AFRL-ML-TY-TR-1998-4602

KINETICS OF AEROBIC COMETABOLISM OF CHLORINATED SOLVENTS

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Contract No. F41624-94-D-8055-0204

July 1998

AIR FORCE RESEARCH LABORATORY
MATERIALS AND MANUFACTURING DIRECTORATE

■ **Air Force Materiel Command** ■ **United States Air Force** ■ **Tyndall Air Force Base, FL 32403-5323**

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

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1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
31-JUL-1998	Thesis	30-JUN-1995 -- 30-JUN-1998

4. TITLE AND SUBTITLE

Kinetics of Aerobic Cometabolism of Chlorinated Solvents

5a. CONTRACT NUMBER

F41624-94-D-8055-0204

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

2103W504

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Air Force Research Laboratory
Materials and Manufacturing Directorate
Airbase Technologies Division
139 Barnes Drive, Suite 2
Tyndall Air Force Base, FL 32403-5323

10. SPONSOR/MONITOR'S ACRONYM(S)

AFRL/MLQL

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

AFRL-ML-TY-TR-1998-4602

12. DISTRIBUTION/AVAILABILITY STATEMENT

Distribution A: Approved for public release; distribution unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT

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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 Michaelis-Menton/Monod half saturation constants (K_{sc} and K_{sg}) as estimated for inhibition constants (K_{isc} and K_{isg}) in 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 generation 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.

15. SUBJECT TERMS

aerobic cometabolism, microorganisms

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Alison Thomas
U	U	U	UU	39	19b. TELEPHONE NUMBER *(Include area code)*

Reset

Standard Form 298 (Rev. 8/98)
Prescribed by ANSI Std. Z39.18

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

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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* remediation. 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 intracellular concentrations of key reaction species.

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 involve oxygenase enzymes, molecular oxygen, and a source of reducing equivalents, typically NAD(P)H (see previous paper in this series by Arp et al.). Following the initial oxygenase reaction, chlorinated solvent oxidation products 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 & Chapman 1990; Harker and 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, the cometabolic degradation of chlorinated solvents by oxygenase-expressing microorganisms may be a function of enzyme inhibition by growth or other cometabolic substrates, chlorinated solvent product toxicity, and reducing energy or NAD(P)H regeneration.

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). Consequently, although cometabolic oxidations can be promoted by the simultaneous degradation of growth substrate by the oxygenase enzyme,

competition between the growth substrate and the cometabolic substrate can result in overall decreased transformation rates of both substrates (competitive inhibition). 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 and McCarty 1990; Strand et al. 1990; Saéz and Rittmann 1991; Broholm et al. 1990; Chang et al. 1993; Speitel et al., 1993; Keener and Arp 1993; Malachowsky et al., 1994; Hyman et al., 1995; Chang and Alvarez-Cohen, 1995b). Further, enzyme competition may occur during the simultaneous degradation of multiple cometabolic substrates, resulting in decreased degradation rates for each compound (Alvarez-Cohen And McCarty, 1991d; Palumbo et al., 1991; Segar and Speitel, 1995; Dolan and McCarty,-1995b; Chang and Alvarez-Cohen, 1997; Aziz, 1997).

Reducing energy consumption

Oxygenase enzymes consume molecular oxygen and NAD(P)H 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 NAD(P)H during subsequent metabolic steps (Anthony, 1982; Dalton and Higgins, 1989), while cometabolic substrates such as chlorinated organics do not (Figure 1b) (Wackett and Gibson, 1988; Dalton and Stirling, 1982). Consequently, the rate and quantity of cometabolic transformation reactions occuring in the absence of primary substrate may be limited by the availability of reducing energy in the form of NAD(P)H. Conversely, both the rate and quantity of cometabolic reactions can be enhanced in the presence of growth or energy-producing substrates due to the regeneration of NAD(P)H.

The simultaneous availability of primary substrate can be beneficial during cometabolism from the viewpoint of regenerating NAD(P)H, 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 NAD(P)H 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 and Alvarez-Cohen, 1995a).

