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Nonlinear Estimation of the Parameters of Monod Kinetics That Best Describe Mineralization of Several Substrate Concentrations by Dissimilar Bacterial Densities

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The kinetics of mineralization of a wide range of concentrations of benzoate, glucose, and benzylamine by *Pseudomonas* sp., *Salmonella typhimurium*, and microorganisms in acclimated sewage was studied. The treatment of initial substrate concentration and population density as independent variables in nonlinear regression analysis permitted the estimation of a single value for each of the parameters of Monod kinetics that best described the mineralization of substrate at each concentration by the pure cultures and the sewage microflora. One value for each of the parameters of Monod kinetics was used for each of the three compounds to produce theoretical curves which lay close to the observed data on mineralization. Statistically significant differences existed in the values of the parameters of Monod kinetics that best described mineralization in cultures differing only in initial substrate concentration and cell density. However, for the compounds tested, the variance left by analyses using one value for each parameter of Monod kinetics was less than double the unexplained variance left by individual analyses of the data from each treatment. Although significant, this increase is small compared with the amount of variance that could be explained using only one value for each parameter of Monod kinetics.

A variety of mathematical models have been proposed to describe the kinetics of metabolism of compounds introduced into pure cultures of bacteria, samples of natural environments, or natural environments. Often a compound reaches a natural environment as a single addition, and the kinetics of its metabolism would resemble more closely the kinetics of substrate use of a batch culture rather than a continuous culture. Many models for batch systems use as variables only the concentration of the introduced substrate and the density of the active organisms. First-order (8), second-order (11), integrated Michaelis-Menten (18), integrated Monod (12), and logistic (14) kinetics have all been proposed as models using these variables. The term logarithmic kinetics has been applied to models in which the bacteria grow exponentially at the expense of a compound provided at concentrations greatly in excess of the half-saturation constant for growth (14). Other researchers have described models of this type (13,17). It has been proposed that all of the above models form a related family of models in that they are special or degenerate forms assumed by the integrated Monod equation at extreme ratios of initial substrate concentration to the half-saturation constant or at extreme ratios of initial cell density to initial substrate concentration (14).

Discussions of models of this type are greatly simplified if an experimental treatment is defined solely in terms of the initial values for substrate concentration (S_0) and population density (B_0). In a mathematical sense, a treatment will be defined as an ordered pair (S_0, B_0). In a practical sense, different treatments for the purposes of this paper differ in at least one of these two variables. Replicates differ in neither. Treatments differing in factors other than initial substrate concentration or population density will not be considered.

All of the aforementioned models have been used to analyze the mineralization of a substrate in individual treatments. Many treatments may be studied as part of a single experiment, but the data from individual treatments are usually analyzed independently. It should be possible, however, to use nonlinear regression analysis of the data from more than one treatment to obtain estimates for the parameters of a model believed to describe the metabolism of a substrate over a wide range of concentrations by bacteria at different initial densities. The patterns of substrate disappearance from replicates of a single treatment could readily be subjected to a nonlinear regression analysis. The combined data from all replicates could be analyzed by using the same computer program that would have been used to analyze the data from any one replicate independently. It is a less straightforward problem to obtain estimates of the parameters of a model believed to describe mineralization in treatments differing in initial cell density or substrate concentration. Nevertheless, models incorporating the variables of cell density and substrate concentration should be able to account for the patterns of mineralization in different treatments. For example, it should be possible to find a single value of a parameter such as K_s, which describes the metabolism of a compound from two treatments receiving different concentrations of the substrate at the beginning of an experiment but which were otherwise identical.

This study was initiated to determine whether it is possible to obtain nonlinear estimates of the parameters of Monod kinetics which describe the patterns of mineralization of organic substrates in cultures containing different cell densities and different concentrations of substrate. If the patterns of mineralization in different treatments cannot be explained adequately using only one value for each of the parameters of Monod kinetics, then the model fails even though Monod kinetics may offer a close fit to the data from any one treatment. In such an event, more complex models

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possibly incorporating multiple uptake systems of variable yield coeficients would be needed. However, it becomes difficult to justify the use of models more complex than Monod kinetics if no evidence can be found for variation of half-saturation constants or yield coefficients between treatments. Although many environmental factors with the potential to affect mineralization kinetics are not included in the integrated Monod equation (12, 14), the model need not necessarily fail. For example, maintenance energy requirements are not included in the model, but maintenance energy is thought to consume only a small fraction (ca. 5%) of the energy used by cells growing aerobically (6). Such a small diversion of cellular resources may not detectably affect the experimentally measured patterns of mineralization.

MATERIALS AND METHODS

Medium and organisms. The inorganic salts solution was described previously (14). [*U*-ring-^{14}C]benzoic acid (specific activity, 130 mCi/mmol), [*U*-^{14}C]glucose (348.2 mCi/mmol), and [7-^{14}C]benzylamine·HCl (56 mCi/mmol) were obtained from Amersham Corp. (Arlington Heights, Ill.).

Pseudomonas sp. was described previously (14). *Salmonella typhimurium* LT2 was obtained from J. M. Calvo, Cornell University, Ithaca, N.Y. Because benzoate-grown *Pseudomonas* sp. organisms readily lost activity on this substrate, 500 ml of a benzoate-grown culture containing 2×10^8 cells per ml was incubated for 3 h with 100 μg of benzoate per ml in 2-liter Erlenmeyer flasks at 23°C without shaking. The cells were collected by centrifugation and washed three times in sterile salts solution. The same procedure was followed in experiments with *S. typhimurium*, but the substrate was glucose, and the flasks were incubated at 29°C on a rotary shaker operating at 90 rpm. Unreplicated 150-ml portions of diluted suspensions of these washed cells were incubated with the substrates in 500-ml Erlenmeyer flasks at 23°C without shaking for *Pseudomonas* sp. and at 29°C on a shaker for *S. typhimurium*.

