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Microbial growth and substrate utilization kinetics

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Microbial growth on and utilization of environmental contaminants as substrates have been studied by many researchers. Most times, substrate utilization results in removal of chemical contaminant, increase in microbial biomass and subsequent biodegradation of the contaminant. These are all aimed at detoxification of the environmental pollutants. Several microbial growth and biodegradation kinetic models have been developed, proposed and used in bioremediation schemes. Some of these models include Monod's, Andrews, Bungay's weighted model, general substrate inhibition models (GSIM) and sum kinetic models. Most research on microbial potentials to degrade chemical pollutants has been performed on a laboratory scale. There is a need to extend such studies to pilot scale as well as to full-scale field applications.

Key words: Microbial growth, substrate utilization, biodegradation, kinetics, detoxification, organic contaminants, models, environmental pollutants.

INTRODUCTION

Contamination of the environment with hazardous and toxic chemicals is one of the major problems facing the industrialized nations today. The petroleum industry is responsible for the generation of high amounts of petroleum hydrocarbons and their derivatives as well as for the pollution of air, soils, rivers, seas and underground water. These compounds undergo modifications by either physico-chemical or biological processes. Diverse metabolic capabilities of microorganisms have been exploited by man in diverse ways in the biodegradation of waste materials.

Microbial activities allowed the mineralization of some petroleum components into carbon dioxide and water, and microbial transformation is considered a major route for complete degradation of petroleum components (Okpokwasili et al., 1986). The potentiality of microbes as agents of degradation of several compounds thus indicates biological treatment as the major promising alternative to attenuate environmental impact caused by pollutants (Nwaeke and Okpokwasili, 2003). Many scientific approaches have been used in the *in situ* and *ex situ* biodegradation of organic pollutants. However, the extent of biodegradation is critically dependent on salinity, temperature, pH, heavy metals surfactants, nutrients and presence of readily assimilable carbon sources (Amanchukwu et al., 1989; Okpokwasili and Odokuma, 1990; Okpokwasili and Nnubia, 1995).

Many methods such as oxidation, precipitation, ion exchange, solvent extraction, enzyme treatment and adsorption have been used for removing both organic and inorganic materials from aqueous and non-aqueous solution. A variety of microbial growth and biodegradation kinetic models have been developed, proposed and used by many researchers (Simkins and Alexander, 1984, 1985; Schmidt et al., 1985). Such models allow prediction of chemicals that remain at a certain time, calculation of the time required to reduce chemical to certain concentration, estimation of how long it will take before a certain chemical concentration will be attained at a certain point (e.g. a case of aquifer, soil or surface water) and design of bioremediation schemes *in situ* or *ex situ* to remove chemical contaminant to a designed concentration. On the other hand, it can be used to predict the amount of biomass production achievable at a given time.

This review gives an overview of the kinetic models as

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applied in the prediction of microbial growth and degradation of organic substances. Substrate inhibition and interactions during biodegradation of pollutant mixtures are also discussed.

MICROBIAL GROWTH KINETICS

The relation between the specific growth rate (μ) of a population of microorganisms and the substrate concentration (S) is a valuable tool in biotechnology. This relationship is represented by a set of empirically derived rate laws referred to as theoretical models. These models are nothing but mathematical expressions generated to describe the behaviour of a given system.

The classical models, which have been applied to microbial population growth, include the Verhulst and Gompertz function (Verhulst, 1845, 1847; Gompertz, 1825). The Gompertz function was originally formulated for actuarial science for fitting human mortality data but it has also been applied deterministically to organ growth (Causton, 1977). The Gompertz function is based on an exponential relationship between specific growth rate and population density. Equation 1 represents one of its parameterization.

$$N_{(t)} = C\exp\{\exp[-B(t-M)]\} \qquad (1)$$

where t = time, $N_{(t)}$= population density at time t, C = upper asymptotic value, that is; the maximum population density, M = time at which the absolute growth rate is maximal, and B = relative growth rate at M. time.

Gibson et al. (1987) modified the Gompertz function to a function which could be applied to the description of cell density versus time in bacterial growth curves in terms of exponential growth rates and lag phase duration (equation 2)

$$\text{Log } N_{(t)} = A + D\exp\{-\exp[-B(t-M)]\} \qquad (2)$$

Where $N_{(t)}$ = population density at time t, A = value of the lower asymptote (Log $N_{(-\infty)}$), D = difference in value of the upper and lower asymptote [Log $N_{(\infty)}$ – log $N_{(-\infty)}$], M = time at which the exponential growth rate is maximal.

The idea of microbial growth kinetics has been dominated by an empirical model (equation 3) originally proposed by Monod (1942). The Monod model introduced the concept of a growth limiting substrate.

$$= \frac{S}{K_s + S}\,\mu_{max} \qquad (3)$$

Where μ = specific growth rate, μ_{max} = maximum specific growth rate, S = substrate concentration, K_S = substrate saturation constant (i.e. substrate concentration at half μ_{max}).

