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CONTENTS

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,

or are growing on an alternative substrate (sometimes called a primary substrate) when conditions for co-metabolism occur.

Based on enzyme kinetics, substrate uptake is defined by

$$-dS/dt = v = k_2 \cdot E_t \cdot S / (K_m + S)$$

where E_t is the total enzyme concentration, K_m is the Michaelis constant, which is the substrate concentration at which the reaction occurs at half of the maximum rate Vmax ($V_{max} = k_2 . E_t$).

In bacterial systems E_t can be assumed to be proportional to the bacterial biomass concentration B

$$E_t = k \cdot B$$

resulting in

$$-dS/dt = k_2 \cdot k \cdot B \cdot S / (K_m + S).$$

The product of k_2 and k can be replaced by one new constant k_2', leading to

$$-dS/dt = k_2' \cdot B \cdot S / (K_m + S)$$

which is comparable to the Monod equation, or identical, when

$$k_2' = \mu_{max} / Y \quad \text{and} \quad K_m = K_s .$$

Where growth is negligible compared to the initial biomass, $k_2' . B$ can be substituted by V_{max} and the equation becomes

$$-dS/dt = V_{max} \cdot S / (K_m + S).$$

Where $S << K_m$ (e.g. $S < 0.1 K_m$), then

$$-dS/dt = V_{max} \cdot S / K_m .$$

Since V_{max} / K_m is assumed to be a constant k_1 for a given substrate the equation simplifies to

$$-dS/dt = k_1 \cdot S$$

which is first order kinetics.

Where $S >> K_m$ (e.g. $S > 10\ K_m$), the equation becomes

$$-dS/dt = V_{max}.$$

It is assumed that all enzyme sites are fully saturated with substrate and thus reaction proceeds at maximum rate independent of substrate concentration (zero-order reaction).

Between the two extremes of $S << K_m$ and $S >> K_m$ where significant growth of biomass occurs mixed order kinetics will be observed producing sigmoidal substrate removal curves. The changed kinetic orders derived from a BOD curve under these conditions are illustrated in Figure 2 (Ramalho, 1977).

2.3. *The Pragmatic Approach - First Order Kinetics*

First order kinetic data (shown above derived from the theoretical Michaelis-Menten equation), have been obtained by many workers adopting the pragmatic approach of determining loss of substrate from a number of aquatic environments without taking into account specific theoretical considerations of cell growth or enzymic activity (Larson, 1979, 1980; Larson and Games, 1981; Larson and Wentler, 1982; Bourquin and Pritchard, 1985). These data have also been used to develop the concept of a biodegradation half-life ($t_{1/2}$) which is the time required to reduce the mass of a chemical to 50% of its original value. The half-life is derived from the first order reaction constant k_1 by

$$t_{1/2} = \ln 2 / k_1$$

This pragmatic approach gives kinetics which are applicable when degraders are present in excess and when the concentration of substrate is low.

A similar approach has also been successfully adopted to describe plug flow first-order kinetics for fixed film reactors such as laboratory rolling tubes and trickling filter sewage treatment units (cf Appendix 4).

3. *SUMMARY OF MATHEMATICAL APPROACHES*

The following scheme summarises the mathematical approaches which are described more fully in the text (for references see Table 1).

Authors' Designation*	Approach	Order with respect to		Overall Kinetic Order
		Substrate	Biomass	
GENERAL				
MONOD	Mo	First	First	Second
MICHAELIS-MENTEN	MM	First	First	Second
SPECIAL CASES				
Pseudo-Zero	Mo/MM	Zero	First	First
no growth	Mo/MM	First	First	Second
Pseudo-First	Mo/MM	First	First	Second
Logistic	Mo/MM	First	First	Second
Exponential	Mo/MM	First	First	Second
PRAGMATIC				
First	MM	First	Zero	First

* The designations given by the authors do not necessarily correspond with the overall kinetic order.

D. METHODS FOR THE MEASUREMENT OF BIODEGRADATION KINETICS

1. *CRITERIA FOR REALISTIC BIODEGRADATION TESTS*

Biodegradation rates are not intrinsic properties. As indicated earlier biodegradation rates are greatly affected by environmental and test conditions. Physical (e.g. temperature, light intensity), chemical (e.g. substrate concentration,oxygen availability, pH) and biological variables (e.g. type and concentration of biomass) all influence kinetics.

To determine biodegradation kinetics that are relevant to natural systems, it is necessary to use test conditions that mimic as closely as possible those systems. Substrate concentration and inoculum type, cometabolism, acclimatisation and temperature merit particular attention.

1.1. *Substrate Concentration and Inocula Type*

The concentration of substrate in water can have a major effect on degradation rate: if high it may be toxic and if low may not induce the enzyme systems within the inocula necessary to achieve biodegradation. Moreover bacteria with different biochemical activity may thrive at different substrate concentrations. In a comprehensive review of biodegradation testing Battersby (1989) indicated that "oligotrophic" bacteria, capable of growing in low nutrient solutions, may be able to degrade low concentrations of test compound, whereas "eutrophic" bacteria adapted to higher nutrient concentrations, may not be able to degrade the same level of test compound.

In studies of phenol degradation in lake water Rubin et al. (1982) suggested that the rate of biodegradation was high at concentrations of 1 pg/l to 1 mg/l, lower between 1 - 100 mg/l and again higher above 100 mg/l, suggesting oligotroph activity below 1 mg/l and eutroph activity at high levels.

Threshold concentrations below which no degradation occurs have also been described, e.g. 10 µg/L for 4-nitrophenol (Schmidt et al., 1987), 2 µg/L

for 2,4-dichlorophenoxy-acetate (Boethling and Alexander, 1979) and < 1µg/L for linear alcohol ethoxylates (Vashon and Schwab, 1982). For many organic pollutants such levels are typical of those found in the environment. Other investigators did not observe such thresholds in the µg range (Larson and Davidson, 1982; Larson et al., 1983). The occurrence of degradation thresholds therefore seems to be substrate and environment specific; they may in reality represent a thresholds for inducing relevant enzymes in unacclimatised systems, rather than biodegradation thresholds.

1.2. Co-metabolism and Alternative Substrates

Many ready biodegradability tests have the test chemical as the sole carbon source. Under these conditions no degradation will occur if the inocula require a cometabolite or additional carbon source for successful growth. In natural environments, particularly sewage treatment systems and post treatment, a variety of other substrates are present, increasing the opportunity for co-metabolism and growth.

1.3. Acclimatisation

Inocula used in biodegradation tests are frequently unadapted (unacclimatised) to the test chemical or the particular concentration present. Depending on the test conditions, biodegradation will then be delayed or may not occur. Conversely, the use of an inoculum fully adapted (acclimatised) to the test chemical and the concentration present should give a maximum degradation rate.

A delay in the onset of detectable biodegradation may be due to: i) the time needed for the induction of enzymes; ii) the time needed for a genetic change (e.g. plasmid transfer) to occur; iii) the time for small populations of competent organisms to multiply sufficiently for detectable biodegradation to occur; or iv) the preferential degradation of other organic compounds before the chemical of interest. Other possibilities have been discussed by Wiggins et al. (1987).

Whether a test system should be preacclimatised to the test material, or the concentration present, depends on the type of information required from an investigation.

1.4. *Temperature*

Whilst biodegradation and bacterial growth rates might be expected to approximately halve with every 10°C decrease in temperature, there is evidence that under certain circumstances a change in temperature, particularly a reduction from 15 - 20°C to 5 - 8°C, can result in a severe reduction in the biodegradation rate of some chemicals (e.g. linear alkyl phenolethoxylates) which is out of all proportion to the temperature fall (Stiff et al., 1973). In the porous pot used as a model activated sludge sewage treatment plant, a similar effect was found for nonylphenol ethoxylate; the critical SRT showed a steep increase from less than 2 days at 11° and 15°C to approximately 6 days at 7°C (Birch, 1991).

1.5. *Overview*

It is evident from the above that tests likely to yield useful results will be those most closely reproducing the conditions in the environment under consideration. For example, if the biodegradation kinetics of a chemical continuously present in an environment are to be evaluated, tests should use acclimatised inocula, realistic substrate concentrations, additional substrate sources where appropriate and a suitable temperature regime. Where the effect of a chemical spillage on an environmental compartment not previously exposed to the chemical is to be evaluated, then the use of an unacclimatised inoculum and higher substrate concentrations may be more appropriate. It should be appreciated that relatively simple laboratory test systems cannot be expected to give precise data on the behaviour of chemicals in a large and complex environment.

2. READY- AND INHERENT BIODEGRADABILITY TESTS

The current tests adopted by the EEC as tests of ready and inherent biodegradability are performed under strictly defined conditions (OECD, 1981; EEC, 1984, 1988). These operating conditions do not mimic any natural environment. Levels of inorganic nutrient, substrate and temperature are elevated compared to the natural environment and the inocula are of different types. The differences are summarised in Table 2. Thus the strictly controlled conditions of the tests severely limit their application in determining relevant biodegradation kinetics. Even though they are designed to measure the extent of biodegradation, kinetic constants are sometimes derived from these systems.

At best the tests offer a simplistic "ranking" procedure for assessing different chemicals and produce differing rates for the same chemical, due to solubility/toxicity problems, lack of co-metabolism and variable inocula source and activity. The kinetics data generated from these tests are thus more likely to be related to the limitations of the test system or of the range of microorganisms introduced than to any specific characteristic of the test chemical.

3. LABORATORY TESTS WHICH SATISFY CRITERIA FOR KINETICS STUDY

Tests which are likely to give realistic kinetic data must be capable of mimicking the environmental compartment into which the test chemical is to be discharged. Consequently it is not appropriate for the tests to have predefined conditions attached to them. Rather, the conditions should be modified to suit the particular circumstances of the study.

Two types of test systems are considered further: those simulating surface waters in which the biomass is relatively low, and those simulating sewage biotreatment systems in which the biomass is high. Both systems may be modelled experimentally with batch tests and the activated sludge sewage systems may also be modelled with continuous (or semi-continuous) tests. In the latter case, hydraulic and sludge residence times may optionally be

controlled. More details about these test systems and the fixed film systems are given in Appendix 5.

3.1. *Tests Simulating Surface Waters*

Batch incubation is normally used to simulate surface-water biodegradation. The surface water of interest is sampled and used as quickly as possibly for testing. Incubations are carried out as closely as possible under the conditions of the sampling site, particularly with respect to temperature and dissolved oxygen. If the test is prolonged the sample will change as bacteria grow, nutrients are used and the pH changes so that results have less relevance to the environmental conditions.

This method has been used extensively to derive first order kinetics data on chemicals in a range of natural waters; for river water (Larson and Games, 1981; Larson and Davidson, 1982; Larson and Wentler, 1982; Larson et al., 1981; Larson, 1984; Hales and Ernst, 1991), for estuary water (Larson et al., 1983; Pfaender et al., 1985) and for groundwater (Ventullo and Larson, 1985). Second order kinetics data can be obtained by also measuring the number of bacteria present in the original water sample. μ_{max} and K_S have also been obtained (Alexander 1981, 1985; Jones and Alexander, 1986; Schmidt and Alexander, 1985; Schmidt et al., 1985, 1987; Simkins and Alexander, 1984, 1985; Simkins et al., 1986; Hales and Ernst, 1991).

It is important to note that most of these studies were conducted using ^{14}C radiolabelled test chemicals, this being the most effective means of following biodegradation at low test substance concentrations. Alternatively a suitable sensitive analytical method may be used. The cost of developing such analytical methods or synthesising radiolabelled chemicals may limit the widespread application of these studies.