To prepare these dilutions, the unlabeled substrate at one concentration and a suspension of the bacterial cells were added to a flask. Dilutions of this suspension were then made in sterile salts solution contained in 500-ml flasks, the cells and substrate thus being diluted simultaneously. To each of the dilutions, a constant amount (less than 1 ng/ml) of the ^{14}C-labeled substrate was added. Because of the small amount of labeled substrate added, its contribution to the total amount of substrate in the cultures was ignored in the analysis.

Freshly collected sewage from the primary settling tank of the sewage treatment plant of Ithaca, N.Y., was passed through Whatman no. 41 filter paper. It was amended with 50 μg of nonradioactive benzylamine per ml or was left unamended, and 750-ml portions of the suspensions were incubated at 23°C for 48 h in 2-liter flasks. The samples were vigorously aerated with compressed air. The sample which had previously received benzylamine was provided again with unlabeled benzylamine and incubated as described above for 3 h. The previously unamended flasks were incubated with no additions. The amended sewage was then centrifuged at 16,300 × *g* for 15 min. At the same time, the unamended sewage was sterilized by passing it through a 0.2-μm polycarbonate membrane filter (Nuclepore Corp., Pleasanton, Calif.). The cells and particles from the amended sewage were washed by centrifugation and resuspension three times in the filter-sterilized, unamended sewage, and the cells from the acclimated sewage were then suspended in a volume of the filter-sterilized unamended sewage equal to the volume from which the cells and other particles were originally removed.

Measurement of mineralization. The method used to measure mineralization of ^{14}C-labeled substrate has been previously described (14). Six milliliters of Liquiscint (National Diagnostics, Inc., Somerville, N.J.) was added to 2 ml of an acidified, out-gassed sample. The experimental treatments, as defined by initial substrate concentration and cell density, were not replicated.

Regression analyses. One of the models used to analyze all of the patterns of substrate disappearance is the integrated Monod equation. The development of this model was described previously (14) and will only be summarized here. The integrated Monod equation reflects two assumptions about the physiology of the cells metabolizing a test substrate. First, the specific growth rate of the active cells is represented as following simple Monod kinetics, which may be expressed as follows:

$$\frac{dB}{dt} \cdot \frac{1}{B} = \frac{\mu_{max} S}{K_s + S} \quad (1)$$

where B is cell density, S is substrate concentration, μ_{max} is the maximum specific growth rate, and K_s is the half-saturation constant. The second assumption is that the yield of organisms per unit amount of substrate taken up does not vary with time and substrate concentration. This assumption is reflected in the following mass-balance relationship:

$$S + qB = S_0 + qB_0 \quad (2)$$

where B_0 is initial population density, S_0 is initial substrate concentration, and q is cell quota. Cell quota is frequently replaced with $1/Y$ where Y is yield (9). If equations 1 and 2 are assumed to offer an accurate representation of the kinetics of growth of the active organisms at the expense of the test substrate, then an expression for substrate concentration as an implicit function of time (the integrated Monod equation) is given (14) by

$$K_s \ln(S/S_0) = (S_0 + qB_0 + K_s)\ln[(S_0 + qB_0 - S)/(qB_0)] - (S_0 + qB_0)\mu_{max} t \quad (3)$$

In equation 3, substrate concentration (S) is the dependent variable, time is an independent variable, and μ_{max}, K_s, and q are parameters.

Nonlinear regression analysis offers a means for generating values of the parameters of a model which minimize the (squared) differences between predicted and experimentally observed values of the dependent variable. Values for variables are provided as data to the analyses, whereas values for the parameters are the output desired from the analyses. The status of the terms B_0 and S_0 in equation 3 is ambiguous. Technically, S_0 is an initial condition (3). Bard (2) recommends that initial conditions be treated as parameters subject to nonlinear estimation when their values are known with only moderate accuracy. However, when highly accurate values for an initial condition are known, it is better treated as an independent variable than as a parameter. It is probably safest also to treat B_0 as an initial condition.

The ability to treat S_0 and B_0 as independent variables offers a means of obtaining estimates for the parameters of Monod kinetics which best describe mineralization in more than one experimental treatment. Data for mineralization from two treatments which differ only in initial population density could be used to estimate values of the parameters of

Monod kinetics which best describe substrate loss in both cultures. It would be necessary only to treat B_0 as an independent variable. An individual observation would be characterized by three values: measured substrate concentration, the time of sampling, and initial population density. Similarly, it would be possible to fit data on substrate disappearance from several treatments which received different additions of the compound.

One problem in fitting a model to the data from treatments differing in B_0 is the difficulty in quantifying low bacterial densities. Methods such as plate counts and most-probable-number techniques have less precision than the method of measuring ^{14}C remaining in solution as used in this study, and it is possible to dilute solutions and suspensions of cells with great accuracy. Consequently, it should at least be possible to specify the relative population densities in a series of treatments that were prepared by quantitative dilution of a single suspension of cells. Therefore, we decided to analyze the data from different treatments by specifying B_0 as a relative quantity rather than an absolute number of cells per milliliter. For this purpose, the unit rlb is introduced for relative biomass. A treatment with $B_0 = 0.5$ rlb would have half the initial cell density of a culture with $B_0 = 1.0$ rlb.

To treat the initial substrate concentration as an independent variable in these experiments, an additional modification of the equations is needed. In the procedures that were used, the concentration of unlabeled compound was varied by dilution, and then each treatment received the same amount of ^{14}C-labeled compound. Thus, the specific activity of the substrate varied between treatments. It is not possible to estimate a single value for a parameter such as K_s in units of disintegrations per minute per milliliter based on the data from treatments in which a value with these units corresponds to different concentrations of substrate in units of nanograms per milliliter. It is possible to transform all measured values for concentrations of substrate remaining to units of nanograms per milliliter before performing regression analyses. However, this is undesirable because the residuals (the differences between observed and predicted values) would be expected to be much smaller for the data from treatments that received low rather than high concentrations of substrate. The data would not be homoscedastic; i.e., the variance would not be independent of the mean. Analyses of the original data are preferable. To overcome the problem of variable specific activity, a term for specific activity, ξ, with units of disintegrations per minute per nanogram can be introduced into the model. To modify the integrated Monod model (equation 3) to incorporate the parameter ξ, every term that has units of substrate concentration (except for S)—that is, S_0, K_s, and qB_0—is multiplied by ξ, resulting (after cancellation) in the following:

$$K_s\ln(S/\xi S_0) = (S_0 + qB_0 + K_s)\ln[(S_0 + qB_0 - S/\xi)/(qB_0)] - (S_0 + qB_0)\mu_{max}t \quad (4)$$

When differences in specific activity (ξ) between treatments are taken into account, equation 4 describes the disappearance of ^{14}C from samples in which mineralization of a ^{14}C-labeled substrate follows the integrated Monod model.

