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Kinetics of aerobic cometabolism of chlorinated solvents

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Abstract

The objectives of this paper are to review the wide range of kinetic models that have been introduced to describe the cometabolic oxidation of chlorinated solvents, to compare modeling approaches and associated experimental data, and to discuss knowledge gaps in the general topic of cometabolism kinetics. To begin, a brief description of the mechanism of oxygenase enzyme metabolism and its qualitative effects on cometabolic degradation kinetics is given. Next, a variety of kinetic expressions that have been used to describe cometabolism, ranging from adaptations of simple metabolic relationships to the development of complex equations that account for intracellular concentrations of key reaction species, are presented. A large number of kinetic coefficients published for a variety of oxygenase populations degrading a broad range of chlorinated solvents are categorized and compared. The discussion section of the paper contains an exploration of knowledge gaps that exist in our understanding of the kinetics of aerobic chlorinated solvent cometabolism. Specific topics covered include:

- the use of half saturation constants (K_{sc} and K_{sg}) as estimates for inhibition constants (K_{isc} and K_{isg}) in saturation modeling expressions,
- the specific nature of chlorinated solvent induced product toxicity and the capability for cells to recover from toxic effects, and
- methods for incorporating reducing energy limitations into cometabolism models

Finally, the applicability of the broad range of kinetic modeling approaches to scale-up and field applications for *in situ* bioremediation of chlorinated solvents is discussed.

Introduction

A wide range of chlorinated solvents can be microbially degraded under aerobic conditions by means of cometabolic transformation reactions. Cometabolic transformations are reactions that are catalyzed by existing microbial enzymes and that yield no carbon or energy benefits to the transforming cells (Horvath 1972). Therefore, a growth substrate must be available at least periodically to grow new cells, provide an energy source, and induce production of the cometabolic enzymes.

Cometabolism may occur relatively slowly in comparison to metabolism of growth substrates (Alexander 1994). Therefore, the kinetics of cometabolism can be an important consideration in bioremediation applications. For example, the likelihood of degradation kinetics, in contrast to mass transfer rates, controlling the overall contaminant removal rate is greater with cometabolism than with metabolism-based treatment schemes. In addition, the requirement for growth substrates in addition to cometabolic substrates and the related stoichiometry make predictions of cometabolic kinetics complex. Appropriate mathematical expressions of the underlying phenomena are essential to accurately describe cometabolism rates.

Biological degradation kinetics can be of major practical importance for application of *in situ* re-

mediation. Project costs and duration can be greatly influenced by the kinetics of the dominant biological reactions. Kinetic expressions are also important components of fate and transport models, which are used to plan and monitor site remediation, and to conduct risk and exposure assessments. The kinetics of cometabolism, however, are not entirely understood and can be quite complex; as such, a variety of kinetic expressions have been used to describe cometabolism, ranging from adaptations of simple metabolism expressions to the development of complex expressions that account for a range of reaction factors.

The objectives of this paper are to review the wide range of kinetic models that have been introduced to describe the aerobic cometabolic degradation of chlorinated solvents, to compare modeling approaches and associated experimental data, and finally to discuss knowledge gaps in the general topic of cometabolism kinetics.

Background

The aerobic cometabolic transformation of chlorinated solvents involves oxygenase enzymes, molecular oxygen, and a source of reducing equivalents, typically NAD(P)H (see extensive discussion of this topic in Arp et al. 2001). The oxygenase reaction generates chlorinated solvent oxidation products that may react with cellular macromolecules or may be hydrolyzed spontaneously into carbon dioxide, chloride, or other non-volatile products that are easily mineralized by microorganisms (Little et al. 1988; Tsien et al. 1989; Oldenhuis et al. 1989; Fox et al. 1990; Nelson et al. 1986, 1987; Rasche et al. 1991).

Chlorinated solvents can be oxidized by a wide range of oxygenase-expressing microorganisms including those that utilize methane (Wilson & Wilson 1985; Strand & Shippert 1986; Fogel et al. 1986; Little et al. 1988; Tsien et al. 1989; Oldenhuis et al. 1989), propane (Fliermans et al. 1988; Wackett et al. 1989; Phelps et al. 1990), propene (Ensign et al. 1992), isoprene (Ewers et al. 1990), isopropylbenzene (Dabrock et al. 1992), toluene (Nelson et al. 1986; Wackett et al. 1988; Zylstra et al. 1989; Shields et al. 1989), phenol (Folsom et al. 1990; Harker & Kim 1990; Segar 1994), butane (Wilson et al. 1988; Kim et al. 1997), and ammonia (Arciero et al. 1989; Vannelli et al. 1990; Rasche et al. 1991) as energy and/or carbon sources.

As is discussed in detail below and depicted in Figure 1, some of the factors that may adversely affect the cometabolic degradation of chlorinated solvents by oxygenase-expressing microorganisms include enzyme inhibition by growth or other cometabolic substrates, chlorinated solvent product toxicity, and reducing energy or reductant shortages.

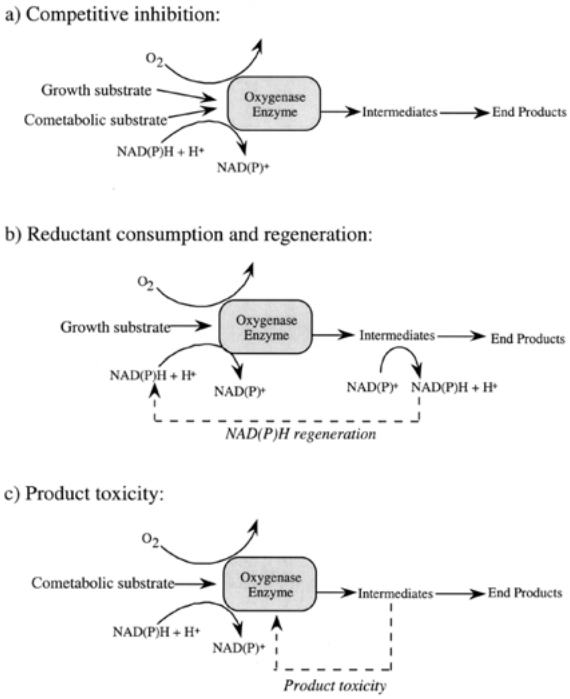

Figure 1. Generic oxygenase enzyme reactions with NAD(P)H serving as reductant. Schamatics illustrate: (a) competitive inhibition between growth and cometabolic substrates, (b) reductant consumption and regeneration during growth substrate metabolism, and (c) product toxicity exerted by transient intermediates of cometabolic substrate oxidation.

Enzyme competition

Since enzymes that catalyze cometabolic reactions have active sites that can react with a number of different substrates, including the primary substrate and perhaps a wide range of cometabolic substrates, competition for the active site may occur when multiple substrates are simultaneously available (Figure 1a), resulting in an apparent decrease in enzyme affinity for each substrate (competitive inhibition). Consequently, competition between growth substrate and cometabolic substrate, or among different growth or cometabolic substrates, can result in overall decreased transformation rates of each substrate. Competitive inhibition between growth substrates and cometabolic substrates has been observed for many oxygenase-utilizing microorganisms (Suzuki et al. 1976; Nelson

et al. 1986; Lanzarone & McCarty 1990; Strand et al. 1990; Saéz & Rittmann 1991; Broholm et al. 1990; Chang et al. 1993; Speitel et al. 1993; Keener & Arp 1993; Malachowsky et al. 1994; Hyman et al. 1995; Chang & Alvarez-Cohen 1995b). Further, enzyme competition during the simultaneous degradation of multiple cometabolic substrates has resulted in decreased degradation rates for each compound (Alvarez-Cohen & McCarty 1991d; Palumbo et al. 1991; Segar & Speitel 1995; Dolan & McCarty 1995b; Chang & Alvarez-Cohen 1997; Aziz et al. 1999). Other types of inhibition have also been observed to occur during the cometabolic oxidation of solvents. Noncompetitive inhibition of growth substrate was observed for an ammonia monooxygenase utilizing pure culture degrading monohalogenated methanes, ethanes and chloropropane (Keener & Arp 1993), and for a propane-oxidizing mixed culture degrading TCE (Kennan et al. 1994). Noncompetitive inhibition involves the independent binding of substrate and inhibitor to different sites on the enzyme causing a decrease in maximum reaction rate without an associated decrease in substrate affinity for the enzyme. Inhibition patterns that did not fit the classical models but that suggested the presence of a secondary binding site were observed for highly chlorinated solvents (carbon tetrachloride and tetrachloroethylene) that were not degraded by the ammonia oxidizer (Keener & Arp 1993).

