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A model for the effects of primary substrates on the kinetics of reductive dehalogenation

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Abstract

A kinetic model that describes substrate interactions during reductive dehalogenation reactions is developed. This model describes how the concentrations of primary electron-donor and -acceptor substrates affect the rates of reductive dehalogenation reactions. A basic model, which considers only exogenous electron-donor and -acceptor substrates, illustrates the fundamental interactions that affect reductive dehalogenation reaction kinetics. Because this basic model cannot accurately describe important phenomena, such as reductive dehalogenation that occurs in the absence of exogenous electron donors, it is expanded to include an endogenous electron donor and additional electron acceptor reactions. This general model more accurately reflects the behavior that has been observed for reductive dehalogenation reactions. Under most conditions, primary electron-donor substrates stimulate the reductive dehalogenation rate, while primary electron acceptors reduce the reaction rate. The effects of primary substrates are incorporated into the kinetic parameters for a Monod-like rate expression. The apparent maximum rate of reductive dehalogenation ($q_{m,ap}$) and the apparent half-saturation concentration (K_{ap}) increase as the electron donor concentration increases. The electron-acceptor concentration does not affect $q_{m,ap}$, but K_{ap} is directly proportional to its concentration.

Introduction

In anaerobic environments, the most important mechanism for the biological transformation of highly halogenated aliphatic hydrocarbons is reductive dehalogenation. Reductive dehalogenation reactions involve transfer of two electrons to the halogenated substrate (Wade & Castro 1973a, b; Kochi 1978) and result in cleavage of a carbon-halogen bond. Because primary electron-donor and -acceptor substrates are the major source and sink for electrons in bacterial metabolism, their concentrations can control the intracellular availability of electrons. Therefore, they can be expected to affect the rate of reductive dehalogenation reactions.

The anaerobic biotransformation of halogenated aliphatic and aromatic hydrocarbons often is affected by electron-donor and -acceptor substrates. These effects include stimulation of reductive dehalogenation by electron donors (Fathepure & Boyd 1988; Krone et al. 1989b; Freedman & Gossett 1989; Mikesell & Boyd 1990; DeWeerd et al. 1991; Dolfing & Tiedje 1991a, b), inhibition by electron acceptors (DeWeerd & Suflita 1990; DeWeerd et al. 1991; Criddle et al. 1990a, b; Gibson & Suflita 1990; Kuhn et al. 1990), alterations in the acclimation times that precede the onset of biodegradation in environmental microcosms (Gibson & Suflita 1990), and shifts in the distribution of products (Kuhn et al. 1990; Criddle et al. 1990b). Responses such as these can be produced by direct or indirect effects of the primary substrates. Direct effects occur by alteration of the intracellular availability of electrons for transfer to the halogenated substrate. Indirect effects can result from changes in the composition of the microbial population, the biomass density, or from changes in gene expression, such as induction or repression of dehalogenase enzyme synthesis.

There are many cases in which the primary substrates probably affected microbially catalyzed reductive dehalogenation reactions by directly influencing the availability of electrons. For example, electron-donor substrates (e.g., methanol or methylamines) were required to support the reductive dechlorination of $CHCl_3$ to CH_2Cl_2 by two strains of *Methanosarcina* sp. (Mikesell & Boyd 1990). In most other cases, reductive dechlorination reactions proceeded in the absence of exogenous electron donors, but the rates were substantially increased by addition of appropriate substrates (Fathepure & Boyd 1988; Krone et al. 1989b; Freedman & Gossett 1989; DeWeerd & Suflita 1990; DeWeerd et al. 1991). The dechlorinating organisms used in these studies apparently had endogenous sources of electrons that could support the reductive dehalogenation reactions that were observed. Reductive dechlorination of 3-chlorobenzoate by *Desulfomonile tiedjei* was inhibited by sulfite and thiosulfite, which serve as primary electron acceptors for this organism (DeWeerd & Suflita 1990; DeWeerd et al. 1991). Inhibition by these substrates might have been due to their effect on the availability of electrons for the dechlorination reaction.