3.2. *Tests Simulating Sewage Systems*

Four types of tests are used for assessing biodegradation in sewage treatment systems. In order of increasing complexity, they are (i) the batch activated sludge test, (ii) the semi-continuous activated sludge

test (SCAS) (iii) the continuous activated sludge test (CAS) with a) semi-continuous or no sludge wastage or b) the continuous activated sludge test with continuous sludge wastage and iv) tests simulating trickling filter sewage systems (cf Appendix 4). All of these systems have been used to derive kinetics data.

i) The batch system resembles the surface water test systems in its principles and has the same weaknesses. It normally generates first-order degradation kinetics data because of the high biomass and has been used extensively on a range of substances (Gilbert and Lee, 1980; Larson, 1979, 1980; Larson and Payne, 1981; Larson and Perry, 1981; Sullivan, 1983). Second order kinetics data can be obtained from this method by measuring the number of bacteria present in the inoculum and at time of sampling by procedures outlined by Hobbie et al. (1977) and Lehmicke et al. (1979).

ii) The semi-continuous system has been modelled and used to derive first-order biodegradation constants (Games et al., 1982). Modifications include the modified SCAS approaches of Larson (Larson and Wentler, 1982).

iii) a) The CAS system with semi-continuous or no sludge wastage is based on the OECD confirmatory test guideline but should preferably use real sewage. This system has been used very widely to simulate the biodegradation kinetics in real-life sewage treatment. Many variations and modifications exist (Painter and King, 1978; King et al., 1980; Streuli, 1980; OECD, 1981; Wierich and Gerike, 1981; EEC, 1982, 1984; Holman and Hopping, 1986).

b) The CAS system with continuous sludge wastage (to give SRT control) is based on the WRC Porous Pot System (Painter and King, 1978); alternatively a modified OECD method (CAS) may be used. Its main feature is the ability to derive K_S and μ_{max} by measuring equilibrium effluent concentrations in plants with SRT controlled at different values (Birch, 1984, 1991; Cech and Chudoba, 1988; Chudoba et al., 1989a-b).

iv) High rate plastic packed towers and conventional trickling filter sewage treatment systems are simulated in the laboratory by two types of test:

a) The rotating tube system (Gloyna et al., 1952) which is now an UK-Dept. of Environment (HMSO, 1982) biodegradability assessment test. The test has been used to develop plug flow first order kinetics, combining a first order biodegradation constant with one taking into account mass transfer limitations (Roberts, 1985). A similar approach has been used by Namkung et al. (1983);

b) Laboratory scale trickling filters were used to derive first order kinetic constants using the Monod approach where $K_s >> S$ and zero order kinetics where $K_s << S$ (Eckenfelder, 1966; Kornegay and Andrews, 1968).

When relevant test conditions are used, simulation tests can satisfactorily predict the fate of chemicals in sewage or industrial treatment plants but extrapolation of data to a wide range of temperatures and to low concentrations may be of uncertain validity (Hales, 1991).

4. *FIELD STUDIES IN ENVIRONMENTAL COMPARTMENTS*

Field studies, establishing kinetic data in the actual environmental compartment of concern, are the ultimate evaluation procedures and may be necessary with certain large tonnage chemicals in order to validate laboratory data obtained using test methods which simplify the biodegradation processes occurring in the environment. So far few field studies have been performed because they are expensive and difficult to perform and the findings obtained are usually difficult to interpret as a result of the complex interactions of and the continuous changes in field parameters. Moreover results are inevitably site specific. Some river water studies have been done in situ (Lewis et al., 1986), but these are rare.

Depending on the results of laboratory tests, more refined estimates of chemical fate and removal by sewage treatment may be required from field studies. There are two approaches: i) the chemical is dosed to a sewage treatment plant or, ii) where the population served by the plant (e.g. for a housing development or small community) is provided with a prototype product containing the chemical of interest. This kind of confirmatory information can only be obtained for primary biodegradation, using a suitable analytical method to measure environmental concentrations. Data are available for detergent chemicals using primary settlers (Eckenfelder, 1966; King et al., 1980), trickling filters (Baumann et al., 1979; King et al., 1980) and activated sludge plants (Hopping, 1978; Sykes et al., 1979; King et al., 1980; Matthijs et al., 1989).

E. FATE MODELS WHICH USE BIODEGRADATION KINETICS DATA

1. *INTRODUCTION*

The fate models described below are designed to predict the exposure level of a chemical as a function of time in a particular environmental compartment. Biodegradation kinetics data generated in laboratory experiments serve as only one input parameter for these models, which also require other parameters (e.g. physico-chemical properties). The number and complexity of these input parameters vary with the complexity of the model.

In surface waters where active biomass concentration is low, other input parameters such as diffusion, volatility etc. may be likely to be more important for the prediction of environmental concentrations. In sewage treatment systems where active biomass concentration is high, biodegradation is frequently the rate limiting process. Biodegradation kinetic parameters can be of value when such models are used to predict effluent concentrations.

2. *FATE MODELS SIMULATING SURFACE WATERS*

Models which can be used for assessing the distribution and concentration of chemicals in the aquatic environment are in a state of active development. Methods range from pragmatic half-life approaches used in simple partition or dilution models needing only a few input parameters, to involved procedures, which encompass many different processes and therefore need more input parameters which characterise the system and the substance under consideration.

In mathematical models of rivers, a first order treatment may be appropriate. It should be noted that the first order constant is a derived parameter equivalent to $\mu_{max} \cdot X_o / K_s$ (cf Chapter C). It has the dimension of 1/time and so its reciprocal is proportional to the half-life of the test chemical. Different quantities of competent organisms may be found in rivers. However, if the concentration of organisms, proportional to X_o, is

determined, then μ_{max}/K_s may be calculated and is known as the "second order" decay constant. Use of this constant combined with data on concentrations of competent organisms should enable estimation of half-lives for a particular substrate in a range of rivers.

One of the simpler models uses first order kinetics as described in Chapter C. It relates the biodegradation half-life ($t_{1/2}$) of a chemical to its residence time (Chemical Residence Time, CRT) in a particular environment (Woltering et al., 1987). The CRT is defined as the time available for biodegradation to occur within a specific environment before environmental organisms or man (via drinking water) can be exposed, e.g. the transport time between two locations (Shimp et al., 1990). These authors have so far applied this model to rivers, ground water, soil, and estuaries.

In recent years many fate models have been developed, which permit a calculation of a concentration or the distribution between different environmental compartments. Two typical representatives of these approaches are EXAMS (Exposure Analysis Modelling System) (Burns et al., 1981) and QWASI (A Quantitative Water, Air, Sediment Interaction Fugacity Model) (Mackay et al., 1983a, b). The EXAMS and 12 other water models were reviewed by OECD (1989). Several of thse models are not relevant to this report as they do not consider biodegradation. Others require an extremely large number of parameters to characterise the environment and are applicable only to very specific situations. Two of these methods remain: EXWAT (Exposure of Surface Water Bodies) (Brueggemann and Muenzer, 1987) and MEXWA (Model of Exposure Assessment in Water) (not published so far). The latter also take biodegradation into account (like EXAMS and QWASI) in assessing the partition and fate of a chemical in rivers. All models need a lot of input data such as substance release, physico-chemical properties, river geometry and degradation kinetics. None of the models use Monod kinetics for the description of biodegradation. QWASI, EXWAT and MEXWA use first order reaction kinetics data (considering only substance concentration as a variable), whereas EXAMS can also apply second order equations. It is implied for all these models that the substance concentration can be kept so low that neither insolubility nor adverse biological effects (inhibition, toxicity) will occur. It is assumed that preacclimatised bacteria are present, enabling further biodegradation without any lag time.

More details about these models are given in Table 3.

3. *MODELS SIMULATING THE FATE OF CHEMICALS IN SEWAGE PLANTS*

These simulation models have been developed for determining the design of treatment plant based on organic load removal (usually expressed as BOD) from a sewage flow of given volume and strength and to determine the effects of SRT and temperature on removal of a chemical from an activated sludge unit.

In principle the same models should be applicable equally to specific chemicals and total organic carbon, provided they are based on satisfactory experimental data and not extrapolated beyond the experimental range.

Inevitably all models have to make a number of gross simplifications regarding, for example, micro-organism type, chemical complexity and the composition of the waste water. These simplifications can give reasonable approximations in practice but require a pragmatic approach. Details of the problems requiring such simplification are described by Simkins et al. (1986) and Namkung and Rittmann (1987).

3.1. *Activated Sludge Systems*

3.1.1. IAWPRC and GFM Models. The most generally accepted approach is that of the IAWPRC (International Association on Water Pollution Research and Control) Task Group (IAWPRC, 1986, 1987) and features the organisation of various interlinking bioprocesses as a matrix of stoichiometric and kinetic equations. This matrix can be simplified or extended to suit the specific requirements or to satisfy the complexity of the system under consideration.

A simplification of the IAWPRC approach is given by Namkung and Rittmann (1987) as the General Fate Model (GFM). It is based on a mass balance for a substance within a completely mixed aeration tank consisting of the influent as the only input and having the outputs of effluent and surplus sludge taking into account biodegradation, sorption and volatilisation.

The resulting equation can be easily solved, when assuming steady state conditions. The necessary input data are:

i) sewage plant parameters: wastewater flow rate, substance concentration in influent, gas volumetric flow rate, temperature, wasted sludge flow rate, volatile solids concentration, active cell concentration;

ii) substance parameters: water solubility, vapour pressure, octanol/water partition coefficient, volume of aeration tank, biodegradation rate constant. The latter parameter may be the most difficult to obtain.

By analogy to EXAMS a biodegradation rate constant is used in the GFM model which depends on the bacterial density. The rate constant is described elsewhere as a second order rate constant but in this model it is called an apparent first order rate constant. The application of the model is very simple.

Both the IAWPRC model and GFM are based on Monod kinetics for the main biodegradation processes.

3.1.2. The SRT Model. A further application of Monod kinetics to laboratory scale activated sludge units has led to consideration of Sludge Retention Time (SRT) as the controlling factor in chemical removal from sewage treatment plants. Data have been reported for nitrification (conversion of ammonia to nitrite and nitrate) (Painter and Loveless, 1983) and more recently for the biodegradation of specific organic chemicals (Birch, 1984; Cech and Chudoba, 1988).

The laboratory studies required to determine the effect of SRT and temperature on the removal of a chemical from an activated sludge unit and an approximation method for establishing critical SRT values, where limited data are available, were given by Birch (1984, 1991). An important conclusion is that the K_m and μ_{max} can be determined for a given chemical under biotreatment conditions. The effluent concentration may be calculated for each SRT and is not affected by the

influent concentration. Data supporting this approach are provided by the author.

Cech and Chudoba (1988), whilst giving no theoretical justification for the use of the SRT concept, produce data demonstrating its use in determining the removal of four organic chemicals in a laboratory activated sludge unit.

3.1.3. Other Models. The literature also contains references to various models not based on Monod kinetics. These are used either in special cases or as pragmatic models to suit unusual processes and are not considered applicable to the general case of the fate of chemicals in sewage treatment processes. For example, Simkins et al. (1986) reviewed the applicability of the "one and a half" order models of Brunner and Focht (1984) to mineralisation kinetics in sewage.

Where simple cases are considered the derivation of biodegradation constants from simple respirometric tests may suffice but in more difficult cases extensive testing may be required. A comprehensive model was attempted by Giona and Annesini (1979) but the correlations developed have not been adequately established.

3.2. Fixed Film Reactors

Trickling filters in which effluent is aerobically treated by flowing over a biological slime attached to an inert support media, represents one of the oldest forms of sewage treatment.

Mass transfer limitations associated with heavy slime formation in such systems, frequently give overall reaction rates of ½ to 1 and first order kinetics are often assumed (Hills and Mecklenburgh, 1981).

Early trickling filter designs using slag or coke support media were joined in the 1960's by organised plastic packing support media and random plastic packings both offering greater control over void space, surface area and freedom from blockage.

Increased interest in trickling filter (fixed film reactor) application to sewage and industrial waste treatment led to the development of a large number of models to describe biodegradation in fixed film reactors (Eckenfelder, 1966; Kornegay and Andrews, 1968; Roberts, 1985). All derive either first order or zero order kinetic constants dependent on substrate concentration. Many include functions to describe sorption, mass transfer and volatility effects.