Reducing energy consumption

Oxygenase enzymes generally consume molecular oxygen and reductants such as NAD(P)H or ubiquinone during the oxidation of both energy generating and cometabolic substrates (Gibson 1970; Large et al. 1983; Burrows et al. 1984; Rasche et al. 1990). Energy generating substrates, however, regenerate the reductant during subsequent metabolic steps (Anthony 1982; Dalton & Higgins 1989), while cometabolic substrates such as chlorinated organics do not (Figure 1b) (Wackett & Gibson 1988; Dalton & Stirling 1982). Consequently, the rate and extent of cometabolic transformation reactions occurring in the absence of primary substrate may be limited by the availability of reductant. Conversely, both the rate and extent of cometabolic reactions can be enhanced in the presence of growth or energy-producing substrates due to the regeneration of reductant.

The simultaneous availability of primary substrate can be beneficial during cometabolism from the viewpoint of regenerating reductant, but primary substrate can also be detrimental to the cometabolism rate because of competitive inhibition between the primary and cometabolic substrates. The conflicting processes of reductant regeneration and competitive inhibition inherent in oxygenase enzyme activity were recently illustrated in a study that showed TCE degradation rates of methane, propane, toluene, and phenol oxidizing cultures increased with addition of low growth substrate concentrations ($<$0.1 mM) over those with no substrate addition, while TCE degradation rates decreased with addition of higher growth substrate concentrations ($>$0.1 mM) (Chang & Alvarez-Cohen 1995a).

Some oxygenase expressing cultures can regenerate reductant by using alternate energy substrates that are not oxidized by the oxygenase enzymes, and therefore do not result in competitive inhibition with either growth or cometabolic substrates (Dawes & Senior 1973; Stirling & Dalton 1979; Oldenhuis et al. 1989; Tsien et al. 1989; Stensel et al. 1992). Examples include catabolic intermediates such as formate for methanotrophs and propanol for propane oxidizers, or internal storage polymers such as poly-β-hydroxybutyrate (PHB). The utilization of an alternate energy substrate for the regeneration of reductant allows cometabolic oxidations to be carried out without limitations due to either reducing energy depletion or competitive inhibition. Eventually, the growth substrate must be provided again, however, because oxygenase enzyme levels may diminish and biosynthesis cannot proceed in the absence of enzyme-inducing growth substrates.

Product toxicity

The cometabolic oxidation of chlorinated solvents by a wide range of oxygenase enzymes can result in product toxicity (Figure 1c) (Wackett & Householder 1989; Alvarez-Cohen & McCarty 1991a,d; Henry & Grbic-Galic 1991; Oldenhuis et al. 1991; Stensel et al. 1992; Ensign et al. 1992; Heald & Jenkins 1994; Rasche et al. 1991). Although the specific chlorinated solvent products responsible for the observed product toxicity are not known, toxic effects have been shown to include damage directly to the oxygenase enzymes (Fox et al. 1990; Ely et al. 1997) as well as to general cellular constituents (Wackett & Householder 1989; Alvarez-Cohen & McCarty 1991d; Oldenhuis et al. 1991; Rasche et al. 1991; Hyman et al. 1995; van Hylckama Vlieg et al.1997). Studies conducted with

a wide range of oxygenase-utilizing cultures suggest that both the extent of product toxicity and the mode of action are highly variable across species and genera (Arp & Hymen 2001). However, for the cultures in which toxicity has been quantified, the attack of toxic products on the enzyme and/or cellular materials has resulted in activity and viability that decreases in proportion to the amount of compound degraded (Oldenhuis et al. 1991; Alvarez-Cohen & McCarty 1991a; Tompson et al. 1994; Fitch et al. 1996; Ely et al. 1997; van Hylcklama Vlieg et al. 1997; Chu & Alvarez-Cohen 1998).

Cometabolism kinetics

Modeling approaches

Saturation kinetics

The kinetics of cometabolic degradation reactions have been described by a number of different models, ranging from simple first-order reaction models to complex multi-substrate mixed order models. The most commonly applied approach to cometabolic modeling involves modifications to the saturation kinetic expression originally derived from Michaelis-Menten enzyme kinetics:

$$r_c = -\frac{k_c X S_c}{K_{sc} + S_c}, \tag{1}$$

where r_c is the rate of cometabolic reaction (μmol or mg $L^{-1}d^{-1}$); S_c, the cometabolic substrate concentration (μmol or mg L^{-1}); k_c, the maximum specific rate of cometabolic substrate degradation (μmol or mg substrate (mg cells)$^{-1}$ d^{-1}); X, the active microbial concentration (mg cells L^{-1}); K_{sc}, the half-saturation constant for the cometabolic substrate (μmol or mg L^{-1}).

With this approach, the rate of cell growth is typically expressed as a function of growth substrate consumption and cell decay as follows:

$$r_x = \mu X = Y r_g - bX = Y\frac{k_g X S_g}{K_{sg} + S_g} - bX, \tag{2}$$

where r_x is the net cellular growth rate (μmol or mg L^{-1} d^{-1}); μ, the net specific cellular growth rate (d^{-1}); r_g, the rate of growth substrate consumption (μmol or mg L^{-1} d^{-1}); Y, the cellular yield of growth substrate (mg cells (μmol or mg growth substrate)$^{-1}$); S_g, the growth substrate concentration (μmol or mg L^{-1}); k_g, the maximum specific rate of growth substrate degradation (μmol or mg substrate (mg cells)$^{-1}$ d^{-1}); K_{sg}, the half-saturation constant for growth substrate (μmol or mg L^{-1}); b, the cell decay rate (d^{-1}).

Pseudo first order model

The pseudo-first order rate model for cometabolism is a simplification of saturation kinetics predicated on the assumption that substrate concentrations (S_c) are significantly lower than half-saturation constants (K_{sc}):

$$r_c = -k_1 X S_c, \tag{3}$$

where k_1 is the pseudo-first-order cometabolic degradation rate constant (L (mg cells)$^{-1}$ d^{-1}).

In this expression, k_1 is equivalent to k_c/K_{sc} in Equation (1). Incorporation of k_c and K_{sc} into a single term can be especially useful in the common situation where these two parameters cannot be determined independently because of toxicity problems associated with the high concentrations needed to achieve saturation kinetics (Anderson & McCarty 1996; Smith & McCarty 1996; Alvarez-Cohen & McCarty 1991b). Many researchers have applied the pseudo-first-order model to describe the cometabolic oxidation of chlorinated solvents when concentrations are relatively low and both competitive inhibition and product toxicity are not of concern.

Modeling multiple substrates (growth substrates and multiple cometabolic substrates)

The enzyme inhibition caused by concurrent degradation of growth substrates and cometabolic substrates or by multiple cometabolic substrates has generally been modeled by including competitive inhibition terms in the saturation kinetic expression for the degradation of the growth and cometabolic substrates respectively as follows (Broholm et al. 1992; Strand et al. 1990):

$$r_g = -\frac{X k_g S_g}{K_{sg}(1 + S_c/K_{isc}) + S_g} \tag{4}$$

$$r_c = -\frac{X k_c S_c}{K_{sc}(1 + S_g/K_{isg}) + S_c}, \tag{5}$$

where K_{isg} is the inhibition coefficient for growth substrate (μmol or mg L^{-1}); K_{isc}, the inhibition coefficient for the cometabolic substrate (μmol or mg L^{-1})

An assumption derived from enzyme kinetics that is commonly applied with respect to the above equations is that $K_{isc} = K_{sc}$ and $K_{isg} = K_{sg}$. This assumption is discussed below.

The simultaneous presence of more than one competitive growth or cometabolic substrate can be modeled in the manner of multiple independently-operating competitive enzyme substrates (Cornish-Bowden 1976), using a separate degradation equation for each compound and replacing the competitive inhibition term $(1 + S_c/K_{isc})$ with the more general term

$$\left(1 + \sum_j \frac{S_c^j}{K_{isc}^j}\right)$$

as described previously for cometabolic oxidation reactions (Alvarez-Cohen 1993).

A number of researchers have used modifications of the above expressions for competitive inhibition to successfully model the degradation of chlorinated solvents in the presence of growth substrate by a range of oxygenase expressing organisms (Chang & Alvarez-Cohen 1995b, 1997; Ely et al. 1995b; van Hylckama Vlieg et al. 1996; Smith et al. 1997; Tschantz et al. 1995; Chang & Criddle 1997; Anderson & McCarty 1994, 1996; Arcangeli & Arvin 1997; Travis & Rosenberg 1997; El-Farhan et al. 1998; Yang et al. 1999; Kelly et al. 2000).

