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Modelling of Microbial Processes that Govern Degradation of Organic Substrates in Soil, with Special Reference to Pesticides [and Discussion]

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Modelling of microbial processes that govern degradation of organic substrates in soil, with special reference to pesticides

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SUMMARY

We tried to develop deterministic models for kinetics of 2,4-D breakdown in the soil based on the following considerations: (i) at low concentrations degradation results from maintenance consumption by a large fraction of the soil microbial population; (ii) at high concentration in addition to the maintenance consumption there is a growth-associated carbon incorporation by a small specific microbial population. Values for the biokinetic parameters are consistent with those commonly found in the literature. Comparison between observed and simulated curves suggests that a non-negligible part of the pesticidal carbon exists as microbial by-products.

INTRODUCTION

Several models for kinetics of biodegradation, or mineralization, of organic compounds have been developed. Most of them are specially adapted to liquid environments with pure or mixed cultures. Recently, Simkins & Alexander (1984) and Schmidt *et al.* (1985) have proposed an integrated view of different models of biodegradation supporting or not bacterial growth. For instance, Simkins & Alexander (1984) have found that the plane defined by initial substrate concentration and bacterial cell density might be divided into six areas where different models based on zero-order, Monod (without growth), first-order, logistic, Monod (with growth), and logarithmic kinetics are expected to apply. These basic formal kinetics probably also apply to more complex natural ecosystems such as the soil even if a variety of factors and processes may alter the shape of the disappearance curves. Focht & Shelton (1987) have found that standard Monod kinetics are applicable to the growth of an inoculant, *Pseudomonas alcaligenes* C—O, degrading 3-chlorobenzoate in the soil. However, when applied to the soil system, theoretical models, including the Monod equation, have a poor descriptive value as shown by Scow *et al.* (1986) particularly when chemicals are present in low concentrations. This is the consequence of both theoretical and methodological constraints. Illustrative of theoretical constraints are the diversity of microbial species able to act upon a xenobiotic compound and the diversity of their carbon substrates. So, the shape of the disappearance curve results from a compromise reflecting the concurrent activities of microorganisms differing in their metabolic performances. Moreover physical and chemical interactions of some organic compounds with soil constituents may alter their biological availability. These are among the main reasons why Brunner & Focht (1984) found that kinetics of biodegradation of carbonaceous substrates in soil have resulted in a descriptive rather than a quantitative approach. Methodological constraints results from our present inability to gain *in situ* suitable estimates of the size of the active microbial populations.

The purpose of this study was to propose a deterministic mathematical model based on the consideration of different microbial processes, and populations, that govern degradation of the herbicide 2,4-D in the soil.

MATERIALS AND METHODS

(*a*) *Preparation of soil samples*

A silty-clay soil (pH 7.8, 1.2 °C, 52% silt and 33% clay) was mixed with a solution of radioactive 2,4-D. We have simultaneously recorded mineralization of the pesticidal carbon and its incorporation into microbial biomass. Samples of 10 g of soil aggregates between 2 and 3 mm were distributed into 20 ml glass vials. Water was added to bring the soil to 72% of the W.H.C. A total of 192 samples were prepared and divided into four groups. Each of the 48 samples of one group received the same concentration of 2,4-D, 0.064 or 4.10 mg kg^{-1}, labelled either in the methylenic carbon or uniformly in the ring. The amounts of radioactivity were the same for all samples of the same group and varied from 3.19 kBq to 3.70 kBq between the different groups. 200 µl of radioactive solution was distributed to each sample bringing the soil to 80% of the W.H.C. Treated samples were incubated in closed 250 ml glass flasks also containing 10 ml of water and 50 ml 0.2 M sodium hydroxide in two scintillation vials. Incubation temperature was 20 ± 0.5 °C.

(*b*) *Measurement of mineralization*

The evolved $^{14}CO_2$ was trapped in the sodium hydroxide solution of the incubation jar. These traps were periodically replaced at a frequency that depended on the rate of $^{14}CO_2$ production. They were added to scintillation cocktail and the radioactivity counted in a scintillation counter. Twelve data points were recorded during a monitoring period of 74 days.