To perform nonlinear regression analyses of the data from treatments with differing specific activity, it is necessary to estimate a value for the parameter ξ that is different for each treatment. One value for each of the parameters K_s, μ_{max}, and q should be capable of describing mineralization in different treatments. However, to statistically compare values estimated for the parameters of Monod kinetics based on the data from different treatments, it is also desirable to be able to estimate separate values for any parameter.

The general form of a model for biodegradation can be formulated as follows:

$$f(s,\mathbf{x},\boldsymbol{\theta},\xi) = 0 \quad (5)$$

where $\boldsymbol{\theta}$ (a vector) represents all parameters in the model except ξ, $\mathbf{x}$ (a vector) represents all independent variables in the model, and ξ is specific activity. To permit the separate estimation of two different values for ξ (i.e., one value for each of two different treatments), artificial, two-state, independent variables (a_i) can be introduced into equation 5 as follows:

$$f[S,\mathbf{x},\boldsymbol{\theta},(a_1\xi_1 + a_2\xi_2)] = 0 \quad (6)$$

An individual experimental observation is described for analysis with equation 6 by the following quantities: (i) measured substrate concentration; (ii) the values for the independent variables, namely, S_0, B_0, and t; and (iii) values for a_1 and a_2. If the concentration of substrate was measured in the first of two treatments, then a_1 would be set to 1 and a_2 would be 0. Setting a_2 equal to 0 has the effect of temporarily removing ξ_2 from the model. Parameters other than ξ may be associated with artificial independent variables in the same way. Thus, with artificial, two-state, independent variables, it is possible to estimate some parameters separately for the data from different treatments while estimating values for other parameters which must be the same for all treatments.

The integrated Monod equation shown in equation 4 was modified for use in nonlinear regression analyses by incorporating a term to correct for the failure of the experimental method to discriminate between radioactivity from ^{14}C in cells and that in solution. This correction, which has been described previously (14), introduces a new parameter (ζ the fraction of substrate taken up that is incorporated into the cells) into the model. It is not possible to present a modified form of equation 4 reflecting the ζ correction because the correction must be applied after the model has been solved numerically for substrate concentration as a function of time.

A computer program, MARQANCOVA, was developed to permit the analysis of substrate disappearance from treatments differing in initial substrate concentration and population density. In this program, S_0 and B_0 are treated as independent variables, and the use of artificial, two-state, independent variables is supported. MARQANCOVA uses the method of Marquardt (2) for the estimation of parameter values that minimize the sum of the squared residuals. For models that have no explicit analytical solution for substrate concentration as a function of time, e.g., the integrated Monod equation, Newton-Raphson iteration (3) is used to solve the model numerically.

The integrated Monod model is the most general model used in this paper. It has been argued that the integrated Monod equation can be closely approximated by five simpler functions when growth is negligible, the substrate concentration is well below K_s, and the substrate concentration greatly exceeds K_s (14). These five models include first-order, zero-order, and integrated Michaelis-Menten (Monod without growth [14]) kinetics. The remaining two models are mirror images of the logistic model for growth, which is used in ecology (10), and of a logarithmic growth curve plotted on arithmetic axes. The data obtained from individual treat-

ments were analyzed by using these models. When the combined data from all treatments were analyzed with MARQANCOVA, only the integrated Monod model was used.

Statistical analyses. The statistical test used is similar to the analysis of covariance (15) that is used with linear models to determine whether two lines differ in slope or intercept. In this study, an analysis of covariance is used to determine whether the value for a parameter of Monod kinetics, such as K_s, differs significantly between two or more treatments. To determine whether a significant difference exists between two values for a parameter which best describe the kinetics of mineralization in two different treatments, two quantities are needed. One quantity is the sum of the squared residuals (SS_i) which remains when the data from the two treatments are independently analyzed using a different estimate of the parameter in question for each culture. The other quantity is the sum of the squared residuals (SS_c) that remains when the combined data from the two treatments must be explained using a single value of the parameter. The difference between the two quantities ($SS_c - SS_i = SS_d$) is associated with 1 degree of freedom, corresponding to the difference in the number of parameters in the two models that were used to produce SS_c and SS_i. An F ratio (15) can be formed from $(SS_d/1)/[SS_i/(n - p - 1)]$, where n is the number of observations made in both treatments and p is the total number of parameters used in the analysis of the data that produced SS_i. In general, p would be twice the number of parameters in the original model because a separate set of parameters of the original model is estimated in each of the independent analyses of the data from the two treatments. A significant value of F indicates a significant difference between the values for the parameter which best explain mineralization in two treatments. This test can be used to evaluate differences in parameter estimates between more than two treatments. It can also be used to determine whether any of a group of parameters differs significantly between treatments.

The statistical test used to determine which of the models derived from the Monod equation offers the most valid description of the kinetics of degradation observed in an individual treatment has been described previously (14).