Keenan et al. (1994) used the following noncompetitive inhibition model to describe the degradation of TCE in the presence of growth substrate by a mixed culture of propane oxidizers:

$$r_c = -\frac{Xk_cS_c}{(K_{sc} + S_c)(1 + S_g/K_{isg})}. \quad (6)$$

A conceptual model that incorporated both competitive and noncompetitive inhibition into cometabolic enzyme kinetics was derived and described by Ely et al. (1995a):

$$r_g = -\frac{Xk_g^*S_g}{K_{sg}(1 + S_c/K_{isc1}) + S_g(1 + S_c/K_{isc2})} \quad (7)$$

$$r_c = -\frac{Xk_c^*S_c}{K_{isc1}(1 + S_g/K_{isg}) + S_c(1 + S_gK_{isc1}/K_{isg}K_{isg2})}, \quad (8)$$

where k_g^* is the maximum specific rate of growth substrate consumption adjusted for enzyme inactivation and recovery (μmol or mg substrate (mg cells)$^{-1}$ d^{-1}) (see Equation (12) for full expression); k_c^*, the maximum specific rate of cometabolic substrate consumption adjusted for enzyme inactivation and recovery (μmol or mg substrate (mg cells)$^{-1}$ d^{-1}) (see Equation (13) for full expression); K_{isc1}, the competitive inhibition coefficient for the cometabolic substrate (μmol or mg L^{-1}); K_{isc2}, the noncompetitive inhibition coefficient for the cometabolic substrate (μmol or mg L^{-1}).

Although the general form of this model included terms to account for either type of inhibition as shown, the model was simplified for analysis and applied to situations with competitive inhibition alone (Ely et al. 1995b; Ely et al. 1997).

Modeling product toxicity

Product toxicity associated with cometabolic oxidations has been observed to cause cell activity to decrease in proportion to the amount of compound degraded. This product *toxicity* is different from classically modeled product *inhibition*, in which cell activity decreases in proportion to the accumulation of inhibitory products (Aiba et al. 1968; Bazua & Wilke 1977). With some exceptions, the products of chlorinated solvent oxidation are transient, so they do not accumulate appreciably. Rather, they exert toxicity either while being formed or immediately after formation (Arp & Hyman 2001). Product toxicity associated with the cometabolic oxidation of chlorinated solvents has been quantified in a number of different ways. Oldenhuis et al. (1991) introduced an inactivation constant, p, that related the change in the maximum rate of cometabolic substrate degradation to the amount of cometabolic substrate being degraded as follows:

$$\frac{dk_c}{dS_c} = p, \quad (9)$$

where p is the inactivation constant (L (mg cells)$^{-1}$ d^{-1}).

The implication of this approach is that the toxic effects function to decrease enzyme activity specifically rather than affecting overall cellular functions.

A similar approach introduced by Alvarez-Cohen & McCarty (1991b) utilized a transformation capacity (T_c) term, a constant representing the amount of compound degraded divided by the amount of cells inactivated :

$$\frac{dS_c}{dX} = T_c, \tag{10}$$

where T_c is the transformation capacity for the cometabolic substrate (μmol or mg cometabolic substrate (mg cells)$^{-1}$); dS_c, the change in cometabolic substrate concentration during the reaction (μmol or mg L^{-1}); dX, the change in active cell concentration during the cometabolic reaction (mg L^{-1})

The implication of this approach is that the toxic effects function to decrease overall cellular functions rather than affecting specific enzyme activity alone.

A related term, the transformation yield (T_y), was defined as the amount of cometabolic substrate degraded prior to cell inactivation divided by the amount of primary substrate required to grow the cells, and is calculated as YT_c, the cellular yield of growth substrate (mg cells (μmol or mg growth substrate)$^{-1}$) multiplied by the transformation capacity (Alvarez-Cohen & McCarty 1991b). T_y is of considerable practical significance in that it provides an indication of the amount of growth substrate needed, which directly affects operating costs.

The distinction between the inactivation constant and transformation capacity approaches is primarily conceptual, with the inactivation constant representing the observed decrease in degradation activity as a decrease in the maximum enzyme degradation rate and the transformation capacity representing it as a decrease in the active cell concentration.

The transformation capacity representation of product toxicity has been incorporated into the saturation kinetic expression to describe cometabolic reaction rates occurring in the presence or absence of growth substrate by defining the net specific cell growth rate (μ) as a function of cell growth due to consumption of growth substrate (S_g) and cell inactivation due to product toxicity and cellular decay as follows (Criddle 1993; Chang & Alvarez-Cohen 1995; Anderson & McCarty 1996):

$$\mu = \frac{r_x}{X} = Y\frac{r_g}{X} - \frac{1}{T_c}\frac{r_c}{X} - b. \tag{11}$$

An important aspect of this modeling expression is that cometabolic oxidations may continue temporarily in the absence of growth substrate until the active cell mass (that is, cells with active oxygenase enzymes) has been depleted due to the combined effects of product toxicity and cell decay. Modifications of the above model incorporating the concept of a transformation capacity have been used by a number of researchers to effectively describe the cometabolic degradation of chlorinated solvents by a number of oxygenase expressing cells both in the presence and absence of growth substrate (Alvarez-Cohen & McCarty 1991b, 1991d; Anderson & McCarty 1994, 1996; Chang & Alvarez-Cohen 1995b, 1997; Chang & Criddle 1997; Tschantz et al. 1995; Smith et al. 1997; Travis & Rosenberg 1997).

Ely et al. (1995a) derived a general cometabolic model from enzyme kinetics that incorporated the concept of an enzyme inactivation constant introduced by Oldenhuis et al. (1991). This model explicitly includes terms representing the potential recovery of enzyme activity following inactivation into the expression for maximum degradation rate (see Equations (7) and (8) for general model form):

$$k_g^* = k_g - k_{\text{inact}}P_2 + k_{\text{rec}}P_1 \tag{12}$$

$$k_c^* = k_c(k_g - k_{\text{inact}}P_2 + k_{\text{rec}}P_1)k_g, \tag{13}$$

where k_{inact} is the specific enzyme inactivation coefficient (μmol or mg substrate L (μmol or mg cometabolic oxidation product)$^{-1}$ (mg cells)$^{-1}$ d^{-1}); k_{rec}, the specific enzyme recovery coefficient (μmol or mg substrate L (μmol or mg growth oxidation product)$^{-1}$ (mg cells)$^{-1}$ d^{-1}); P_1, the product of growth substrate oxidation (μmol or mg L^{-1}); P_2, the product of cometabolic substrate oxidation (μmol or mg L^{-1}).

This model was used to effectively describe the degradation of a range of chlorinated solvents by ammonia oxidizers in the presence of growth substrate (Ely et al. 1995b, 1997). A simplification of this model was also used by Yang et al. (1999) to describe TCE degradation by a nitrifying enrichment culture. Although this model explicitly includes a term for enzyme recovery from toxic damage, it does not include an expression for cell growth. Interestingly, while the model was designed to incorporate cell repair rather than cell regrowth in response to product toxicity, the resulting recovery term is mathematically equivalent to the cell yield term used for cell growth in the previously described models. For example, when Equation (11) is used to incorporate cell growth and product toxicity into the competitive inhibition model (Chang & Alvarez-Cohen 1995), the resulting expressions for substrate degradation (r_g and r_c) are the same as those of the Ely model with the cell yield replacing the enzyme recovery expression and the transformation capacity replacing the enzyme inactivation expression:

$$\frac{Xk_{\text{rec}}}{k_g} \sim Y \tag{14}$$

$$\frac{k_g}{Xk_{\text{inact}}} \sim T_c. \tag{15}$$

This mathematical equivalency led both groups to define similar expressions for the ratio of growth and cometabolic substrate concentrations required to sustain long-term cometabolic reactions that balance product toxicity with enzyme recovery or cellular growth. The substrate ratio in the respective notations is given as follows (Ely et al. 1997; Chang & Alvarez-Cohen 1995):

$$\frac{S_g}{S_c} \geqslant \frac{k_c K_{sg} k_{\text{inact}}}{k_g K_{sc} k_{\text{rec}}} \approx \frac{k_c K_{sg}}{Y T_c k_g K_{sc}}. \tag{16}$$

Despite the mathematical similarity, the major conceptual difference between the Ely et al. (1995a) approach and the other models is that enzyme recovery is de-coupled from cellular growth, allowing for the possibility that enzyme recovery following toxic inactivation may occur more rapidly and with less energy consumption than cellular regrowth.

Tompson et al. (1994) took a slightly different approach to modeling cometabolic oxidation reactions. They used the general saturation kinetic expression (Equation (1)) modified with an exponential rather than linear toxicity term to model TCE degradation by methanotrophs in porous media. That is, instead of describing the inactivation of cells due to product toxicity as a zero-order function as described above (Equation (10)), they used the following first-order cellular decay expression:

$$\frac{\mathrm{d}S_c}{\mathrm{d}X} = \frac{X_o}{\upsilon X}, \tag{17}$$

where υ is the a positive dimensionless constant and X_0 is the initial active microbial concentration (mg cells L^{-1}).