In Monod's model, the growth rate is related to the concentration of a single growth-limiting substrate through the parameters μ_{max} and K_S. In addition to this, Monod also related the yield coefficient ($Y_{x/s}$) (equation 4) to the specific rate of biomass growth (μ) and the specific rate of substrate utilization (q) (equation 5).

$$Y_{x/s} = \frac{dx}{ds} \qquad (4)$$

$$= \frac{Y_{X/S}}{X} \cdot \frac{ds}{dt} \cong Y_{X/S}\, q \qquad (5)$$

Derivatives of the Monod kinetic model

In 1912, Penfold and Norris proposed the first kinetic principle for microbial growth. They stated that the relationship between μ and S is best described by a "saturation" type of curve where at high concentration of substrate, the organism grows at a maximum rate (μ_{max}) independent of the substrate concentration (Penfold and Norris, 1912). Monod's model satisfies this requirement, but it has been criticized particularly because of derivations of μ at low substrate concentration (Powell, 1967; Kovárová-Kovar and Egli, 1998).

Owing to the limitations of the Monod's model, a number of structured and unstructured kinetic expressions were put forward to describe the hyperbolic curve characteristic of microbial growth. However, the development of structured models had suffered serious setback due to the complexity of cell growth. Thus, most proposed growth models are unstructured. Three approaches were used to develop the equations for growth kinetics of cells in suspension:

(1) Describing the influence of physicochemical factors on Monod growth parameters (Gibson et al., 1987; Ratkowsky et al., 1983; Kovárová et al., 1996).

(2) Inclusion of additional constants into the original Monod model to correct for substrate or product inhibition, substrate diffusion, maintenance or effects of cell density on μ_{max} (Andrews, 1968; Boethling and Alexander, 1979; Rittmann and McCarty, 1980; Schmidt et al., 1985; Tros et al., 1996; Contois, 1959; Dabes et al., 1973; Heijnen and Romein, 1995; Mulchandani and Luong, 1989; Pirt. 1975; Shehata and Marr, 1971; Simkins and Alexander, 1985).

(3) Proposing different kinetic theories, which result in both empirical (Heijnen and Romein, 1995; Tan et al., 1994; Westerhoff et al., 1982) and mechanistic (Kooijman et al., 1991; Nielsen and Villadsen, 1992) models.

Like the Monod kinetics, Gompertz function has also been modified to generate models that describe the effect of intrinsic factors such as temperature and oxygen availability on microbial growth parameters. Studies have been carried out on the combined effects of several controlling factors on bacterial growth for example papers by Sutherland et al. (1994), McMeekin et al. (1987), Wijtzes et al. (1992,1995) and Adams et al. (1991). These models are mostly used to predict the change in quality of a food over time and can therefore be applied to estimate the shelf-life of foods.

KINETICS OF BIODEGRADATION

The basic hypothesis of biodegradation kinetics is that substrates are consumed via catalyzed reactions carried out only by the organisms with the requisite enzymes. Therefore, rates of substrate degradation are generally proportional to the catalyst concentration (concentration of organisms able to degrade the substrate) and dependent on substrate concentration characteristic of saturation kinetics (e.g. Michaelis-Menten and Monod kinetics). Saturation kinetics suggests that at low substrate concentrations (relative to the half-saturation constant), rates are approximately proportional to substrate concentration (first order in substrate concentration), while at high substrate concentrations, rates are independent of substrate concentration (zero-order in substrate concentration). In the case of substrates that contribute to the growth of the organisms, rates of substrate degradation are linked to rates of growth (i.e. the concentration of the biomass increases with substrate depletion). The mathematical analysis of such growth-linked systems is more complex than those situations where growth can be ignored. There are a number of situations where it may not be possible to quantify the concentration of substrate-degrading organisms in a heterogeneous microbial community. However, the rate of substrate depletion can be measured. There are also situations in which the organism concentration remains essentially constant even as the substrate is degraded (i.e. no growth situation). Given these various features of biodegradation kinetics, different models including first-order, zero-order, logistic, Monod (with and without growth) and logarithmic models can be used to describe biodegradation.

Biodegradation kinetics is used to predict concentrations of chemical substances remaining at a given time during *ex situ* and *in situ* bioremediation processes. In most cases, information is based on loss of parent molecule targeted in the process. The key interest is frequently the decrease in toxicity concentration. Nevertheless, toxicity measurements require bioassays, which are always very difficult and tedious. Therefore, efficacy of biodegradation is based on chemical measurements, e.g. disappearance of parent molecule, appearance of mineralization products or disappearance of other compounds used stoichiometrically during biodegradation of a compound, for instance, electron acceptors. There are several scenarios by which a compound can be transformed biologically. This includes when the compounds serve as:

(1) Carbon and energy source
(2) Electron acceptor
(3) Source of other cell components.