(*c*) *Measurement of the incorporation of radioactivity*

Every time mineralization was measured, four samples from each treatment were removed for incorporation measurement. The experimental procedure was a modification of the method described by Soulas *et al.* (1984). Briefly, soil samples were fumigated 16 h in saturating vapours of chloroform. After vacuum elimination of traces of fumigant left in the soil, soil samples were reincubated for 28 days at 28±0.5 °C. Kinetics of $^{14}CO_2$ evolution were fitted by the model proposed by Chaussod *et al.* (1986):

$$y = b(1-e^{-k_b t}) + ct, \quad (1)$$

where y is the percentage of initial radioactivity evolved as $^{14}CO_2$ at time t, b the percentage of radioactivity incorporated in the labile microbial constituents, k_b the rate constant for their mineralization and c the rate of mineralization of labelled soil organic matter. Total radioactivity incorporated by the soil biomass is given by:

$$R_b = b/0.41. \quad (2)$$

(*d*) *Theoretical considerations*

These have been extensively discussed by Soulas (1982) in his attempt to develop a theoretical model of pesticide degradation in the soil. Briefly, two main microbial processes are responsible for incorporation of pesticidal carbon, and radioactivity by the soil microbial population. At low concentration it results essentially from maintenance consumption. As the concentration of 2,4-D increases, a growth-associated incorporation process becomes significant (figure 1).

To estimate the biokinetic parameters of the degrading populations it was considered that at low concentration only maintenance incorporation was effective. The following assumptions have been formulated: (i) active soil biomass is at a steady-state. There is no net carbon accumulation in the microbial compartment; (ii) only soil biomass governs the rate of carbon consumption and 2,4-D is used in proportion of its 'partial concentration'; (iii) 2,4-D does not exhibit inhibiting effects.

If m is the maintenance coefficient of the active biomass B, C_s and C_t the concentrations of pesticidal carbon and total organic carbon, f_b the fraction of the pesticidal carbon that is actually incorporated into cell components (incorporation yield), a_s and a_b the specific activities of the herbicide and the soil biomass, then the net rate of increase of microbial radioactivity will be:

$$dq_b/dt = mB\frac{C_s}{C_t}f_b a_s - mBf_b a_b, \quad (3)$$

or, in percentage of initially added radioactivity:

$$dR_b/d_t = Kf_b R_s - mf_b R_b, \quad \left(K = \frac{mB}{C_t}\right). \quad (4)$$

The rates of ^{14}C-substrate disappearance (R_s) and of $^{14}CO_2$ production (R_c) will conform to equations:

$$dR_s/dt = -KR_s, \quad (5)$$

$$dR_c/dt = Kf_c R_s + mf_b R_b, \quad (6)$$

where f_c is the fraction of the substrate mineralized. Equations (4), (5) and (6) define what will be called model 1.

A second 'maintenance' model (model 2) based on consideration of two compartments in the soil biomass was also tested. These two compartments distinguish between labile protoplasmic constituents (b_1) and more stable cellular structures (b_2). Equation (4) must be replaced by the following:

$$dR_{b1}/dt = Kf_{b1} R_s - mf_{b1} R_{b1} - k_1 R_{b1} + k_2 R_{b2}, \quad (7)$$

$$dR_{b2}/dt = k_1 R_{b1} - k_2 R_{b2}. \quad (8)$$

Equations (5) and (6) are left unchanged with R_b replaced by R_{b1}.

When the 2,4-D concentration is high enough, a small microbial population, B_m, is able to grow at the expense of the herbicide. To establish the corresponding model (model 3), the following assumptions have been made: (i) the rate of increase in carbon content of the growing population, C_m, was considered as first-order with respect to both the size of that population and the concentration of the herbicide; (ii) maintenance energy requirements of that population was regarded as independent of the specific growth rate. This energy is partly derived from the degradation of the herbicide in a proportion that depends on its remaining fraction. The other part is accounted for by intracellular recycling of biochemical (or storage) products. This is the so-called 'endogenous metabolism', which results in a decrease, or death, of the biomass. Such an approach is in agreement with both concepts of maintenance consumption and endogenous metabolism; (iii) all the carbon that had been taken up was either present in microbial constituents or mineralized. The corresponding model 3 is written as:

$$\frac{dR_m}{dt} = \mu_m R_m \frac{R_s}{100} C_s^\circ - k_e R_m \left[1 - \frac{R_s}{100}\right], \quad (9)$$

$$\frac{dR_s}{dt} = -\frac{\mu_m}{Y_m} R_m \frac{R_s}{100} C_s^\circ - \frac{k_e}{Y_m} R_m \frac{R_s}{100}, \quad (10)$$

$$\frac{dR_c}{dt} = \frac{1-Y_m}{Y_m} \mu_m R_m \frac{R_s}{100} C_s^\circ + k_e R_m \left[1 + \frac{1-Y_m}{Y_m}\frac{R_s}{100}\right], \quad (11)$$

where μ_m is the specific growth rate, Y_m the maximum growth yield, k_e the specific rate of endogenous metabolism, C_s° the initial herbicide concentration, and R_m, R_s and R_c the respective percentages of radioactivity in the biomass, in the remaining substrate and in $^{14}CO_2$.