Some oxygenase expressing cultures can regenerate NAD(P)H 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 and 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 NAD(P)H 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 biosynthesis cannot proceed with some alternate energy substrates and oxygenase enzyme levels may diminish in the absence of the 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 and Householder 1989; Alvarez-Cohen and McCarty 1991a,d; Henry and Grbic-Galic 1991; Oldenhuis et al., 1991; Stensel et al., 1992; Ensign et al., 1992; Heald and 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) as well as to general cellular constitiuents (Wackett and Householder, 1989; Alvarez-Cohen and McCarty, 1991d; Oldenhuis et al., 1991; Hyman et al., 1995; van Hylckama Vlieg et al.,1997). The attack of toxic products on the enzyme and/or cellular materials results in cell activity and viability that decreases in proportion to the amount of compound degraded (Oldenhuis et al., 1991; Alvarez-Cohen and McCarty, 1991a; Tompson et al., 1994; Fitch et al., 1996; van Hylcklama Vlieg et al., 1997; Chu and Alvarez-Cohen, 1998).

COMETABOLISM KINETICS

Modeling Approaches

Michaelis-Menten/Monod

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 approaches to cometabolic models involve simplifications and modifications to the Michaelis-Menten/Monod expression given here:

$$r_c = - \frac{k_c X S_c}{K_{sc} + S_c} \qquad (1)$$

where

r_c = rate of cometabolic reaction (μmol or mg/L/d)
S_c = cometabolic substrate concentration (μmol or mg/L)
k_c = maximum rate of cometabolic substrate degradation (μmol or mg S_c/mg cells/d)
X = active microbial concentration (mg cells/L)
K_{sc} = half-saturation constant for S_c (μmol or mg/L)

Pseudo first order

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

$$r_c = -k_1 X S_c \qquad (2)$$

where

k_1 = pseudo-first-order cometabolic degradation rate constant (L/mg cells/d)

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 when these two parameters cannot be determined independently because of toxicity problems associated with the high concentrations needed to achieve saturation kinetics (Anderson and McCarty, 1996, Smith and McCarty, 1996?, Alvarez-Cohen and 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.

Transformation capacity and yield

Product toxicity associated with cometabolic oxidations has been observed to cause cell activity to decrease in proportion to the amount of compound degraded. This phenomenon can be quantified by a transformation capacity (T_c) term, a constant representing the amount of compound degraded divided by the amount of cells inactivated (Alvarez-Cohen and McCarty, 1991b):

$$\frac{dS_c}{dX} = T_c \qquad (3)$$

where:

T_c = transformation capacity for the cometabolic substrate (μmol or mg S_c/mg cells)

dS_c = change in cometabolic substrate concentration during the reaction (μmol or mg/L)

dX = cells inactivated during the cometabolic reaction (mg/L)

A related term, the transformation yield (T_y), is 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) multiplied by the transformation capacity (Alvarez-Cohen and McCarty, 1991b). The linear representation of product toxicity can be incorporated into modified Michaelis-Menten/Monod kinetics to describe cometabolic reaction rates occuring 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 and Alvarez-Cohen, 1995):

$$\mu = \frac{r_x}{X} = Y \frac{r_g}{X} - \frac{1}{T_c} \frac{r_c}{X} - b \qquad (4)$$

where:

μ = net specific cellular growth rate (d^{-1})
r_x = net cellular growth rate (μmol or mg/L-d)
r_g = rate of growth substrate consumption (μmol or mg/L-d)
Y = cellular yield of growth substrate (mg cells/μmol or mg growth substrate),
b = cell decay rate (d^{-1}).

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

Ely et al. (1995a) introduced a model that incorporated the concept of a linear transformation capacity as proposed by others (Criddle, 1993; Chang and Alvarez-Cohen, 1995b) and also explicitly included a term representing the potential recovery of cells following inactivation. This model, however, does not include a mechanism for incorporating cell growth into the kinetic expression; therefore, their recovery term is equivalent to the cell yield term used for cell growth in the other models, making Ely's model mathematically identical to the other approaches. However, the explicit inclusion in this model of cell repair rather than cell regrowth in response to product toxicity is novel.

