MAINTENANCE ENERGY MODEL FOR MICROBIAL DEGRADATION OF TOXIC CHEMICALS IN SOIL

THOMAS F. HESS,* STEVEN K. SCHMIDT" and GREGORY M. COLORES*
Department of Biological and Agricultural Engineering, University of Idaho, Moscow, ID 83844-0904, U.S.A. and Department of Environmental, Population and Organismic Biology, University of Colorado, Boulder, CO 80309-0334, U.S.A.

(Accepted 13 February 1996)

Summary—A new kinetic model incorporating maintenance energy requirements is developed for use in the description of microbial degradation of toxic chemicals in soil. This model better describes extended, long-term CO₂ respiration data that typically occur when substrate concentrations are low. The model is tested with data from the respiration of 2,4-dinitrophenol (DNP) and pentachlorophenol (PCP) to CO₂, and is shown to statistically fit better than an associated model without maintenance considerations.

INTRODUCTION

Over the past 10 years there have been major advances in our understanding of the factors controlling the rate of biodegradation of toxic compounds in soil. Of particular interest to our investigation were studies that attempted to model the mineralization of organic compounds by growing and non-growing soil microorganisms (Brunner and Focht, 1984; Scow et al., 1986; Scow et al., 1989; Scow and Hutson, 1992; Schmidt, 1992; Hess and Schmidt, 1995). These studies demonstrated the importance of mass transfer constraints in the soil matrix including the effects of sorption and diffusion (Scow and Hutson, 1992) as well as the role of other limiting factors and microbial population dynamics (Schmidt, 1992). One factor that was not considered in most of these studies was the role of the maintenance energy requirements of the microorganisms degrading toxic organic chemicals in soil.

Energy demand for essential, non-growth-related processes is called maintenance energy (Neidhardt et al., 1990). Maintenance energy considerations would be especially important during long-term degradation of chemicals or during the later phases of degradation of easily-degraded compounds. Many researchers have noted an extended slow phase of substrate mineralization following the initial growth-related phase of degradation of compounds added to soil (Malik and Haider, 1982; Brunner and Focht, 1984; Scow et al., 1986; Van de Werf and Verstraete, 1987). It is our contention that this slow phase of mineralization is often the result of maintenance-related microbial activity.

Maintenance-related activity would be especially evident in studies in which respiration of CO₂ is used to monitor mineralization of added substrates. In such studies turnover of cell mass accounts for a portion of the CO₂ emitted by soil and this fraction increases proportionately as the added substrate is degraded. Once all of the added substrate is gone, cell turnover continues in the absence of growth and the CO₂ emitted is substantially due to the so-called slow phase of mineralization. It should be noted that maintenance processes result mainly in the production of CO₂, and that the substrate that feeds these processes can be added substrate, cell energy reserves, or, under starvation conditions, even structural components and enzymes can be degraded (Morita, 1988).

Another situation in which maintenance would play a major role in mineralization kinetics is when initial substrate concentration is too low to support bacterial growth but high enough to meet the maintenance needs of the standing biomass (Schmidt et al., 1985a). This type of substrate use could also contribute to the slow second phase of mineralization if the initial phase of substrate mineralization reduced concentrations of the substrate to the threshold concentration for growth.

In the present study, we propose a model that incorporates maintenance-related microbial activity into a pre-existing model of growth-related biodegradation kinetics. The new model is tested using data from studies of 2,4-dinitrophenol (DNP) mineralization by microorganisms indigenous to soil and pentachlorophenol (PCP) mineralization by bacteria inoculated into soil.

METHODS AND MATERIALS

Soil incubations

For the DNP incubations, uniformly labeled [U-¹⁴C] DNP (Sigma Chemical, St Louis, MO) was
added with unlabeled, reagent grade DNP (99% pure, Fluka Chemical Corporation, Ronkonkoma, NY) to 30 g soil (dry weight) to obtain final concentrations of 0.1 or 1.0 µg DNP g⁻¹ soil. The soil used was an organic soil from a coniferous forest near the University of Colorado Mountain Research Station in Boulder County, Colorado. The soil had a pH of 5.5 with 6.7% organic matter, and additional abiotic characteristics as given in Schmidt and Gier (1990). For the PCP incubations, uniformly labeled [U-¹³C] PCP was added with unlabeled, reagent grade PCP (99% pure, Fluka Chemical Corporation, Ronkonkoma, NY) to 44.5 g soil (dry weight) to give a final concentration of 300 µg PCP g⁻¹ soil. The soil used was a sandy loam from a meadow near the University of Colorado campus, Boulder, Colorado. This soil had a pH of 7.0, 5.0% organic matter, with additional soil characteristics given in Schmidt and Gier (1990).