Other scenarios are the transformation of a compound by non- growing cells (the compound does not support growth) and the transformation of a compound by co-metabolism, that is; transformation of a compound by cells growing on other substrate. The simplest case is where the compound serves as source of carbon and energy for the growth of a single bacterial species. The compound is assumed to be water-soluble, non-toxic and other substrates or growth factors are limiting.

In the case of single-substrate limited process, the Monod equation (equations 3 and 6) is often used to describe microbial growth and biodegradation processes.

$$\mu = \frac{\mu_{max} S}{K_s + S} \qquad (3)$$

$$q = \frac{q_{max} S}{K_s + S} \qquad (6)$$

where μ = specific growth rate (1/X.dX/dt), q = specific substrate utilization/removal rate (1/X .dS/dt), and $\mu = Yq$, with Y = true growth yield [mass of biomass (X) synthesized per unit of substrate (S) utilized or removed], S = aqueous phase concentration of the compound, K_S = affinity constant or half saturation constant for the compound (meaning the concentration of compound when μ or q is maximum).

The hyperbolic equation proposed by Monod was modified by Lawrence and McCarty (1970) to describe the effects of substrate concentration (S) on the rate at which a given microbial concentration (X) removes the target substrate (-dS/dt) (equation 7). Alternatively, Monod equation can be written in terms of microbial growth by incorporating the net yield coefficient (Y) (equation 8).

$$\frac{dS}{dt} = -\frac{q_{max} SX}{K_s + S} \qquad (7)$$

$$\frac{dX}{dt} = -Y \frac{dS}{dt} = \frac{Yq_{max} SX}{K_s + S} \qquad (8)$$

The Monod equation has frequently been simplified to an equation, which is either zero or first order in substrate concentration and the kinetics, has been widely used to describe biodegradation of organic contaminations in aquifer systems (Alvarez et al., 1991, 1994; Borden and Bedient, 1986; Chen et al., 1992; Widdowson et al., 1988). The versatility of Monod's equation is attributed to its ability to describe biodegradation rates that follow zero- to first-order kinetics with respect to the concentration of the target substrate. Moreso, Monod's model describes the dependence of biodegradation rate on the concentration of biomass.

SUBSTRATE INHIBITION OF BIODEGRADATION

When a substrate inhibits its own biodegradation, the original Monod model becomes unsatisfactory. In this case, Monod derivatives that provided corrections for substrate inhibition (by incorporating the inhibition constant K_i) can be used to describe the growth-linked biodegradation kinetics. Among the substrate inhibition models, the Andrew's equation (equation 9 and 10) is most widely used (Sokol, 1986; Tang and Fan, 1987; Grady et al., 1999). It is also a good representation of experimental data sets examined in the study of Goudar et al. (2000).

$$\mu = \mu_{\max} \frac{S}{K_s + S + \frac{S_2}{K_i}} \qquad (9)$$

$$q = q_{\max} \frac{S}{K_s + S + \frac{S^2}{K_i}} \qquad (10)$$

A generalized Monod type model (equation 11) originally proposed by Han and Levenspiel (1988) has been used to account for substrate stimulation at low concentration and substrate inhibition at high concentration.

$$q = \frac{\left(\frac{S}{S_m}\right)^n}{S + K_s \left(1 - \frac{S}{S_m}\right)^m} \qquad (11)$$

where μ = specific substrate consumption rate of cells, q_{max} = maximum consumption rate constant, S = substrate concentration, K_S = the Monod constant, S_m = critical inhibitor concentration above which reaction stops, n and m are constants.

Information available on the substrate inhibition of biodegradation are mostly those that described microbial degradation of phenol. Thus in this review, substrate inhibition of biodegradation is discussed with particular reference to phenol. The inhibitory nature of phenol at high concentrations is well known, and the kinetics of pure and mixed culture microbial growth on phenol have been described by a variety of substrate inhibition models (Livingstone and Chase, 1989; Pawlowsky and Howell, 1973; Seker et al., 1997; Yang and Humphrey, 1975). Most of these models are empirical. However, they are able to provide satisfactory description of phenol biodegradation data, thus providing a convenient means of modelling phenol biodegradation. Rozich et al. (1985) examined 113 microbial curves and reported that among 5 different models, Andrew's provided the best description of observed data. However, the superiority of the Andrew's equation is not a consistent feature in literatures. Pawlowsky and Howell (1973) observed statistically insignificant difference between 5 inhibition models. Yang and Humphrey (1975) made similar observation with Andrew's equation and 2 other models in describing phenol degradation by *Pseudomonas putida* and *Trichosporon cutaneum*. A two-parameter derivative of Andrew's equation was reported to be a better representation of experimental data obtained from mixed culture biodegradation of phenol.

