APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 1987, p. 1064–1068
0099-2240/87/051064-05$02.00/0

Vol. 53, No. 5

Alternative Nonlinear Model for Estimating Second-Order Rate Coefficients for Biodegradation

JOSEPH M. SUFLITA,[1]* WALTER J. SMOLENSKI,[2] AND JOSEPH A. ROBINSON[2]

Department of Botany and Microbiology, University of Oklahoma, Norman, Oklahoma 73019,[1] and Agricultural Division, The Upjohn Company, Kalamazoo, Michigan 49001[2]

Received 16 October 1986/Accepted 22 January 1987

A modification of the second-order model for biodegradation was derived, applied to an example data set, and shown to be superior for describing the anaerobic biodegradation of *p*-cresol by an enriched bacterial consortium. The modified model circumvents the no-growth assumption implicit in the use of the second-order rate equation, but still requires the assumption of first-order kinetics over the course of substrate depletion. Violation of the no-growth assumption is particularly important since overestimates of the pseudo-first-order rate coefficient lead to underestimates of the time required for the removal of a xenobiotic chemical from a contaminated environment. Our calculations show that the errors introduced into the pseudo-first-order rate coefficient (and the resulting estimates of the second-order rate coefficient) approach 100% if one doubling occurs in activity over the course of substrate depletion. For an exemplary data set, use of a first-order model resulted in a 100% overestimate of the first-order decay coefficient, which would in turn lead to a corresponding overestimate of the second-order rate coefficient. The modified model we describe is a potential alternative to the pseudo-first-order model for the routine estimation of second-order rate coefficients.

The second-order rate model has received considerable attention as a tool for quantitatively predicting the disappearance of xenobiotic chemicals from natural environments (1, 7–10). While attractive in its simplicity, the model suffers from several practical and theoretical constraints. The major focus of this manuscript is to quantitatively examine the errors associated with the second-order rate assumptions and to lay a theoretical foundation for a modified version of the model which circumvents some of the theoretical difficulties.

The second-order rate model can be derived from the differential form of the Monod equation. Published mixed-culture data support this derivation (7). Unfortunately, this model has never been applied to substrate depletion data obtained with pure cultures (or defined mixed cultures) sampled from chemostats at various growth rates to check the general validity of this derivation. Such an experiment is essential since the previous growth rate history of an organism is known to influence growth and uptake kinetic parameters determined in batch systems (11). Despite this, the second-order rate model for biodegradation has been used to describe the fate of a limited number of xenobiotic compounds to a statistically appropriate degree (7–10).

Only one of the two assumptions associated with the use of the second-order rate expression compromises its utility in a nonconservative way. The first assumption is that first-order kinetics is obeyed over the course of substrate depletion. This allows a first-order model to be fit to the data and the second-order rate constant to be calculated if a suitable estimate of active biomass is available. When incorrect, this assumption leads to an underestimate of the second-order rate coefficient and a corresponding overestimate of the time required for a xenobiotic compound to reach acceptable levels in natural ecosystems. From a regulatory perspective, overestimating the time required to reach this substrate concentration is conservative and, thus, acceptable. In contrast, the assumption that microbial growth and thus activity changes are negligible can lead to overestimates of the second-order rate constant and to unwarranted optimism regarding the rate of pollutant elimination from a contaminated environment. Violation of this second assumption may occur when the requisite microorganisms grow at the expense of substrates other than the pollutant under investigation. Also, significant microbial growth can occur when the xenobiotic substrate concentration is in the mixed-order or zero-order region.

In the present manuscript we describe the derivation and use of a modified version of the second-order rate model that circumvents the need for the no-growth or no-changes-in-activity assumption. This modified model still makes the first-order assumption but allows the active biomass to increase over the course of substrate depletion. The model allows the estimation of the second-order rate coefficient and the slope of the Monod equation in the first-order region (μ_{max}/K_S). We illustrate the use of this new model by fitting it to a substrate depletion curve for the anaerobic biodegradation of *p*-cresol. It is clear from the discrimination techniques used that the new model is an improvement statistically in the description of this data set over the second-order rate expression.