Before use, each soil was sieved (2 mm) and the water holding capacity (WHC) was determined gravimetrically by saturating soils, allowing them to drain overnight, weighing and re-weighing after oven drying at 100°C for 24 h. One day prior to an incubation, 30 g or 44.5 g of soil, for DNP or PCP experiments, respectively, was placed in 250-ml biometer flasks (Bartha and Pramer, 1965) with enough deionized water to bring the soil to 50% WHC. The flasks were conditioned for 12 h at 24°C ±2°C to allow the soils to adjust to the temperature and higher water content. After 12 h, the toxic chemical and deionized water solution was added to achieve the desired chemical concentration and a moisture content equivalent to 70% WHC. In the PCP-amended soils, a non-indigenous, PCP-mineralizing Sphingomonas sp. was added (Colores et al., 1995). A total of 10.25 ml or 5.6 ml solution was added to the DNP- or PCP-amended soils, respectively. Subsequent collection and measurement of CO₂ was based on methods adapted from the work of Scow et al. (1986, 1989) and Schmidt and Gier (1989). Evolved ¹⁴CO₂ was trapped in 1.0 ml of 0.5 N NaOH contained in the side-arm of each biometer flask. The NaOH was added to 2.5 ml of Scintiverse II (Fisher Scientific, Fair Lawn, NJ) scintillation cocktail contained in 4-ml Omnivials (Wheaton Scientific, Millville, NJ) and the radio-activity was counted in a liquid scintillation counter (LKB Wallac, 1209 RackBeta, Turku, Finland). Counting efficiency was corrected using an external standard ratio. The incubations were carried out and monitored for up to 3 months.

Theory

The models used to analyze the data in this study were those from the group of Monod-based models of Simkins and Alexander (1984); the dual-substrate models described by Schmidt et al. (1985b), including Model 1, shown to best fit DNP degradation data of Schmidt and Gier (1989) at concentrations of 10 and 100 µg DNP g⁻¹ soil (Hess and Schmidt, 1995); the three-halves order model proposed by Brunner and Focht (1984); and a modification of the two-compartment model proposed by Williams (1967) for use in describing chemostat and batch systems. The modification of Williams’ two-compartment model was made by the addition of cellular maintenance, as developed below, and gave the best fit to the data sets used in our study.

Two-compartment models have been useful in describing biodegradation kinetics in response to substrate diffusion and sorption in soil (Scow et al., 1986; Scow and Hutson, 1992). While the original model of Williams (1967) was based on the assumption of substrate interactions between two cellular compartments, a synthetic portion and a structural–genetic portion, it is postulated that the compartments can also represent interactions of substrate between cells and the surrounding soil environment. The present model was formulated as a two-compartment, bimolecular reaction (after Williams, 1967), but with the addition of a feed-back term to account for microbial maintenance. General statements of the functions used to describe the disappearance of substrate and growth of cells based on the model are the following:

\[ \frac{dS}{dt} = k_S X + mX \]
\[ \frac{dX}{dt} = k_S S \]

where \( S \) = concentration of substrate, \( X \) = concentration of biomass in terms of substrate, \( k_S \) = overall reaction rate constant, \( k_2 \) = growth rate constant and \( m \) = maintenance energy coefficient. To show biomass concentration changes due to changes in substrate concentration, a mass balance can be used (Simkins and Alexander, 1984):

\[ S + X = S_0 + X_0 \]

where \( S_0 \) and \( X_0 \) are the initial substrate concentration and biomass concentration at time \( t = 0 \), respectively, and \( S \) and \( X \) are substrate and biomass concentrations at any time \( t \), respectively. Both \( X \) and \( X_0 \) are products of cell quota or inverse yield, \( q \), and cell population density, \( B \) and \( B_0 \), respectively (Simkins and Alexander, 1984). This conversion allows us to express biomass concentration (with included yield) in terms of substrate concentration. Additionally, the maximum achievable biomass concentration, \( X_{\text{max}} \), will always be a function of the initial biomass present, \( X_0 \), (both with included yield) plus the initial substrate present, \( S_0 \), that can be used during growth to create new cells (Hess et al., 1990):

\[ X_{\text{max}} = X_0 + S_0 \]
\[
\frac{dX}{dt} = k_2 X_{\text{max}} X - k_3 X^2
\]  
(5)