Observations such as these suggest that, although biodegradation kinetics are commonly analyzed by assuming that the reaction rate is controlled only by the concentrations of the target substrate and biomass, the concentrations of the primary substrates might also be important for reductive dehalogenation reactions. In this paper, we present structured kinetic models that describe the direct effects of electron-donor and -acceptor substrates on the rate of cometabolic reductive dehalogenation reactions. These models, which are based on fundamental characteristics of the reductive dehalogenation reaction mechanism, can be applied to pure or mixed microbial cultures as long as the electron-donating and -accepting substrates are used directly by the dehalogenating organisms. Although these models were developed for halogenated aliphatic substrates, they should apply equally well for dehalogenation of halogenated aromatic compounds.

Conceptual model

In our modeling, we assume that reductive dehalogenation is catalyzed by a metalloenzyme dehalogenase, because metal-containing cofactors are frequently implicated in reductive dehalogenation reactions (Wade & Castro 1973a, b; Klecka & Gonsior 1984; Krone et al. 1989a, b; Gantzer & Wackett 1991). The specific biochemical characteristics of the dehalogenase are not crucial for this model, however. Reductive dehalogenation occurs when a halogenated-aliphatic substrate, RX, reacts with the reduced dehalogenase, $E\text{-}M^n$. During the course of the reaction, two electrons are transferred from the enzyme to the halogenated substrate, resulting in formation of a reduced, dehalogenated, aliphatic product (RH) and an oxidized dehalogenase ($E\text{-}M^{n+2}$). The stoichiometry of the reductive dehalogenation reaction is:

$$RX + E\text{-}M^n + H_2O \rightarrow RH + E\text{-}M^{n+2} + X^- + OH^- \quad \text{(R1)}$$

The reductive dehalogenation reaction, as written above, involves the reduced dehalogenase as a reactant. Because the oxidized enzyme is incapable of reacting with another halogenated substrate, the enzyme can function as a true catalyst only when this reaction is coupled to a second reaction in which the oxidized dehalogenase becomes reduced. This can occur if electrons derived from oxidation of the primary electron donor, generically designated at $DH_{2\beta}$, can be transferred to the oxidized enzyme through another metabolic pathway. Oxidation of the electron donor and reduction of the dehalogenase need not be directly coupled, and the metabolic pathway can involve intermediate electron carriers (e.g., NADH and reduced ferredoxin). For the purposes of this model, only the net reaction must be considered. The stoichiometry of the overall reaction that links oxidation of the electron donor to reduction of the oxidized dehalogenase is:

$$DH_{2\beta} + \beta E\text{-}M^{n+2} \rightarrow D + \beta E\text{-}M^n + 2\beta H^+ \quad \text{(R2)}$$

where β is a stoichiometric coefficient for the net reaction, and D represents the products of oxidation of the electron donor. (All model parameters and their units are defined in the 'Definitions for model parameters' section of this paper.)

If the dehalogenase is a component of the normal metabolic pathyways for energy generation, the reduced enzyme can become oxidized in reactions that do not involve reductive dehalogenation. Instead, oxidation of the reduced dehalogenase can be coupled to reduction of the primary electron-acceptor substrate. Although oxidation of the dehalogenase and reduction of the primary electron acceptor can be linked via a complicated pathway that involves intermediate electron carriers, only the net reaction must be considered in this model. The stoichiometry of this reaction is:

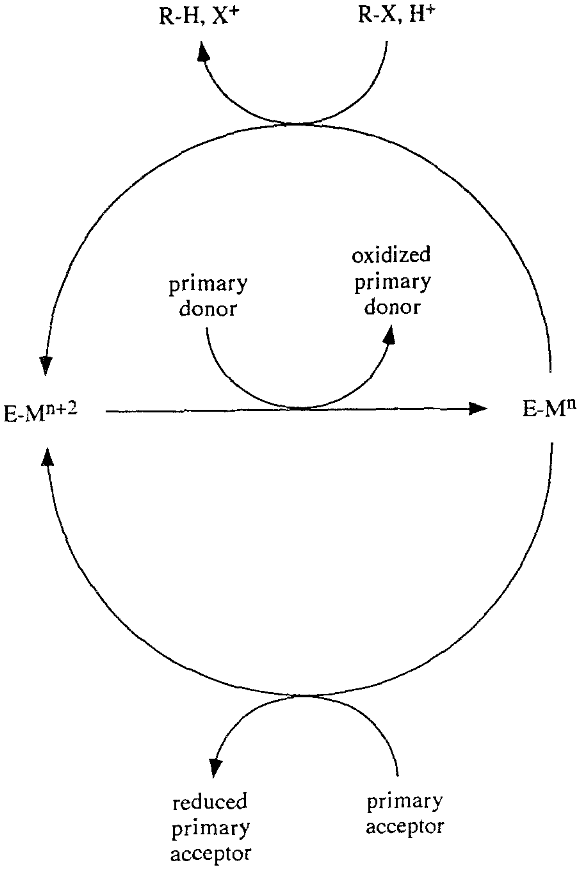

Fig. 1. Basic interactions that occur among the primary electron-donor, primary electron-acceptor, and halogenated-aliphatic substrates during reductive dehalogenation reactions. E-M^{n+2} represents the oxidized dehalogenase, and E-M^n represents the reduced dehalogenase.

$$A + \delta E\text{-}M^n + 2\delta H^+ \rightarrow AH_{2\delta} + \delta E\text{-}M^{n+2} \qquad (R3)$$

in which A is the primary electron acceptor, $AH_{2\delta}$ is its reduced product, and δ is a stoichiometric coefficient for this reaction.

These three processes – reductive dehalogenation, oxidation of the primary electron donor, and reduction of the primary electron acceptor – all involve the metalloenzyme that functions as the reductive dehalogenase. Thus, the effects of primary substrates on the rate of reductive dehalogenation can be mediated through their effects on the steady-state concentration of the reduced form of the dehalogenase. The central role of the dehalogenase in these three processes provides a means by which the effects of primary substrates can be explicitly considered in the reductive dehalogenation rate equation. The relationships among these reactions are shown in Fig. 1.

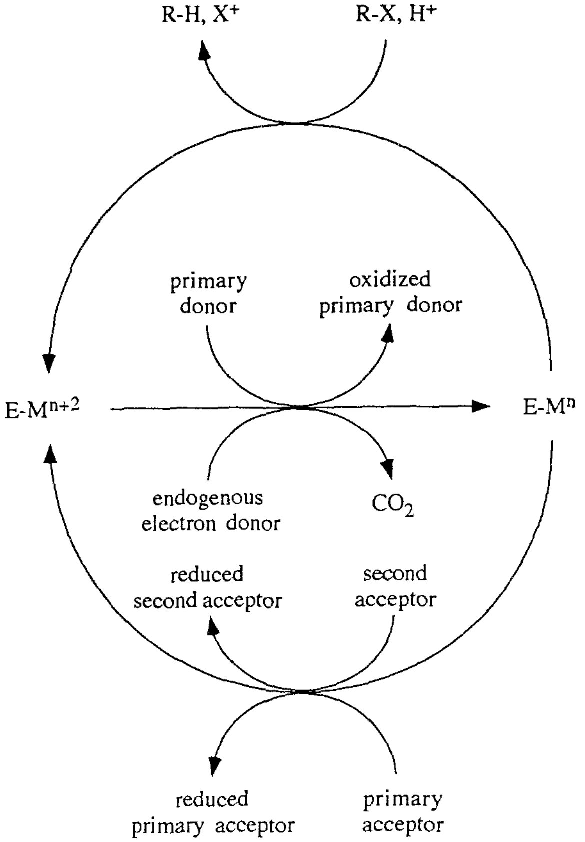

Fig. 2. Conceptual model for substrate interactions that includes exogenous and endogenous electron donors, multiple exogenous electron acceptors, and reductive dehalogenation.

Although the three reactions presented above are sufficient to develop a model that provides a quantitative description of the effects of primary substrates on reductive dehalogenation, they do not adequately describe all electron sources and sinks that are available to microbes that catalyze these reactions. Therefore, a more general model for the substrate interactions that occur during reductive dehalogenation should consider two additional reactions: (1) reduction of the dehalogenase in a reaction that is coupled to oxidation of an endogenous electron donor, and (2) oxidation of the dehalogenase coupled to reduction of a second primary electron acceptor. The interactions considered in the general model are shown in Fig. 2.