Tompson et al. (1994) used the general Michaelis-Menton/Monod expression modified with an exponential rather than linear toxicity effect to model TCE degradation by methanotrophs in porous media. That is, instead of describing the inactivation of cells due to product toxicity as a linear function of the transformation capacity as a given by equations 3 and 4 above, they used the following exponential cellular decay expression:

$$\frac{dS_c}{dX} = \frac{1}{vX} \qquad (5)$$

where:

v = a positive dimensionless constant

Multiple substrates (growth substrates and multiple cometabolic substrates)

The competitive inhibition between growth substrates and cometabolic substrates has been modeled by including competitive inhibition terms in the Michaelis-Menton/Monod expression for the degradation of the growth and cometabolic substrates respectively as follows (Broholm et al. 1992; Strand et al., 1990):

$$r_g = - X k_g \left(\frac{S_g}{K_{sg} (1+S_c/K_{isc}) + S_g} \right) \qquad (6)$$

$$r_c = - X k_c \left(\frac{S_c}{K_{sc} (1+S_g/K_{isg}) + S_c} \right) \qquad (7)$$

where:

S_g = growth substrate concentration (μmol or mg/L)
k_g = maximum rate of growth substrate degradation (μmol or mg S_g/mg cells/d)
K_{sg} = half saturation coefficient for S_g (μmol or mg/L)
K_{isg} = inhibition coefficient for S_g (μmol or mg/L)
K_{isc} = inhibition coefficient for S_c (μmol or mg/L)

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 growth or cometabolic substrate can be modeled 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 (Alvarez-Cohen, 1993).

A number of researchers have used the above expressions for competitive inhibition coupled with linear product toxicity to successfully model the degradation of chlorinated solvents in the presence of growth substrate by a number of oxygenase expressing organisms (Chang and Alvarez-Cohen, 1995b and 1997; Ely et al, 1995b; Smith et al., 1997, Chang and Criddle, 1997; Anderson and McCarty, 1994 and 1996).

Reducing Energy

The Michaelis-Menten/Monod expression for enzymatic degradation reactions 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), NAD(P)H, and molecular oxygen (Figure 1). Assuming for simplicity that molecular oxygen is present in excess, Chang and Alvarez-Cohen (1995b) proposed that the Michaelis-Menten/Monod expression be adjusted to incorporate the potential effects imposed by either substrate or NAD(P)H as a limiting reactant as follows:

$$r_s = - k X \left(\frac{S}{K_S + S}\right)\left(\frac{R}{K_R + R}\right) \qquad (8)$$

where:

r_s = rate of substrate degradation (μmol or mg/L-d)
k = maximum substrate degradation rate (μmol or mg substrate/mg cells/day),
S = growth substrate (S_g) or cometabolic substrate (S_c) concentration (μmol or mg/L),
K_S = half-saturation constant of substrate (μmol or mg/L),
R = reducing energy (NAD(P)H) electron equivalent concentration (mmol e^-/L),
K_R = half-saturation constant of reducing energy (NAD(P)H) (mmol e^-/L),

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

NAD(P)H can be regenerated in cells by means of growth substrate or energy substrate mineralization, utilization of internal energy storage polymers such as PHB, and/or endogenous metabolism. The following expression has been introduced for use with equation (8) in order to account for NAD(P)H consumption and regeneration over time during cometabolic reactions (Chang and Alvarez-Cohen, 1995b):

$$r_R = \alpha_g (-r_g) - \alpha_c (-r_c) \qquad (9)$$

where:

r_R = rate of reducing energy production or consumption (mmol e^-/L/d)
r_g = rate of growth or energy substrate consumption (μmol or mg/L/d),
r_c = rate of cometabolic reaction (μmol or mg/L/d)
α_g = net stoichiometric coefficient of NAD(P)H regeneration from the degradation of growth or energy substrate (mmol e^-/μmol S_r),
α_c = net stoichiometric coefficient of NAD(P)H consumption from the oxidization of S_c (mmol e^-/μmol S_c).