Equation (5) is easier to integrate if we let \( k' = k_2 X_{\text{max}} \). This substitution results in the following modification:

\[
\frac{dX}{dt} = k' X - \frac{k'}{X_{\text{max}}} X^2
\]  
(6)

Equation (6) is a form of the logistic equation and can be integrated to solve for \( X \) with initial conditions \( X = X_0 \) at \( t = 0 \) (Schmidt et al., 1985b):

\[
X = \frac{X_{\text{max}}}{1 + \frac{X_{\text{max}} - X_0}{X_0} e^{-k't}}
\]  
(7)

When Equation (7) is substituted into Equation (1), the two-compartment, maintenance-based model results:

\[
- \frac{dS}{dt} = (k_1 S + m) \left[ \frac{X_{\text{max}}}{1 + \frac{X_{\text{max}} - X_0}{X_0} e^{-k't}} \right]
\]  
(8)

In the two-compartment model with maintenance [TCM model, Equation 8], \( k_1 \) the overall reaction rate constant, represents the maximum rate at which substrate is converted to biomass. It is proposed that \( k_1 \) can be treated as a lumped rate parameter representing a combination of enzyme induction rate, cellular growth rate and any other environmentally-influenced rate, such as substrate diffusion.

Equation (8) was modified for use with a system respiring the substrate of interest to CO\(_2\), as follows:

\[
- \frac{dS}{dt} = k_0 S \left[ \phi \left( e^{k't} - 1 \right) + 1 \right]^{-\left(\frac{1}{\psi} - 1\right) e^{-k't}} + \frac{m_k}{1 + \left( \frac{1}{\psi} - 1 \right) e^{-k't}}
\]  
(9)

where \( S_x \), final amount of substrate respired to CO\(_2\), \( S = \) unutilized portion of \( S_x \), at time \( t \), \( k_0 = k_1 X_{\text{max}} \), and \( m_k = m X_{\text{max}} \). \( \phi = X_0/X_{\text{max}} \) and all other parameters are described above. The differences between Equation (8) and (9) arise from the combination of several parameters which are not exclusively identifiable using nonlinear regression techniques (e.g. \( k_1 \), \( X_{\text{max}} \), \( m \), and \( X_0 \)) and the use of \( S_x \), similar to a previous description of 2,4-dinitrophenol degradation in soil (Hess and Schmidt, 1995).

The TCM model was compared to Model I of Schmidt et al. (1985b), among the other models previously mentioned, and both model fits were plotted for use in our work. Model I in its differential form, as redeveloped in Hess and Schmidt (1995), is shown below:

\[
- \frac{dS}{dt} = k_0 S \left[ \phi \left( e^{k't} - 1 \right) + 1 \right]^{-\left(\frac{1}{\phi} - 1\right) e^{k't}}
\]  
(10)

Other types of maintenance models may be useful for describing kinetics of toxic compound mineralization in soil. Schmidt et al. (1985b) postulated several dual-substrate models of microbial growth and substrate mineralization which were subsequently used to describe the kinetics of biodegradation of mixtures of substrates in soil (Scow et al., 1989). It is therefore informative to look at how Equation (8) (TCM model) relates to a dual-substrate model with maintenance. Development of the model begins with the general expression for cell growth and substrate depletion accounting for maintenance (Marr et al., 1963):

\[
\frac{dB}{dt} + mB = - Y \frac{dS}{dt}
\]  
(11)

where \( B \) is the population density, \( S \) is the substrate concentration, \( Y \) is the yield coefficient and \( m \) is the maintenance coefficient. Additionally, Michaelis–Menten kinetics can be used to express mineralization of the substrate being measured as follows (Simkins and Alexander, 1984):

\[
\frac{dB}{dt} \frac{1}{B} = \frac{V_{\text{max}} S}{K_m + S}
\]  
(12)

where \( V_{\text{max}} \) is the maximum specific reaction rate, \( K_m \) is the half-saturation constant and all other parameters were previously defined. Equation (12) can be solved for \( dB/dt \) and substituted into Equation (11) to obtain:

\[
\frac{V_{\text{max}} S B}{K_m + S} = Y \frac{dS}{dt} - mB
\]  
(13)