When different substrate inhibition models are used to describe experimental data, it becomes difficult to compare kinetic parameters across different studies. This complicates the application of laboratory kinetic information in the design of biological treatment systems for inhibitory waste. In the study of Goudar et al. (2000), a theoretical basis for selection of an appropriate substrate inhibition model (to solve this problem) was discussed. In this regard, the generalized substrate inhibition model (GSIM) of Tan et al. (1996), which describes substrate inhibition of microbial growth using a statistical thermodynamics, was used.

ESTIMATION OF MODEL KINETIC PARAMETERS

Kinetic equations, which describe the activity of an enzyme or a microorganism on a particular substrate, are crucial in understanding many phenomena in biotechnological processes. Quantitative experimental data is required for the design and optimization of biological transformation processes. A variety of mathematical models have been proposed to describe the dynamics of metabolism of compounds exposed to pure cultures of microorgainsms or microbial populations of natural environment. The Monod equation has been widely used to describe growth-linked substrate utilization (Corman and Pave, 1983; Naziruddin et al., 1995; Smith et al., 1997, Robinson and Tiedje, 1983). Characterization of the enzyme or microbe-substrate

interactions involves estimation of several parameters in the kinetic models from experimental data. In order to describe the true behavior of the system, it is important to obtain accurate estimates of the kinetic parameters in these models.

Both derivative and integrated forms of equations derived for enzyme catalyzed reactions have been used to estimate kinetic parameters of microbiological processes. Estimates of kinetic parameters V_{max} and K_m have been calculated by fitting data to either integrated (Robinson and Characklis, 1984; Betlach and Tiedje, 1981; Counotte and Prins, 1979; Gouder and Delvin, 2001; Duggleby and Morrison, 1977; Robinson and Tiedje, 1982; Strayer and Tiedje, 1978; Suflita et al., 1983) or derivative (Betlach et al., 1981; Robinson and Tiedje, 1982; Strayer and Tiedje, 1978) forms of Michaelis-Menten and Monod equation. Different approaches have been proposed for estimating the kinetic parameters, but progress curve analysis is the most popular because substrate depletion or product formation data from a single experiment are enough for parameter estimation (Duggleby and Wood, 1989; Zimmerle and Frieden, 1989). In this approach, substrate depletion or product formation-time course is used in the integrated form of the kinetic model for parameter estimation. Some of these differential and integral equations can be found in the papers of Gouder and Delvin (2000), Schmidt et al. (1985) and Simkins and Alexander (1984). Estimates of kinetic parameters obtained from some biodegradation studies are shown in Tables 1 and 2.

It is important to note that most kinetic models and their integrated forms are nonlinear. This makes parameter estimation relatively difficult. However, some of these models can be linearized. Various linearized forms of the integrated expressions have been used for parameter estimation (Robinson, 1985). However, the use of linearized expression is limited because it transforms the error associated with the dependent variable making it not to be normally distributed, thus inaccurate parameter estimates. Therefore, nonlinear least- squares regression is often used to estimate kinetic parameters from nonlinear expressions. However, the application of nonlinear least-squares regression to the integrated forms of the kinetic expressions is complicated. This problem and solutions were discussed by Goudar and Delvin (2001) . The parameter estimates obtained from the linearized kinetic expressions can be used as initial estimates in the iterative nonlinear least-squares regression using the Levenberg-Marquardt method (Marquardt, 1963).

The kinetic parameters of the Andrew's equation (μ_{max}, q_{max}, K_S or K_i) can be estimated with the application of reduced form of the generalized substrate inhibition model (GLIM), reduced to the form of Andrews equation. The linearized expression of this model was used to obtain initial parameter estimates for use in nonlinear regression (for detail see the paper of Goudar et al., 2000).

MULTIPLE SUBSTRATE-CONTROLLED BIODEGRADATION: SUBSTRATE INTERACTIONS

Wastewaters from industrial and municipal sources are characterized by presence of mixtures of chemicals. Pollutant mixtures may contain only organic chemicals or may also include inorganic substances such as heavy metals. Co-contamination of natural environments with mixtures of pollutants is an important problem. In biodegradation or bioremediation investigations and projects, it is important to understand and be able to model the fate of specific chemicals. Development of treatment strategies for soil or water contamination requires consideration of interactions among substrates to control the concentration of individual pollutants to meet regulatory standards. Single substrate kinetic parameters alone cannot describe the phenomena observed with degradation of mixtures. It is important therefore to predict the biodegradation kinetics of pollutant mixtures in a given system.