Criddle (1993) and Chang and Criddle (1997) introduced a cometabolic model that incorporated reducing energy considerations into a modification of the Michaelis-Menton/Monod kinetic model. The cometabolic model included competitive inhibition and a linear transformation capacity 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 consumption. The theoretical T_y' was incorporated into the cometabolic degradation expression as follows:

$$r_c = -(T_y' r_g + k_c) \frac{XS_c}{K_{sc} + S_c} \qquad (10)$$

where:

T_y' = stoichiometric transformation yield (μmol S_c/μmol S_g)

This equation was developed to account for the observed increase in cometabolic rates in the presence of a growth or energy substrate. $T_y^{'}$ 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 susbtrate consumption by the rate of growth substrate consumption: $T_y^{'} = r_c/r_g$. The model incorporating $T_y^{'}$ was used by Chang and Criddle (1997) to describe the degradation of TCE in the presence of methane by a mixed methane oxidizing culture.

Finally, Sáez and 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 endogenous metabolism as follows:

$$r_c = T_y^{'} r_g - \beta b X \qquad (11)$$

where:

β = mass of S_c oxidized per mass of biomass oxidized (μmol/μmol)

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

$$r_g = - \frac{k_g X S_g}{K_{sg} + S_g + \frac{S_g^2}{K_I}} \qquad (12)$$

where:

K_I = inhibition constant for S_g (μmol or mg/L)

This model also included competitive and non-competitive 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.

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 Monod maximum specific utilization rate (k_c), the Monod 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 and McCarty, 1991 a,b,d; Chang and Alvarez-Cohen, 1996) generally have the highest reported rate constants. *M. trichosporium* OB3b expressing pMMO has a substantially smaller rate constant, ranging from non-measureable to about 10% of that found with sMMO. Lontoh and 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 and Wittman, 1979); therefore, slow pMMO cometabolism rates would be expected.

The majority of known methanotrophs cannot produce sMMO (Murrell, 1992; Hanson and 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 and 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 power, illustrating the importance of adequate reducing power in cometabolism. The presence of the growth substrate, methane, in any appreciable concentration can cause a substantial decrease in the apparent k_1, because of enzyme competition (Speitel et al., 1993).

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 and 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 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. Correlations between T_c values and initial TCE degradation rates have generally not been observed for methanotrophs (Oldenhuis et al., 1991; Chang and Alvarez-Cohen, 1996; Smith et al., 1997; Chu and Alvarez-Cohen, 1998). In fact, a specific correlation between these two parameters should not be expected since Tc is a measure of the cumulative effects of product

toxicity while the initial TCE degradation rate is a kinetic measurement of enzyme efficiency. Even though enzyme efficiency can be significantly impacted by product toxicity, *initial* rate measurements should not reflect this and should therefore be independent of toxic effects.

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.

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. 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. 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, 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 with propane degraders, and one with nitrifiers, all the research has been conducted with methanotrophs. The kinetic data for chlorinated solvents other than TCE are listed in Table 4. Cometabolism of the three dichloroethylene (DCE) isomers is possible, 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. For all organisms, however, the transformation capacity for 1,1-DCE is quite small, indicating that 1,1-DCE cometabolism produces a very toxic intermediate. 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 and

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 to 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 to 30% and the transformation capacity was 10 to 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, 1997). Likewise, Semprini and 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. Although not listed in Table 4 because of the absence of published rate constants, several reports in the literature indicate that organisms growing on butane can cometabolize 1,1,1-TCA (Wilson et al., 1988; Kim et al., 1997).