In addition to the derivation and application of this new model, we discuss the quantitative implications of the two assumptions discussed above on estimates of the second-order rate coefficient. Using theoretically derived error plots, we show that (i) even modest increases in activity (which may be undetectable depending on how biomass is measured) can lead to significant overestimates of the second-order rate coefficient and (ii) the substrate concentration must be at least 10-fold below the half-saturation constant to produce acceptably small errors (<10%) in estimates of the

---

* Corresponding author.

second-order rate coefficient when this parameter is determined assuming first-order kinetics.

THEORY

Model derivations. The second-order rate model and the model we describe in this manuscript may each be derived from the differential form of the Monod equation (1, 13, 17):

$$dS/dt = -[(\mu_{\max} \times S)/(K_S + S)] \times (B/Y) \quad (1)$$

where $\mu_{\max}$ = the maximum specific growth rate, K_S = the half-saturation constant for growth on rate-limiting substrate S, and Y = a yield coefficient for the conversion of substrate carbon into biomass carbon B. If S is in the first-order region, then the differential form of the Monod model simplifies to

$$dS/dt = -[(\mu_{\max} \times S)/K_S] \times (B/Y) \quad (2)$$

This model may be re-expressed as

$$dS/dt = -k_2 \times S \times B \quad (3)$$

which is the second-order model for biodegradation where saturation is presumed negligible and with $k_2 = \mu_{\max}/(K_S Y)$.

Experimentally, the second-order rate model is used in the following reparameterized form:

$$dS/dt = -k_1 S \quad (4)$$

with $k_1 = k_2 \times B_0$. k_1 is referred to as a pseudo-first-order rate constant because its value is proportional to the concentration of biomass present at $t = 0$ (B_0). By using this form, the net change in biomass or activity over the course of a substrate decay experiment is assumed to be negligible. The derivation of the modified model begins again with the differential form of the Monod equation, equation 1. Instead of assuming that B is constant over the progress curve and equal to B_0, it is allowed to change according to a mass-balance relationship,

$$B = Y \times (S_0 - S) + B_0 \quad (5)$$

where S_0 equals S at $t = 0$. Assuming first-order kinetics leads to

$$dS/dt = -[(\mu_{\max} \times S)/K_S] \times [(S_0 - S) + (B_0/Y)] \quad (6)$$

If we group terms in equation 6 and simplify, the following expression is obtained:

$$dS/dt = (k \times S^2) - [(k \times S_0) + k_1] \times S \quad (7)$$

where $k = \mu_{\max}/K_S$ and k_1 equals the pseudo-first-order rate coefficient. Thus, by fitting data to equation 7, we can estimate both the pseudo-first-order rate coefficient, k_1, and an additional parameter, k, that equals the slope of the Monod equation in the first-order region ($\mu_{\max}/K_S$). The second-order rate coefficient k_2 may be estimated by dividing k_1 in equation 7 by the estimate of active B present at the start of the substrate depletion experiment (B_0). This approach is analogous to the standard method of estimating k_2 from the value of k_1 determined by fitting a progress curve to equation 4, only the no-growth assumption is not required.

Error curves for k_1 and k_2. The impact of the first-order and no-growth assumptions on estimates of k_1 (and on k_2 since errors in this parameter are directly proportional to errors in k_1) can be theoretically quantified. Assuming that active microbial growth occurs according to equation 7, the apparent estimate of k_1 determined with equation 4 depends linearly on the fraction of substrate carbon converted into biomass. We can express this dependence relative to the initial amount of active biomass carbon present, B_0 (Fig. 1). The curve shown in Fig. 1 does not depend on the yield coefficient, Y, since the x axis incorporates all possible Y values. One can conclude from Fig. 1 that the magnitude of the overestimate of k_1 and, hence, k_2 will approach 100% if one doubling in activity occurs during a time series kinetic experiment.

An error curve for violation of the first-order assumption may be derived by considering the error in the predicted rate of substrate depletion for a saturable process as approximated by first-order kinetics. This curve is mathematically defined as the difference between rate predictions made by first-order versus Michaelis-Menten models divided by the rates predicted from the Michaelian model, as a function of substrate concentration. This relationship shows that a substrate concentration 10-fold below the half-saturation constant produces errors (<10%) that are acceptable from a practical perspective (Fig. 2). The absolute error in k_1, and k_2 estimates derived from k_1, approach 50% as the substrate concentration approaches the half-saturation constant, K_m.