The removal of one component may be inhibited by other components in the mixture and different conditions may be required to degrade different compounds within the mixture. Biodegradation patterns of a compound as component of pollutant mixture and as a single component have been shown to be different (Alvarez and Vogel, 1991; Arvin et al., 1989; Reardon et al., 2000; Smith et al., 1991). Strong interactions among components of a pollutant mixture have been reported (Egli, 1995; Kle ka and Maier, 1988; Meyer et al., 1984; Saéz and Rittmann, 1993). In the case of homologous mixture (mixture of substrates serving the same purpose) of carbon and energy substrates, the effect of other compounds in a mixture can be positive (Alvarez and Vogel, 1991; McCarty et al., 1984; Schmidt and Alexander, 1985) or negative due to competitive inhibition (Arvin et al., 1989; Bielefeldt and Stensel, 1999; Chang et al., 1993; Cort and Bielefeldt, 2002; Deschenes et al., 1996; Goudar et al., 1999; Haller and Finn, 1999; Saez and Rittmann, 1993), toxicity (Haigler et al., 1992), and the formation of toxic intermediates by non- specific enzymes (Bartels et al., 1984; Kle ka and Gibson, 1981).

The utilization pattern can change with different mixture compositions, depending on the chemical nature and concentration of the substrate, oxygen concentration and microbial growth rates. Arvin et al. (1989) observed both substrate inhibition and stimulation interactions during the aerobic degradation of mixtures of benzene, toluene and *o*-xylene. When toluene or *o*-xylene was degraded in the presence of benzene, the degradative ability of toluene and *o*-xylene by the microorganisms was stimulated. When *p*-xylene and toluene were both present, an inhibition effect on benzene degradation was observed.

Table 1. Some kinetic parameters during microbial degradation of organic substrates.

K_s	Y	$_{max}$	q_{max}	Comments/Conditions	Reference
2 (M)	1 (g protein/mol H_2)	0.06	-	Measurement of hydrogen depletion for H_2-limited batch growth of *Desulfovibrio* sp. Strain G11	Robinson and Tiedje (1983)
0.45 ± 0.58 (g/ml)	-	7.2 ± 0.6 ($x10^{-3}min^{-1}$)	-	Metabolism of [U-ring-^{14}C] benzoate by the *Pseudomonas* sp. at 3.2 g/ml initial substrate concentration.	Simkins and Alexander (1984)
13.8 ± 0.9 (mg/l)	1.28 ± 0.13 (g/g)	0.86 ± 0.01 (h^{-1})	-	Aerobic biodegradation of 43 mg/l toluene by batch culture of *Pseudomonas putida* F1 at 30^{O}C	Reardon et al. (2000) Reardon et al. (2002)
0.1 (mg/l)	1.2 (g/g)	0.504 (h^{-1})	-	Aerobic biodegradation of 4 mg/l toluene by batch culture of *Pseudomonas putida* K1 at 25^{O}C	Pederson et al. (1997)
1.96 ± 1.26 (mg/l)	1.22 ± 0.1 (g/g)	0.543 ± 0.076 (h^{-1})	-	Aerobic biodegradation of 10 mg/l toluene by batch culture of *Pseudomonas fragi* B1 at room temperature	Chang et al. (1993)
1.88 ± 1.26 (mg/l)	0.99 ± 0.25 (g/g)	0.452 ± 0.115 (h^{-1})	-	Aerobic biodegradation of 10mg/l toluene by batch culture of *Pseudomonas* sp. X1 at room temperature	Chang et al. (1993)
0.12 ± 0.02 (mg/l)	1.20 ± 0.05 (mg/l)	0.73 ± 0.03 (h^{-1})	-	Aerobic biodegradation of benzene by batch culture of *Pseudomonas putida* F1	Reardon et al. (2002)
32.0 ± 2.4 (mg/l)	0.80 ± 0.07 (g/g)	0.11 ± 0.01 (h^{-1})	-	Aerobic biodegradation of phenol by batch culture of *Pseudomonas putida* F1	Reardon et al. (2002)
0.181 ± 0.168046 (mM) [degradation]	1.44 ± 0.162 (cells/mmol) $x10^{-10}$	0.284 ± 0.022 (h^{-1})	1.975 ± 0.162 (mmol/cell h) $x\ 10^{11}$	Aerobic biodegradation of toluene by suspended cells of *Pseudomonas* strain K3-2	Shreve and Vogel (1993)
0.00064 ± 0.00063 (mM) [growth]					
0.1904 ± 0.168 (mM) [degradation]	3.482 ± 1.484 (cells/mmol) $x10^{-8}$	3.485 ± 1.188 (day^{-1})	1.01 ± 0.256 (mmol/cell day) $x10^{8}$	Aerobic biodegradation of 2,4-D by suspended culture of *Pseudomonas* strain K3-2	Shreve and Vogel (1993)
0.5929 ± 0.585 (mM) [growth]					
96.181± 18.839 (mg/l)	0.516 ± 0.011 (g/g)	0.927 ± 0.086 (h^{-1})	-	Biodegradation of sodium dodecyl sulphate (SDS) by microbial population of activated sludge	Gouder et al. (1999)
31.919 ± 1.1415 (mg/l)	0.646± 0.007 (g/g)	0.414 ± 0.011 (h^{-1})	-	Biodegradation of T-Maz-80 by microbial population of activated sludge	Gouder et al. (1999)

Table 1. contd.