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 *Nitrosomas europea* 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 *Nitrosomas europea*, 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 chlorinated 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 -

There is currently some debate about the use of Michaelis-Menton/Monod half saturation constants (K_{sc} and K_{sg}) as estimates for the inhibition constants (K_{isc} and K_{isg}) in the expressions describing competitive inhibition (equation 6 and 7). Although this practice is certainly appropriate when dealing with pure enzyme kinetics, it may not be applicable for whole cell kinetics due to the transport issues and other cell dynamics that may be involved. 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 and McCarty, 1991d; Chang and Alvarez-Cohen, 1995b; Broholm, 1992, Anderson and McCarty, 1994; Hyman et al., 1995; Ely et al., 1995a and 1995b, Aziz, 1997), while a study with methane oxidizers and one with toluene degraders have shown the substitution to be inadequate (Chang and 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

There is still not 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, it is not known 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 and McCarty, 1991d; Heald and Jenkins, 1994; Hyman et al., 1995; Fitch et al., 1996; van Hylckama Vlieg et al., 1997; Chu and 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. From a modeling point of view, it may not actually matter whether specific cells within a culture are recovering or whether new cells are being generated, since both processes require consumption of growth substrate. In fact, the mathematical similarity between the Ely et al. (1995a) model (which explicitly incorporates cell recovery) and the Chang and Alvarez-Cohen (1995b) model without the reducing energy considerations (which explicitly incorporates cell regrowth) verify this. However, it is not known whether the same amount of growth substrate is required for cell growth and cell recovery, so the amount of growth substrate consumption required to counter toxic effects may not be reliably estimated in the absence of direct experimental measurements. In application, it would follow that sustained cometabolism of chlorinated solvents could be achieved by operating at solvent loadings that are well enough below the governing tranformation capacities to ensure that some proportion of active cells are retained allowing both cell recovery and synthesis to occur.

In contrast to the potential for cell recovery, the issue of whether chlorinated solvent product toxicity causes linear or non-linear cell inactivation is one that needs to be resolved from

a modeling point of view. A large number of studies have reported linear inactivation of cells due to chlorinated solvent oxidation (Alvarez-Cohen and McCarty 1991b; Oldenhuis et al., 1991; Anderson and McCarty, 1994 and 1996; Ely et al. 1995b; Chang and Alvarez-Cohen, 1995b; Chang and 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 and Alvarez-Cohen, 1998).

In contradiction, two studies have reported non-linear cell decay due to chlorinated solvent oxidation. In work with methanotrophs, van Hylcklama Vlieg et al. (1997) suggested that although cell *activity* as measured by chlorinated solvent oxidation followed a linear decrease with the amount of chlorinated solvent degraded, cell *viability* as measured by plate counts followed an exponential decrease with degradation. 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 methanotrophs using plate counts (Hanson andHanson, 1996). Tompson et al. (1994) showed that both linear and exponential decay models adequately fit their experimental data describing the methanotrophic degradation of TCE by resting cells 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 NADH, and endogenous cell decay. Therefore, the mathematical simplicity of the linear decay model and its demonstrated utility under a variety of conditions strongly point toward it as the expression of choice for practical application of cometabolism models.

Methods for Incorporating Energy Generation into Cometabolism Models

The problem of incorporating reducing energy generation into cometabolism models is more subtle than the inhibition and toxicity issues discussed above. In fact, when comabolism occurs in the presence of sufficient growth substrate, the issue of reducing energy generation is not important since growth substrate metabolism provides reducing energy to drive the cometabolic reaction. So it is only in cases of low or no growth substrate availability that the

issue of kinetic limitation due to energy generation is important. The three approaches that have been introduced to address this issue (Criddle, 1993; Chang and Alvarez-Cohen, 1995b; and Saez and Rittmann, 1993) are all based upon the assumption that growth substrate, internal storage polymers and endogenous metabolism are capable of generating reducing energy in the form of NAD(P)H. The difference in the models lies mainly in their implementation. The Chang and Alvarez-Cohen (1995b) model is based upon a mechanistic analysis of the enzyme reaction and reducing energy as a potential limiting substrate while both the Criddle (1993) and Saez and Rittmann (1993) models utilize a stoichiometric transformation yield that couples the degradation of growth or energy substrate to the degradation rate of cometabolic substrate. Each of the three models is capable of describing cometabolic degradations in the presence of growth substrates. The major limitations associated with all three models, however, are the large number of modeling parameters required (8-9), some of which are easily measured in laboratory experiments and some of which must be estimated by curve fitting. Fortunately, each of the three 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 three 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. In particular, a focus on chemicals that methanotrophs do not cometabolize well (e.g., 1,1,1-TCA) would be helpful. 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 chemical, 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.

