacteria has also been reported. It is not clear, however, whether the existence of two K_s values for the microbial community of lake water reflects two K_s values for a single population or two populations with dissimilar K_s values.

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