From the foregoing it is clear that at higher concentration the general model for biodegradation of

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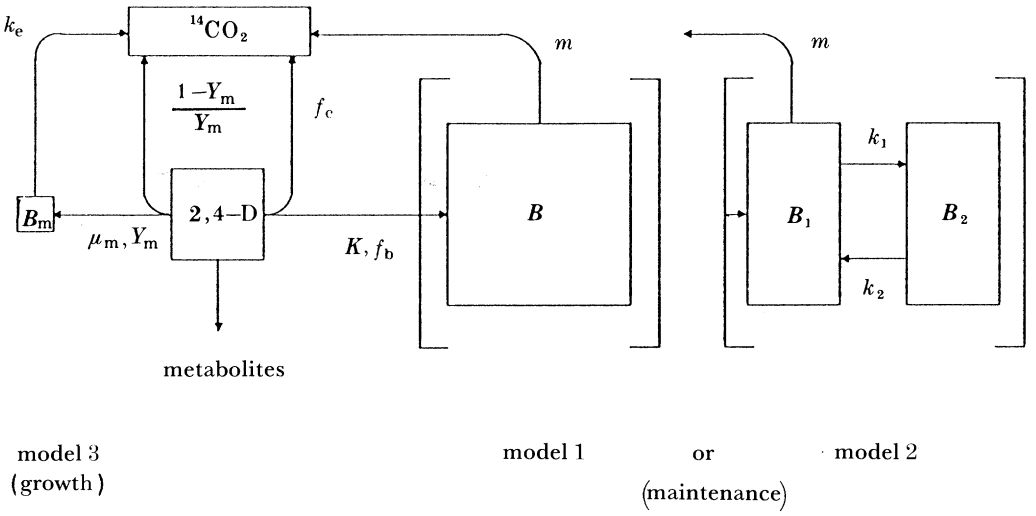

Figure 1. Definition of the different compartments on which the three models have been formulated (see text for explanation of the symbols).

2,4-D is a combination of model 1 (or model 2) and model 3. In that case the number of parameters to be estimated (7) is high as compared to the number of experimental data points (12). We have proceeded by considering separately the sets of parameters relative to each submodel. One set of parameters was adjusted while keeping the other at fixed values. The non-linear parameter estimation was based on the Gauss-Marquardt algorithm.

RESULTS AND DISCUSSION

Figure 2 is a synoptic of the fitness of simulated curves to experimental data points for the kinetics of incorporation and mineralization. Table 1 gives the corresponding values of the parameters.

Model 2 is more appropriate than model 1 to describe the incorporation process when degradation proceeds exclusively via maintenance consumption (figure 2*a*). This might indicate that radioactivity, and then pesticidal carbon, should first locate on labile cellular constituents before becoming evenly distributed throughout the cell. Values for the maintenance coefficient are intermediate between those found under *in situ* conditions for metabolically activated soil biomass (Anderson & Domsch 1985*a*) and for dormant microbial populations (Anderson & Domsch 1985*b*). Simulated curves for mineralization (figure 2*b*) are not in good agreement with experimental ones. Discrepancies essentially arise from the delay that affects observed mineralization compared with simulated. Two explanations are equally possible. From a theoretical point of view, the working assumption of a constant incorporation yield, f_b, may not be valid. Initial treatment of soil reactivates the soil microflora. It is possible that parameter f_b should be regarded as dependent on the state of activity of the soil microflora in the same way it has been found that the growth yield was dependent on the specific growth rate. On another hand, from a technical point of view, trapping of $^{14}CO_2$ is not instantaneous and a loss of respired carbon should result in underestimates of mineralization that reflects the frequency of sampling operations, more numerous at the beginning of the experiment.

Because we did not try to estimate jointly all parameters of the general degradation model that holds at the higher concentration, care must be taken in considering values in table 1 that are only good- and not best-fit values. For that reason definitive conclusions cannot be drawn. Nevertheless, some trends are in accordance with well-known experimental observations. For instance, the slow decrease of the growing population (figure 2*c*) is consistent with persistent accelerated degradation of some pesticides (2,4-D, carbofuran ...) that occurs in soil 'enriched' with adapted microorganisms (Walker & Suett 1986). 2,4-D is a poor growth substrate for soil microorganisms. The parameter Y_m is an order of magnitude less than parameter f_b for the maintenance consumption. Simulated mineralization curves (figure 2*d*) greatly exceeds observed kinetics for $^{14}CO_2$ evolution. This is an indication that our assumption on the fate of pesticidal carbon is wrong. Part of it probably exists as microbial by-products whose formation seems mainly growth-associated.