RESULTS

The inoculum of *Pseudomonas* sp. was derived from a culture that had been grown for 48 h in the salts solution with 100 μg of benzoate per ml. The cells were collected by centrifugation, washed, incubated with the substrate for 3 h, and then washed free of the substrate. The washed cells were resuspended in a volume of the sterile salts solution equal to the volume in which they were originally grown. This cell suspension was then diluted, and portions of the diluted suspension were added to 250 ml of sterile salts solution containing 400 ng of benzoate per ml. The number of cells per milliliter present was approximately equal to the number that would have been produced at the expense of 4 ng of benzoate per ml. Portions of this mixture of cells and benzoate were diluted in sterile salts solution contained in sterile flasks so that the flasks in a series contained 400, 200, 100, 50, and 25 ng of benzoate per ml and cell densities that could have been produced by 4, 2, 1, 0.5, and 0.25 ng of benzoate per ml, respectively.

The patterns of benzoate mineralization by *Pseudomonas* sp. are shown in Fig. 1. The solid curves were fit by separate regression analyses of the data from each treatment, and the dotted curves were fit by analyses using only one value each for K_s, μ_{max}, and q. The dotted curves are shown only if they

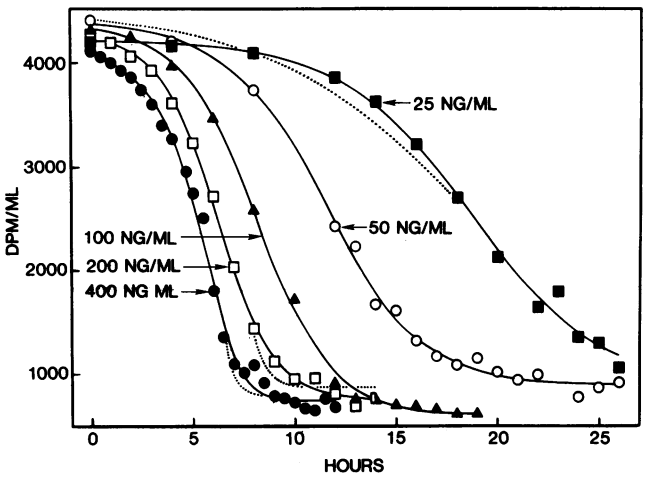

FIG. 1. Mineralization by *Pseudomonas* sp. of benzoate at five concentrations.

are distinguishable from the solid curves. All patterns are S-shaped, and the data were analyzed by using regression fits to the integrated Monod equation with growth (equation 3) and the logistic equation (14). The disappearance of 400 ng of benzoate per ml was better described by the integrated Monod equation than by the logistic equation. However, the integrated Monod equation failed to provide a fit to the data for mineralization at the other concentrations that was significantly better than a logistic fit at the $P = 0.05$ level. Therefore, the solid curve in Fig. 1 shown for the disappearance of 400 ng of benzoate per ml was fit by using the integrated Monod equation, and the remaining solid curves were produced with the logistic equation.

The regression estimates for parameters of the model selected as being most appropriate for the analysis of the data from each of the six flasks appear in Table 1. The results of individual analyses of the data from the different treatments are given in columns 2 through 6. Estimates of the parameters of the integrated Monod equation based on an analysis by MARQANCOVA of the combined data from all treatments are given in the last column. The estimates of the parameters afforded by the single regressions vary widely only for the specific activity, ξ, of the benzoate used in the five treatments. The specific activity would be expected to vary between the treatments because different amounts of unlabeled benzoate were added. Estimates for parameters that would not be expected to vary between treatments are in closer agreement. The estimates of k_4 of the logistic model varied inversely with S_0, whereas values estimated for q varied directly with S_0. Because the amount of substrate added to each treatment was ca. 100 times greater than the amount that would have been required to produce the number of cells used as inoculum for each treatment, considerable growth of the cells would be expected in all cases. The integrated Monod and logistic models are postulated to apply when growth of the active organisms is appreciable, and the logistic and integrated Monod models are thought to apply to the mineralization of concentrations of substrate that are low and high, respectively, relative to K_s (14). Thus, the models statistically chosen as the most appropriate descriptions of the kinetics are consistent with expectation.

Comparisons of the estimates of the parameters were performed with the analysis of covariance test described above. The apparent differences in the values of ζ shown in

TABLE 1. S_0, B_0, and the parameters with associated asymptotic standard deviations of the Monod equation with growth and of the logistic equation fit to data on the mineralization of benzoate by *Pseudomonas* sp.

Regression model	S_0 (ng/ml)	B_0 (rlb/ml)	Rate constant	K_s (ng/ml)	q (ng/rlb)	ξ (dpm/ng)	ζ
Monod	400	4	0.56 ± 0.15[a]	44 ± 3	4 ± 2	10.02 ± 0.1	0.225 ± 0.012
Logistic	200	2	3.6 ± 0.2[b]	NA[c]	2.2 ± 0.5	21.1 ± 0.2	0.177 ± 0.009
Logistic	100	1	5.4 ± 0.3[b]	NA	1.08 ± 0.28	43.1 ± 0.4	0.140 ± 0.007
Logistic	50	0.5	8.2 ± 0.7[b]	NA	0.84 ± 0.32	87.3 ± 1.2	0.204 ± 0.009
Logistic	25	0.25	12.6 ± 1.9[b]	NA	0.28 ± 0.20	168 ± 3	0.208 ± 0.051
Monod[d]	All	All	0.66 ± 0.03[a]	33 ± 1	1.3 ± 0.2	SE[e]	SE

[a] The rate constant is μ_{max} with units of per hour.
[b] The rate constant is $k_4 = \mu_{max}/K_s$ with units of milliliters per picogram per hour.
[c] NA, Not applicable.
[d] The combined data from all treatments were used to estimate a single value for each parameter, except for ξ and ζ.
[e] Values for ξ and ζ were separately estimated (hence designated SE) for each curve in the combined regression. The estimated values differ little from the estimates derived from analyses of single curves.