Of all the varied (although basically similar) approaches, one involving a combination of a biochemical rate constant and a mass transfer constant has given a satisfactory approach from which, if all other test conditions are constant, the biochemical reaction terms can be determined and compared for different compounds (Roberts, 1985).

The details of this approach and a summary of other trickling filter models are given in Appendix 4.

F. UTILITY OF BIODEGRADATION KINETICS

1. INTRODUCTION

Previous chapters of this report have indicated the basis for biodegradation kinetics, the methods used to obtain laboratory data and the way such data can be applied to predicting the fate of a chemical in a number of natural environments using models of increasing complexity.

The following conclusions are evident from the data reviewed. The utility of the kinetic data derived increases the closer the laboratory studies mimic the actual environment. First order and pseudo-first order reaction constants are widely used in natural water models with good results. Second order rate constants can likewise be successfully applied to sewage treatment plant models.

The limitations of this approach and the alternatives are discussed further.

2. RIVERS, ESTUARIES AND MARINE ENVIRONMENTS

2.1. First order Kinetics

At the simplest level, if all that is needed is an estimate of the rate of biodegradation of a particular substance within a specific particular environment this can be determined reasonably accurately by sampling the water and, while fresh, adding a sample of the test material at a realistic concentration. If biodegradation is fairly rapid, first order kinetics will adequately describe the rate of biodegradation. This approach may well be the most suitable for studying the biodegradation of secondary substrates in bacterial populations found post-sewage-treatment and has been applied successfully in studying biodegradation of individual organic chemicals in rivers (Larson, 1980; Larson and Payne, 1981; Larson and Perry, 1981; Larson et al., 1983; Larson, 1984; Shimp et al., 1990), estuaries (Vashon and Schwab, 1982; Larson et al., 1983; Larson and Ventullo, 1986); and in marine environments (Shimp et al., 1990).

Results from such studies are used to predict test substance half-lives appropriate to the specific test conditions in which they were derived. This half-life is obviously not an intrinsic property of the chemical since widely varying values can be obtained for the same chemical in different environmental situations.

2.2. *Fate Models using Biodegradation Kinetics Data*

A more complex approach to determine the transport and loss of a chemical involves the use of models requiring more than a consideration of biodegradation rates. From the four fate models reviewed in this report EXAMS is most broadly used, whereas little experience exists on the use of QWASI, EXWAT and especially MEXWA. The different approaches have been developed with different objectives. MEXWA, QWASI and particularly EXAMS give an assessment of absolute concentrations of a chemical in a defined environment, whereas EXWAT was primarily designed for the comparative assessment of environmental hazards of existing chemicals. EXAMS is the most refined of these models and one of its advantages is its ability to use second order biodegradation kinetics data (the others use only first order kinetics). Hence the degradation velocity of a chemical can be related not only to the substance concentration but also to the concentration of the active biomass. Additional merits of EXAMS are the optional consideration of the influence of temperature on degradation kinetics and the possibility of calculating simultaneously the fate and transport behaviour of a chemical and its transformation products.

2.2.1. Sensitivity of Fate Models to Variations in the Input Parameters.

Only a few papers have been published on the influence of parameter changes on the model outputs. Most of them used EXAMS for different substances in rivers or lakes.

Slimak and Delos (1982) showed that the surface-to-volume ratio (depth) and the turbulence of a river had the largest effect on the fate calculations of the relatively volatile tetrachloroethylene. The behaviour of pentachlorophenol in coastal plain rivers was most influenced by changes in the photolysis rate coefficient, whereas calculations for phenol were most sensitive to changes in the

biodegradation rate constant. Honeycutt and Ballantine (1983) found that changes of the input load influence to a large extent the calculated results of the concentration of the insect growth inhibitor CGA-72662 in water and sediments of ponds/lakes. Reinert et al. (1987) ascertained that changes of the dilution rate (flow) had a large effect on calculation of the half-life of the herbicide endothelial in a lake.

Brueggemann and Muenzer (1987) found, when using EXWAT, that the dilution rate (flow), the biodegradation rate, the Henry-constant and the octanol/water partition coefficient K_{OC} represented the most important parameters for describing the fate of o-chloronitrobenzene in a river.

Therefore, it can be concluded that with respect to the substance and ambient environmental conditions no single parameter has an overriding influence on the model results in all cases. When one or two major processes are operative, the variability of the obtained results is closely related to the accuracy of the relevant inputs.

2.2.2. Transferability of Kinetics Data taken from Laboratory Tests to the Fate Models. A few publications show a more or less successful application of fate models to the environmental behaviour assessment of different chemicals in surface water systems, for example Games (1982), Rodgers et al. (1983), Pollard and Hern (1985), Holysh et al. (1986), Reinert and Rodgers (1986), Brueggemann et al. (1987) and Schramm et al. (1988).

Two groups, Games (1982) and Holysh et al. (1986), used EXAMS or QWASI respectively, to calculate the concentration of LAS in a small river downstream from a municipal sewage treatment plant. They found a fairly good correspondence between the measured and calculated concentrations by using only an arbitrary set of parameters like dispersion coefficient, sediment-water mass transfer coefficient and effective sediment bed depth. However, both groups wrongly used a rate constant determined by Larson and Payne (1981) which described the $^{14}CO_2$-evolution velocity of only partly (ring) labelled LAS incubated in river water, as a first order biodegradation rate constant of the parent substance. They also used a rate constant, determined in water systems

containing only 0.5 g/l of sediment as the sediment degradation rate constant.

Furthermore, the soil degradation rate constant used by Holysh et al. (1986) was derived in an even more doubtful manner: they used data from Kawashima and Takeno (1982) to extrapolate a first order degradation rate constant, although the latter authors had observed only 12 to 23% mineralisation of radiolabelled LAS in several soils within 12 days. Taking this into account it has to be considered that the fairly good agreement between measured and calculated LAS-concentrations may have resulted from the values selected for other important parameters like dispersion coefficient, sediment-water mass transfer coefficient and sediment bed depth.

Therefore data presented in these publications do not seem adequate to validate the river models used. For this purpose these parameters will have to be determined in independent experiments using material from the environment under consideration and well known reference substances for model calibration purposes.

The above observations serve once again to underline the need for all degradation rate constants to be determined from experiments closely related to the environmental compartments under consideration. Not only substance and active biomass concentrations but also pH and temperature have to be considered. Models like EXAMS using second order biodegradation kinetics suppose that the active biomass concentration within a compartment or its segments remains constant. Therefore it is important to ensure that in experiments to obtain kinetic parameters the biomass concentration is constant throughout. As it is the concentrations of the parent material that are of concern, the substance concentrations have preferably to be followed by specific analysis rather than summary or product evolution parameters like COD, DOC, BOD, dissolved radioactivity or CO_2. For this reason, when using radioactive material, it is important to recognise that the position of the labelled ^{14}C can strongly influence the numerical value of the rate constants calculated from radioactivity measurements.

2.3. *Sewage Treatment Systems*

First order kinetics have been employed successfully in determining biodegradation constants from batch activated sludge studies (Lee and Ryan, 1979; Pritchard et al., 1979; Johnson, 1980; Larson and Payne, 1981; Paris et al., 1981;) and can also be used to predict the biodegradation of secondary substrates in the continuous activated sludge process (Games et al., 1982). In both cases sorption and volatilisation constants for the test chemical should be taken into account.

In the General Fate Model (GFM) (Namkung and Rittman, 1987), a pseudo-first order approach is used. Here the first order rate constant k_1 is substituted for μ_{max}/K_S in the second order equation giving:

$$-dS/dt = - k_1 \cdot B_o \cdot S$$

This model gives a good, pragmatic estimate of biodegradation and removal of secondary substrates in the continuous activated sludge process. A disadvantage of this approach is that it predicts that effluent concentration is proportional to influent concentration. Observations of real-life sewage treatment plants show that this is not the case (Matthijs et al., 1989); instead the concentration of effluent increases disproportionally to and to a lesser extent than increases in the influent concentration, until the influent concentration reaches a value at which break-through occurs. The use of a second order rate constant which is dependent on both substrate and microbial concentration would produce a more realistic model.

Pseudo-first order kinetics are also used in fixed film (trickling filter) models and have been successful in predicting the fate of test chemicals in such systems under closely defined conditions of temperature and test substance concentration (Roberts, 1985).

Perhaps the most successful and comprehensive approach to activated sludge modelling is based on Monod kinetics and is known as the SRT approach. In this the critical control parameter is taken to be the sludge retention time (SRT). Other parameters affecting effluent concentrations refer to

the competent degrading bacteria and the substrate affinity constant (K_s), the decay rate (K_d) and the maximum specific growth rate (μ_{max}) (Birch, 1984, 1991). As with all other models there is a high level of temperature dependency (working mainly through μ_{max}). For biodegradable materials, effluent concentration is predicted to be independent of influent concentration. Compared with other approaches the SRT approach demands a more extensive test programme to determine the critical parameters.

G. CONCLUSIONS AND RECOMMENDATIONS

The mathematical treatment of biodegradation kinetics has produced a wide range of kinetic models based on either Monod or Michaelis-Menten equations. These models have been successfully applied in describing the biodegradation rates of chemicals in laboratory systems under a range of specific conditions.

Where biodegradation is the dominant factor influencing the fate of a chemical these models were also successfully applied to surface waters. They differ from the more complex simulation and fate models in using only a few biokinetic parameters and do not take into account additional physico-chemical properties of the substance or test system.

Selection of the most appropriate kinetic models will depend mainly on the nature of the chemical under test, its concentration, the diversity and density of microbial population and the type of environmental compartment. It is recommended to use first order kinetics (half-life) for surface waters derived from studies conducted under relevant environmental conditions. Second order SRT approach should be used to model activated sludge systems.

Kinetic constants obtained cannot be regarded as universal parameters and will change if test conditions change. They only apply to a restricted and defined environmental situation. Realistic kinetic constants can therefore only be reliably obtained when tests are used which closely mimic the environmental compartment under consideration.

Future work should be aimed at improving recovery and analytical techniques for surface water tests and optimising the number and range of studies needed to derive temperature related kinetic constants from SRT studies.

Great care should be taken when using biodegradation kinetic data in exposure modelling.

TABLE 1

Summary of Principal Literature on Biodegradation Kinetics

Authors (date)	Model (1)	Order (2)	Substance type or subject	Substance conc. (3)	Inoculum type (4)	Biomass conc. (5)	Environ. Comp. (6)	Test Method Type (7)	Test Method Analysis(8)
Aelion et al., 1987			various		soil		W/*		RA
Alexander, 1981			No kinetics, biodegradation review						
Alexander, 1985	M	*,1,2	review						
Anderson et al., 1988			No kinetics, enumeration of organisms						
Balaz et al., 1989		*,1	QSAR;log P/biodegradation rate						
Banerjee et al., 1984	M	2	various, mainly chlorophenolics	5 - 150	S, M	H	F		SA
Bartholomew and Pfaender, 1983	MM	1,2	various aromatics		M	L	E, M, F		RA
Battersby, 1989									
Baughmann and Burns, 1980									
Baughmann et al., 1980	M	2	various, pesticides/phthalates		M		F, S		SA
Berry et al., 1987									
Birch, 1984	M	*	LAEO, NTA, di-Cl.phenol	5 - 25	M	H	AS	C	SA
Birch, 1991	M	*	surfactants, di-Cl.phenol, NTA	5 - 25	M	H	AS	C	SA
Bishop et al., 1987									
Bouwer, 1985	M	1	various, hydrocarbons and halogen. derivatives	0.01	M	H	TF		SA
Boyd and Shelton, 1984			Anaerobic degradation						
Braha and Hafner, 1987	M	2	industrial waste	100 - 300	M	H	AS	B	TOC
Brown et al., 1990	M	2	phenols (Haldane inhibition model)	20 - 100	M	L	AS	B	R
Christensen, 1984			SRB and sediments, no kinetics						
Chudoba et al., 1989a	MM	1	morpholine, sulfanilic acid, NTA	50 - 130	M	H	AS	C	SA
Chudoba et al., 1989b	MM	1	2,4-dichlorophenol	89	M	H	AS	C	SA
Costerton et al., 1978			surface growth, no kinetics						
Crane et al., 1981			Hc concentrations, no kinetics						
Cripe et al., 1987		1	various	0,2	M	H	F, S		SA
Dalton and Stirling, 1982									
Dickson et al., 1982			Math modelling environmental concentrations						
DoE, 1979			Environmental concentrations						
Games et al., 1982	M	1	cationic surfactants	0.1 - 20	M	L, H	AS	B	R, RA, SA
Hales and Ernst, 1991	M	*,1,2	NTA	0.001 - 1	M	L	F, M	B	RA
Hales, 1991	M		DASS	1 - 20	M	H	AS	C	RA
Hao and Lau, 1988	M	2	glucose	1000	S	L	AS	C	SA
Harder and Dijkhuizen, 1982			mixed substrate use / Pseudomonas	high	S		F	B	

TABLE 1 (cont.)