Following the development of Simkins and Alexander (1984), \( Y \) can be considered invariant with time and substrate concentration, and therefore \( B/Y \) is replaced with \( X \), the amount of substrate required to produce a population density \( B \). Equation (13), after rearranging can then be expressed as:

\[
- \frac{dS}{dt} = \frac{V_{\text{max}} S X}{K_m + S} + mX
\]  
(14)

Two special cases of Equation (14) can now be considered.

1) With regard to the substrate being measured, when \( S \ll K_m \), a pseudo-first order approximation of the expression can be used (Schmidt et al., 1985b). Equation (14) can be modified as below:

\[
- \frac{dS}{dt} = \frac{V_{\text{max}} S X}{K_m} + mX
\]  
(15)

2) Logistic growth on a second uncharacterized substrate can be considered when the original second substrate concentration is less than the half saturation constant for the substrate. The cell population, \( X \), can be expressed similar to Equation (6), above, and substituted into Equation (15), resulting in the combined dual substrate model of logistic growth on an uncharacterized substrate with
simultaneous mineralization of low concentrations of measured substrate and additionally accounting for maintenance:

\[
\frac{dS}{dt} = \left( \frac{V_{\text{max}} S}{K_m} + m \right) \left[ \frac{X_{\text{max}} - X_S}{1 + X_{\text{max}} - X_S e^{-k t}} \right]
\]

(16)

The above model is similar to Model I of Schmidt et al. (1985b) with the addition of the maintenance parameter. It can be seen that Equation (16) is similar in form to Equation (8), the TCM model, if we let \( V_{\text{max}}/K_m = k_1 \).

Model evaluation and comparison

All original discretized data sets were analyzed with SOILWT (Hess and Schmidt, 1995), a non-linear regression program using differential model forms, discretized rate-based data and a biweight function (Mosteller and Tukey, 1977) to account for the non-uniform variability of \(^{14}\)CO\(_2\) evolution data. This method of analysis provided statistically valid estimates of model parameters and their uncertainties and has been described by Hess and Schmidt (1995).

Discretization of data was done in a manner similar to that outlined by Bates and Watts (1988) where rates of \(^{14}\)CO\(_2\) appearance were based on the measured \(^{14}\)CO\(_2\) concentration divided by the time between two successive \(^{14}\)CO\(_2\) measurements, and plotted at the midpoint between the two data collection times.

Discrimination between two different models used in the present analyses was based on three methodologies; the F-test for significance, the Akaike Information Criterion (AIC) and Pearson's correlation coefficient (r). The model of best fit was determined by meeting all three criteria as described below. A particular model with the lowest residual sum of squares for a given data set was considered the model of best fit in the standard F-test if the difference between it and the model with fewer parameters was significant at the 95% confidence level (\(P = 0.05\)) (Robinson, 1985). The second criteria considered the model of best fit to have the lowest AIC value as described by Akaike (1974). The final discrimination factor was based on calculations of a Pearson correlation coefficient, r (Bailey, 1981). Observed data, as the independent variables, were linearly regressed against calculated values from the models in question to obtain the correlation coefficient. Models were additionally judged for goodness of fit based on their standard error of the estimated parameters. Only models whose standard error was less than 50% of the estimated parameter were considered acceptable (Robinson, 1985).