More experimentation is needed to further validate the different models that have been presented. One point would be to find a technical procedure to selectively measure, and model, the accumulation of metabolites and by-products to get a better description of the kinetics of mineralization specially as growth becomes more effective in the incorporation process. A second point is concerned with the demonstration that 2,4-D is degraded by two microbial communities acting together. So far, metabolizing microbes have been assumed to play the major role in the breakdown of 2,4-D. Substantiating involvement of non-specialized co-metabolizing species could be made by tentatively stimulating their 2,4-D mineralization activity by addition to the soil of easily degradable carbon substrates.

Application of the models with other chemicals would depend on the particular microbial and physicochemical processes that are involved. For instance,

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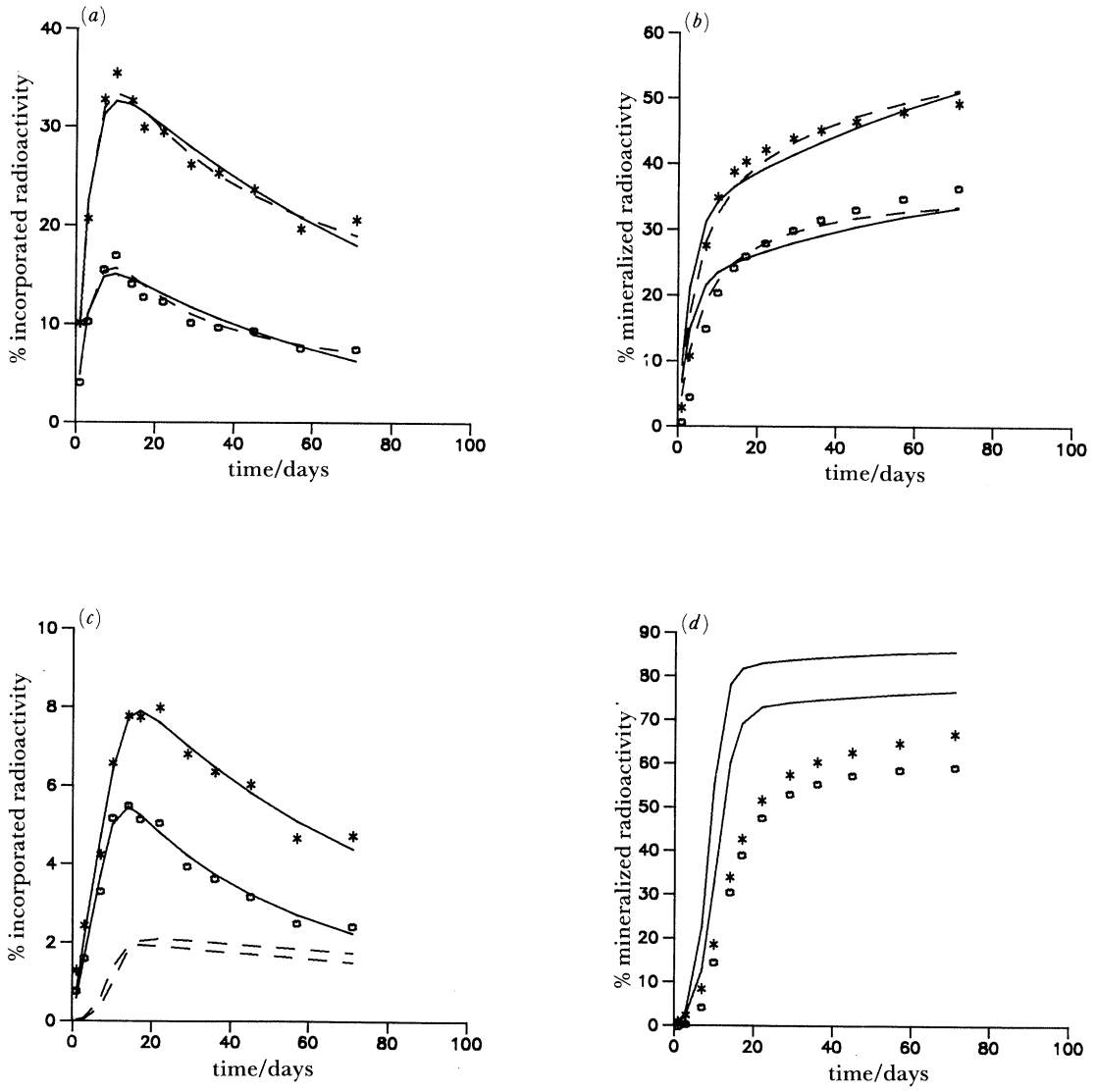