Authors (date)	Model (1)	Order (2)	Substance type or subject	Substance conc. (3)	Inoculum type (4)	Biomass conc. (5)	Environ. Comp. (6)	Test Method Type (7)	Test Method Analysis(8)
Howard, 1985			review of weaknesses of methods						
Howard and Banerjee, 1984	M/MM		biodeg. tests: kinetic interp. and relevance						
Hwang et al., 1989	MM	1	organics	0.0001-1000	M	L	F	B	RA
Jacobsen, 1987	M	*	review of SRT models		M	H	AS	C	
Jannasch, 1967	M	*	lactate, glycerol, D-Glucose	0.5 - 100	M	L	M	C	cell count
Jones and Alexander, 1986	M	*,1,2	Phenol	0.0005 -- 1	M	L	F	B	RA
Kennedy et al., 1990		2	4-chlorophenol (Haldane inhibition model)	20 - 160	M	H	S, AS	B	SA
Kim and Suidan, 1989			biofilm model	high	M	H	AS	C	
Klecka and Maier, 1988	M	*	pentachlorophenol	0.25 - 0.5	M	L	F	B, C	SA
Kollig et al., 1987		1,2	2,4-DBE; p-Cresol	0.1 - 0.2	M	L	F	B	SA
Ladd et al., 1982	MM		Glutamic acid; Phenylalanine; Glycolic acid	0.0002-0.016	M	L	F, GW	B	RA
Lapat-Polasko et al., 1984		*	Methylene chloride; sodium acetate	0.01 - 5	S	L	F	B, C	SA, RA
Larson, 1980	MM	1	Glucose; Surfactants; NTA	0.05 - 10	M	L	F	B	RA
Larson, 1983		*	STAC; DSDMAC; LAS; NTA	0.05 - 50	M	L	F	B	RA
Larson, 1984	M	1	NTA, Detergents	0.001 - 1	M	L	F, GW	C	RA
Larson and Payne, 1981		*	LAS	0.05 - 10	M	L	F	B	RA, IL-MBAS
Larson and Perry, 1981		*	LAS; CTAC; C12E07	5-80	M	L	F	B	R
Larson and Vashon, 1983	M	1	cationic surfactants	0.001 - 0.1	M	L	F	B	R, RA
Larson and Ventullo, 1986	MM	1	NTA	0.003 - 9	M	L	E	B	RA
Lehmicke et al., 1979			14C-Most-Probable-Number Method						
Lewandowski, 1990		2	phenols (Haldane inhibition model)	20 - 100	M	H	AS	B	S
Lewis and Gattie, 1988		1,2,*	2,4-DME; 2,4-DBE	0.1	M	L	F	B	SA
Lewis and Holm, 1981		1,2	Methylparathion (MP); Diethylphthalate (DEP)	0.1 - 0.3	M	L	F	B	SA
Lewis et al., 1984	MM	1,2	MP; DEP; 2,4-DBE; p-Cresol	0.1 - 10	S, M	L	F	B	
Lewis et al., 1988	MM	1	Glucose; Phenol; p-Cresol; Butanol; ...	0.01 - 80	M	L	F	B	RA
Mackay, 1979			Fugacity Models						
Mackay and Patterson, 1981			Fugacity Models						
Maria and Ognean, 1989	M	2	organics	100	M	H	AS	C	TOC
Monod, 1949	M		Growth Model						
Moos et al., 1983	M	1	pentachlorophenol	0.1	M	H	AS	C	RA
Namkung and Rittmann, 1987	M	1	volatile organic compounds		M	H	AS	C	
Nanqi and Zijie, 1990		* - 2.5	tannery wastewater in ponds	high	M	L	*	B,C	R
Orhon et al., 1989	M	2	wastewater (model for activated sludge process)	high	M	H	AS	C	
Padukone and Andrews, 1989	M	2	wastewater (model for activated sludge process)	high	M	H	AS	C	

TABLE 1 (cont.)

Authors (date)	Model (1)	Order (2)	Substance type or subject	Substance conc. (3)	Inoculum type (4)	Biomass conc. (5)	Environ. Comp. (6)	Test Method Type (7)	Test Method Analysis(8)
Painter and King, 1985	M	*,2							
Paris et al., 1981	M	2	Pesticides	0.01 - 1	M	L	F	B	SA
Paris et al., 1982a	M	*,1,2	organics		M	L, H	F, AS	B	R, RA, SA
Paris et al., 1982b	M	1,2	phenols		M	L, H	F, AS	B	SA
Paris et al., 1983	M	1,2	phenols		M	L	F	B	SA
Paris et al., 1984	M	1,2	esters of chloroacids		M	L	F	B	SA
Parsons and Strickland, 1962	M	1,2	organics		M	L	E, *	B	SA
Parsons and Govers, 1990	M/MM	1,2	QSAR; molecular structure/biodegr. rate						
Pavlou, 1980			Thermodynamics		M	L	F, S	B	SA
Peng, 1977			Sample preparation in scintillation counting						RA
Pfaender and Bartholomew, 1982	MM	1	organics		M	L	F	B	RA
Rogers et al., 1984	M/MM	1,2	phenols, quinoline ...		M	L	F	B	SA
Rubin et al., 1982	M/MM	1,2	aromatics	trace	M	L, H	F, AS	B	R, RA
San, 1989	M	2	waste waters	60 - 1000	M	H	AS	C	
Schmidt and Alexander, 1985	M	*,1,2	organics	low	S, M	L	F, AS	B	R, RA
Schmidt et al., 1985	M	*,1,2	phenol, arabinose, glucose	low	S	L	F, AS	B	R, SA
Schmidt et al., 1987	M	*,1	p-nitrophenol	low	S	L	F, AS	B	R, RA, SA
Schwartz et al., 1979			phthalate esters analysis	low	M	L	S	B	SA
Shelton et al., 1984			phthalate esters (anaerobic)	low	M	M	AS	B	R, RA, SA
Shimp and Young, 1987	MM	1	benzoic acid	0.05 - 20	M	L	E	B	R, RA, SA
Shimp and Young, 1988	MM	1	cationic surfactant, phenol	low	M	L	S, S+F	B	RA, SA
Simkins and Alexander, 1984	M	1,2	sodium benzoate	0.1 - 10	S, M	L	F, AS	B	RA
Simkins and Alexander, 1985	M/MM	1,2	organics	0.1	S, M	L	F, AS	B	RA
Simkins et al., 1986	M	2	organics	0.0003-0.03	M	L	AS	B	RA
Spain et al., 1984		1	p-nitrophenol	0.1	M	L	F	B	RA
Speitel and DiGiano, 1988	M	2	phenol	0.5	M	L	AS	B	RA
Steen and Collette, 1989		2	amides in ponds	0.5	M	L	*	B	SA
Strand et al., 1990	M/MM	1,2	trichlorethylene, trichlorethane	0.5 - 2	M	H	AS + GW	B	R
Subba-Rao et al., 1982		1	organics	0.000001	M	L	F	B	RA
Swindoll et al., 1988	MM	1	organics	0.01 - 600	M	H	GW	B	RA
Templeton and Grady, 1988	M	2	2-chlorophenol	100	S	L		B	SA

TABLE 1 (cont.)

Authors (date)	Model (1)	Order (2)	Substance type or subject	Substance conc. (3)	Inoculum type (4)	Biomass conc. (5)	Environ. Comp. (6)	Test Method Type (7)	Test Method Analysis(8)
Vaishnav and Babeu, 1987		1	organics	1	M	L	F, GW	B	R
Vashon and Schwab, 1982	M/MM	*,1,2	LAE, LAES	0.001 - 100	M	L	E	B	RA
Ventullo and Larson, 1985	M/MM	1,2	organics	0.001 - 1	M	L	GW	B	RA
Wright and Hobbie, 1965	MM	1	Glucose, Acetate	1	M	L	F	B	RA

Notes: (1) : M = Monod, MM = Michaelis-Menten; (2) : 1 = 1st order, 2 = 2nd order, * = other; (3) : mg/l; (4) : S = single organism, M = mixed culture; (5) : L = low concentration, H = high; (6) : F = fresh water, S = sediment, M = marine water, GW = ground water, AS = activated sludge, TF = trickling filter, E = estuarine water, * = other; (7) : B = batch, C = continuous; (8) : R = respiratory, RA = radioactive, SA = specific analysis

2,4-DBE	2,4-Dichlorophenoxyacetic acid butoxyethyl ester
2,4-DME	2,4-Dichlorophenoxyacetic acid methyl ester
C12EO7	Dodecylheptylethoxylate
C12EO9	Dodecylnonylethoxylate
CTAC	Cetyltrimethylammonium chloride
DEP	Diethylphthalate
DSDMAC	Distearyldimethylammonium chloride
DTMAC	Dodecyltrimethylammonium chloride
IL-MBAS	Interference-limited MBAS method
LAE	Linear alcohol ethoxylate
LAES	Linear alcohol ethoxysulfate
LAS	Linear alkylbenzene sulfonate
MP	Methylparathion
NTA	Nitrilotriacetic acid
STAC	Stearyltrimethylammonium chloride

TABLE 2

Comparison of Typical Standard Biodegradability Test Conditions with Typical European Aquatic Environmental Conditions
(after Battersby, 1989)

Parameter	Typical standard tests		Typical environmental conditions		
	Screening tests	Activated sludge simulation tests	River water	Sea water	Sewage treatment
Concentration of synthetic chemical	2 - 100 mg/l	10 - 50 mg/l	ng-µg/l	ng-µg/l	<µg-mg/l
Concentration of other substrates	1 mg C/l (nominally absent)	200 mg C/l	1 - 20 mg C/l*	0.3 - 3 mg C/l*	200 mg C/l*
Temperature range	20 - 25 °C	20 - 25 °C	2 - 21 °C	3 - 15 °C	8 - 20 °C
Concentration of phosphate	0.4 - 8 mM	0.2 - 0.7 mM	<3 - 70 µM*	0.03 - 0.7 µM*	0.7 mM*
Concentration of ammonia	0.03 - 4 mM	3 mM	5 - 350 µM*	<0.1 - 4 µM*	3 mM*
Buffer system	$H_2PO_4^-$ - HPO_4^{2-}	HCO_3^--CO2	HCO_3^--CO2	HCO_3^--CO2	HCO_3^--CO2
pH	7.2 - 7.5	7.0 - 7.8	7.1 - 8.0	7.8 - 8.2	7.2 - 7.8**
Nature of system	Static	Dynamic	Dynamic	Dynamic	Dynamic
Organisms favoured	Eutrophic (high µmax & Ks)	Oligotrophic (low µmax & Ks)	Oligotrophic (low µmax & Ks)	Oligotrophic (low µmax & Ks)	Oligotrophic (low µmax & Ks)
Predominant microbial population	Planktonic	Flocs	Attached	Attached	Planktonic Flocs*** Attached****