This modeling approach was shown to fit the degradation of TCE by a pure culture of methane oxidizers in the absence of growth substrate; however, unlike the previously described models, it has not yet been tested over a wide range of conditions.

Modeling reducing energy effects

Criddle (1993) and Chang & Criddle (1997) introduced a cometabolic model that incorporated reducing energy considerations into a modification of the saturation kinetic model. This model employs a stoichiometric coefficient to couple the generation of reductant to the consumption of growth substrate. The comprehensive cometabolic model includes competitive inhibition and a transformation capacity coefficient as well as a growth substrate transformation capacity (later referred to as the theoretical transformation yield), T_y', defined as the stoichiometric mass of cometabolic substrate consumed per mass of growth substrate consumed. The theoretical T_y' was incorporated into the cometabolic degradation expression as follows:

$$r_c = -(T_y' r_g + k_c)\frac{X S_c}{K_{sc} + S_c}, \tag{18}$$

where T_y' is the stoichiometric transformation yield (μmol cometabolic substrate (μmol growth substrate)$^{-1}$)

This equation is based on the assumption that cometabolic degradation rates are enhanced by the generation of reductants caused by the degradation of growth substrates. T_y' differs from the previously described transformation yield, T_y, in that it represents only the stoichiometric amount of growth substrate consumed during a cometabolic degradation reaction rather than the amount required to initially grow the cells. The observed T_y' was approximated by dividing the rate of cometabolic substrate consumption by the rate of growth substrate consumption: $T_g' = r_c/r_g$. The model incorporating T_y' was used by Chang and Criddle (1997) to describe the degradation of TCE in the presence of methane at several concentrations by a mixed methane-oxidizing culture.

Sáez & Rittmann (1993) also incorporated reducing energy consumption into cometabolism kinetics by using a stoichiometric transformation yield that linked growth substrate consumption to reducing energy generation, but added an explicit term for the reducing energy generated by biomass oxidation as follows:

$$r_c = T_y' r_g - \beta b X, \tag{19}$$

where β is the mass of cometabolic substrate oxidized per mass of biomass oxidized (μmol cometabolic substrate (mg cells)$^{-1}$).

This expression was incorporated into a model that included a Haldane type of substrate toxicity term for the growth substrate:

$$r_g = -\frac{k_g X S_g}{K_{sg} + S_g + \frac{S_g^2}{K_I}}, \tag{20}$$

where K_I is the inhibition constant for growth substrate (μmol or mg L^{-1}).

This model also included competitive and noncompetitive inhibition, but did not include either substrate or product toxicity for the cometabolic substrate. The model was used to describe the cometabolic degradation of 4-chlorophenol in the presence of phenol by *Pseudomonas putida PpG4*, an aromatic degrading bacterium.

Chang & Alvarez-Cohen (1995b) modeled reducing energy effects by considering reductant to be a potentially limiting substrate. That is, the saturation kinetic expression for enzymatic degradation reactions (Equation (1)) is based upon the concentration of the limiting reactant. However, for oxygenase catalyzed reactions, three reactants are actually involved, any of which could be present in limiting concentrations. The three reactants required for an oxygenase reaction are the substrate (growth or cometabolic), reductant, and molecular oxygen (Figure 1). Assuming for simplicity that molecular oxygen is present in excess, the saturation kinetic expression can be adjusted to incorporate the potential effects imposed by either substrate or reductant as a limiting reactant as follows (Bailey & Ollis 1986):

$$r_s = -kX\left(\frac{S}{K_s + S}\right)\left(\frac{R}{K_R + R}\right), \qquad (21)$$

where r_s is the rate of substrate degradation (μmol or mg L^{-1} d^{-1}); k, the maximum specific substrate degradation rate (μmol or mg substrate (mg cells)$^{-1}$ d^{-1}); S, the growth substrate (S_g) or cometabolic substrate (S_c) concentration (μmol or mg L^{-1}); K_s, the half-saturation constant of substrate (μmol or mg L^{-1}); R, the reductant electron equivalent concentration (mmol e^- L^{-1}); K_R, the half-saturation constant of reductant (mmol e^- L^{-1}).

Implicit in this expression is the assumption that the substrate and reductant react with the enzyme at two distinct active sites and that the binding of each reactant is independent of the other.

Reductant can be regenerated in cells by means of growth substrate or energy substrate mineralization or by utilization of internal energy storage polymers such as PHB. The following expression has been introduced for use with Equation (21) in order to account for reductant consumption due to cometabolic oxygenase reactions and regeneration due to degradation of growth or energy substrate over time during cometabolic reactions (Chang & Alvarez-Cohen 1995b):

$$r_R = \alpha_g(-r_g) - \alpha_c(-r_c), \qquad (22)$$

where r_R is the rate of reducing energy production or consumption (mmol e^- L^{-1} d^{-1}); r_g, the rate of growth or energy substrate consumption (μmol or mg L^{-1} d^{-1}); r_c, the rate of cometabolic reaction (μmol or mg L^{-1} d^{-1}); α_g, the net stoichiometric coefficient of reductant regeneration from the degradation of growth or energy substrate (mmol e^- (μmol substrate)$^{-1}$); α_c, the net stoichiometric coefficient of reductant consumption from the oxidization of cometabolic substrate (mmol e^- (μmol substrate)$^{-1}$).

This model was modified to incorporate competitive inhibition between growth and cometabolic substrates and product toxicity using the transformation capacity approach, and applied to describe the degradation of TCE in the presence and absence of methane and formate by a mixed methane-oxidizing culture.

Arcangeli & Arvin (1997) also employed an expression for a second reactant as the limiting substrate in order to incorporate reducing energy effects into competitive inhibition kinetics. They modeled the observed increase in TCE degradation rates, caused by small amounts of growth substrate, by making the growth substrate a potentially limiting substrate to the cometabolic substrate saturation kinetic expression as follows:

$$r_c = -\frac{Xk_cS_c}{K_{sc}(1 + S_g/K_{isg}) + S_c}\left(\frac{S_g}{K_{sg} + S_g}\right). \qquad (23)$$

Product toxicity was not incorporated into this model. The Arcangeli and Arvin (1997) model was used to describe the degradation of TCE by a toluene-enriched biofilm operating over a range of TCE and toluene concentrations. Although the model adequately described general trends in degradation behavior, it was incapable of predicting TCE removal in the absence of growth substrate.

Kinetic coefficients

By far, trichloroethylene is the most widely studied chlorinated solvent in aerobic cometabolism, and methanotrophs are the most widely studied bacteria. Extensive work in particular has been done on the methanotroph *Methylosinus trichosporium* OB3b and its mutants. Methane monooxygenase (MMO) is the non-specific enzyme in methanotrophs that catalyzes cometabolism of chlorinated solvents. A number of

methanotrophs, including *M. trichosporium* OB3b, produce two forms of MMO: soluble MMO (sMMO) and membrane bound or particulate MMO (pMMO). sMMO has been shown to catalyze much more rapid cometabolic degradation rates than pMMO; however, sMMO is only produced in the wild type organism at very low copper concentrations (<16 μg/L) (Tsien et al. 1989).

A sampling of kinetic coefficients for TCE cometabolism by methanotrophs is provided in Table 1. The maximum specific degradation rate (k_c), the half saturation coefficient (K_{sc}), the pseudo-first-order rate constant ($k_1 = k_c/K_{sc}$), the transformation capacity (T_c), and the transformation yield (T_y) are listed, as reported in or calculated from various studies. TCE degradation has most often been characterized by pseudo-first-order kinetics, so examination of k_1 values is the easiest way to make comparisons among organisms. On this basis, *M. trichosporium* OB3b, its copper-resistant mutants (e.g., PP358), and mixed cultures having substantial genetic similarity to *M. trichosporium* OB3b (Alvarez-Cohen & McCarty 1991a,b,d; Chang & Alvarez-Cohen 1996) generally have the highest reported rate constants. *M. trichosporium* OB3b expressing pMMO has a substantially smaller rate constant, ranging from nonmeasurable to about 10% of that found with sMMO. Lontoh & Semrau (1998) recently demonstrated that copper plays an important role in pMMO cometabolism of TCE, in addition to its well-known role in controlling sMMO expression. At copper concentrations of 50 to 300 μg/L, TCE cometabolism was quite slow; however, the cometabolism rate increased considerably at a copper concentration of 1.3 mg/L. In most natural environments, the copper concentration is less than 150 μg/L (Forstner & Wittman 1979); therefore, slow pMMO cometabolism rates would be expected.