Note that a dimensionless substrate concentration (S/K_m) is plotted in Fig. 2. Thus, this error curve does not depend on the specific value of K_m since this parameter is incorporated in the dimensionless variable S/K_m.

MATERIALS AND METHODS

Experimental data set. Progress curves for the anaerobic biodegradation of *p*-cresol were used to evaluate equations 7 and 4 for estimating the pseudo-first-order rate coefficient k_1. Details on the experimental conditions and methods of analysis can be found elsewhere (19). Briefly, bacterial cells were enriched from a shallow anoxic aquifer for their ability to degrade *p*-cresol under sulfate-reducing conditions. The enrichment was grown in a RAMM mineral salts medium (14) supplemented with 20 mM Na_2SO_4 and 300 μM *p*-cresol as the carbon source. The enrichment was anaerobically transferred to incubation flasks which were in turn connected to an automated high-pressure liquid chromatography

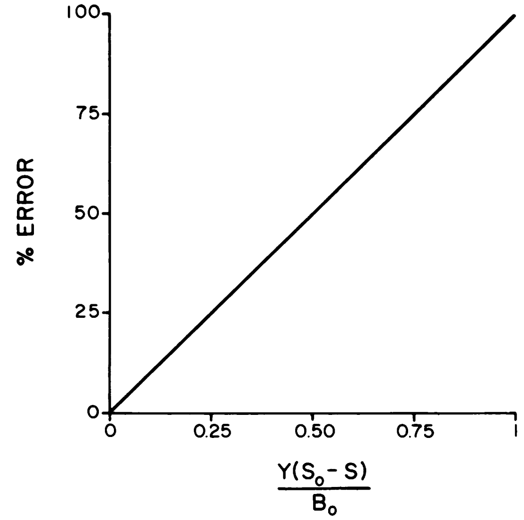

FIG. 1. Errors introduced into the pseudo-first-order rate coefficient (and estimates of the second-order rate constant derived from it) by violation of the no-growth assumption. The x axis equals the fraction of substrate converted into biomass relative to the initial biomass. It thus incorporates all possible Y values.

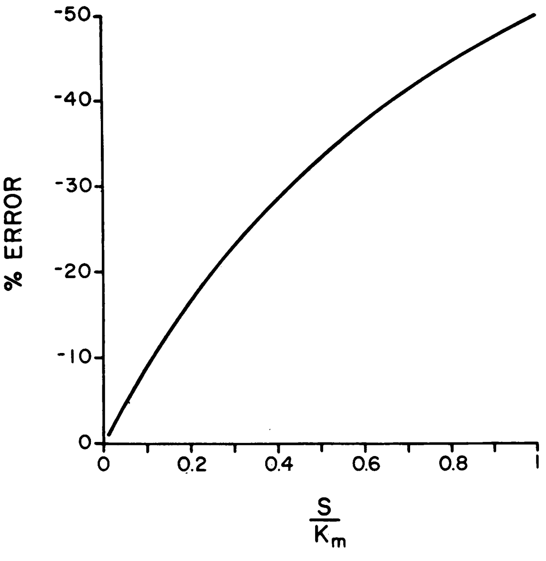

FIG. 2. Errors introduced into the pseudo-first-order rate coefficient (and estimates of the second-order rate constant derived from it) by violation of the first-order kinetic assumption. The dimensionless substrate concentration S/K_m is plotted on the x axis; thus this curve is a general one and independent of the K_m value.

system designed for the unattended collection of time series kinetic data. The enrichment was amended with *p*-cresol, and substrate disappearance was monitored by high-pressure liquid chromatography.

Regression method. The *p*-cresol decay curve was fit to both equations 4 and 7, using Hartley's modification of the Gaussian method (5). The standard errors of the parameters were approximated by using the covariance matrix of the parameters (12). These standard errors are optimistic since they are based on within-assay rather than between-assay variation.