* Considerable variations occur

** Dependent on hardness of water supply

*** Activated sludge

**** Attached to particulate matter and solid supports (e.g. trickling filters, rotating biological contactors)

TABLE 3

Surface Water Fate Models

Model	Aquatic Systems	Mathematical Dimensions / Solution Techniques	Input Data (major)	Source	Reaction Order of Degradation Processes	Level of Application	Reference
EXAMS (version 2.92)	various types	1, 2, 3 numerical	chemical loadings, physico-chemical data, environmental characteristics, system	continuous intermittend single multiple diffuse	second	intermediate	Burns et al. (1981)
QWASI (fugacity)	river lake	1 analytical	source data, physico-chemical data, environmental characteristics	continuous	first	intermediate	Mackay et al. (1983)
EXWAT (fugacity)	river	1 analytical	source data, physico-chemical data, environmental characteristics, geometry of fluid and sediment compartments	continuous	first	screening intermediate	Brueggemann and Muenzer (1987)
MEXWA	river	2 analytical	source data, physico-chemical data, river characteristics	continuous single	first	intermediate	OECD (1989)

FIGURE 1

Kinetic Models as a Function of Initial Substrate Concentration and Bacterial Cell Density (Simkins and Alexander, 1984)

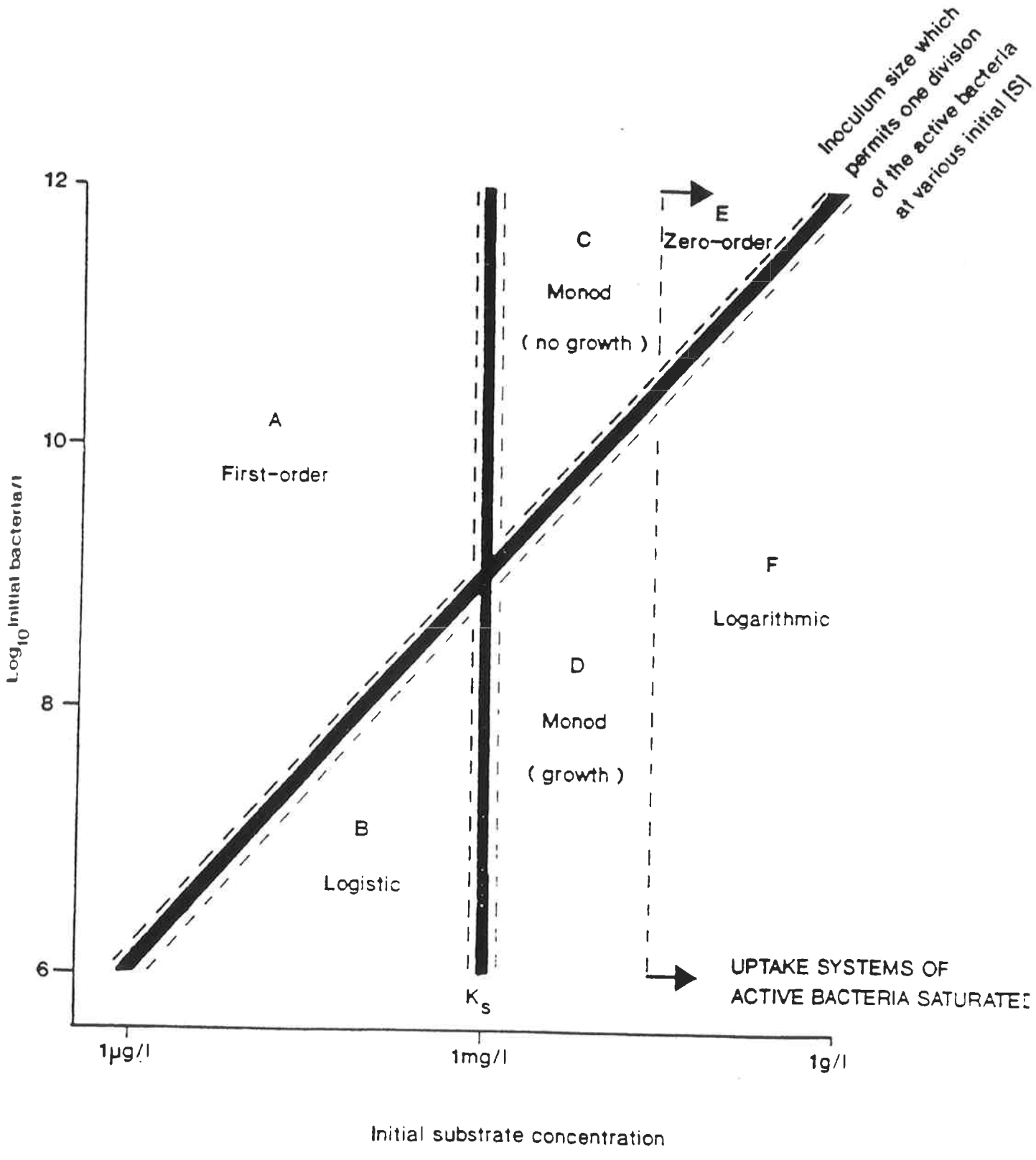
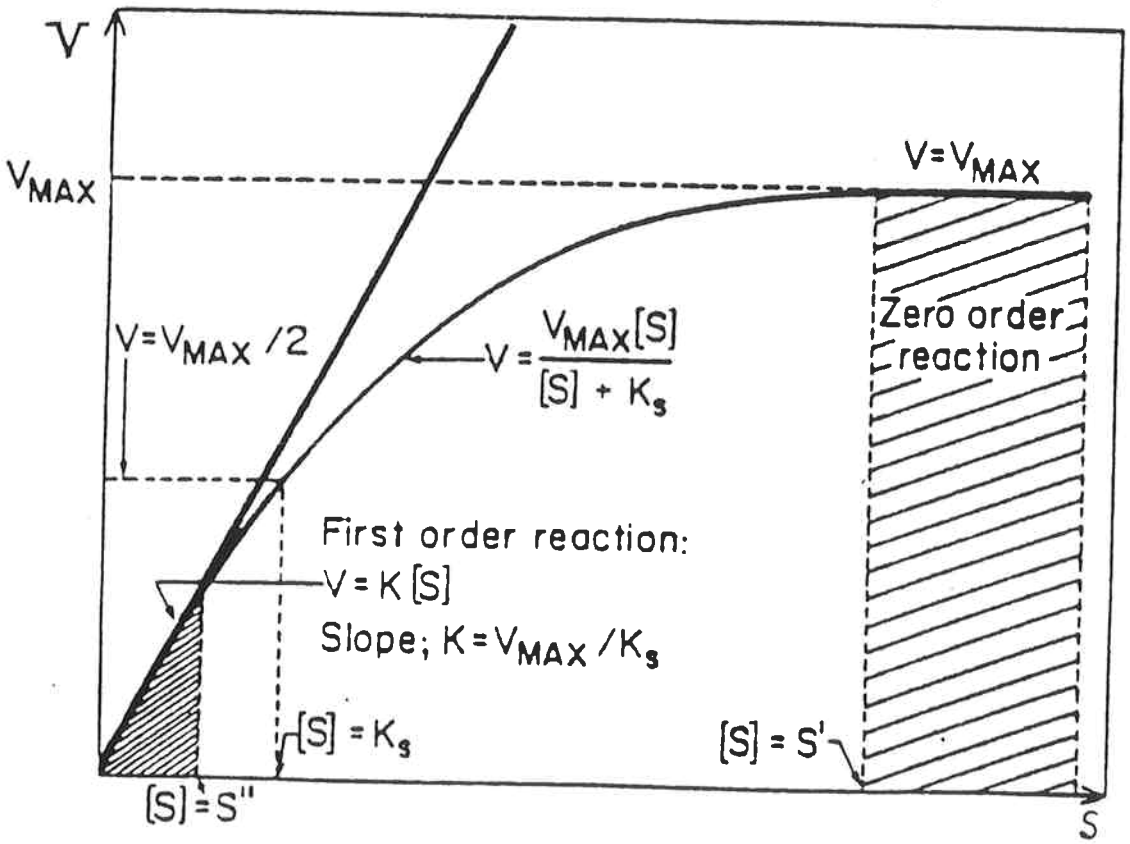


FIGURE 2

Relationship between BOD Rate and Substrate Concentration



S = Limiting substrate concentration

V = Reaction velocity in day $^{-1}$

V_{max} = Maximum velocity

K_S = Saturation constant

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I. APPENDICES

APPENDIX 1

GLOSSARY OF TERMS

- *Abiotic Degradation.* Degradation of a substance not resulting from the action of any living organism.

- *Acclimatisation.* The process by which a test system is modified by exposure to a material which enables it better to degrade that material. This may be caused by bacterial growth, selection of competent bacteria, mutation of bacteria or other processes.

- *Biodegradability.* The ability of an organic substance to undergo biodegradation, hence "inherently biodegradable" and "readily biodegradable". See Biodegradation.

- *Biodegradation.* Molecular degradation of a substance, resulting from the complex action of living organism.

- *Biodegradation kinetics.* The mathematical expression of the rate of biodegradation derived through the study of systems in which biodegradation is taking place.

- *Biomass.* The total mass of living organisms in a defined area or volume of habitat.

- *BOD (Biochemical Oxygen Demand).* The amount of oxygen consumed by micro-organisms when metabolising a substrate.

- *COD (Chemical Oxygen Demand).* The amount of oxygen consumed during oxidation of a substrate with hot acid dichromate or other strong oxidants. It provides a measure of the oxidisable matter in a given solution.

- *Complete mineralisation.* A theoretical concept involving complete breakdown of an organic compound into inorganic compounds. However, over the time-scale of a biodegradability test, ultimate biodegradation rather than complete mineralisation will be observed because a proportion of the compounds will be utilised for the synthesis of new cell material. In practice, these natural products will themselves eventually undergo biodegradation and the terms "ultimate biodegradation" and "complete mineralisation" are often used interchangeably.

- *Critical Sludge Retention Time.* The critical sludge retention time (SRT_c) is the SRT below which the competent micro-organisms will be washed out of sewage treatment plants and biodegradation will cease.

- *Degradation.* The reduction of the complexity of a chemical substance to form simpler molecules by physical, chemical and/or biological processes.

- *Exposure.* The presence in the environment, i.e. the availability at a site or location where effects might be observed. The concept includes elements of both concentration and time.

- *Half-life.* The time necessary to reduce the concentration of a chemical to half the starting value.

- *Load.* Is usually expressed as the food to microorganism ratio or as the ratio of the daily nutrient input to the existing biomass in the sewage plant.

- *Persistence.* The ability of a substance to remain in the environment in a chemically unchanged state.

- *Primary Biodegradation.* A discrete alteration to the structure of a chemical such that basic physico-chemical properties are lost.

- *Sludge retention time (SRT).* The mean age of the sludge in a biodegradation system.

- *Ultimate Biodegradation.* A transformation of a chemical to its inorganic constituents such as carbon dioxide (CO_2) and water (H_2O).