The majority of known methanotrophs cannot produce sMMO (Murrell 1992; Hanson & Hanson 1996). Therefore, the uncharacterized mixed cultures reported in Table 1 may be expressing pMMO, especially the ones grown in the presence of significant amounts of copper. Although some exceptions are listed in Table 1, relatively small rate constants are typical of pMMO cometabolism (DiSpirito et al. 1992; Lohtoh & Semrau 1998). Rate constants on the order of 0.1 to 1% of the maximum values measured for *M. trichosporium* OB3b are typical.

The presence of formate generally leads to higher rate constants relative to no external source of reducing energy, illustrating the importance of adequate reductant in cometabolism. The presence of the growth substrate, methane, can cause complex effects due to the duel phenomena of enzyme inhibition and generation of reductant. That is, the presence of methane generally causes the apparent k_1 to decrease due to competitive inhibition (Speitel et al. 1993); however, this effect may be partially or entirely counteracted by the rate increase caused by the generation of reductant and production of new enzymes and cells caused by degradation of the growth substrate.

With respect to *in situ* cometabolism of TCE, k_1 values at the lower end of the range reported in Table 1 are probably most realistic for typical field conditions given the usual concentrations of copper encountered in the environment and the likelihood that organisms will be expressing pMMO. For example, Semprini & McCarty (1992) used a k_1 of 0.025 L/mg-day to model TCE removal observed during field tests at Moffett Field. The large rate constants obtained with specialized organisms and highly controlled environmental conditions might be possible in engineered reactors, but are unlikely in the less controlled environment associated with *in situ* bioremediation.

The TCE transformation capacity generally ranges from 25 to 150 μg TCE/mg cells (Table 1), although some larger values have been measured for *M. trichosporium* OB3b and similar mixed cultures. Since T_c is a measure of the cumulative effects of product toxicity while initial degradation rates are kinetic measurements of enzyme efficiency, correlation between these two parameters should not be expected. In fact, correlations between T_c values and initial TCE degradation rates (those that are the least affected by product toxicity) have generally not been observed for methanotrophs (Oldenhuis et al. 1991; Chang & Alvarez-Cohen 1996; Smith et al. 1997; Chu & Alvarez-Cohen 1998). However, measured degradation rates can be severely impacted by the diminishing effects of product toxicity. Therefore, it is important to consider product toxicity when reporting reaction kinetics by either reporting initial rate measurements or by incorporating toxic effects into the kinetic applied kinetic model.

As expected, measured transformation yields are also quite consistent ranging from 15 to 50 μg TCE/mg methane for a variety of cultures. As with the transformation capacity, the transformation yield for *M. trichosporium* OB3b and a genetically similar mixed culture is considerably larger than for the other cultures.

Table 1. Selected kinetic coefficients for methanotrophic cometabolism of TCE

Organism/condition	Initial TCE conc. (mg/L)	Additional substrate	Temperature (°C)	k_c (mg/mg-day)[1]	K_{Sc} (mg/L)	k_1 (L/mg-day)[1]	T_c (μg/mg)	T_y (μg/mg)[1]	Reference
M. trichosporium OB3b, sMMO	4–90	Formate	30	55	19	2.9	290	150	a
M. trichosporium OB3b, sMMO	2.6	None	30	3.8	7.2	0.53			b
M. trichosporium OB3b, sMMO	4	None		1.0	10	0.1	320		c
M. trichosporium OB3b, pMMO	0.13–8.5	None	24	0.24	4.7	0.05			d
M. trichosporium OB3b, pMMO	0.13–8.5	Formate	24	0.39	1.0	0.37			d
M. trichosporium OB3b, PP358, sMMO	0.06–8	Formate	23	21	11	1.4	150		e, f
Mixed culture	0.15	Methane	25			0.0052			g
Mixed culture	4	None	20			0.008		29	h
Mixed culture, biofilm	0.9	None	20–24			0.0029		34	i
Mixed culture, 0.06 μM Cu^{2+}	0.03–0.07	None	21			0.041			j
Mixed culture, no Cu^{2+}	0.03–0.07	None	21			0.62			j
Methlyomonas sp. MM2, pMMO	0.03–0.07	None	21	0.046	1.4	0.033			j
Methlyomonas sp. MM2, pMMO	0.03–0.07	EDTA	21	0.29	0.51	0.57			j
Methlyomonas sp. MM2, pMMO	0.03–0.06	Formate	21			2.3	28		p
Mixed culture, sMMO	15	None	21	0.84	0.69	1.2	43	15	k
Mixed culture, sMMO	0.4–25	Formate	21	7.6	8.2	0.93	80	28	k
Mixed culture, sMMO, N_2 fixing	2.2	None	20			0.28	56	21	l
Mixed culture, sMMO, N_2 fixing	2.2	Formate	20			0.54	140	52	l
Mixed culture, sMMO	0.5–60	Formate	20	9.6	6.2	1.6	540	180	m
Mixed culture, sMMO	0.5–30	None	20	1.0	3.8	0.27	50	17	n
Mixed culture, sMMO	0.5–30	Formate	20	4.2	7.0	0.6	100	34	n
Mixed culture, sMMO	0.5–9	Methane	21	0.15	1.9	0.078	60		o
Mixed culture, sMMO	5.9	None	25			0.35	210		t
Mixed culture, pMMO	1	Methane	20	>0.012	0.13	>0.092	25	16	q, r
Methylomonas methanica 68-1, sMMO	6.6–66	Formate	25	3.7	30	0.12			s
Average[2]				6.8	7.0	0.61	150	52	
Standard deviation				14	7.8	0.76	150	57	

a. Oldenhuis et al. 1991; b. Tsien et al. 1989; c. Tompson et al. 1994; d. Lontoh & Semrau 1998; e. Aziz et al. 1999; f. Fitch et al. 1996; g. Leeson & Bouwer 1989; h. Strand et al. 1991; i. Arvin 1991; j. Henry & Grbic-Galic 1990; k. Alvarez-Cohen & McCarty 1991b; l. Chu & Alvarez-Cohen 1996; m. Chang & Alvarez-Cohen 1996; n. Chang & Alvarez-Cohen 1995a; o. Chang & Criddle 1997; p. Henry & Grbic-Galic 1991; q. Anderson & McCarty 1996; r. Anderson & McCarty 1997; s. Koh et al. 1993; t. Smith et al. 1997.

[1] Biomass reported in mg dry cell mass; units conversions assume dry cell mass is 50% protein.

[2] Average and standard deviation computed by ignoring ">" signs and by assuming a range can be approximated by taking one sample value at the top and one at the bottom of the range.

Table 2. Selected kinetic coefficients for TCE cometabolism by aromatic degraders

Organism/condition	Initial TCE conc. (mg/L)	Additional substrate	Temperature (°C)	k_c (mg/mg-day)[1]	K_{Sc} (mg/L)	k_1 (L/mg-day)[1]	T_c (μg/mg)	T_y (μg/mg)[1]	Reference
Pseudomonas cepacia G4, phenol	0.66–6.6	None	26–28	1.5	0.39	3.8	34		a, b
Pseudomonas cepacia G4, toluene	0–10	Toluene	28	0.94	0.79	1.2		14	h
Pseudomonas pudita, toluene	2.6	Toluene	30			0.19	5.2		n
Pseudomonas pudita B2 & TVA8, toluene	5	Toluene	28	1.3	6.4	0.20			o
Mixed culture, chemostat, phenol	0.1	None	23			0.012–0.48	>15		e
Mixed culture, chemostat, phenol	1–20	None	20	0.21	2.04	0.10	3.1	1.7	f
Mixed culture, chemostat, phenol	20	Phenol	20				3.4	1.9	f
Mixed culture, chemostat, phenol	27	None	21				240	110	j
Mixed culture, chemostat, phenol	1–25	None	20	0.33	11	0.030	82	52–222	m
Mixed culture, chemostat, toluene	1–30	None	20	0.17	8.64	0.020	7.3	2.1	f
Mixed culture, chemostat, toluene	30	Toluene	20				8.5	2.5	f
Mixed culture, biofilm, phenol	0.1–2	None	23	0.038–0.15	0.23–0.67	0.055–0.18	13–29		c, d
Mixed culture, biofilm, toluene	40–135	Toluene	20	0.38	0.17	2.2			g
Mixed culture, soil slurry, phenol	0.66	Phenol	20			0.08–0.13			i
Mixed culture, soil slurry, toluene	0.66	Toluene	20			0.07–0.15			i
Mixed culture, semi-batch, phenol	25	None	20	0.18			>510		k
Mixed culture, microcosm, phenol		Phenol	–					170	l
Average[2]				0.52	3.4	0.56	86	64	
Standard deviation				0.53	4.2	1.0	160	84	

a. Folsom et al. 1990; b. Folsom & Chapman 1991; c. Segar 1994; d. Segar et al. 1995; e. Speitel et al. 1990; f. Chang & Alvarez-Cohen 1996; g. Arcanlegi & Arvin 1997; h. Landa et al. 1994; i. Jenal-Wanner & McCarty 1997; j. Hopkins et al. 1993; k. Bielefeldt et al. 1995; l. Tovanabootr et al. 1997; m. Shurtliff et al. 1996; n. Heald & Jenkins 1994; o. Kelly et al. 2000.