To calculate \(m\), the maintenance coefficient, for comparison to literature values, it was necessary to convert cell number (cells g\(^{-1}\) dry weight of soil) from MPN (Most Probable Number) values in the literature (Schmidt and Gier, 1989) to the same units as \(X_{\text{max}}\) (\(\mu g\) g\(^{-1}\) dry weight soil). This was done assuming a yield coefficient of 50% and that \(10^8\) soil microorganisms were equivalent to 1 mg of biomass (Soulas et al., 1984). The resulting value of \(m\) was further converted from \(\mu g\) DNP \(\mu g^{-1}\) cells h\(^{-1}\) to \(\mu g\) DNP-C \(\mu g^{-1}\) biomass-C h\(^{-1}\) assuming that DNP is composed of 39% C and biomass is composed of approximately 53% C (Grady and Lim, 1980).

RESULTS

Metabolism of 2,4-DNP

The two-compartment maintenance model (TCM model) was evaluated using data from the study of Schmidt and Gier (1989). The data were originally presented as accumulated curves of \(^{14}\)CO\(_2\) evolution from mineralization of 1.0 and 0.1 \(\mu g\) \([^{14}\text{C}]\text{DNP} \text{ g}^{-1}\) soil and fit by integrated forms of kinetic models. Subsequent research has shown that analysis using differential model forms and discretized data is the correct method to obtain statistically-valid parameter estimates of microbial kinetic models (Hess and Schmidt, 1995). The data for this study were analyzed using such a methodology in conjunction with the newly developed TCM model.

Fig. 1 and Fig. 2 show both Model I and the TCM model fit to discretized data from incubations of 1.0 and 0.1 \(\mu g\) DNP g\(^{-1}\) soil, respectively. The TCM model was shown to be the model of best fit for both DNP incubations based on F test results (\(P = 0.05\)) and lowest AIC value. Model I and a pseudo first-order model were reported to give the best fit to

<table>
<thead>
<tr>
<th>DNP concentration ((\mu g \text{ g}^{-1}))</th>
<th>Model</th>
<th>(k') ((\text{h}^{-1}))</th>
<th>(k_w) ((\text{h}^{-1}))</th>
<th>(\phi)</th>
<th>(S, (\mu g \text{ g}^{-1}))</th>
<th>(m, (\mu g \text{ g}^{-1} \text{ h}^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (\mu g \text{ g}^{-1})</td>
<td>Model I*</td>
<td>0.4463 ± 0.0271</td>
<td>0.1667 ± 0.0087</td>
<td>0.1682 ± 0.0078</td>
<td>0.2720 ± 0.0055</td>
<td>0.0017 ± 0.0002</td>
</tr>
<tr>
<td>1 (\mu g \text{ g}^{-1})</td>
<td>Model 1</td>
<td>0.5785 ± 0.0626</td>
<td>0.1151 ± 0.0044</td>
<td>0.1899 ± 0.0233</td>
<td>0.3350 ± 0.0056</td>
<td>NA</td>
</tr>
<tr>
<td>0.1 (\mu g \text{ g}^{-1})</td>
<td>Model I*</td>
<td>1.3979 ± 0.0510</td>
<td>0.2344 ± 0.0059</td>
<td>0.1409 ± 0.0073</td>
<td>0.0231 ± 0.0003</td>
<td>0.0002 ± 0.0001</td>
</tr>
<tr>
<td>0.1 (\mu g \text{ g}^{-1})</td>
<td>Model 1</td>
<td>1.5230 ± 0.1888</td>
<td>0.1951 ± 0.0105</td>
<td>0.1372 ± 0.0252</td>
<td>0.0270 + 0.0007</td>
<td>NA</td>
</tr>
</tbody>
</table>

*Model of best fit.
NA = not applicable.
Microbial degradation of toxic chemicals in soil

Fig. 1. Non-accumulated rate data from the mineralization of 1.0 µg DNP g⁻¹ soil. The curves were fit to the data using non-linear regression and Model I and the two-compartment maintenance (TCM) model in their differential forms.

Fig. 2. Non-accumulated rate data from the mineralization of 0.1 µg DNP g⁻¹ soil. The curves were fit to the data using non-linear regression and Model I and the two-compartment maintenance (TCM) model in their differential forms.
of 1.0 μg DNP g⁻¹ soil, the Pearson correlation coefficient, r, equalled 0.996 and 0.990 for the TCM model and Model I, respectively. The correlation coefficient for the incubation of 0.1 μg DNP g⁻¹ soil equalled 0.996 and 0.988 for the TCM model and Model I, respectively. Values of the maintenance coefficient, m, were calculated from values of the population-based maintenance rate, m₀, from Table 1, divided by population size, Xₐₚ, determined from the original study (Schmidt and Gier, 1989). The resulting calculated values of m for incubations of 1.0 and 0.1 μg DNP g⁻¹ soil equalled 0.0008 ± 0.0002 h⁻¹ and 0.0012 ± 0.0002 h⁻¹, respectively.