Model discrimination. Goodness-of-fit for model 4 versus model 7 was assessed in two ways. First, an F-test for model discrimination (12) was used to determine if the decrease in the residual mean square for model 7 was significantly lower than that obtained for the fit of the *p*-cresol depletion data to model 4. Since the integrated versions of the models being compared are nonlinear with respect to their parameters, the significance of the F-test is only approximate (12). Second, lack-of-fit errors (that is, errors due to use of an incorrect model) were checked for by using residuals plots (4, 12). It is more instructive to construct residuals plots than to merely plot the collected data versus the best-fit nonlinear model since deviations from the fitted equation are magnified in residuals plots (3).

RESULTS AND DISCUSSION

The objectives of this work were twofold. First, we wished to quantify and emphasize the influence of the two assumptions implicit in the use of the second-order model for biodegradation, namely, the first-order kinetics and no-growth assumptions. Second, we wished to show, through the use of an alternative nonlinear model, that the second-order rate coefficient (derived from the pseudo-first-order rate coefficient and a suitable estimate of active biomass at time zero) can be determined without assuming that growth is negligible during first-order substrate depletion.

Violation of the no-growth assumption can be serious if the interest in estimating k_2 lies with making regulatory decisions since estimates of pollutant decay rates will be nonconservative. That is, violation of this assumption leads to an underestimate of the time needed for removal of a pollutant since k_1, and k_2 in turn, are overestimated. The magnitude of these errors can be large even for modest increases in biomass (e.g., 25%) during pseudo-first-order substrate depletion (Fig. 1). For the anaerobic *p*-cresol depletion curve, we found that fitting the data to equation 4 resulted in an approximately 100% larger value for k_1 than that obtained with model 7 (Table 1). Substituting equation 7 in place of 4 for routine estimation of k_1 and k_2 would eliminate the need for the no-growth assumption, but the general validity of equation 7 needs to be established experimentally before a specific recommendation can be made.

If an underestimate of the pseudo-first-order rate coefficient (and the corresponding value of k_2) is obtained, then the time required for a xenobiotic substrate to reach acceptable concentrations in a given habitat will be overestimated. This type of error can occur when zero- or mixed- rather than first-order substrate disappearance data are incorrectly fit to the pseudo-first-order model (equation 4). But, this error is a conservative one. Its quantitative impact has heretofore not received specific expression, yet this type of error deserves some attention since overestimating the time required for pollutant decomposition could have economic consequences.

Equation 7 did a better job of describing the anaerobic degradation of *p*-cresol than did equation 4. The residual mean square for model 7 was significantly lower than that obtained for equation 4 at the 1% level of statistical significance (Table 1). In addition, the residuals for equation 7 (Fig. 3) showed less systematic behavior than those obtained for equation 4 (Fig. 4). However, the existence of several apparently nonrandom regions of the residuals suggest that even model 7 is not entirely correct. The presence of visually apparent lack-of-fit errors (Fig. 3) may be due to a violation of the assumption that first-order kinetics was obeyed during *p*-cresol decay. Some systematic behavior in the residuals is expected even if model 7 is biologically correct given that (i) residuals are generally correlated since there are only $n - p$

TABLE 1. Results of fitting *p*-cresol test data set to equation 4 versus 7

Equation	k_1 (h^{-1})	SE of k_1	k ($h^{-1} \cdot \mu M^{-1}$)	SE of k	df[a]	RMS[b]
4	2.0×10^{-2}	6.6×10^{-4}	NE[c]	NE	43	24.80
7	1.0×10^{-2}	1.2×10^{-3}	2.4×10^{-4}	3.8×10^{-5}	42	19.95**[d]

[a] Number of data pairs minus number of parameters in model.

[b] RMS, Residual mean square (sum of squared residuals divided by degrees of freedom).

[c] NE, Not estimable; the ratio of μ_{max}/K_S ($= k$) cannot be estimated by fitting model 4 to a first-order progress curve without prior knowledge of Y.

[d] ** indicates that reduction in the residual mean square for model 7 versus model 4 is significant at the 1% level of statistical significance; note that the significance level is only approximate since the models are nonlinear.

