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SUMMARY

There is an increasing interest in the quantitative prediction of the environmental fate of chemicals and hence in determining their potential for biodegradation in biotreatment processes.

This report reviews literature on the determination of biodegradation rates and other kinetic constants and their use in predicting the environmental concentration of chemicals in aerobic freshwater and in biotreatment processes. The various mathematical approaches based on Monod, Michaelis Menten and first order kinetics are discussed and their application to environmental fate models and biotreatment systems assessed.

It is concluded that current experimental procedures for determining biodegradation rates produce highly variable kinetic data that are strongly dependent on test conditions such as temperature, inoculum type, substrate concentration, nutrient levels and the opportunity for co-metabolism. Consequently no single test procedure can accurately predict the rate of degradation of test chemicals in a variety of aquatic environments. The present methods measure as much the properties of the environmental compartment as the chemical itself. Experimental data on biodegradation kinetics are relatively limited. It is recognised that environmentally relevant kinetic data can only be produced when test methods closely reflect the specific aquatic environment under consideration.

For data relevant to surface waters further development of test methods will depend on the use of refined analytical techniques to measure environmentally relevant levels of test compounds; these techniques are likely to rely on the use of ^{14}C radiotracers. Biotreatment test procedures may not require such techniques since higher concentrations of test chemical are used. For predicting behaviour during treatment by the activated sludge process, an approach in which the influence of Sludge Retention Time (SRT) and temperature is studied, is considered to be worth further development.

In view of the uncertainties in deriving kinetic data and the difficulties of extrapolation to other environmental compartments, a single pragmatic approach is recommended. It is suggested that modelling should be based on first order (half-life) kinetics for surface waters and the SRT method for activated sludge systems. Constants obtained are not universal parameters but apply only to limited and defined environmental situations.

A. INTRODUCTION

Many schemes for regulating the production and use of chemicals (EEC, 1967; 1979) require information on their biodegradability so that an estimate of their likely persistence in the environment or their potential for biodegradation in biotreatment processes can be made.

In previous publications (ECETOC, 1983; Blok et al., 1985), the applicability and limitations of biodegradability test methods given in the OECD Guidelines (1981) and Annex V of the 6th Amendment (EEC, 1984) were assessed. Recommendations for improvements were outlined.

It was recognised that, even when improved, current test methods determine only the percentage elimination of the test chemical after a specified test period and are not designed for making detailed kinetic interpretations. Whilst adequate for classifying chemicals as easily degradable or resistant test results have only a limited value in predicting the actual behaviour and the degree of persistence of chemicals in the wide variety of environmental compartments, from treatment plants to the open sea.

An ability to predict environmental fate for chemicals is becoming increasingly important. An earlier report (ECETOC, 1983) concluded that knowledge of biodegradation kinetics (the mathematical expression of the rate of breakdown of a chemical by biological means) in various environmental compartments would be extremely valuable for predicting fate.

Over the last decade many publications have given data on biodegradation rates and kinetic constants for selected chemicals in defined environments, notably constituents in natural waters and biotreatment plants. Limited attempts have been made to review such data and to investigate whether biodegradation kinetics and the development of appropriate test methods could have a wider application in predicting the environmental fate of industrial chemicals.

A Task Force was therefore established to review the present status and future application of biodegradation kinetics in assessing the environmental fate of chemicals, under the following Terms of Reference:

1. review the literature on the determination of biodegradation rates and kinetic constants, and on the use of this information in predicting environmental concentrations of chemicals;

2. critically assess the validity of the various approaches reported;

3. identify those approaches which might form an acceptable basis for further development.

B. BACKGROUND

Many studies on biodegradation kinetics have used pure bacterial cultures growing on single substrates under strictly controlled conditions (Monod, 1949). The mathematical approaches established in these studies have been shown to apply also in many cases of mixed culture growth (Simkins and Alexander, 1984).

The application of laboratory studies to predict biodegradation rates in the natural environment poses considerable difficulties. Biodegradation in the natural environment not only depends on the nature and concentration of the chemicals to be degraded but also on environmental conditions, including pH, salinity, temperature, redox potential, availability of growth factors and nutrients, the presence of other substrates and the number of degradative organisms present.