-	0.22 ± 0.03 (mg/mg)	-	-	Aerobic biodegradation of phenol at high concentration by batch culture of acclimated phenol-degrading organisms obtained from activated sludge	Yoong et al. (1997)
0.3 (mg/l) 0.9 (mg/l) 13.7 (mg/l)	0.731 (g/g) 0.571 (g/g) 0.323 (g/g)	0.121 (h^{-1}) 0.519 (h^{-1}) 0.391 (h^{-1})	0.0397(g/g h) 0.0703(g/g h) 0.0811(g/g h)	Multiple phase aerobic biodegradation of phenol by acclimated *Pseudomonas putida* cells in the presence of glucose	Mamma et al. (2004)
-	-	0.058± 0.034 (h^{-1})	-	Biodegradation of SDS by epilithic and planktonic microbial population of a river	Anderson et al. (1990)
-	0.53 ± 0.02 0.42 ± 0.04 (g/g)	-	-	Aerobic growth of *Saccharomyces pombe* on diesel and kerosene	Amanchukwu et al. (1989)

Table 2. Kinetic parameter estimates for substrate inhibition of biodegradation.

K_s	K_i	q_{max}	S_m	$_{max}$	Model	Comments/Conditions	Reference
8.5 (M)	454 (M)	466 (nmol/mg protein/ min)	-	-	Andrews	Phenol disappearance assay in 10 ml volume containing *Pseudomonas cepacia* G4 suspension	Folsom et al. (1990)
3 (M)	-	8 (nmol/mg protein/ min)	-	-	Monods	No-headspace bottle assay for trichloroethylene degradation by *P. cepacia* G4 at 26^{O}C	Folsom et al. (1990)
56.7 (g/l)	249.08 (mg/l)	-	-	0.27 (h^{-1})	Andrews	Aerobic biodegradation of phenol by batch culture of a pseudomonad at 30^{O}C	Polymenakou and Stephanou (2005)
0.01 1 (g/l)	0.348 (g/l)	-	-	0.25 1 (h^{-1})	Andrews	Aerobic shake flask biodegradation of phenol by	Goudar et al. (2000)
3.5 1.7 (mM)	4.5 1.3 (mM)	10.5 0.8 (M/m g protein/h)	4.7 1.5	- -	Han and Levenspiel	Sole carbon and energy source utilization of 3,4- and 2,4-dimethylphenol by a mixed culture	Acuña-Argüelles et al. (2003)

Similar inhibition and stimulation of biodegradation have been observed with mixtures of benzene, toluene and *p*-xylene (Alvarez and Vogel, 1991).

In addition to biodegradation stimulation due to increased growth at low substrate concentrations, stimulation of one compound by another in a mixture can be by induction of catabolic enzymes required for degradation of the second pollutant (Arvin et al., 1989). This mechanism produces simultaneous degradation of pollutants in mixtures and has been reported for pentachlorophenol and chlorinated aromatics (Kle ka and Gibson, 1981), toluene and *p*-xylene (Lee et al., 1993), and toluene (Pettigrew et al., 1991). Moreso, one component of a mixture can be degraded in the presence of another by co-metabolism (Saéz and Rittmann, 1993; Alvarez-Cohen and McCarty, 1991a, b; Criddle, 1993; Ely et al., 1995a, b).

In the literature, most studies on the kinetics of biodegradation were on single substrate utilization. However, models of mixed homologous substrate utilization and microbial growth have been proposed (Biolefeldt and Stensel, 1999; Kle ka and Maier, 1988; Kompala et al., 1986; Lendenmann et al., 1996; Nikolajsen et al., 1991; Tsao and Hanson, 1975; Yoon et al., 1997). Most of these models have been tested with only two substrates. However, in recent times, models have been proposed and tested for larger mixtures. Typical examples include the growth of *Escherichia coli* on six sugars (Lendenmann et al., 1996), the growth of a mixed culture on five BTEX compounds (Bielefeldt and Stensel, 1999), and the biodegradation of three polycyclic aromatic hydrocarbons (Guha et al., 1999).