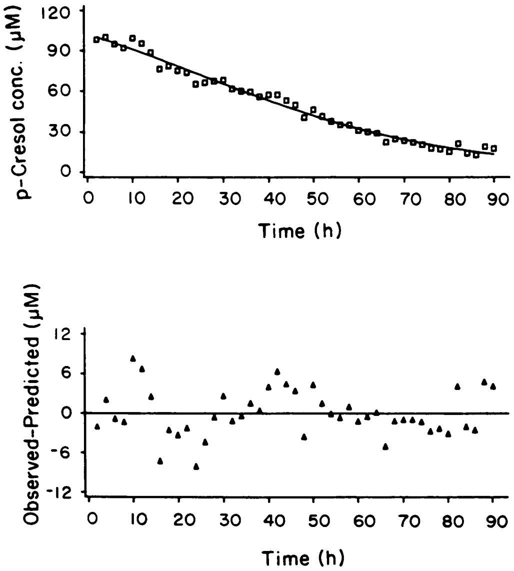

FIG. 3. Best-fit curve and residuals for the fit of the *p*-cresol data to model 7.

degrees of freedom among them (4) and (ii) intensive sampling alone often results in correlated residuals (6).

Model 7 is mathematically similar to two other biodegradation kinetic models derived by Brunner and Focht (2). These authors described 3/2-order models in which substrate depletion was not explicitly linked to growth. Equation 7 may be considered similar in that it depends both on S^2 and S, but substrate depletion is explicitly linked to growth in equation 7.

Recently, a number of nonlinear models have been proposed to describe the biodegradation of compounds under various conditions (2, 14, 16–18). The model (equation 7) described in this work is less complicated than most of these and includes one more parameter than the simplest biodegradation model, equation 4. The use of increasingly more complicated models to describe the kinetic patterns of biodegradation presents difficulties since the number of parameters which may be uniquely estimated is limited. Practically, it is difficult to uniquely determine (that is, identify) the parameters of nonlinear models with five or more parameters from a given biodegradation progress curve. Thus, it is important that attempts to make models more general by increasing their complexity be tempered with statistical judgment. We believe model 7 is a compromise between the unrealistic simplicity of the first-order model and more complex multiparameter models which may be difficult to both identify and discriminate among statistically.

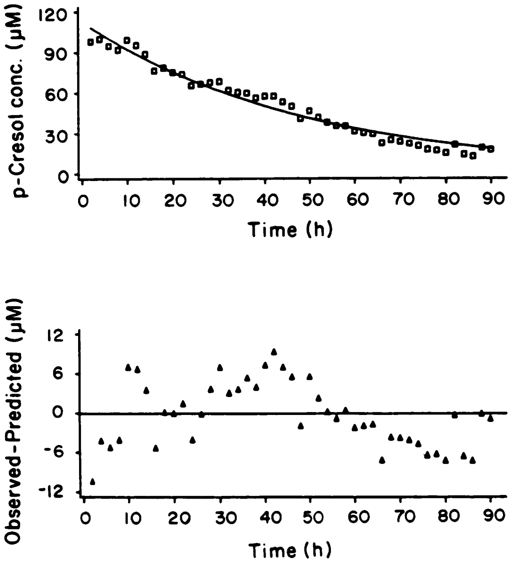

FIG. 4. Best-fit curve and residuals for the fit of the *p*-cresol data to model 4.

ACKNOWLEDGMENTS

This work has been supported in part by the U.S. Environmental Protection Agency through assistance agreements CR-812808 and CR-811146 to the University of Oklahoma.

ADDENDUM IN PROOF

Estimating k and k_1 (given B_0) by fitting progress curve data to equation 7 requires a nonlinear regression algorithm capable of numerically integrating this model to obtain the best fit S versus t curve. This is not always practical since many commercial regression routines do not permit fitting data to differential equations directly. To overcome this practical difficulty, the integrated version of equation 7 is

$$S = [P \times S_0 \times \exp(P \times t)]/[P + (Q \times S_0) - (Q \times S_0) \times \exp(P \times t)] \qquad (8)$$

where $P = -[(k \times S_0) + k_1]$, $Q = k$, and exp indicates exponentiation. Fitting data to equation 8 still requires a nonlinear regression algorithm, but not one capable of numerically estimating differential equations (i.e., equation 7).