Table 1 were statistically significant ($P < 0.005$). When the results from the individual treatments were analyzed separately with the models in Table 1, a total residual variance of 464,000 (dpm/ml)^2 could not be explained by the models. For analysis of the regression of the combined data from all treatments, separate values of ξ and ζ were estimated for each treatment, but a single value for each of the parameters of Monod kinetics was required to explain the data from all treatments. This analysis left a value of 812,300 (dpm/ml)^2 unexplained. The variation left unexplained by the analysis of the combined data with a minimum parameter set was significantly greater ($P < 0.01$) than the variation remaining when the data from the five treatments were analyzed individually. Thus, at least one of the three parameters of Monod kinetics must differ significantly between treatments. If the integrated Monod equation completely described mineralization kinetics in this experiment, then no significant differences in μ_{max}, K_s, or q would be expected. Therefore, a factor not accounted for in the model is affecting the kinetics of mineralization by *Pseudomonas* sp.

Although the analysis of covariance indicated that a single value for each of μ_{max}, K_s, and q did not account adequately for the patterns of mineralization, one value for each parameter accounted qualitatively for much of the observed kinetics. The solid curves in Fig. 1 were generated by the five individual regressions. However, the combined regression in which all estimates for the parameters were the same for all curves (except for ξ and ζ) produced curves of best fit which generally lie so close to the individual regression curves that the two sets of curves cannot be distinguished readily from one another. When the two sets of curves of best fit were separated by a distance greater than half of the size of the circles used for the data points in Fig. 1, a dotted curve was used to indicate the best fit that could be achieved using only one value for each of the parameters K_s, μ_{max}, and q. For the mineralization of 100 and 50 ng of benzoate per ml, only the curves generated by the individual regressions are shown, since the dotted curves of best fit were so close to the solid curves that they could not be distinguished. In terms of variance, the integrated Monod model could account for more than 9×10^7 (dpm/ml)^2 using only one value for each of the parameters of Monod kinetics. Thus, the additional amount of variation that can be explained using different values for μ_{max}, K_s, and q is less than 1% of the variance explained using only one value for each of these parameters. Not only the shapes of individual curves showing mineralization, but also much of the change in their shapes as related to initial substrate concentration or population density apparently can be explained by simple Monod kinetics.

In tests with *S. typhimurium*, the initial glucose concentrations and cell densities were varied by dilution. The initial number of cells was approximately 1% of the number that could be produced from the substrate initially present. The patterns of glucose mineralization are shown in Fig. 2. The last four data points in the mineralization of 0.10 μg of glucose per ml are not shown, but they were included in the analyses. The seven patterns were analyzed separately by nonlinear regression; the solid curves were fit by analyses of the data from individual treatments, and the dotted curves were fit by analyses with only one value each for K_s, μ_{max}, and q. The dotted curves are shown only when they can be distinguished from the solid curves.

The logarithmic and logistic models provided the best descriptions of the mineralization of 100 and 0.1 μg of glucose per ml, respectively. The patterns of mineralization of all intermediate concentrations were best described by the integrated Monod equation. These models were used to generate the solid curves of best fit shown in Fig. 2. When expressed in terms of percentage of substrate carbon left in solution, the patterns of mineralization of 10, 32, and 100 μg of glucose per ml were nearly superimposable. In contrast, the time required for essentially complete mineralization of 0.10, 0.32, and 1.0 μg of glucose per ml increased with decreasing substrate concentration, presumably because the growth rate was governed by substrate levels present at less than saturating levels.

The values for S_0 and B_0 provided to the regressions for each curve and the values of ξ and ζ estimated by the regression analyses for each curve are given in Table 2. The values for ζ have associated asymptotic standard deviations which are about 1% of the values. Therefore, the increase in ζ from 0.569 to 0.689 with increasing initial glucose concentrations from 0.32 to 100 μg/ml may be significant. An analysis of covariance showed that the apparent differences were statistically significant ($P < 0.005$).

The estimates of μ_{max}, K_s, and q are given in Table 3. It is evident that differences existed in some of the values for the parameters of Monod kinetics that best describe glucose mineralization at different substrate concentrations. To test the significance of these differences, the combined data from all treatments were subjected to an analysis in which one value for each of K_s, μ_{max}, and q was estimated. In this regression, individual values of ξ and ζ for each treatment (Table 2) were estimated. Unexplained variation equal to 334,100 (dpm/ml)^2 was left by the combined regression.

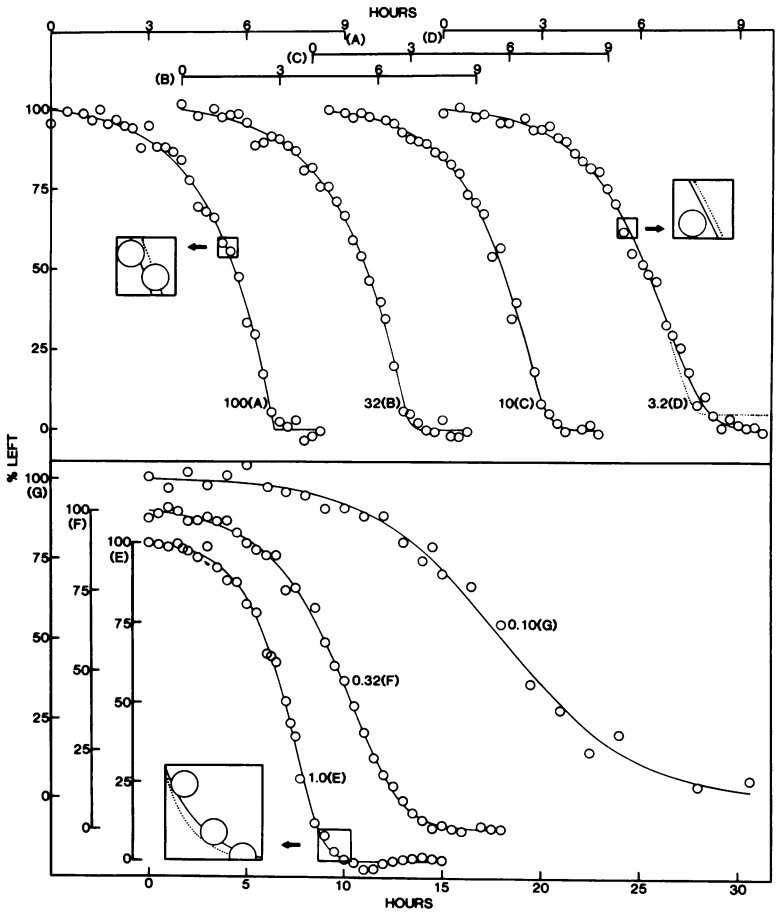

FIG. 2. Mineralization by *S. typhimurium* of glucose at seven concentrations. The curves at the top are horizontally displaced and those at the bottom are vertically displaced for clarity.