Reductive dehalogenation frequently occurs in the absence of exogenous electron donors (Fathepure & Boyd 1988; Krone et al. 1989b; DeWeerd & Suflita 1990; DeWeerd et al. 1991; Freedman & Gossett

1989; Wrenn & Rittmann submitted). Therefore, a second reaction by which the dehalogenase can become reduced must be considered. In this model, biomass decay is assumed to be the source of these endogenous electrons:

$$C_5H_7O_2N + 13H_2O + 10E\text{-}M^{n+2} \rightarrow 5HCO_3^- + NH_4^+ + 24H^+ + 10E\text{-}M^n \tag{R4}$$

where $C_5H_7O_2N$ is used to represent the empirical elemental composition of biomass (McCarty 1972).

A second electron-acceptor reaction is included in this kinetic model for practical reasons that will be discussed later. Its inclusion is theoretically justified, however, because common constituents of aqueous media (such as H^+ and CO_2) can serve as electron acceptors for many anaerobes (Gottschalk 1986). These substrates are always present, even when they are not explicitly added to the culture medium. Also, many sulfate-reducing bacteria can reduce oxidized sulfur compounds that may be present in anaerobic culture media due to oxidation of sulfide (Gottschalk 1986; Widdel 1988), which is frequently added as a reducing agent. Therefore, a reaction that involves a second electron acceptor, A2, is incorporated into the general model:

$$A2 + \kappa E\text{-}M^n + 2\kappa H^+ \rightarrow A2H_{2\kappa} + \kappa E\text{-}M^{n+2} \tag{R5}$$

where κ is the stoichiometric coefficient that relates oxidation of the dehalogenase to reduction of the second electron acceptor.

Mathematical models

Mathematical modeling is developed in two steps. First, the basic model, corresponding to Fig. 1, is derived to illustrate the main effects of the primary substrates on reductive dehalogenation rate. Then, the general model, corresponding to Fig. 2, is derived to emphasize how the endogenous electron donor and a secondary electron acceptor alter these effects.

The same approach is followed in the derivation of the basic and general models. Both models assume that the concentrations of the reduced and oxidized forms of the dehalogenase achieve steady-state. Since all of the reactions considered in these models involve the dehalogenase, rate equations that are functions of the concentrations of its reduced or oxidized forms can be written. The steady-state concentration of the reduced dehalogenase can be obtained by solving the appropriate mass-balance equation, and this concentration is substituted into the rate equation for reductive dehalogenation. The result is an expression that gives the rate of reductive dehalogenation as a function of the concentrations of the primary substrates, the halogenated substrate, and the dehalogenase.

Basic model

Figure 1 shows the minimum interactions that must occur among the reactions that produce and consume the reduced form of the reductive dehalogenase. If the concentrations of the primary substrates and the halogenated aliphatic substrate remain relatively constant, then the concentrations of the reduced and oxidized forms of the dehalogenase will reach a steady state.

For the basic model, the steady-state mass balance on the reduced dehalogenase has the following form:

$$\frac{d[E\text{-}M^n]}{dt} = \beta r_{d1} - \delta r_{a1} - \alpha r_{RX} = 0 \tag{1}$$

where r_{d1} is the rate of oxidation of the primary exogenous electron donor, r_{a1} is the rate of reduction of the primary exogenous electron acceptor, r_{RX} is the rate of the reductive dehalogenation reaction, and α is the stoichiometric coefficient for reaction Rl. Since one mole of RX is assumed to react with one mole of the reduced dehalogenase, α equals one.

All of the reactions are assumed to follow first-order kinetics with respect to each substrate (i.e., mixed second order overall). The appropriate rate equations are as follows:

$$r_{RX} = k_{RX}[E\text{-}M^n][RX] \tag{2}$$

$$r_{d1} = k_{d1}[E\text{-}M^{n+2}][DH_{2\beta}] \tag{3}$$

$$r_{a1} = k_{a1}[E\text{-}M^n][A] \tag{4}$$

where k_{RX}, k_{d1}, and k_{a1} are mixed-second-order rate coefficients.

The steady-state concentration of the reduced metalloenzyme can be obtained by substituting Eqs. 2, 3, and 4 into Eq. 1. The following mass balance on total dehalogenase ($[E\text{-}M_t]$) is required to solve the resulting equation:

$$[E\text{-}M]_t = [E\text{-}M^n] + [E\text{-}M^{n+2}] \tag{5}$$

In general, the total concentration of the dehalogenase is not readily measurable. In many cases, its concentration cannot be measured, because the dehalogenase is not identified. Even when the identity of the dehalogenase is known, however, routine estimation of its concentration is often unnecessary, because the enzyme concentration can be related to an easily measurable property of the system, such as biomass. This can be done by assuming that the dehalogenase represents a constant fraction, f, of the total biomass:

$$[\mathrm{E\text{-}M}]_t = f\mathrm{X} \tag{6}$$

where X is the biomass concentration. Because f is assumed to be constant, this model cannot be applied directly to those cases in which the expression of the dehalogenase gene or the proportion of dehalogenating bacteria in a mixed microbial population changes during the course of an experiment.