These conditions vary in different environmental compartments such as soil, anaerobic or aerobic sediments, freshwater, sea-water and sewage treatment plants and thus are likely to produce different biodegradation rates in each environmental compartment. This variability alone has made it difficult for chemical fate predictions to be transferred from one environmental compartment to another and may limit the application of laboratory derived data unless the studies have been carried out under conditions relevant to each compartment.

In this report the principles of biodegradation kinetics are reviewed (Chapter C) and the biodegradation tests that may be used for either freshwater or sewage treatment are indicated (Chapter D and Appendix 5). The review of test methods and application of biodegradation kinetics has been confined to aerobic systems. It is recognised that anaerobic processes may play an important role in the environment but, to date, few standard test methods have been established (Birch et al., 1989).

Much data reviewed by the Task Force demonstrated a high variability in results, often associated with toxicity, volatility or low solubility of test compounds. Whilst recognising that these factors would be significant when assessing the fate of specific chemicals, the Task Force attempted to confine

the current review to studies on compounds not exhibiting these characteristics. Most attention was also paid to kinetic data obtained with mixed cultures and mixed substrates, where biodegradation was indicated as the major degradative pathway.

Kinetic parameters derived for the breakdown of any chemical would be specific to the environmental compartment studied. In this report their use in environmental fate models has been limited to two environmental compartments, namely freshwater and aerobic sewage treatment plants. Sewage treatment plants would often be the first compartment exposed to a widely used chemical and freshwaters represent a major receiving environment for chemicals with a dispersed end use.

The possibilities and limitations of using biodegradation kinetic data and their application to predictive models are discussed in Chapter F and overall conclusions from the review are drawn in Chapter G together with indications for future work.

Table 1 summarises the most important literature used by the Task Force. A glossary of terms is included in Appendix 1. More specific details relating to the different chapters are given in Appendices 2 to 5.

C. PRINCIPLES OF BIODEGRADATION KINETICS

1. *DEFINING BIODEGRADATION KINETICS*

The study of biodegradation kinetics enables rates of biodegradation to be represented by a mathematical expression which may then be used to describe and predict quantitatively the breakdown of a chemical.

Biodegradation processes have been conveniently grouped into two categories: primary biodegradation and ultimate biodegradation. Primary biodegradation occurs when a discrete alteration is made to the structure of a chemical such that basic physico-chemical properties are lost (OECD, 1984; Larson, 1984). Primary biodegradation is generally determined by a specific analytical technique which measures the rate of disappearance of parent material. Ultimate biodegradation occurs when a chemical is broken down to simple inorganic molecules such as carbon dioxide (CO_2), water (H_2O) and to biomass. This biomass may in turn degrade leading to complete mineralisation.

Primary and ultimate biodegradation are in fact, parts of the same biodegradation pathway. The kinetic principles that govern biodegradation apply to the whole pathway including biomass formation and decay, and hence the principles and mathematical approaches described below apply equally to primary and ultimate biodegradation and mineralisation but will nevertheless lead to different kinetic values.

A consistent set of symbols has been used in this report. These may differ from those used in the original literature.

2. *MATHEMATICAL APPROACHES TO BIODEGRADATION KINETICS*

Most kinetic models for cell growth and substrate removal have been based on the classical Monod equation (Monod, 1949) for cell growth or on those used to describe enzyme kinetics, in particular the Michaelis-Menten equation (Michaelis and Menten, 1913).

Where the model has been based on enzyme kinetics it has normally been assumed that, whereas cells may be regarded as complex reactors involving many interactive enzyme-catalysed substrate reactions, one reaction step will be the slowest. This step will effectively control the overall rate of the reaction, substrate loss and cell growth. Under these conditions the rate of cell growth can be represented mathematically in terms of the effect of substrate concentration on this one enzyme reaction step.

The most commonly applied enzyme/substrate reaction rate equation is the Michaelis-Menten expression first developed in 1913 to describe Invertase catalysed reactions (Michaelis and Menten, 1913).

$$v = \frac{k_2 \cdot S}{K_m + S} \cdot E_t$$

where v = reaction velocity
k_2 = rate constant (second order)
E_t = total enzyme concentration
K_m = Michaelis constant
S = limiting substrate concentration
($k_2 . E_t$ = maximum velocity (V_{max}))

The development of this equation and its application to biodegradation kinetics are described more fully in Section 2.2 below.