Like with the homologous substrates, some efforts have also been made to develop kinetic models that

described multiple-nutrient-controlled growth with heterologous substrate (substrate that serves different purposes, e.g. carbon and nitrogen mixtures) (Baltzis and Fredrickson, 1988; Mankad and Bungay, 1988). The heterologous substrate concept assumes that the growth rate can be affected simultaneously by more than one substrate. A "Double Monod" model (equation 12) originally proposed by McGee et al. (1972) was used to describe this phenomenon.

$$\mu = \mu_{\max} \frac{S_1}{K_1 S_1} \frac{S_2}{K_s + S_2} \qquad (12)$$

Where 1 and 2 represent the substrates. However, this multiplicative model has narrow range of utility (Bader, 1978, 1982). Mankad and Bungay (1988) had expressed growth rates under dual substrate limitation in terms of weighted average of rates under individual nutrient limitations (equation 13)

$$\frac{}{\max} = (W1)\frac{S_1}{K_1 + S_1} + (W2)\frac{S_2}{K_2 + S_2} \qquad (13)$$

Where W (*i*) is the weight assigned to nutrient *i*. substituting the functional dependence for the weight functions W (*i*) (equation 14) into equation 13 yields the Mankad and Bungay's expression for growth rate (equation 17)

$$W1 = \frac{\frac{K_1}{S_1}}{\frac{K_1}{S_1} + \frac{K_2}{S_2}} ; W2 = \frac{\frac{K_2}{S_2}}{\frac{K_1}{S_1} + \frac{K_2}{S_2}} \qquad (14)$$

$$\frac{}{} = \frac{\frac{K_1}{S_1}}{\frac{K_1}{S_1} + \frac{K_2}{S_2}} \frac{S_1}{K_1 + S_1} + \frac{\frac{K_2}{S_2}}{\frac{K_1}{S_1} + \frac{K_2}{S_2}} \frac{S_2}{K_2 + S_2} \qquad (15)$$

For the homologous substrate, sum kinetic model incorporating purely competitive substrate kinetics was proposed by Yoon et al. (1977) (equation 16).

$$\mu(S_1, S_2) = \mu_{\max} \frac{\mu_{\max,1} S_1 / K_{s,1}}{K_{s,1} + S_1 + \frac{K_{s,1}}{K_{s,2}} S_2} + \frac{\mu_{ma,2\,x}\, S_2 / K}{K_{s2} + S_2 + \frac{K_{s,2}}{K_{s,1}} S_1} \qquad (16)$$

Equation 16 indicates that each substrate exhibits a competitive inhibition effect on the utilization of the other substrate. The competitive substrate kinetics can be used to describe simultaneous and sequential substrate consumption for mixtures of substrate.

Another form of dual- substrate interaction with an enzyme is noncompetitive inhibition, characterized by the formation of a non-reactive complex when both substrates are simultaneously bound to the enzyme (Segel, 1975). The cell growth model based on this type of interaction is expressed mathematically (equation 17).

$$= \frac{\max 1 \; S_1}{(K_{s1} + S_1)\left(1 + \frac{S_2}{K_{s2}}\right)} + \frac{\max 2 \; S_2}{(K_{s2} + S_2)\left(1 + \frac{S_1}{K_{s1}}\right)} \qquad (17)$$

Uncompetitive enzyme inhibition model has also been used to describe dual substrate interaction. It differs from non- competitive inhibition in that one of the compounds (the inhibitor) can bind only to the enzyme substrate complex and not the free enzyme (Segel, 1975). A cell growth model based on uncompetitive substrate interaction is (equation 18):

$$= \frac{\max 1 \; S_1}{K_{s1} + S_1\left(1 + \frac{S_2}{K_{s2}}\right)} + \frac{\max 2 \; S_2}{K_{s2} + S_2\left(1 + \frac{S_1}{K_{s1}}\right)} \qquad (18)$$

In the sum kinetic models, kinetic parameters determined in the single substrate experiments are used for curve fitting. These models were evaluated by Reardon et al. (2000) for biodegradation of benzene, toluene and phenol mixtures, and found that the interactions between these substrates could not be described by sum kinetics models using only parameters determined in a single substrate experiment. An alternative model was formulated by adding an unspecified type of interaction into the sum kinetics model to produce the sum kinetics with interaction parameter (SKIP) model first proposed by Yoon et al. (1977) (equation 19).

$$= \frac{\max 1 \; S_1}{K_{s1} + S_1 + I_{2,1} S} + \frac{\max 2 \; S_2}{K_{s2} + S_2 I_{1,2} S_1} \quad (19)$$

The interaction parameter Ii, j indicates the degree to which substrate i affects the biodegradation of substrate j. The larger the value, the stronger the inhibition. The SKIP model form for a three-compound mixture is (equation 20):

$$= \frac{\max 1 \; S_1}{K_{s3} + S_1 + I_{2,1} S_2 + I_{3,1} S_3} + \frac{\max 2 \; S_2}{K_{s2} + S_2 I_{1,2} S_1 + I_{3,2} S_3} + \frac{\max 3 \; S_3}{K_{s3} + S_3 I_{1,3} S_1 + I_{2,3} S_2} \quad (20)$$

where the subscripts 1, 2, and 3 denote parameters for three different substrates. The extended SKIP model for N substrates is expressed as (equation 21):

$$= S_1 S_2 \ldots, S_N = \sum_{i=1}^{N} \frac{\max,i \; S_i}{K_{si} + S_i + \sum_{j=1, j \neq 1}^{N} I_{j,i} S_j} \quad (21)$$

The effect of one substrate on the degradation of another is given by the $S_j I_{j,i}$ terms. The values of the interaction coefficients, $I_{j,i}$, represent the degree of inhibition exerted by substrate j on substrate i. In a dual-substrate system, sequential substrate utilization is represented by a large value of $I_{1,2}$ and a small value of $I_{2,1}$. The SKIP model satisfactorily described simultaneous (Rogers and Reardon, 2000) and sequential (Reardon et al., 2000) degradation patterns in two different biological systems.