LITERATURE CITED

1. **Boethling, R.** 1985. The "second-order" model—an overview, p. 40–48. *In* A. W. Bourquin, P. H. Pritchard, W. W. Walker, and R. Parrish (ed.), Proceedings of the workshop: biodegradations kinetics. EPA/600/9-85/018. U.S. Environmental Protection Agency, Washington, D.C.
2. **Brunner, W., and D. D. Focht.** 1984. Deterministic three-half-order kinetic model for microbial degradation of added carbon substrates in soil. Appl. Environ. Microbiol. **47:**1211–1216.
3. **Cleveland, W. S., and R. McGill.** 1985. Graphical perception and graphical methods for analyzing scientific data. Science **229:**828–833.
4. **Cook, R. D., and S. Weisberg.** 1982. Residuals and influence in regression. Chapman and Hall Ltd., New York.
5. **Hartley, H. O.** 1961. The modified Gauss-Newton method for the fitting of nonlinear regression functions by least-squares. Technometrics **3:**269–280.
6. **Jonsson, J. A., J. Vejrosta, and J. Novak.** 1982. Systematic errors occurring with the use of gas-sampling loop injectors in gas chromatography. J. Chromatogr. **235:**307–312.
7. **Paris, D. F., D. L. Lewis, and N. L. Wolfe.** 1975. Rates of degradation of malathion by bacteria isolated from aquatic systems. Environ. Sci. Technol. **9:**135–138.
8. **Paris, D. F., and J. E. Rogers.** 1986. Kinetic concepts for measuring microbial rate constants: effects of nutrients on rate constants. Appl. Environ. Microbiol. **51:**221–225.
9. **Paris, D. F., W. C. Steen, G. L. Baughman, and J. T. Barnett, Jr.** 1981. Second-order model to predict microbial degradation of organic compounds in natural waters. Appl. Environ. Microbiol. **41:**603–609.

10. **Paris, D. F., N. L. Wolfe, and W. C. Steen.** 1982. Structure-activity relationships in microbial transformations of phenols. Appl. Environ. Microbiol. **44:**153–158.
11. **Powell, E. O.** 1967. The growth rate of microorganisms as a function of substrate concentration, p. 34–56. *In* E. O. Powell, C. G. T. Evans, R. E. Strange, and D. W. Tempest (ed.), Microbial physiology and continuous culture. Her Majesty's Stationary Office, London.
12. **Robinson, J. A.** 1985. Determining microbial kinetic parameters using nonlinear regression analysis: advantages and limitations in microbial ecology. Adv. Microb. Ecol. **8:**61–114.
13. **Robinson, J. A., and J. M. Tiedje.** 1983. Nonlinear estimation of Monod growth kinetic parameters from a single substrate depletion curve. Appl. Environ. Microbiol. **45:**1453–1458.
14. **Schmidt, S. K., S. Simkins, and M. Alexander.** 1985. Models for the kinetics of biodegradation of organic compounds not supporting growth. Appl. Environ. Microbiol. **50:**323–331.
15. **Shelton, D. R., and J. M. Tiedje.** 1984. Isolation and partial characterization of bacteria in an anaerobic consortium that mineralizes 3-chlorobenzoic acid. Appl. Environ. Microbiol. **48:**840–848.
16. **Simkins, S., and M. Alexander.** 1984. Models for mineralization kinetics with the variables of substrate concentration and population density. Appl. Environ. Microbiol. **47:**1299–1306.
17. **Simkins, S., and M. Alexander.** 1985. Nonlinear estimation of the parameters of Monod kinetics that best describe mineralization of several substrate concentrations by dissimilar bacterial densities. Appl. Environ. Microbiol. **50:**816–824.
18. **Simkins, S., R. Mukherjee, and M. Alexander.** 1986. Two approaches to modeling kinetics of biodegradation by growing cells and application of a two-compartmental model for mineralization kinetics in sewage. Appl. Environ. Microbiol. **51:** 1153–1160.
19. **Smolenski, W. J., and J. M. Suflita.** 1987. An HPLC system for the automatic sampling and analysis of time-series kinetic studies. J. Microbiol. Methods **6:**71–79.