Metabolism of PCP

For comparative purposes we also analyzed a data set for which we believed that neither Model I nor the TCM model should adequately describe the data. The model inadequacy was hypothesized to be due either to the population dynamics of the added organism responsible for PCP degradation or sorption of PCP and its resultant unavailability. Figure 3 shows the model fits to data obtained from an incubation of 10 μg PCP g⁻¹ soil in which a non-indigenous *Sphingomonas* sp. was added to a soil that showed little indigenous PCP-mineralizing capacity. Statistical analysis of the non-linear regression results showed that Model I best fit the data obtained from an incubation of 10 μg PCP g⁻¹ soil as compared to either Model I or the pseudo first-order model used previously (Schmidt and Gier, 1989). All three statistical criteria, the F-test, AIC and Pearson's correlation coefficient, indicated that the TCM model best fit the data. Statistical tools such as these are the current best methods for a posteriori model discrimination (Robinson, 1985; Hess et al., 1990).

In addition to providing a good fit to the data, models of mineralization kinetics must also make biological sense. If they do not, then efforts at analysis using models are merely curve-fitting exercises and are of little benefit to microbiologists investigating mechanisms of degradation or to engineers interested in degradation rate constants. Based on the concept of constant maintenance energy discussed by Pirt (1982), the present TCM model makes biological sense in describing the data for DNP mineralization in soil 1. In the proposed TCM model, product formation (¹⁴CO₂) due to substrate mineralization and maintenance, is comparable to the
modeling development of Brunner and Focht (1984), although their additive parameter was termed the "rate of indigenous mineralization of soil organic matter or of residual 14C that has become synthesized into new humus" rather than maintenance. Their indigenous mineralization rate is analogous to a maintenance rate as evidenced by their finding of a constant value for the parameter, independent of added substrate concentration (Brunner and Focht, 1984). Values for the maintenance coefficient, \( m \), in the present study, for incubations of 1.0 and 0.1 \( \mu \)g DNP \( g^{-1} \) soil, were not statistically different, further validating the use of the idea of constant maintenance energy. This also reflects the idea that the substrate for maintenance can be anything from internal cell reserves to the contents of lysing cells and that \( m \) is determined by the metabolic state of the cell and not by initial substrate concentrations.

The effects of maintenance energy requirements in batch culture and soil incubations may not show up as being statistically significant unless either initial substrate concentrations are low or experiments are conducted for extended periods. Based on Fig. 1 and Fig. 2, maintenance effects were not evident until after 15 and 25 h, respectively. Indeed, when the data sets were terminated at those respective times and analyzed, the TCM model did not statistically fit the data better than Model I (data not shown). Other authors (Ong, 1983; Andrews, 1984) have noted that the effects of maintenance or endogenous decay are small and can be ignored except in batch situations when initial substrate concentration is low or the cells are growing slowly.

The values for specific maintenance rate obtained in our study for the DNP-mineralizing soil population fall in the range of some specific maintenance rates previously reported in the literature. Tijhuis et al. (1993) reported on a compilation of maintenance rates for numerous axenic organisms aerobically degrading glucose as ranging from 0.008 h\(^{-1}\) to 0.082 h\(^{-1}\). Pipyn and Verstraete (1978) related maintenance values of axenic cultures measured in continuous culture between 0.05 h\(^{-1}\) and 1.0 h\(^{-1}\). They further pointed out that mixed culture activated sludge communities have maintenance values between 0.001 h\(^{-1}\) and 0.01 h\(^{-1}\) and assumed that maintenance requirements decrease with decreasing growth rates. Chang and Criddle (1995) reported on endogenous decay and yield values of mixed cultures from aquifer materials degrading TCE. Based on their values, a calculated maintenance rate for the mixed community of organisms is 0.0012 h\(^{-1}\).