APPENDIX 2

LIST OF SYMBOLS

Symbol	Definition	Dimension
B	bacterial biomass concentration	bacterial biomass amount/ volume
E_t	total enzyme concentration	enzyme amount / volume
k	rate constant	1 / time
k_1	first order rate constant	1 / time
k_2	second order rate constant	1 / (time . biomass concentration)
K_d	bacterial decay rate	1 / time
K_m	Michaelis constant	substrate concentration
K_{oc}	octanol-water partition coefficient	dimensionless
K_s	saturation constant	substrate concentration
μ	specific growth rate	1 / time
μ_{max}	maximum specific growth rate	1 / time
r_B	cell growth rate	biomass concentration / time
S	limiting substrate concentration	substrate amount / volume
S_{eff}	equilibrium effluent concentration	substrate amount / volume
SRT_c	critical sludge retention time	time
SRT	sludge retention time	time
t	time	time
$t_{1/2}$	half life	time
v	reaction velocity (rate)	substrate concentration / time
V_{max}	maximum reaction velocity	substrate concentration / time
X	B / Y (Simkins and Alexander)	substrate concentration
Y	yield coefficient biomass conc. / substrate conc.	dimensionless

APPENDIX 3

USEFUL EQUATIONS

1. *SUBSTRATE DISAPPEARANCE*

In addition to the differential forms of the Monod equation and its simplifications the integral forms may be useful in curve fitting substrate disappearance data. These are as follows:

V General Monod $K_S.\ln(S/S_o) = (S_o+X_o+K_S).\ln(X/X_o)-S_o+X_o.\mu_{max}$

I Zero Order $S = S_o-[\mu_{max}.X_o].t$

II Monod, no growth $K_S.\ln(S/S_o)+S-S_o = -[\mu_{max}.X_o].t$

III First Order $S = S_o.\exp(-[\mu_{max}.X_o/K_S].t)$

IV Logistic $$S = \frac{(S_o+X_o)}{1+(X_o/S_o).\exp([\mu_{max}/K_s].(S_o+X_o))}$$

VI Exponential $S = S_o+X_o(1-\exp.(\mu_{max}.t))$

where square brackets surround a constant value (cf. Simkins and Alexander, 1984).

2. *PRODUCT APPEARANCE*

First order product curves can be expressed as:

$$P = P_o\ (1 - \exp\ (-k_1 t))$$

Where P_o is the maximum amount of product produced and P is the product at time t.

In a similar way, first order kinetics can be applied to CO_2 evolution curves by using the integrated form of the generalised form of the logistics function first described by Richards (1959)..

The equation describing the production of CO_2 is:

$$P = P_o \ (1 - b \ . \ \exp \ (-k_1 t)) \ ^{-1/n}$$

Where: P is the percentage of CO_2 observed at time t (days)
P_o is the upper asymptote of CO_2 production (percent)
b is a coordinate scaling factor associated with the constant of integration (dimensionless)
n is an empirical constant.

APPENDIX 4

MODELLING A ROLLING TUBE OR TRICKLING FILTER

General Case

$$\frac{dS}{dZ} = \frac{-\mu_{max} \cdot A_s}{F}, \quad \frac{S}{K_s + S}$$ at steady state for a "mature" filter

$$\text{then} \quad K_s \ln \frac{S_e}{S_o} + (S_e - S_o) = \frac{\mu_{max} A_s}{F} \cdot Z$$ Kornegay Type Model (1968)

if First order kinetics, $K_s >> S$

$$\text{then} \quad \frac{ds}{dz} = \frac{-\mu_{max} . A_S}{F} \cdot \frac{S}{K_s}$$

$$\ln \frac{S_e}{S_o} = \frac{-\mu_{max} . A_S . Z}{K_S . F}$$ Eckenfelder type (1966)

$$\text{or} \quad \frac{S_e}{S_o} = \exp \left(\frac{-K_1 . 2}{F} \right) \qquad K_1 = \frac{\mu_{max} A_s}{K_s}, \quad K_1 \equiv \frac{m^2}{hr}$$

if Zero order kinetics, $S >> K_s$

$$\text{then} \quad \frac{ds}{dz} = \frac{\mu_{max} A_S}{F}$$

$$S_o - S_e = \frac{\mu_{max} A_S . Z}{F} \qquad K_2 \equiv \mu_{max} A_S, \quad K_2 \equiv \frac{kg}{m.hr}$$

Nomenclature

A_s = Wetted surface area of packing or rolling tube (m^2)
F = Flowrate (m^3/hr)
K_s = Monod coefficient (kg/m^3)
S = Substrate concentration (kg/m^3)
Z = Packed depth or rolling tube length (m)
μ_{max} = Maximum specific growth rate (kg/hr)

A different approach, implying first order kinetics ($K_s >> S$) can be made following the Grady-Lim model, adapted by Roberts (1985).

here $\frac{dS}{dZ} = \frac{-K_m . S}{F}$ K_m = overall mass transfer coefficient

where $\frac{1}{K_m} = \frac{1}{K_L} + \frac{1}{K_B}$ K_B = biochemical reaction term
K_L = liquid phase mass transfer term

and $K_B = \frac{a}{S^m}$ a = coefficient describing mature biomass
m = exponent dependent of concentration

substitution and re-arranging

$$\frac{ds}{S} = \frac{K_L K_B}{(K_L + K_B)} \cdot \frac{dz}{F}$$

now substituting for K_B and re-arranging

$$\left[\frac{K_L . S^{m-1}}{a} + \frac{1}{K_L S} \right] \cdot ds = - \frac{dZ}{F}$$

Integrating

if $m = 0$ $\frac{S_e}{S_o} = \exp \left[\frac{-K_L . a}{K_L + a} \cdot \frac{Z}{F} \right] = \exp \left[\frac{-K_m Z}{F} \right]$ Eckenfelder type

and

if $m = 1$ $\frac{K_L}{a} (S_e - S_o) = \ln \frac{S_e}{S_o} = \frac{-K_L Z}{F}$ Kornegay type

From laboratory experimental data, $K_L \propto F^{1/3}$ for low irrigation rate
$\propto F^{1/2}$ for high rates

Given 5-10 data points from a statistical design run for a test compound from a rolling tube or model trickling filter evaluation, K_B the biochemical reaction term can be determined and interpreted by comparison with other compounds (Roberts, 1985).

APPENDIX 5

EXPERIMENTAL METHODS PRODUCING DATA MEANINGFUL FOR BIODEGRADATION KINETICS

This appendix gives a brief description of test designs which have successfully been used to derive meaningful kinetics in realistic systems.

1. *BIODEGRADATION IN NATURAL WATERS (RIVER WATER DIE AWAY TEST)*

In this method various low concentrations of ^{14}C-labelled test material are added to river, lake, estuary or even sea water. Testing is conducted in 1 or 2 litre Erlenmeyer flasks containing 250 ml to 1 l of natural water sample. No additional nutrients are added to the system for the duration of the experiment.

The ultimate biodegradation of the test material is determined by using ^{14}C-labelled derivatives, and analysing the amount of ^{14}C-labelled CO_2 produced and/or the amount of ^{14}C-label remaining in the water.

Less usually the primary biodegradation of unlabelled test material may be determined by specific analysis.

Three variations on this theme have been used in determining ultimate biodegradability:

i) The "Alexander" method involves incubating ^{14}C test material in surface water without any CO_2 trapping. Periodically, small samples of the medium are taken and centrifuged and acidified; these samples are then scintillation counted. By difference, the amount of radiolabel converted to CO_2, incorporated into bacteria and remaining in solution may be calculated.

ii) As in (i) the ^{14}C test material is incubated in surface water, but in a Gledhill flask (Gledhill, 1975). In this sealed apparatus an open reservoir, side arm or well containing alkali is included in the body of the flask. The alkali is periodically removed, replaced with fresh, and the $^{14}CO_2$ produced is determined by scintillation counting of the alkali. Hence the amount of radiolabel incorporated into CO_2 may be calculated. In addition the medium may be sampled and treated as in (i) to give additional information.

iii) As in (i) and (ii) the test material is incubated in surface water. Air is bubbled through the sealed test vessel and then through alkali traps. The first alkali trap is periodically removed and the second moved along and replaced with a fresh trap. The $^{14}CO_2$ produced is determined by scintillation counting of the alkali. Hence the amount of radiolabel incorporated into CO_2 may be calculated. In addition the

medium may be sampled and treated as in (i) to give additional information.

2. ACTIVATED SLUDGE TEST SYSTEMS

2.1. Biodegradation - Batch Activated Sludge Test

The principle of this batch system is identical to that described in the previous sections.

i) This variation has not been used to date for activated sludge, however, variations (ii) and (iii) have been used:

ii) In this version the Gledhill flask (Gledhill, 1975) (figure A5-1) is again used. The open reservoir or well containing alkali is now suspended over (normally) 0.5 l of activated sludge from a predominantly domestic sewage treatment plant, in a 2 l Ehrlenmeyer flask or equivalent. This activated sludge can either be used directly, or acclimatised to the test chemical in a laboratory semi-continuous activated sludge test for a period of 2 weeks to several months (Saeger, 1983). The sludge is usually sieved through a 2 mm screen to remove large solids, then adjusted with water to 2000-3000 mg-l mixed liquor suspended solids (MLSS).

The activated sludge is spiked with a realistic concentration of ^{14}C-labelled test material and shaken. No additional nutrients are given to the system for the rest of the experiment. Sampling is achieved by removing the old KOH and replacing it with new KOH. During sampling the activated sludge is aerated vigorously with oxygen for 5 minutes.

The ultimate biodegradation of the test material is determined by measuring the $^{14}CO_2$ trapped in the KOH by scintillation counting (Larson, 1980). Biodegradation kinetics information is obtained by analysing the $^{14}CO_2$ evolution data using non-linear regression models (Larson, 1979; Larson and Payne, 1981; Larson and Perry, 1981).

Primary biodegradation can also be followed, by taking samples of the activated sludge over time and analysing with a specific analytical technique, e.g. HPLC with ^{14}C-detection (Howard et al., 1975).

iii) An air flow through version of this method has also been used to derive first order kinetics (Sullivan, 1983).

Second order kinetics data can be obtained from this method by measuring the number of bacteria present in the inoculum and at time of sampling by a procedure outlined by Hobbie et al. (1977).

2.2. *Biodegradation and Removal - Semi-Continuous Activated Sludge Test*

The semi-continuous activated sludge test (OECD, 1981) is a simulation of the most common secondary wastewater treatment process, the aerobic reactor. In the OECD guidelines this test is described as a test of inherent ultimate biodegradability. This test can not only measure the biodegradation but also the removal of a chemical in this system. Removal can be defined as the ability of the apparatus to remove the test chemical from wastewater. This is achieved by the combination of the mechanisms of: biodegradation, adsorption (to activated sludge or other suspended material), volatilisation, precipitation, hydrolysis and oxidation.

In this method activated sludge from a sewage treatment plant is placed in a semi-continuous activated sludge (SCAS) unit (figure A5-2). The test compound and settled domestic sewage are added and the mixture is aerated for 23 hours. The aeration is then stopped, the sludge allowed to settle and the supernatant liquor is removed. The sludge remaining in the aeration chamber is then mixed with a fresh aliquot of test compound and sewage and the cycle is repeated (Snow, 1965; Saeger, 1983).

Normally ultimate biodegradation is determined by measuring dissolved organic carbon (DOC-analysis) in the daily effluent samples. Biodegradation kinetics are determined by analysing the amount of DOC daily during acclimatisation. A half-life may be obtained from the time to 50% removal. Instead of or as well as DOC analysis, ^{14}C-labelled materials may be used and biodegradation kinetics can be determined by analysing the amount of ^{14}C label remaining after evolution of CO_2 - non-linear regression may be used for more sophisticated analysis. ^{14}C is a more specific analytical procedure than DOC. The equations are described in Larson (1979), Larson and Payne (1981), Larson and Perry (1981). A third alternative is for primary biodegradation to be followed by specific analysis.

2.3. *Biodegradation, Removal and Treatability - Continuous Activated Sludge Test*

The continuous activated sludge test (also called the OECD confirmatory test) is a laboratory scale model of a sewage treatment plant (Fig. A5-3). It can measure the biodegradation, removal and treatability of a chemical. Treatability can be defined as the ability of a chemical to be removed under the operating conditions of a specific sewage treatment process, whether by biodegradation or by another removal mechanisms.

The main weaknesses of the method as described in the OECD guidelines, are the use of a poor artificial sewage, the absence of sludge retention time

control and a disproportionately short sludge residence period in the settler. These issues may be addressed by using a domestic sewage feed, wasting the solids daily or continuously, and pumping the sludge intermittently or more slowly back into the aeration basin.