Monod (1949) developed a generalised model in which cell growth was described in relation to the concentration of a growth-limiting substrate giving the following equation:

$$r_B = \frac{\mu_{max} \cdot S}{K_s + S} .B$$

where r_B = rate of cell growth
μ_{max} = max specific growth rate
S = limiting substrate concentration

K_s = saturation constant
B = viable cell concentration

The application of this equation is described in detail in Section 2.1 below.

It is interesting to note in comparing the above equations, that they may under certain conditions be analogous, if it is assumed that E_t is proportional to cell concentration (B) and $k_2.E_t$ is the maximum rate the reaction can proceed (and is commonly written V_{max}).

At substrate concentrations << K_m or <<Ks, the two equations simplify to:

$$v = \frac{k_2 \cdot S}{K_m} \cdot E_t \qquad \text{and} \qquad r_B = \frac{\mu_{max} \cdot S}{K_s} \cdot B$$

Since $K_2.E_t = V_{max}$, the ratio V_{max}/K_m is seen to be a first order rate constant, whereas μ_{max}/K_s is a second order rate constant. Since however, V_{max} includes E_t (proportional to viable cell concentration) under conditions where growth is significant (i.e. E_t is variable) k_2/K_m is clearly seen as a second order rate constant. In practice under study conditions where the increase in cell growth during the reaction period is low compared to the starting cell concentration, the expression approximates to a first order constant.

It is worth reiterating that there are certain limitations in applying either enzyme or growth kinetics to biodegradation of chemicals in the aquatic environment. Whilst giving valid data under conditions where the substrate is the sole carbon source and under laboratory conditions with pure cultures, variations in the natural environment such as alternative carbon sources, variable substrate concentrations and microbial consortia can limit the relevance of laboratory-derived constants to practical situations.

More complicated models have been developed to take into account the secondary substrates and inhibition effects (Schmidt and Alexander, 1985).

These equations do not give sufficient additional insight to justify the considerable increase in complexity of the experimental design and therefore were not considered further in this report.

2.1. *Monod Kinetics*

Although the Monod model (1949) was developed for pure cultures of bacteria growing on a single substrate, it also provides a good approximation with growth of mixed cultures (Simkins and Alexander, 1984). It gives a good, but indirect, description of the disappearance of the growth-limiting substrate. This is also the case when little growth occurs. In addition, simplifications of the model (such as the first-order approximation) can give a good description of observed biodegradation patterns under certain specific conditions.

The bacterial growth rate is given by

$$dB/dt = \mu.B$$

where B is the bacterial biomass concentration and μ is the specific growth rate expressed as 1/time. If Y is the yield, i.e. the proportion of original substrate converted to biomass and which is assumed to remain constant during biodegradation, the disappearance of substrate is defined by:

$$-dS/dt = 1/Y \; . \; dB/dt$$

where S is the substrate concentration. These rates may be calculated by the Monod equation:

$$\mu = \mu_{max} \; . \; S \; / \; (K_S + S)$$

leading to

$$dB/dt = \mu_{max}.S.B \; / \; (K_S+S)$$

where μ_{max} is the maximum specific growth rate and K_S is the substrate saturation constant (substrate concentration). Hence the disappearance of substrate may be represented by:

$$-dS/dt = \mu_{max}.S.B / ((K_S+S).Y)$$

This is the generally accepted form of the Monod model. Measuring B can be difficult (it refers to the number of competent organisms) but it is not needed for determination of μ_{max} and K_S and so B/Y may be replaced by X, the amount of substrate needed to produce a bacterial population density of B.

Hence

$$-dS/dt = dX/dt = \mu_{max}.S.X / (K_S+S)$$

The Monod model can be applied to batch or continuous systems with certain simplifications under controlled conditions.

i) Batch system

In a batch system at any time, (e.g. a typical ready biodegradation test) it is assumed that the sum of the concentration of the substrate and B/Y is a constant

$$S + X = S_o + X_o$$

This is called the conservation equation. where the subscript o means, concentration at time zero, then we obtain the differential equation

$$-dS/dt = \mu_{max}.S.(S_o+X_o-S) / (K_S+S)$$

This model has been used by Alexander and his group to determine values of μ_{max} and K_S from single degradation curves. This approach ignores the cell decay process and may lead to erroneous conclusions at low substrate or high biomass concentrations. Nevertheless the following series of simplified models have been used to describe the biodegradation curve in batch systems under a range of specific conditions (Simkins and Alexander, 1984; Alexander, 1985).