Finally, in previous soil studies values for \( m \) have ranged from 0.0004 h\(^{-1}\) (Smith et al., 1986) to 0.001 h\(^{-1}\) (Babiuk and Paul, 1970) and 0.01 h\(^{-1}\) (Anderson and Domsch, 1985). It should be stressed, however, that most previous soil studies have tried to determine a maintenance rate for the entire microbial biomass over a whole year. In contrast, our study was focused on specific microbial functional groups that degrade toxic organic substances. Such organisms would be expected to have a higher maintenance requirement due to the added stresses of metabolizing a toxic substrate. For example, both DNP and PCP are uncouplers of oxidative phosphorylation (Weinbach and Garbus, 1965) and researchers have demonstrated increased maintenance demands by pure cultures of bacteria in the presence of DNP (Neijssel, 1977).

In our study, the TCM model did not satisfactorily describe the mineralization of PCP in soil inoculated with a PCP mineralizing *Sphingomonas* sp. One possible reason for this is that *Sphingomonas* sp. was not indigenous to the soil used and may die out quickly once the PCP is depleted from the soil. This *Sphingomonas* sp. was subjected to severe selection pressure in culture to ensure that it could mineralize high concentrations of PCP. As a result it has a limited ability to utilize other C sources it might encounter in soil (Radehaus and Schmidt, 1992). Senoo et al. (1992) compared the survival patterns of a hexachlorohexane-degrading *Sphingomonas* sp. as an indigenous species in soil and as an introduced species in the same type of soil. When the bacterium was inoculated into contaminated soil, it declined rapidly after depletion of the contaminant. While in experiments with soil where the organism was indigenous, the population remained constant regardless of the contaminant concentration. Similarly, we have observed that the population of the PCP-mineralizing bacterium rapidly declines once the initial phase of PCP mineralization is complete. Thus, the present form of the TCM model probably failed to describe the PCP data because the *Sphingomonas* sp. was in severe decline and died out before significant maintenance respiration could be measured.

There are other cases in which the present TCM model will probably fail. One example would be a situation in which the rate of mass transfer of the substrate through the soil is much lower than the maintenance requirements of the organism degrading the substrate. Thus, the slow rate of degradation of highly-sorbed chemicals is probably not attributable to maintenance metabolism but rather to the rate of supply of the substrate to the cells. For instance, in our experimentation, PCP was assumed to be more highly sorbed to the soil than DNP, based on \( K_{oc} \) (octanol–water partition coefficients) values of 5.02 and 1.54, respectively (Verschueren, 1983). In some cases, it may be very difficult to tell the difference between maintenance metabolism and metabolism that is limited by diffusion. This will be especially true for the slow second phase of mineralization often noted in soil respiration studies (e.g. Scow et al., 1986; Moorman, 1990). One way to determine if this slow phase of mineralization is due to maintenance metabolism or mass transfer limitation is to determine if the rate of this second phase of mineralization is dependent on substrate concentration. If the rate is dependent on initial substrate concentration, then it...
is probably the result of mass transfer limitations. If on the other hand, the rate is independent of initial substrate concentration (as was the case with DNP) then it is probably the result of some biological property of the biomass such as maintenance metabolism.

In conclusion, maintenance energy requirements need to be considered to understand and model the degradation of low concentrations of weakly-sorbed substrates added to soil. The estimates of \( m \) obtained from the present study agree quite well with literature values, but more work is needed to better understand the interactions between substrate availability and maintenance requirements in the soil environment.

Acknowledgements—We thank R. Crawford and R. Rynk for thoughtful reviews of this manuscript. This work was supported by grants from the U.S. National Science Foundation, the U.S. Environmental Protection Agency and the U.S. Department of Agriculture.

REFERENCES


Mosteller F. and Tukey J. W. Data Analysis and Regression. Addison-Wesley, Reading.


Neijssel O. M. (1977) The effect of 2,4-dinitrophenol on the growth of Klebsiella aerogenes NCTC 418 in aerobic cultures. FEMS Microbiology Letters 1, 47 50.


Simkins S. and Alexander M. (1984) Models for mineralization kinetics with the variables of substrate...
concentration and population density. Applied and Environmental Microbiology 47, 1299–1306.