Separate analyses of the data from each treatment left residual variation equal to 221,800 $(dpm/ml)^2$. The difference between the two amounts of unexplained variation can be attributed to differences in the values of K_s, μ_{max}, and q that best explain the data from individual treatments. The difference was statistically significant ($P < 0.01$).

The curves in Fig. 2 shown as solid lines were generated by the individual regressions. For the patterns of mineralization of glucose at six of the seven concentrations, the curves of best fit generated by the combined regression lie too close to the curves provided by the individual fits to be resolved readily from one another. Only for the mineralization of 3.2 μg of glucose per ml was a portion of the curve produced by the combined regression further away from the curve generated by the individual regression than one-half of the diameter of the circles denoting individual points. This

TABLE 2. Independent variables and the parameters ξ and ζ with associated asymptotic standard deviations estimated by nonlinear regression analysis of patterns of mineralization of glucose by *S. typhimurium*

Variables[a]		ξ[b] (dpm/μg)	ξ[c] (dpm/μg)	ζ[b]	ζ[c]
S_0 (μg/ml)	B_0 (rlb)				
100	1.0	36.4 ± 0.1	36.2 ± 0.1	0.689 ± 0.007[d]	0.686 ± 0.005
32	0.32	121.0 ± 0.4	121.9 ± 0.3	0.645 ± 0.004	0.647 ± 0.004
10	0.10	372 ± 2	373 ± 1	0.613 ± 0.005	0.615 ± 0.005
3.2	0.032	1,165 ± 5	1,183 ± 4	0.578 ± 0.006	0.600 ± 0.004
1.0	0.010	3,800 ± 10	3,820 ± 10	0.571 ± 0.003	0.569 ± 0.004
0.32	0.0032	11,620 ± 40	11,800 ± 40	0.569 ± 0.008	0.574 ± 0.004
0.10	0.0010	37,300 ± 200	37,700 ± 200	0.589 ± 0.007	0.579 ± 0.005

[a] Both individual and combined regressions use the same values for S_0 and B_0.
[b] The data from individual treatments were analyzed to obtain the values shown.
[c] The data from all treatments were analyzed to obtain the values shown.
[d] The logarithmic model best described the data. The value shown was calculated manually because the logistic equation does not apply after mineralization is essentially complete.

TABLE 3. Values estimated by nonlinear regression for some of the parameters of three models for the mineralization of glucose by *S. typhimurium*

S_0 (μg/ml)	Model	Rate constants	K_s (μg/ml)	q (μg/rlb)
100	Logarithmic	μ_{max} = 0.56 ± 0.02	NA[a]	2.2 ± 0.3
32	Monod	μ_{max} = 0.55 ± 0.05	0.7 ± 0.8	2.5 ± 0.6
10	Monod	μ_{max} = 0.74 ± 0.12	1.5 ± 0.8	1.2 ± 0.5
3.2	Monod	μ_{max} = 1.05 ± 0.28	1.8 ± 0.8	1.0 ± 0.4
1.0	Monod	μ_{max} = 0.79 ± 0.13	0.36 ± 0.13	1.1 ± 0.3
0.32	Monod	μ_{max} = 0.61 ± 0.13	0.19 ± 0.07	1.6 ± 0.4
0.10	Logistic	k_4 = 3.0 ± 0.2[b]	NA	0.45 ± 0.15
All[c]	Monod	μ_{max} = 0.567 ± 0.012	0.182 ± 0.003	2.02 ± 0.16

[a] NA, Not applicable.
[b] k_4 has units of milliliters per microgram per hour.
[c] The data from treatments with all concentrations were analyzed. The values are those that best describe the disappearance of glucose at all concentrations.

portion of the curve generated by the combined regression is shown as a dotted line. To illustrate the small differences between the curves generated by individual and combined regressions, portions of the curves and the circles representing data points have been magnified threefold in the inserts in Fig. 2. The curves of best fit produced by analyses of the data from each of the treatments (solid lines) and of the combined data from all treatments (dotted lines) are evident in the inserts. The integrated Monod equation accounted for the patterns of substrate disappearance and the variation in shapes with initial substrate concentrations using only one value for each of the parameters of the model, except for ξ and ζ. However, statistically significant variation remains.

The mineralization of 10 and 100 ng of benzylamine per ml by acclimated sewage microorganisms is shown in Fig. 3. The patterns of metabolism of 10 and 100 ng of benzylamine per ml were best described by the first-order model and by integrated Michaelis-Menten kinetics, respectively. The mineralization of four higher concentrations of benzylamine by sewage microorganisms is shown in Fig. 4. The mineralization of 1.0 and 3.2 μg of benzylamine was best fit by the integrated Monod equation. Mineralization of the two highest concentrations followed logarithmic kinetics. The solid curves of best fit shown in Fig. 3 and 4 were fit by the models deemed most appropriate (at the $P < 0.05$ level) for the analysis of the data when the results from different treatments were analyzed individually. Because little mineralization occurred in the test period in sewage samples that received 100 μg of benzylamine per ml, these data were not further analyzed.

The estimates for ξ and ζ are given in Table 4. The same values of S_0 were used for both independent and combined regressions. Independent and combined regressions were carried out with the data from individual and all treatments, respectively. In the combined regression, a single value of ζ equal to 0.202 ± 0.003 described the results of all tests. The values estimated for ζ by independent regression analyses of the data from individual treatments varied among the treatments from 0.159 to 0.213. The asymptotic standard deviations associated with the values for ζ are 1 order of magnitude smaller than the difference between the largest and the smallest values of ζ.