[1] Biomass reported in mg dry cell mass; units conversions assume dry cell mass is 50% protein. [2] Average and standard deviation computed by ignoring ">" signs and by assuming a range can be approximated by taking one sample value at the top and one at the bottom of the range.

Table 3. Selected kinetic coefficients for TCE cometabolism by other bacteria

Organism/condition	Initial TCE conc. (mg/L)	Additional substrate	Temperature (°C)	k_c (mg/mg-day)[1]	K_{Sc} (mg/L)	k_1 (L/mg-day)[1]	T_c (μg/mg)	T_y (μg/mg)[1]	Reference
Mixed culture, chemostat, propane	0.5–16	None	20	0.45	5.22	0.086	6.5	5.6	a
Mixed culture, chemostat, propane	16	Propane	20				13.9	11.9	a
Mycobacterium vaccae JOB5, propane		None	30	0.057	0.58	0.098			b
Mycobacterium vaccae JOB5, propane	0.03–5.4	Propane	25			0.014			c
Mixed culture, chemostat, propane	3	None	20	0.038	0.6	0.064			d
Nitrosomonas europaea, ammonia	0–3.3	Ammonia	22	1.0	1.4	0.74	8		e
Nitrosomonas europaea, ammonia	2.1	Ammonia	22	1.6	1.6	1.02	13		f
Rhodococcus erythropolis BD1, isopropylbenzene	3.3–26	None	30			0.018			g

a. Chang & Alvarez-Cohen 1995a; b. Wackett et al. 1989; c. Wilcox et al. 1995; d. Keenan et al. 1994; e. Ely et al. 1995b; f. Ely et al. 1997; g. Dabrock et al. 1992.
[1] Biomass reported in mg dry cell mass; units conversions assume dry cell mass is 50% protein.

Bacteria growing on simple aromatic chemicals (e.g., phenol, toluene) are the second most widely studied group of organisms for cometabolizing chlorinated solvents. Non-specific mono- or dioxygenases that initiate the degradation of the aromatic chemicals are responsible for chlorinated solvent cometabolism (Nelson et al. 1986; Wackett et al. 1988; Zylstra et al. 1989; Shields et al. 1989; Folsom et al. 1990; Harker & Kim 1990; Dabrock et al. 1992; Segar 1994; Olsen et al. 1994; Newman & Wackett 1997). Kinetic parameters for TCE cometabolism by aromatic degraders are listed in Table 2. As with the methanotrophs, a pure culture, *Pseudomonas cepacia* G4, has shown the most rapid degradation kinetics, with a k_1 for TCE that is comparable to that of *M. trichosporium* OB3b. All mixed cultures reported except one have much smaller k_1 values, which are comparable to mid-range values reported for methanotrophic mixed cultures. Although the k_1 values for aromatic degraders and methanotrophs are similar, the maximum cometabolic substrate degradation rate (k_c) tends to be smaller for aromatic degraders relative to methanotrophs. Measured transformation capacities for aromatic degraders vary widely, ranging from 3 to >500 μg TCE/mg cells, suggesting considerable diversity among these organisms in their ability to withstand the toxic intermediates produced during TCE cometabolism. This result is not surprising given the great diversity that exists among aromatic oxygenases (a variety of known mono-oxygenases as well as dioxygenases) and the microorganisms that express them. In fact, working with a filamentous phenol-degrading enrichment, Bielefeldt et al. (1995) showed no toxic effects from intermediates up to the maximum loading studied of 510 μg TCE/mg cells. The transformation yields for aromatic degraders also vary more widely than with methanotrophs. Reported values range over two orders of magnitude, while the range for methanotrophs is generally over one order of magnitude.

Organisms other than methanotrophs and aromatic degraders have been evaluated for TCE cometabolism; however, very few kinetic studies with other organisms have been reported. The available data are reported in Table 3. The kinetic coefficients for the propane and isopropylbenzene degraders are comparable to those for mixed cultures of aromatic degraders. The limited data for nitrifiers show their kinetics to be somewhat faster and comparable to those of the pure cultures of aromatic degraders. The relatively small transformation capacities reported for these cultures,

Table 4. Selected kinetic coefficients for cometabolism of chlorinated solvents other than TCE

Organism/condition	Initial solvent conc. (mg/L)	Additional substrate	Temperature (°C)	k_c (mg/mg-day)[1]	K_{Sc} (mg/L)	k_1 (L/mg-day)[1]	T_c (μg/mg)	T_y (μg/mg)[1]	Reference
***trans*-Dichloroethylene**									
M. trichosporium OB3b, sMMO	0.5–24	Formate	30	46.2[a]	14.4[a]	3.2[a]	776[b]		a,b
M. trichosporium OB3b, PP358, sMMO	0.05–37	Formate	23	24.8	6.4	3.9	497		c
M. trichosporium OB3b, pMMO	0.4–12	Formate	30			1.3			d
Mixed culture, pMMO	1	Methane	20	>4.2	0.17	>25	3400	4600	e, f
Mixed culture, batch, methane		Methane				0.0062			m
Mixed-culture, chemostat, methane	60	Formate	20				490		g
Mixed culture, biofilm, phenol	1.2	None	23	0.015–0.043	0.066	0.23–0.65	5.3–11.2		h
***cis*-Dichloroethylene**									
M. trichosporium OB3b, sMMO	0.5–24	Formate	30	25.4[a]	2.9[a]	8.8[a]	252[b]		a, b
M. trichosporium OB3b, PP358, sMMO	0.05–5.6	Formate	23	9.5	1.1	8.6			c
M. trichosporium OB3b, pMMO	0.4–12	Formate	30			0.09			d
Mixed-culture, chemostat, methane	40	Formate	20				250		g
Mixed culture, biofilm, phenol	1.2	None	23	0.8–1.0	0.99	0.8–1.0	138–143		h
Mixed culture, semi-batch, phenol	25	None	20	0.27–1.5			>260		o
1,1-Dichloroethylene									
M. trichosporium OB3b, sMMO	0.5–24	Formate	30	0.84[a]	0.49[a]	1.7[a]	34.9[b]		a, b
M. trichosporium OB3b, PP358, sMMO	0.01–3.4	Formate	23	>7.5	>3.4	2.0–2.7	34.9		c
M. trichosporium OB3b, pMMO	0.4–12	Formate	30			<0.04			d
Mixed-culture, chemostat, methane	0.7	Formate	20				10	4	g
Mixed culture, biofilm, phenol	0.08–1.1	None	23	0.016–0.085	0.006	2.7–14	0.44–6.0		h
Nitrosomas europaea, ammonia	0.097–0.74	Ammonia	22	1.0	0.89	1.13	2.3–4.4		n
Mixed culture, butane	0–3.9	None	20	3.0	0.15	21			p
Vinyl chloride									
M. trichosporium OB3b, sMMO	0.25–7.5	Formate	30			11[d]	700[b]		b, d
M. trichosporium OB3b, pMMO	0.25–7.5	Formate	30			2.7			d
Mixed culture, chemostat, methane	22	Formate	30	5.7	3.6	1.6	368		b
Mixed-culture, chemostat, methane	16	Formate	20				140	50	g
Mixed culture, microcosm, methane			20					1000–3500	g
Mixed culture, biofilm, methane	5.7	None	20	0.025				25	i

Table 4. (Continued)