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 and Alvarez-Cohen (1995b) (Eq. 8 and 9), as well as the more simplified kinetic expressions discussed above (Eq. 1-4, 6 and 7). 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 kinetic measurements can be extrapolated directly from field measurements when possible, or if necessary, from laboratory column experiments or batch 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 psuedo-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 heterogenieties 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 psuedo-first-order kinetics (deBlanc, 1998). In addition, the more sophisticated kinetic expressions can complicate the modeling effort under some conditons. For example, the half-saturation constant of reducing energy (K_R in Eq. 8) and the stoichiometric transformation yield (T_y' in Eq. 9 and 10) are intrinsic properties of the biomass. If the model permits movement of biomass in the subsurface, either through detachment or bioaugmentation, both the biomass concentration and these properties of various fractions 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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Table 1. Selected Kinetic Coefficients for Methotrophic 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 and Semrau, 1998; e. Aziz, 1997; f. Fitch et al., 1996; g. Leeson and Bouwer, 1989: h. Strand et al., 1991; i. Arvin, 1991; j. Henry and Grbic-Galic, 1990; k.Alvarez-Cohen and McCarty, 1991b; l. Chu and Alvarez-Cohen, 1996; m. Chang and Alvarez-Cohen, 1996; n. Chang and Alvarez-Cohen, 1995a; o. Chang and Criddle, 1997; p. Henry and Grbic-Galic, 1991; q. Anderson and McCarty, 1996; r. Anderson and 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_c (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
Mixed culure, 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 culure, 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]				0.42	3.0	0.61	86	64	
Standard Deviation				0.49	4.3	1.1	160	84	

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

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_c (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
Nitrosomas euorpaea, ammonia	0 – 3.3	ammonia	22	1.0	1.4	0.74	8		e
Nitrosomas euorpaea, 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 and 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.

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_c (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 culure, 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 culure, 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 culure, biofilm, phenol	0.08 – 1.1	none	23	0.016 – 0.085	0.006	2.7 - 14	0.44 – 6.0		h
Nitrosomas euor paea, ammonia	0.097-0.74	ammonia	22	1.0	0.89	1.13	2.3-4.4		n
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

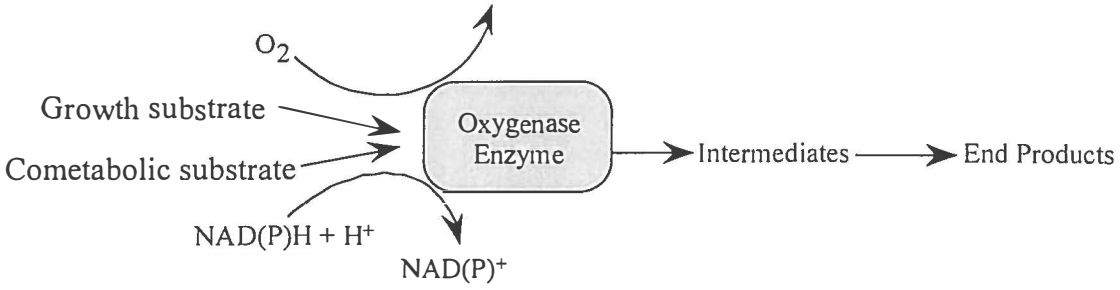
Table 4. (continued) Selected Kinetic Coefficients for Cometabolism of Chlorinated Solvents Other Than TCE

Organism/Condition	Initial Conc. (mg/L)	Additional Substrate	Temperature (°C)	k_c (mg/mg-day) [1]	K_c (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 culure, biofilm, phenol	1.2	none	23			0.0031			h
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 culure, biofilm, phenol	1.3	none	23			0.0064			h
Mixed culture, chemostat, propane	0-20	none	20			0.074			l
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 culure, biofilm, phenol	1.0	none	23			0.0005			h
Nitrosomas euorpaea, ammonia	2.5-140	ammonia	22	36	99	0.37	350-∞		n