The estimates of K_s, q, and the rate constants associated with benzylamine mineralization in samples of sewage are given in Table 5. It is evident that many of the values of μ_{max},

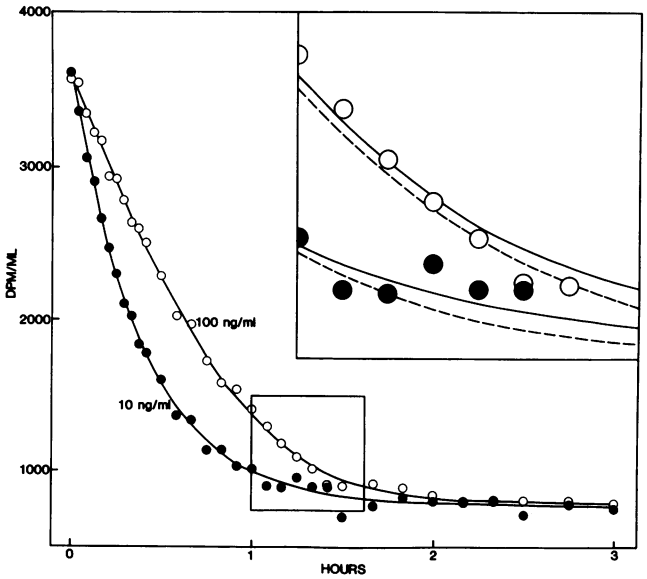

FIG. 3. Mineralization of 10 and 100 ng of benzylamine per ml by sewage microorganisms.

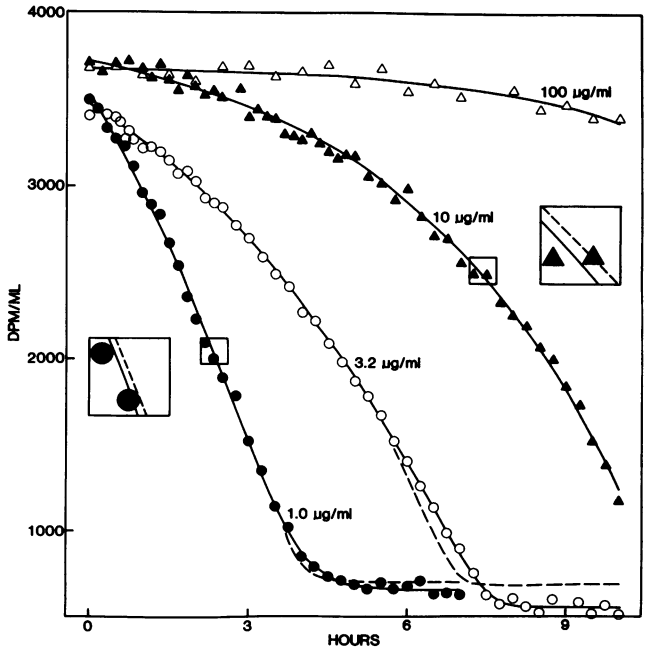

FIG. 4. Mineralization of 1.0, 3.2, 10, and 100 μg of benzylamine per ml by sewage microorganisms.

TABLE 4. Values for ξ and ζ with associated asymptotic standard deviations estimated by nonlinear regression analysis of patterns of mineralization of benzylamine in samples of sewage

S_0 (μg/ml)	ξ (dpm/ng)		ζ (independent regression)
	Independent regression	Combined regression	
0.010	358 ± 3	364 ± 2	0.213 ± 0.003
0.10	36.5 ± 0.2	36.1 ± 0.2	0.219 ± 0.003
1.0	3.50 ± 0.04	3.53 ± 0.02	0.186 ± 0.004
3.2	1.077 ± 0.005	1.082 ± 0.003	0.159 ± 0.003
10	0.368 ± 0.001	0.372 ± 0.001	NA[a]

[a] NA, Not applicable.

K_s, and q differed at different substrate concentrations by more than two asymptotic standard deviations.

The significance of the apparent difference between the values of these parameters was investigated. Estimates were made of one value each for the five parameters of the Monod model (except for ξ) to provide curves that best fit data on the mineralization of benzylamine at all concentrations (Tables 4 and 5). The curves of best fit generated using these estimates are shown as broken curves in Fig. 3 and 4. These curves are shown only when they are separated from the solid curves by a distance greater than the diameter of the symbols used to represent individual observations. In the inserts in Fig. 3 and 4, portions of the curves are enlarged threefold to illustrate the differences between the solid and the broken curves.

An analysis of covariance showed a highly significant difference among the values for ζ that best describe the kinetics of mineralization of various concentrations of benzylamine in sewage. When the combined data from all treatments were analyzed using only one value of ζ and all other parameters (except ξ), a variance equal to 661,800 $(dpm/ml)^2$ remained. When ζ was estimated individually for each treatment but one value for each of the other parameters of the integrated Monod model was required, an unexplained variance equal to 480,900 $(dpm/ml)^2$ remained. Although the reduction in variance achieved by estimating ζ separately for each treatment was highly significant, the further reduction [to 245,100 $(dpm/ml)^2$] that was achieved by individually estimating all parameters for each treatment was even larger. In the experiments with *Pseudomonas* sp. and *S. typhimurium*, the requiring of a single value of ζ for all curves resulted in an increase in unexplained variation that was at least four times greater than the increase in variation when all other parameters of the model (except ξ) were required to be the same for all curves. Consequently, ζ was not treated differently from other parameters, as was done in the studies of the two bacteria, and the broken curves in Fig. 4 and 5 were drawn by using a single value for ζ.