Organism/condition	Initial solvent conc. (mg/L)	Additional substrate	Temperature (°C)	k_c (mg/mg-day)[1]	K_{Sc} (mg/L)	k_1 (L/mg-day)[1]	T_c (μg/mg)	T_y (μg/mg)[1]	Reference
Chloroform									
M. trichosporium OB3b, sMMO	0.6–30	Formate	30			1.9[d]	99[b]		b, d
M. trichosporium OB3b, PP358, sMMO	0.13–15	Formate	23	3.1	3.1	1.0	101		c
M. trichosporium OB3b, pMMO	0.5–14	Formate	30			<0.04			d
Mixed culture, batch, methane		Methane				0.014			m
Mixed culture, chemostat, methane	22	None	21	0.84	1.5	0.56	8.3		k
Mixed culture, biofilm, phenol	1.2	None	23			0.0031			h
Nitrosomonas europaea, ammonia	0.65–1.6	Ammonia	22	10	32	0.32	11–18		n
1,1,1-Trichloroethane									
M. trichosporium OB3b, sMMO	0.6–30	Formate	30	4.6	28.5	0.16[a]	7[b]		a, b
Mixed culture, batch, methane	4.5	Methane	–			0.0021			j
Mixed culture, batch, methane		Methane				0.0013			m
Mixed culture, biofilm, phenol	1.3	None	23			0.0064			h
Mixed culture, chemostat, propane	0–20	None	20			0.074			l
Mixed culture, butane	0–40	None	20	0.61	1.6	0.38			p
1,2-Dichloroethane									
M. trichosporium OB3b, sMMO	0.5–25	Formate	30	9.3	7.6	1.2[a]	2900[b]		a,b
M. trichosporium OB3b, pMMO	0.4–12	Formate	30			<0.04			d
Mixed culture, biofilm, phenol	1.0	None	23			0.0005			h
Nitrosomonas europaea, ammonia	2.5–140	Ammonia	22	36	99	0.37	350–∞		n

a. Oldenhuis et al. 1991; b. Chang & Alvarez-Cohen 1996; c. Aziz et al. 1999; d. van Hylckama Vlieg et al. 1996; e. Anderson & McCarty 1996; f. Anderson & McCarty 1997; g. Dolan & McCarty 1995b; h. Segar 1994; i. Nelson & Jewell 1993; j. Strand et al. 1990; k. Alvarez-Cohen & McCarty 1991d; l. Keenan et al. 1994; m. Leeson & Bouwer 1989; n. Ely et al. 1997; o. Bielefeldt et al. 1995; p. Kim 2000. [1] Biomass reported in mg dry cell mass; units conversions assume dry cell mass is 50% protein.

however, may make application of these organisms in engineered systems problematic.

The kinetics of cometabolism have been studied to a much smaller extent for chlorinated solvents other than TCE. With the exception of two studies with phenol degraders, one each with propane degraders, butane degraders, and with nitrifiers, all the research has been conducted with methanotrophs. The kinetic data for chlorinated solvents other than TCE are listed in Table 4.

Cometabolic oxidation of the three dichloroethylene (DCE) isomers occurs, although some significant differences among the isomers exist. For the phenol degraders and *M. trichosporium* OB3b producing sMMO, *cis*-DCE is degraded more rapidly than *trans*-DCE, which has a k_1 comparable to that of TCE. *trans*-DCE, however, is degraded much more rapidly than *cis*-DCE with *M. trichosporium* OB3b producing pMMO. A mixed methanotrophic culture producing pMMO also showed rapid *trans*-DCE kinetics. For *M. trichosporium* OB3b producing sMMO, the kinetics of 1,1-DCE are slower than for the other two DCE isomers, although k_1 is still quite large (Table 4). For the phenol degraders, k_1 for 1,1-DCE is substantially greater than the k_1 for the other two DCE isomers. The kinetic coefficients for 1,1-DCE degradation with nitrifiers were comparable to those of the methanotrophs and phenol degraders, while the most rapid kinetics among all organisms were observed for a mixed culture of butane degraders. The transformation capacity for 1,1-DCE is quite small, however, for all organisms, indicating that 1,1-DCE oxidation generates significant product toxidicty. In contrast, the transformation capacity for *trans*-DCE is quite large, while that of *cis*-DCE is comparable to or somewhat greater than that of TCE.

Kinetic data on vinyl chloride cometabolism is only available for methanotrophs. In general, vinyl chloride is rapidly cometabolized by both sMMO and pMMO with large transformation capacities and yields. The one report of relatively slow kinetics (Nelson & Jewell 1993) was for an expanded-bed, biofilm reactor operating at a high biomass concentration. A considerable amount of this biomass may have been inactive, which would produce a low apparent rate constant.

Chloroform is cometabolized quite rapidly by sMMO-producing methanotrophs, with k_1 on the order of 30–60% of that observed with TCE (Table 4). The transformation capacity likewise is similar to that of TCE. Very slow kinetics are associated with pMMO-producing methanotrophs and phenol degraders. The results with phenol degraders are typical of aromatic degraders, which in general cometabolize only chlorinated ethenes at appreciable rates (Segar 1994). The k_1 for the nitrifiers was 15–30% and the transformation capacity was 10–20% of that measured with *M. trichosporium* OB3b.

1,1,1-trichloroethane (1,1,1-TCA) is cometabolized very slowly, if at all, by oxygenase expressing organisms. Kinetic coefficients for *M. trichosporium* OB3b are reported in Table 4; however, some studies have observed no degradation of 1,1,1-TCA by *M. trichosporium* OB3b, so the performance of this organism on 1,1,1-TCA probably should be considered inconsistent (Aziz et al. 1999). Likewise, Semprini & McCarty (1992) observed no transformation of 1,1,1-TCA in methanotrophic field studies at Moffett Field. The transformation capacity for 1,1,1-TCA also is quite small. Limited data for propane degraders suggest that they may perform better than methanotrophs, but degradation rates still may be quite slow. The most rapid 1,1,1-TCA degradation kinetics have been reported with a mixed culture of butane degraders.

In contrast to 1,1,1-TCA, sMMO-producing *M. trichosporium* OB3b is much more active with the less heavily chlorinated 1,2-dichloroethane (1,2-DCA). The transformation capacity is also very large. As with 1,1,1-TCA, pMMO producing *M. trichosporium* OB3b and phenol degraders are unable to cometabolize 1,2-DCA at appreciable rates. As with 1,1-DCE, the k_1 for nitrifiers was approximately 30% of that measured for *M. trichosporium* OB3b.

Although detailed kinetic studies were not conducted, Vannelli et al. (1990) reported that *Nitrosomonas europaea* could cometabolize all the chlorinated solvents listed in Table 4. Very limited data suggest degradation rates relative to TCE ranging from 33% for 1,1,1-TCA to 850% for vinyl chloride. Degradation rates for the other chlorinated solvents listed in Table 4 were within ±50% of that measured for TCE. Rasche et al. (1991) reported that all the chlorinated solvents listed in Table 4 produced intermediates that were toxic to *Nitrosomonas europaea*, but the transformation capacity for each chemical was not quantified.

Discussion

Although a large amount of recent research has expanded our understanding of the kinetics of aerobic chlor-

inated solvent cometabolism, a number of knowledge gaps still exist that should be addressed. The following is a discussion of several of the most compelling knowledge gaps in this area followed by an evaluation of approaches to scale-up and field applications of the presented models for *in situ* bioremediation of chlorinated solvents.

Competitive inhibition constants

The use of half saturation constants (K_{sc} and K_{sg}) as estimates for the inhibition constants (K_{isc} and K_{isg}) in the saturation kinetic expressions describing competitive inhibition (Equations (4) and (5)) is currently under debate. Although this practice is mechanistically correct when dealing with pure enzyme kinetics with appropriate assumptions, it may not be applicable for whole cell kinetics due to the transport issues and other cell dynamics that may be involved (Blanch & Clark 1996). A number of studies with methane oxidizers and ammonia oxidizers have shown that solvent degradation kinetics can be adequately predicted using this substitution (Strand et al. 1990; Alvarez-Cohen & McCarty, 1991d; Chang & Alvarez-Cohen, 1995b; Broholm 1992; Anderson & McCarty 1994; Hyman et al. 1995; Ely et al. 1995a, 1995b; Aziz et al. 1999), while a study with methane oxidizers and one with toluene degraders have shown the substitution to be inadequate (Chang & Criddle 1997; Landa et al. 1994). Systematic research specifically addressing the adequacy of this substitution for the various oxygenase enzyme systems would be helpful for improving modeling approaches when both primary and cometabolic substrates are present.

The nature of product toxicity and cell recovery

We still lack a clear understanding of the specific nature of chlorinated solvent induced product toxicity and the capability for cells to recover from these toxic effects. That is, we do not know whether individual cells are capable of recovering from chlorinated solvent induced product toxicity or whether the synthesis of new cells is required. A number of studies have shown that cell damage caused by product toxicity is clearly not limited to the responsible enzymes alone, but is of a more general nature, affecting general cellular metabolism (Oldenhuis et al. 1991; Alvarez-Cohen & McCarty 1991d; Rasche et al. 1991; Heald & Jenkins 1994; Hyman et al. 1995; Fitch et al. 1996; van Hylckama Vlieg et al. 1997; Chu & Alvarez-Cohen 1998). Because of this, it would logically follow that some cells will be capable of recovery while others will not, and that the extent of toxicity will dictate the ratio of these two. Studies with the ammonia oxidizer *Nitrosomonas europaea*, suggested that recovery from toxicity involves de novo protein synthesis but does not require growth of new cells (Rasche et al. 1991). In fact, the model developed by Ely et al. (1995a) to describe cometabolic oxidations by this organism did not include a cell growth expression, but did contain enzyme recovery expressions as described above (Equations (7), (8), (12) and (13)). Although it may be that cell recovery requires less energy than cell regrowth, distinguishing between these two processes in a practical manner is problematic at best. However, from a modeling perspective it may not actually matter whether enzyme recovery within specific cells is occurring or whether new cells are being generated since both processes require consumption of growth substrate. That is, as long as the stoichiometric ratio of growth substrate and cometabolic substrate concentrations required to sustain long-term cometabolic reactions can be measured for a specific culture of interest, it is not important whether recovery or regrowth occurs (see Equation (16)). In fact, the mathematical similarity between the model that explicitly incorporates cell recovery and the model that incorporates cell regrowth verify this (Equations (14) and (15)). In application, sustained cometabolism of chlorinated solvents will be most reliably achievable by operating at solvent loadings that are below the ratio of growth and cometabolic substrate concentrations required to balance product toxicity with enzyme recovery or cellular growth (Equation (16)), in order to ensure that a significant proportion of active cells are always available (Ely et al. (1997), Chang & Alvarez-Cohen (1995)).