Activated sludge, taken from a real sewage treatment plant, is put in a laboratory-scale, completely-mixed-flow aeration basin. The model is run with a continuous flow of settled sewage and test chemical, and the treated effluent passes through a settler before exiting. Activated sludge is continuously or semi-continuously removed directly from the aeration basin, in order to maintain MLSS in a range of 2000-3000 mg-l.

The level of test material in effluent is determined, as are the MLSS, sludge wastage rate and level of test material in influent. Together with flow rate data a mass balance is performed (Namkung and Rittmann, 1987) and a first order biodegradation constant may be calculated.

2.4. *Biodegradation and Treatability - Continuous Activated Sludge Test with Sludge Retention Time (SRT) Control*

Any continuous activated sludge model can be used which provides a completely mixed system, facilitates the continuous wastage of a representative fraction of the mixed liquor and ensures that uncontrolled losses of solids in the effluent are insignificant. An OECD Confirmatory vessel can be used, but can present problems as there is a need to assume a constant proportion of the sludge solids are in the settler at any one time to enable the SRT to be calculated. A modified version of the WRC porous pot procedure (Fig. A5-4) is preferred, as this makes the separate control of SRT relatively easy (Fig. A5-5) (Birch, 1984). In this method the solids are prevented from exiting with the effluent by a porous liner and may be easily, continuously wasted through the base.

Typically, a series of plants would be run with all parameters identical except for the SRT. The level of test material is analysed in the effluent of each plant. Plotting the SRT against the effluent concentration enables the K_s and μ_{max} of the system to be estimated using the model detailed above. The critical sludge retention time may also be estimated if the results span the appropriate range. Note that the effluent concentration is predicted to be independent of the influent concentration, and that the MLSS and active micro-organisms will self adjust at equilibrium.

3. *BIODEGRADATION AND TREATABILITY - SIMULATION OF BIOLOGICAL FILTRATION*

Simulation of biological filtration in the laboratory uses a rotating tube apparatus originally described by Gloyna et al. (1952). A layer of microorganisms, similar to those on the surface of biological sewage treatment filters, is encouraged to grow by the continuous supply of sewage, on the inner surface of slowly rotating perspex tubes.

The apparatus (Fig. A5-6) consists of a bank of perspex tubes 305 mm long by 50 mm internal diameter. These are supported at each end on rubber-rimmed wheels at an angle of 1° to induce a mean residence time of 125 +/- 12.5 sec for the effluent in the tubes. The tubes have an outside lip approximately 5 mm deep to retain their position on the wheels and an internal lip of 5 mm at the influent end to retain the liquid. Their internal surface is roughened by abrasion with coarse wire wool. The wheels rotate continuously at 18 +/- 2 rpm and the apparatus is housed in a laboratory maintained at 18° to 25°C.

The tubes are usually commissioned for 2 to 6 weeks prior to the trial period by feeding the tubes with sewage and sewage plus test material, to the control and test tubes respectively, at a rate of 250 +/- 25 ml/h. No inoculum is normally required and sludge wastage occurs naturally by excessive biomass slime breaking away from the surface and draining out of the tube with the final effluent.

FIGURE A5-1

The Batch Activated Sludge Test Apparatus

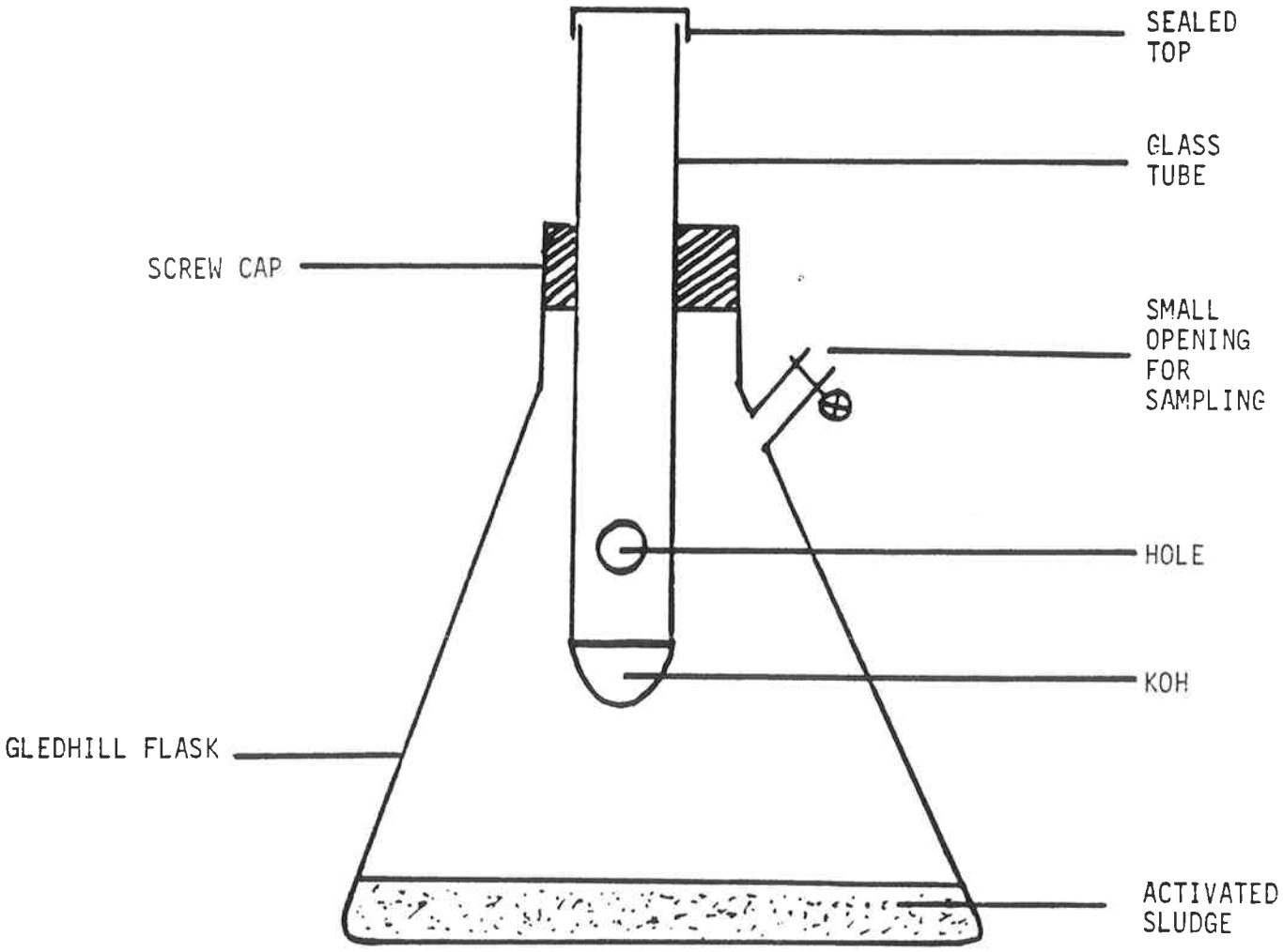


FIGURE A5-2

The Semi-Continuous Activated Sludge Test Apparatus

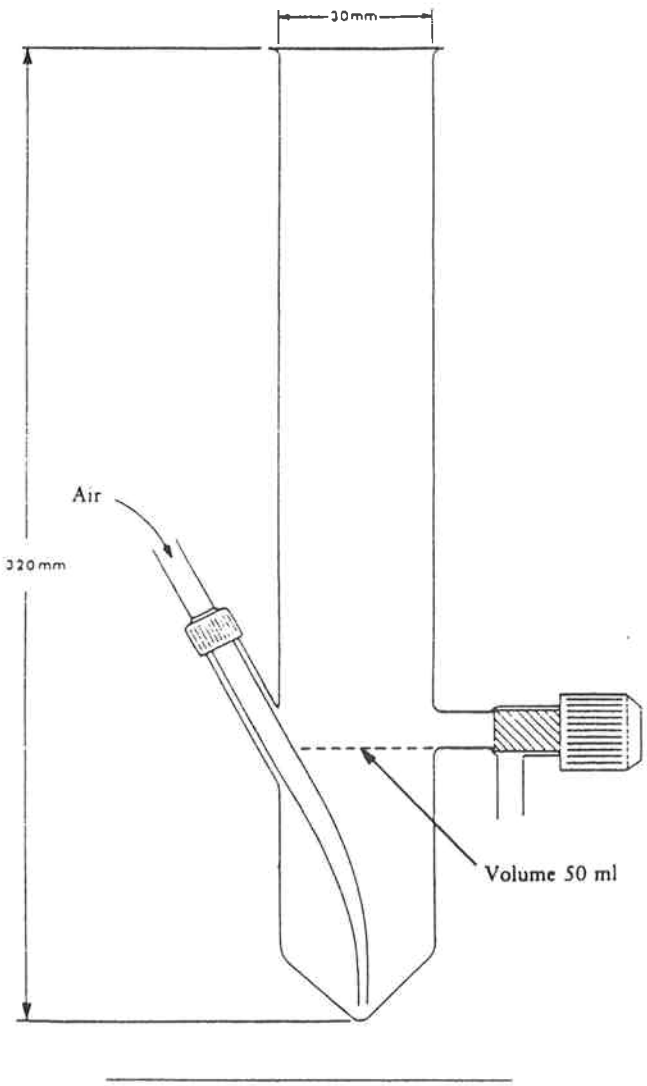
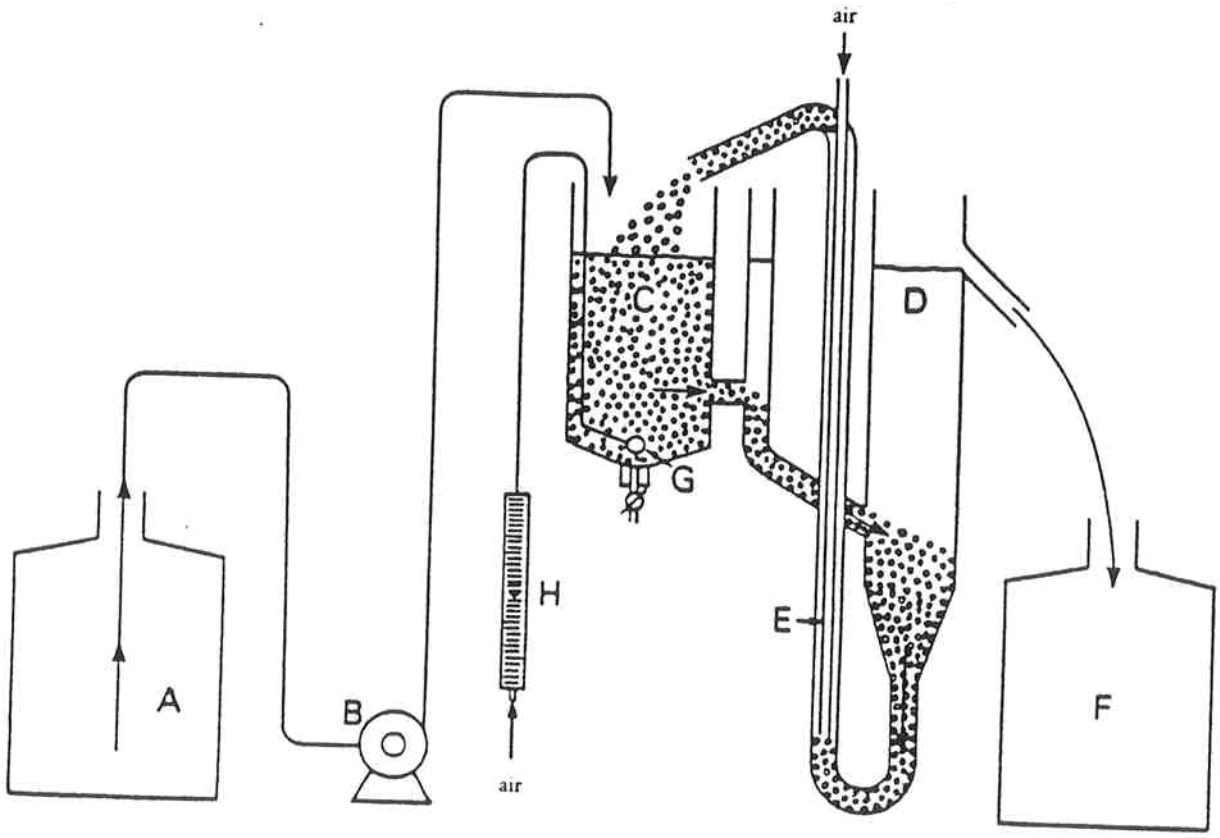