PESTICIDE BIODEGRADATION KINETICS

Increased agricultural practice and pesticide application had resulted in the contamination of natural environments with different kinds of pesticides. Wolt et al. (2001) described the design and interpretation of biodegradation studies conducted globally for the purpose of regulatory decision making with respect to pesticide use. Emphasis was placed on the various approaches utilized for addressing degradation studies in soil and the variability in pesticide soil fate parameters.

Understanding pesticide risks requires characterizing pesticide exposure within the environment in a manner that can be broadly generalized across widely varied and soil degradation are especially important for understanding the potential environmental exposure of pesticides. The data obtained from degradation studies are inherently variable and when limited in extent, lend uncertainty to exposure characterization and risk assessment (Wolt et al., 2001).

Pesticide decline in soils reflects dynamically coupled processes of sorption and degradation that add complexity to the treatment of soil biodegradation data from a kinetic perspective. Additional complexity arises from study design limitations that may not fully account for the decline in microbial activity of test systems or that may be inadequate for considerations of all potential dissipation routes for a given pesticide. Accordingly, kinetic treatment of data must accommodate a variety of differing approaches starting with very simple assumptions as to reaction dynamics and extending to more involved treatments if warranted by the available experimental data. Selection of the appropriate kinetic model to describe pesticide degradation should rely on statistical evaluation of the data fit to ensure that the models used are not over parameterized. Recognizing the effects of experimental conditions and methods for kinetic treatment of degradation data is critical for making appropriate comparisions among pesticide biodegradation data sets (Wolt et al., 2001).

Statistical evaluation of measures of central tendency for multisoil kinetic studies shows that geometric means better represent the distribution in soil half-lives than do the arithmetic or harmonic means (Wolt et al., 2001).

METAL INHIBITION OF BIODEGRADATION AND PREDICTION OF METAL SPECIATION

In sites co-contaminated with metals and organic compounds, metal toxicity inhibits the activity of organic-degrading microorganisms, impacting both their physiology and ecology, thus reducing the rate of biodegradation of the organic compounds (Said and Lewis, 1991; Roane et al., 2001; Maslin and Maier, 2000). Metal inhibition of a broad range of microbial processes including methane metabolism, growth, nitrogen and sulphur conversions, dehalogenation and reductive processes in general is well documented. Thorough reviews of the impacts of metals on many of these processes are available (Baath, 1989; Sandrin and Maier, 2003).

The toxicity of metals to microorganisms is dependent on its bioavailabity. Quantification of bioavailable metal concentration is an important step in the process of standardizing experiments to determine the impact of metals on organic pollutant biodegradation. Concentrations of bioavailable metals (metal speciation) can be estimated from solution phase using ion-selective electrodes and atomic absorption spectroscopy. Biological systems involving immunoassay (Blake et al., 1998; Khosraviani et al., 1998) or bioreporters (Rouch et al., 1995; Selifonova et al., 1993) have been used for mercury. However, the use of immunoassay and bioreporters is limited because of variation in measurements depending on the metal resistance of the bioreporter system used.

The application and limitations of immunoassay and bioreporters for metal detection have been reviewed by Neilson and Maier (2001).

As the alternative, bioavailable metal concentrations as a function of pH and ionic strength can be predicted using geochemical modeling software's (e.g. MINTEQA 2 MINEQL+) (Pardue et al., 1996). A number of computational models have been developed to predict the impact of metal on organic biodegradation (Amor et al., 2001; Jin and Bhattacharya, 1996; Nakamura and Sawada, 2000). These models accounted for metal inhibition by incorporating metal inhibition constant (K_i) to conventional growth or degradation model. For example, Amor et al. (2001) used a form of the Andrew's equation (originally used to describe substrate inhibition of microbial growth or substrate degradation) to model the effect of cadmium, zinc and nickel on rates of alkyl benzene biodegradation.

CONCLUSION

This review highlighted microbial utilization of, and growth on, organic chemicals. It examined the various kinetic models applied in the prediction of microbial removal of organic contaminants from the environment. It shows that the success of any treatment protocol depends on optimization of several controlling factors and this is only possible through modeling of the factors that determine process rate. The ability to model these processes is desirable in order to facilitate understanding and management of contaminated sites and industrial effluents.

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