When the results from separate treatments are analyzed by using individual values for ζ (as well as ξ), the curves of best fit generated using a single value for all other parameters of the integrated Monod equation lie so close to the curves generated by separate nonlinear regression analyses of the data from individual treatments that they cannot be distinguished readily from one another. In this respect, the integrated Monod equation adequately describes the kinetics of mineralization of benzylamine in sewage. However, in all of the experiments performed with pure cultures and sewage, significant differences were observed in the values for the parameters of Monod kinetics that best describe the patterns of mineralization in different treatments.

DISCUSSION

The patterns of mineralization of benzylamine in sewage resembled the curves of benzoate mineralization by *Pseudomonas* sp. (14). In both this and the previous study, the kinetics of mineralization of the lowest concentrations of substrate were best described by the first-order model. In order of increasing substrate concentration, the integrated Michaelis-Menten, integrated Monod, and logarithmic models were most appropriate for the description of the mineralization of higher levels of substrate.

In the previous study of benzoate mineralization by *Pseudomonas* sp., the values of μ_{max} were between 0.34 and 0.44 h^{-1} (14). These values are close to the values for μ_{max} (0.56 to 0.66 h^{-1}) estimated for the same organism in the present investigation. The values estimated for K_s in the two studies differed substantially, however, ranging from 33 to 44 ng/ml in this study and from 130 to 450 ng/ml for the same substrate and organism in the previous report (14). In addition, significant differences were found in the present study in the values for the parameters of Monod kinetics that best described mineralization in treatments differing in initial substrate levels and cell densities. Although such differences both between and within studies may result from the lack of inclusion in the models of important variables affecting mineralization kinetics, it is also possible that such parameters as μ_{max}, K_s, and q may, in fact, vary in the course of an experiment. Evidence for changes in yield ($1/q$ in our model) with substrate concentration has been found in studies of continuous cultures (7, 16). However, in the studies reported here, any change in q appears to be opposite from that which one would expect from considerations of maintenance energy. A tendency is evident in Table 1 for cell quota to decrease with decreasing initial concentration of benzoate

TABLE 5. Values estimated by nonlinear regression for some of the parameters and associated asymptotic standard deviations of models of mineralization of benzylamine in samples of sewage

S_0 (μg/ml)	Model	Rate constants[a]	K_s (ng/ml)	q (ng/rlb)
0.010	First order	k_3 = 2.55 ± 0.03	NA[b]	NA
0.10	Michaelis-Menten	k_1 = 160 ± 15	51 ± 9	NA
1.0	Monod	μ_{max} = 0.39 ± 0.11	230 ± 82	530 ± 150
3.2	Monod	μ_{max} = 0.20 ± 0.02	150 ± 47	1,120 ± 120
10	Logarithmic	μ_{max} = 0.234 ± 0.006	NA	900 ± 60
All[c]	Monod	μ_{max} = 0.256 ± 0.004	72 ± 3	710 ± 25

[a] The first-order rate constant, $k_3 = \mu_{max}q/K_s$, has units of per hour per unit of relative biomass. The zero-order rate constant, $k_1 = \mu_{max}q$, has units of nanograms per milliliter per hour per unit of relative biomass. The units for μ_{max} are per hour.

[b] NA, Not applicable.

[c] The combined data from all treatments were used to estimate a minimum set of parameters.

and density of *Pseudomonas* sp. cells. Because the substrate was used more slowly when provided at low levels to small populations, one would expect maintenance costs to consume a greater fraction of the cell's energy, thus increasing the cell quota. If there is any trend for change in q with initial glucose concentration and numbers of *S. typhimurium* (Table 3), it too is a tendency for q to decrease with lower S_0 and B_0. Thus, if the apparent differences in q are real, it is unlikely that maintenance energy could account for them. The possibility exists, however, that the differences in q are not real. As can be seen from the sensitivity equations of the integrated Monod equation (12), yield (and hence q) is highly correlated both with K_s and with μ_{max}. Real differences in either of these two parameters could manifest themselves as apparent changes in q.

K_s may also vary with substrate concentration if multiple uptake systems with differing affinities for the compound are present. The activity of high-affinity transport systems would be expected to increase relative to uptake by systems of lower affinity as substrate concentration decreases. In addition, organisms capable of producing different uptake systems may change the relative rates at which high- and low-affinity systems are synthesized as a function of substrate concentration. Presumably, over several generations of growth at low substrate concentrations, progeny cells would be enriched for high-affinity transport systems. In either case, the value of K_s estimated by nonlinear regression might be expected to decrease with declining substrate concentration. In the present study, the estimated values of K_s for the growth of *S. typhimurium* on glucose appear in general to decrease with initial substrate concentration. Because $k_4 = \mu_{max}/K_s$ (14), increasing values for k_4 with decreasing benzoate concentrations (Table 1) could also result from lowered K_s values for growth of *Pseudomonas* sp. at lower concentrations of benzoate. *Escherichia coli* has been reported to possess more than one uptake system for many substrates, including glucose (5) and ribose (9). Strains of *Pseudomonas* often have multiple, overlapping uptake systems for amino acids (4). Azam and Hodson (1) found evidence for the presence of multiple uptake systems for glucose in populations of marine microorganisms. The development of models more elaborate than integrated Monod kinetics may be necessary to provide an adequate description of mineralization of a substrate in different treatments by organisms with more than one uptake system.

To predict the fate of synthetic chemicals introduced into natural environments, models more elaborate than the integrated Monod equation often may be unnecessary. Although evidence was found for differences in the parameters of Monod kinetics between treatments, the integrated Monod equation gave a close description of mineralization in different treatments with only a single value for each parameter. The theoretical curves produced with a minimum set of parameters were indistinguishable in these studies from curves of best fit generated with a different value for each parameter for each treatment.

If the integrated Monod equation is to be useful in describing the kinetics of degradation in natural environments, reliable estimates of the values of its parameters are needed. The treatment of initial substrate concentration and population density as independent variables for regression analyses offers a means for obtaining values for μ_{max}, K_s, and q that best describe mineralization of a substrate over a wide range of concentrations.

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