Figure 2. Comparison between observed and simulated kinetics of radioactivity incorporation or mineralization. (*a*) Incorporation (maintenance); (*b*) mineralization (maintenance); (*c*) incorporation (growth); (*d*) mineralization (growth). In (*a*) and (*b*) 2,4-D concentration is 0.064 mg kg^{-1}. Only maintenance consumption is effective (models 1 or 2). Chain-label (*) and ring-label (○), observed. Model 1 (——) and model 2 (– – – –), simulated. In (*c*) and (*d*) the 2,4-D concentration is 4.1 mg kg^{-1}. Maintenance and growth consumption are acting together. Chain-label (*) and ring-label (○), observed. General model (model 1 + model 3, ——), simulated and, for figure (*c*), growth-associated part (– – – –) for incorporated radioactivity. (See text for definition of models 1, 2 and 3 and table 1 for the best-fit values of the parameters.)

Table 1. *Best-fit values of the biokinetic parameters*

	model 1		model 2		model 3	
para-meter[a]	chain-label	ring-label	chain-label	ring-label	chain-label	ring-label
K	0.328	0.352	0.273	0.267	0.031	0.023
f_b	0.366	0.173	0.414	0.211	0.240	0.254
m	0.027	0.087	0.055	0.171	0.062	0.110
f_c	0.330	0.223	0.291	0.211	0.200[b]	0.200[b]
k_1	—	—	0.027	0.024	—	—
k_2	—	—	0.015	0.100	—	—
μ_m	—	—	—	—	0.322	0.364
Y_m	—	—	—	—	0.031	0.025
k_c	—	—	—	—	0.004	0.005

[a] All parameters are in day^{-1}; f_b, f_c and Y_m are dimensionless.
[b] Fixed values.

pesticides are often degraded co-metabolically. In these situations 'maintenance' models 1 or 2 are likely to apply. On another side, chemicals frequently interact with soil constituents that alter their biological availability. Development of a 'physical' submodel allowing for adsorption desorption processes could contribute to a better description of their fate in the soil.

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Discussion

D. J. Greenwood (*AFRC Institute of Horticultural Research, Wellesbourne, U.K.*). I wonder whether the problem of obtaining more frequent measurements of the pesticide concentrations could be obtained by following the degradation in a soil percolator? Perhaps measurements of the concentration in soil solution could be corrected for adsorption on the soil surface, to give a measure of the total amount of pesticide in the system? The procedure would have the advantage that it would be non-destructive.

G. Soulas. Soil percolation has the theoretical advantage of permitting more frequent measurements of the residual concentration of the pesticide. Such an advantage is largely offset by the different shortcomings of the procedure, particularly in relation to excessive soil water content, limitation of oxygen transfer and leaching of organic carbon. Also, chemical analysis of the pesticide may require long extraction and purification procedures making it difficult to increase the frequency of obtaining data points when compared to classical radiorespirometry.

P. H. Nye (*Department of Plant Sciences, University of Oxford, U.K.*), I do not think it is possible to use the flowing solution or soil percolation techniques to simulate the microbial decomposition kinetics that occur in an incubated moist soil. The solute concentrations in the soil pores will be affected by the flowing solution; and it is not possible to maintain a concentration that is the same within inter- and intra-aggregate pores by these methods.

D. J. Greenwood. I accept what Professor Nye says, but I wonder whether for mobile non-absorbed materials there is an appreciable difference between the solution concentrations within the aggregates and in the percolate, and thus whether useful information cannot still be obtained by the use of percolation techniques.

I. J. Graham-Bryce (*Shell, The Netherlands*). In most of the examples you have presented, the agreement between model predictions and experimental results is reasonably good. As with many models, however, this could be expected from the mathematical nature of the model whether or not the underlying concept is valid, particularly as the experimental curves are relatively simple in form. What further experimental studies would you regard as particularly helpful in seeking to validate the basic concepts?

G. Soulas. It is the inherent nature of all prospective mathematical models to have a demonstrative value only when the processes it takes into account are not operative.

Our model for kinetics of 2,4-D breakdown in the soil is based on the consideration that two microbial populations are involved in the degradation process. If the participation of specific microbial strains (metabolizing microorganisms) has long been recognized the demonstration that a large fraction of the soil microflora could be active specially at low concentrations has yet to be made. A further experimental study could be to try to stimulate the degrading activity of such a non-growing population by adding to the soil easily mineralizable carbon substrates that have been proved inhibitory at higher concentrations when growth of specialized microorganisms can take place.