FIGURE A5-3

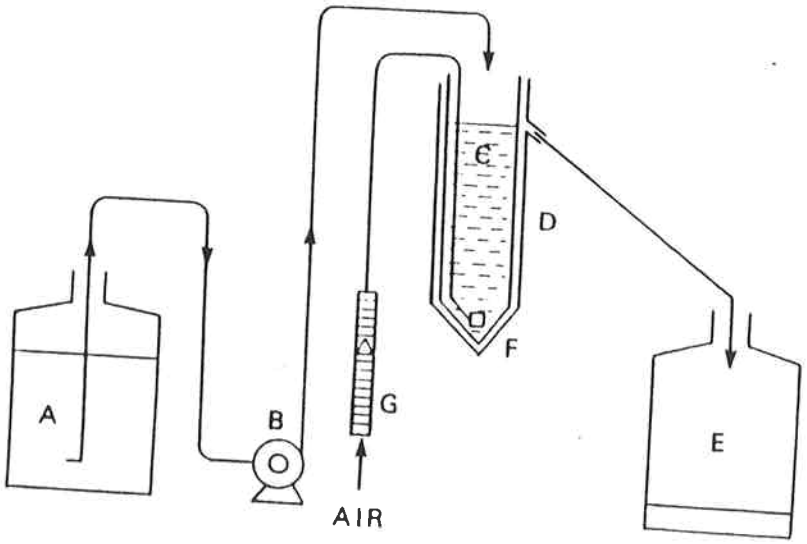
The Continuous Activated Sludge Apparatus



Key: A = storage vessel;
B = dosing device;
C = aeration chamber (3 l capacity);
D = settling vessel;
E = air lift;
F = collector;
G = aerator;
H = air flow meter (optional).

FIGURE A5-4

WRC Porous Pot



A, STORAGE VESSEL; B, DOSING PUMP; C, POROUS AERATION VESSEL
D, OUTER IMPERMEABLE VESSEL; E, EFFLUENT COLLECTION VESSEL
F, DIFFUSER-STONE AERATOR; G, ROTAMETER

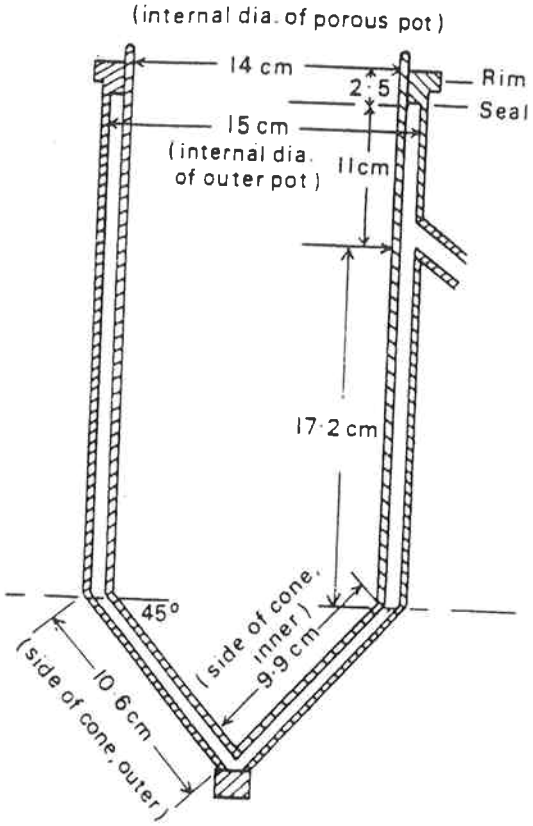


FIGURE A5-5

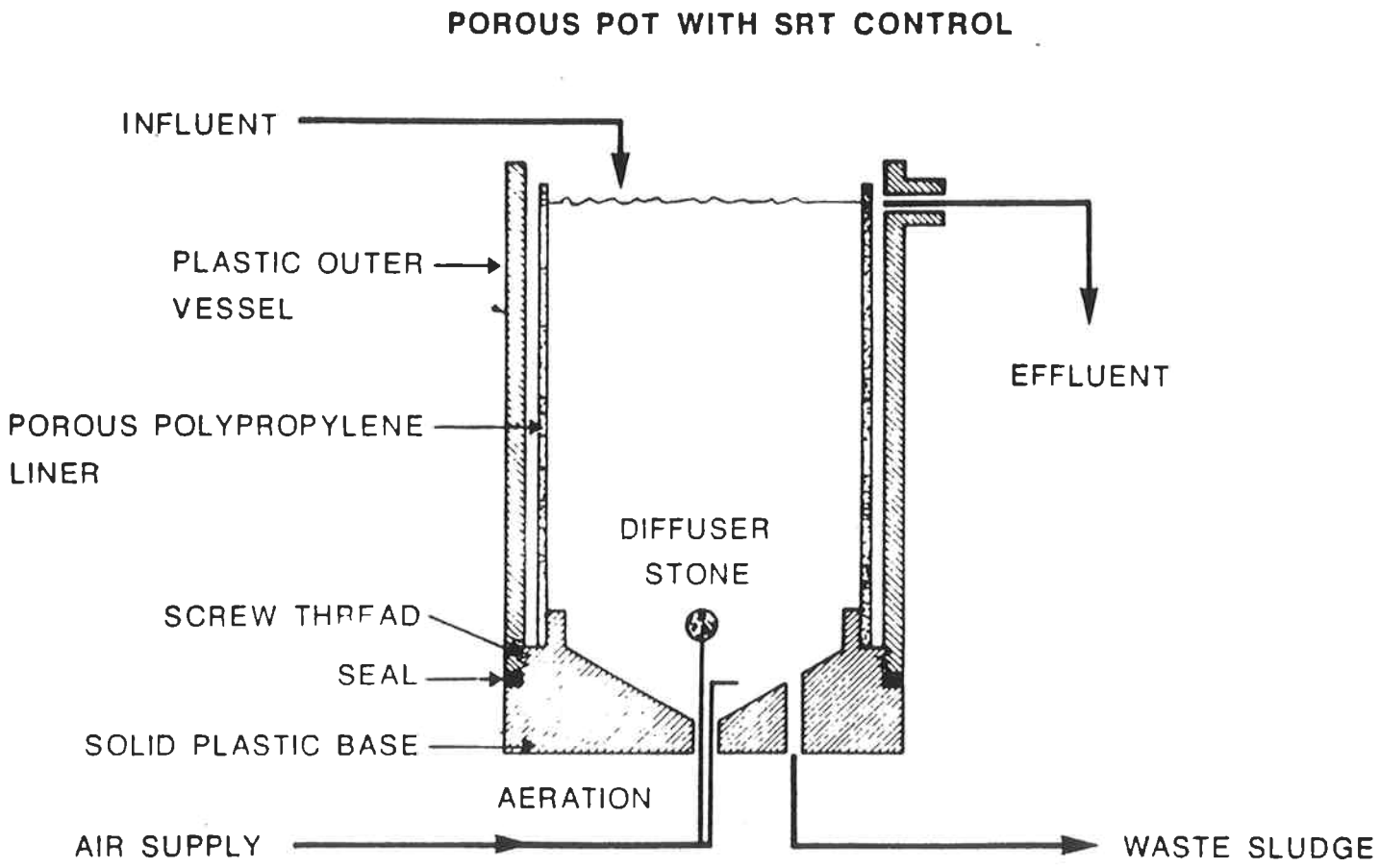
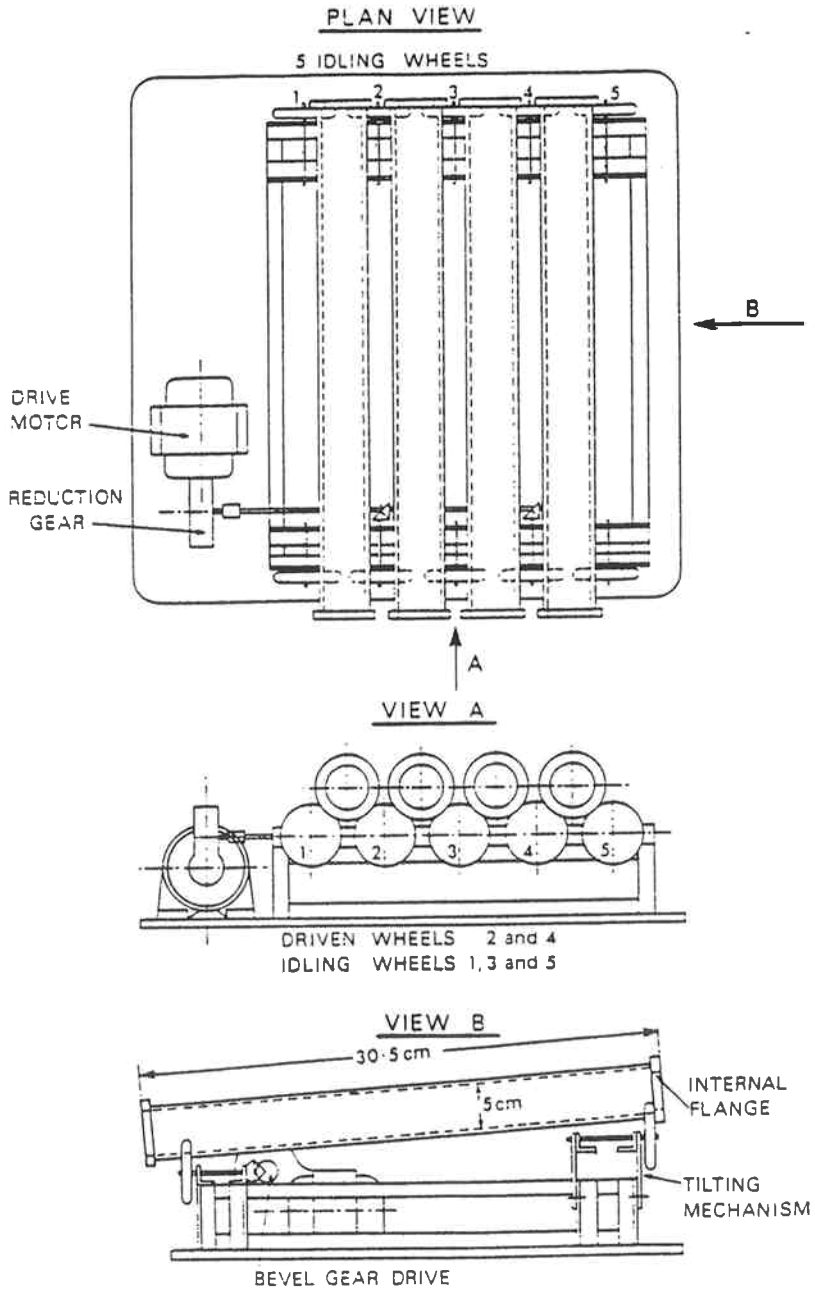

POROUS POT WITH SRT CONTROL
INFLUENT
PLASTIC OUTER VESSEL
POROUS POLYPROPYLENE LINER
SCREW THREAD
SEAL
SOLID PLASTIC BASE
AIR SUPPLY
DIFFUSER STONE
AERATION
EFFLUENT
WASTE SLUDGE

FIGURE A5-6

Rotating Tubes Apparatus



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