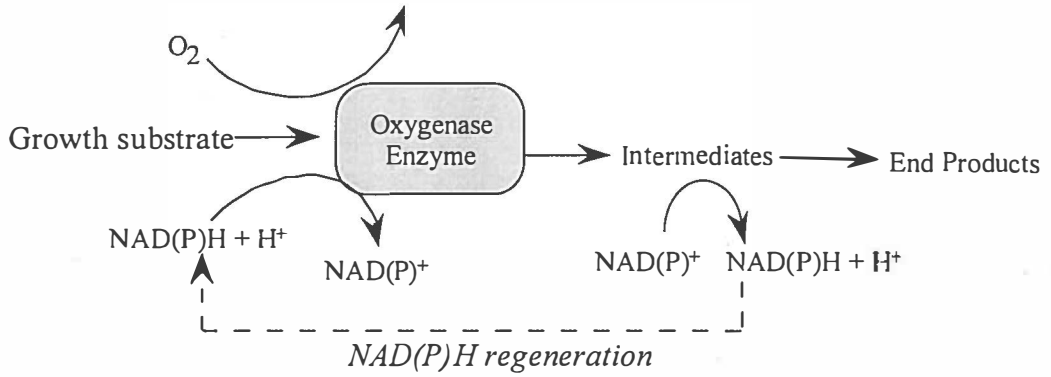
a. Oldenhuis, et al., 1991; b. Chang and Alvarez-Cohen, 1996; c. Aziz, 1997; d. van Hylckama Vlieg et al., 1996; e. Anderson and McCarty, 1996; f. Anderson and McCarty, 1997; g. Dolan and McCarty, 1995b; h. Segar, 1994; i. Nelson and Jewell, 1993; j. Strand et al., 1990; k. Alvarez-Cohen and McCarty, 1991d; l. Keenan et al., 1994; m. Leeson and Bouwer, 1989; n. Ely et al., 1997; o Bielefeldt et al., 1995.

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

a) Competitive Inhibition:



b) NAD(P)H consumption and regeneration:



c) Product Toxicity:

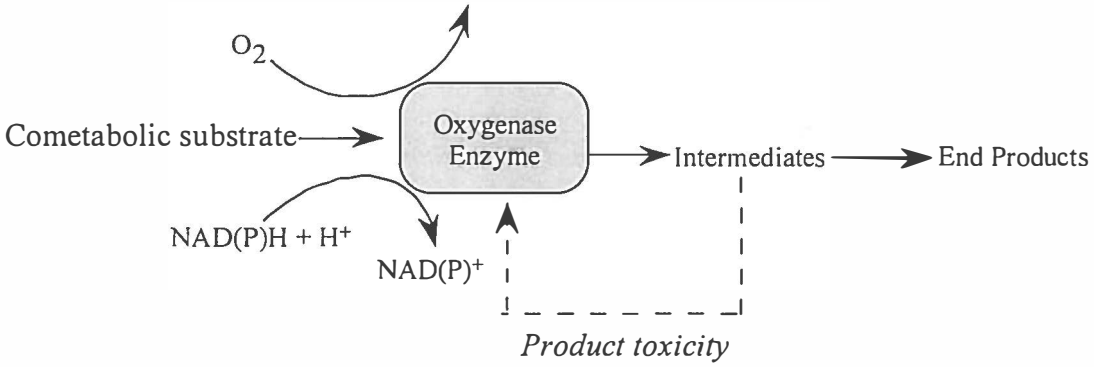


Figure 1. Generic oxygenase enzyme reactions illustrating: a) competitive inhibition between growth and cometabolic substrates, b) reducing energy (NAD(P)H) consumption and regeneration during growth substrate metabolism, and c) product toxicity exerted by transient intermediates of cometabolic substrate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