Model	Conditions	Equation*
General Monod (batch system) (V)	none	$-dS/dt = \mu_{max}.(S_o+X_o-S).S\ /(K_s+S)$
Zero Order (I)	$X_o>>S_o$ and $S_o>>K_s$	$-dS/dt = \mu_{max}.X_o$
Monod, no growth (II)	$X_o>>S_o$	$-dS/dt = \mu_{max}.X_o.S\ /\ (K_s+S)$
First Order (III)	$X_o>>S_o$ and $S_o<<K$	$-dS/dt = \mu max.X_o.S\ /\ K_s$
Logistic (IV)	$S_o<<K_s$	$-dS/dt = \mu_{max}.(S_o+X_o-S).S\ /\ K_s$
Exponential (VI)	$S_o>>K_s$	$-dS/dt = \mu_{max}.(S_o+X_o-S)$

* These equations are approximations from the general Monod (V)

It is possible to determine μ_{max} only from fitting the Monod and exponential models to a biodegradation curve. K_s may be determined only from fitting the Monod no growth and Monod models. Some of the above equations give derived parameters, as well as μ_{max} and K_s, which may be treated as constants.

Model	Derived parameter	Dimension
First Order	$\mu_{max}.X_o/K_s$	1/time
Monod no growth	$\mu_{max}.X_o$	concentration/time
Zero Order	$\mu_{max}.X_o$	concentration/time
Logistic	μ_{max}/K_s	time/concentration

The relationship between substrate and biomass concentrations under which these models fit observed conditions is shown in Figure 1. Under most environmental conditions of low bacterial and substrate

concentrations, the relevant equations are the first order, logistic and, less frequently, the Monod models.

Both the biodegradation and bacterial growth rates would be expected to follow the Arrhenius relationship within the normal temperature range (0 to 30°C), and this has indeed been demonstrated for nitrilotriacetic acid (NTA) and linear alkylbenzene sulfonate (LAS) (Larson, 1980). This is an exponential relationship which in practice results in an approximate doubling of these rates with a ten degree temperature increase (although with activated sludge a doubling for every 14 to 16 degrees has been reported (Roberts, 1990)). In practice this theoretical assumption does not always hold over wide temperature ranges due to changes in the populations involved in biodegradation. The result of this is that all rate related constants (e.g. μ_{max}, first order constant) would show temperature dependency, but other constants (e.g. K_s) would not.

ii) Continuous Systems

Under conditions of continuous culture, as in biotreatment systems, the conservation equation

$$S + X = S_o + X_o$$

does not apply and the two processes of substrate uptake and biomass decay must be considered separately. This leads to the development of the following equation incorporating a biomass decay factor (Birch, 1984):

$$dB/dt = B/SRT - S = \mu_{max} \cdot S_{eff} \cdot B \; / \; (K_s + S_{eff}) \; - K_d \cdot B$$

Hence

$$S_{eff} = \frac{K_s \; (1 + K_d \cdot SRT)}{SRT \; (\mu_{max} - K_d) - 1}$$

where S_{eff} is the equilibrium effluent concentration of substrate, K_d is the bacterial decay rate in units of 1/time and SRT is the sludge

retention time (1/dilution rate). In theory then, K_S and μ_{max} values determined with the appropriate mixed cultures in batch systems may be used to predict the effluent concentrations in sewage treatment plants. When used in this manner it is important to note that the following three conditions apply:

a) the effluent concentration is independent of the inflow concentration;

b) the only plant control parameter affecting the concentration of substrate in the effluent is the mean sludge retention time, which can be directly related to "load" (an expression relating daily nutrient input to biomass);

c) for any given effluent concentration there will be a critical sludge retention time (SRT_c) given by

$$\frac{1}{SRT_c} = \frac{\mu_{max} \cdot S_o}{K_S + S_o} - K_d$$

below which the competent micro-organism will be washed out of the plant and biodegradation will cease.

The application of this model to sewage treatment is discussed further in Section E 3.2.

2.2. *Michaelis - Menten Kinetics*

Another generalised approach to enzyme-catalysed reactions kinetics was put forward by Michaelis and Menten (1913) and is often described as Michaelis-Menten kinetics. In this approach the rate of growth is described by reference only to the concentration of rate limiting growth substrate. It gives an extremely good approximation when describing the biodegradation of a trace substrate (sometimes called a secondary substrate) by a large excess of competent bacteria which are not growing,