In contrast to the potential for cell recovery, the issue of whether chlorinated solvent product toxicity causes linearly proportional or exponential cell inactivation is one that needs to be resolved from a modeling point of view. However, it must be recognized that a wide range of organisms with widely differing oxygenase enzymes cometabolically oxidize chlorinated solvents. Consequently, this issue may be resolved differently for differing systems.

A number of studies conducted with methanotrophs have reported linearly proportional inactivation of cells due to chlorinated solvent oxidation (Alvarez-Cohen & McCarty 1991b; Oldenhuis et al. 1991; Anderson & McCarty, 1994, 1996; Ely et al. 1995b; Chang & Alvarez-Cohen, 1995b; Chang & Criddle

1997), including a recent study that specifically measured methanotrophic inactivation due to TCE degradation using three individual activity assays: methane uptake, naphthalene oxidation, and respiratory activity (Chu & Alvarez-Cohen 1998). In addition, linearly proportional enzyme inactivation was reported in studies with *Nitrosomonas europaea* (Ely et al. 1995b).

In contradiction, two studies have reported exponential inactivation due to chlorinated solvent oxidation by methanotrophs. In work with *M. trichosporium* OB3b, van Hylckama Vlieg et al. (1997) suggested that although cell activity, as measured by chlorinated solvent oxidation rates, followed a linearly proportional decrease with the amount of chlorinated solvent degraded, cell *viability*, as measured by plate counts, followed an exponential decrease with the amount degraded. It is difficult to reconcile a linear decrease in cell "activity" with an exponential decrease in "viability". These results are perhaps an artifact of the substantial difficulty associated with enumerating methanotrophic viability using plate counts (Hanson & Hanson 1996). Tompson et al. (1994) showed that both linear and exponential inactivation models adequately fit their experimental data describing the degradation of TCE by resting cells of *M. trichosporium* OB3b during two-week experiments. Due to the longevity of the experiments and the use of resting cells, measured cell inactivation was most likely not solely due to toxicity, but also due to starvation, deprivation of reductant, and endogenous cell decay.

Methods for incorporating energy limitations into cometabolism models

The problem of incorporating reducing energy limitations into cometabolism models is more subtle than the inhibition and toxicity issues discussed above. In fact, when cometabolism occurs in the presence of sufficient growth substrate, the issue of reducing energy limitation is not important since growth substrate metabolism provides sufficient reducing energy to drive the cometabolic reaction. So, only in cases of low or no growth substrate availability is the issue of kinetic limitation due to energy generation important. The four approaches that have been introduced to address this issue (Criddle 1993; Saez & Rittmann 1993; Chang & Alvarez-Cohen 1995b; and Arcangeli & Arvin 1997) are all based upon the assumption that growth substrate is capable of generating reductant which is a required reactant in the cometabolic oxidations. The Saez & Rittmann (1993) model also allows for reductant generation from biomass oxidation. The major difference in the models lies in their implementation. Both the Criddle (1993) and Saez & Rittmann (1993) models utilize a stoichiometric transformation yield that couples the degradation of growth or energy substrates to the degradation rate of cometabolic substrate, while the Chang & Alvarez-Cohen (1995b) model and the Arcangeli & Arvin (1997) model are based upon a mechanistic analysis of the enzyme reaction with reducing energy or growth substrate as potential limiting substrates, respectively. Each of these models is capable of describing cometabolic degradations in the presence of growth substrates, and all but the Arcangeli & Arvin (1997) model are capable of predicting cometabolic degradation in the absence of growth substrate. The major limitations associated with all four models, however, are the large number of modeling parameters required, some of which are easily measured in laboratory experiments and some of which must be estimated by curve fitting. Fortunately, each of the models can be simplified to forms that require measurement of fewer parameters and that are more suitable to field applications. Although some of the simplified forms of these models are mathematically indistinguishable, further research with respect to the specific applicability of these kinetic models and their intricacies with respect to the various oxygenase systems would be helpful for improving our design of bioremediation processes.

Additional kinetic information

Additional kinetic studies on organisms other than methanotrophs would be useful for more clearly delineating the cometabolic capabilities of the different oxygenase systems. The paucity of kinetic data on nitrifiers, propane degraders, and butane degraders especially limits a complete assessment of these organisms and our ability to predictively model their growth and degradative behavior.

With respect to chemicals, the primary focus on TCE has provided extensive data on this compound, as well as a bench mark for comparing the kinetics of various cultures. More kinetic data on chemicals other than TCE is now needed to provide a fuller understanding of the capabilities of aerobic cometabolism. In particular, a focus on solvents that methanotrophs do not cometabolize well (e.g., 1,1,1-TCA) would be helpful.

Applicability of modeling approaches to scale-up and field applications

deBlanc et al. (1996a) reviewed and summarized the various subsurface biodegradation models available. Of the models developed before 1995, several are able to account for cometabolism and competitive inhibition, but not transformation capacity and reducing energy availability. deBlanc et al. (1996b) developed a three-dimensional, multiphase-flow model that includes the transformation capacity and reducing energy kinetic expressions of Chang & Alvarez-Cohen (1995b) (Equations (21) and (22)), as well as the more simplified kinetic expressions discussed above (Equations (1)–(5), (10) and (11)). Travis & Roberts (1997) incorporated competitive inhibition and product toxicity into a multiphase transport model in order to describe the in situ bioremediation of TCE by methanotrophs at DOE's Savannah River site. Application of their model suggested that significant TCE degradation was occurring and that product toxicity and predation of methanotrophs by protozoa were important limiting factors of bioremediation.

The kinetic expression appropriate for a given modeling effort is a function of the goals of the modeling, chemical concentrations, data availability, and computational resources. Initial feasibility testing might be conducted using simple kinetic expressions and typical values of kinetic coefficients from the literature. A full-scale remediation design, however, requires a much greater level of effort to ensure that the modeling provides useful information. Samples should be collected from the field so that kinetic measurements can be extrapolated directly from field measurements when possible, or if necessary, from laboratory column experiments or microcosm experiments conducted with field samples.

Sensitivity analyses to judge the significance of individual kinetic parameters and to select appropriate kinetic expressions also can be very helpful. For example, low concentrations of chlorinated solvents might permit the use of pseudo-first-order kinetics, while ignoring competitive inhibition and transformation capacity terms, or less complicated kinetic expressions may be acceptable as time or distance from the source increase. Verifying that laboratory-measured kinetic coefficients are representative of field conditions can be quite difficult because of subsurface heterogeneities and the resulting flow field complexities, which again indicates the importance of sensitivity analyses.

The kinetic expression selected can also affect computation times significantly; therefore, the level of complexity should be appropriate to the circumstances. For example, the computation time might be as much as an order of magnitude greater in a multi-chemical modeling effort if transformation capacity and reducing energy concentration need to be accounted for, versus pseudo-first-order kinetics (deBlanc 1998). In addition, the more sophisticated kinetic expressions can complicate the modeling effort under some conditions. For example, the specific enzyme inactivation coefficient (k_{inact} in Equations (12) and (13)), the stoichiometric transformation yield (T'_y in Equations (18) and (19)), and the half-saturation constant of reducing energy (K_R in Equation (21)) are intrinsic properties of specific cells. If the model permits movement of biomass in the subsurface, either through detachment or bioaugmentation, both the biomass concentration and these properties of various components of the biomass may have to be tracked with time and position, thereby greatly increasing the complexity of the modeling effort.

In summary, our knowledge of the kinetics governing the aerobic cometabolism of chlorinated solvents has been significantly enriched over the past 15 years because of a large amount of basic and applied research. However, we still have an incomplete understanding of how to most effectively model these complex biological reactions in environmental applications. Additional research with respect to modeling approaches, kinetic coefficients, and scale-up methods would improve our abilities to predict the in situ bioremediation of chlorinated solvents by aerobic cometabolism.

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