For the basic model, the steady-state concentration of the reduced dehalogenase that results from simultaneous solution of Eqs. 1 to 6 is:

$$[\mathrm{E\text{-}M}^n]_{ss} = \frac{\beta f k_{d1}[\mathrm{DH}_{2\beta}]\mathrm{X}}{\beta k_{d1}[\mathrm{DH}_{2\beta}] + \delta k_{a1}[\mathrm{A}] + \alpha k_{RX}[\mathrm{RX}]} \tag{7}$$

Substitution of Eq. 7 into Eq. 2 puts the rate of the dehalogenation reaction in the following form:

$$r_{RX} = \frac{q_{m,ap}\mathrm{X}[\mathrm{RX}]}{K_{ap} + [\mathrm{RX}]} \tag{8}$$

where $q_{m,ap}$ is the apparent maximum specific rate of reductive dehalogenation, and K_{ap} is the apparent half-saturation concentration.

Equation 8 is formally similar to the Monod equation, which is frequently used to describe biodegradation kinetics, except that $q_{m,ap}$ and K_{ap} are not constants. Instead, they are functions of the concentrations of the primary substrates. For the basic model, these kinetics coefficients are defined as follows:

$$q_{m,ap} = f\frac{\beta}{\alpha}k_{d1}[\mathrm{DH}_{2\beta}] \tag{9}$$

and

$$K_{ap} = \frac{\beta k_{d1}}{\alpha k_{RX}}[\mathrm{DH}_{2\beta}] + \frac{\delta k_{a1}}{\alpha k_{RX}}[\mathrm{A}] \tag{10}$$

When the concentration of the halogenated substrate is much less than the apparent half-saturation coefficient, the dehalogenation kinetics become first-order with respect to the concentration of RX. Under these conditions, the pseudo-first-order rate coefficient, k_{ap}, is a function of the ratio of the concentrations of the primary substrates:

$$k_{ap} = \frac{f k_{RX}}{1 + \frac{\delta k_{a1}}{\beta k_{d1}}\frac{[\mathrm{A}]}{[\mathrm{DH}_{2\beta}]}} \tag{11}$$

If this ratio remains constant as the concentrations of the primary substrates vary, k_{ap} will not change.

The concentrations of the primary electron-donor and -acceptor substrates can co-vary in some important situations in anaerobic environments. For example, a single substrate serves as the electron donor and electron acceptor during methanogenesis from acetate, formate, and methanol (Jones et al. 1987) and during acetogenesis from formate and methanol (Zeikus et al. 1985). When one substrate has both roles, the concentrations of the electron donor and electron acceptor cannot vary independently. Similar behavior can result when the electron acceptor is derived from the electron donor, such as occurs during fermentation of glucose to lactate, ethanol, and butyrate. In these cases, glucose is the electron donor and pyruvate or acetate, which are products of glucose metabolism, are the electron acceptors (Gottschalk 1986). When the electron acceptor is derived from the electron donor, it may be possible – at least in principle – to vary the concentration of the electron acceptor independently from the electron donor, but this would usually not occur.

Figures 3, 4, and 5 illustrate how the basic model describes reductive-dehalogenation kinetics. The kinetic and stoichiometric parameters that were used in these, and subsequent, simulations are presented in Tables 1 and 2, respectively. Although the same trends result from any set of parameter values, these parameters were chosen from the literature to correspond to a hypothetical situation in which CCl_4 is reductively dechlorinated to $CHCl_3$ by a pure culture of formate-oxidizing, sulfate-reducing bacteria. The dehalogenase is assumed to be a corrinoid-containing enzyme. When kinetic parameters for sulfate-reducing bacteria could not be found, they were assumed to be equal to those reported for H_2- or formate-oxidizing methanogens.

The rates of reductive dehalogenation for this solution to the model are plotted as a function of the concentration of the halogenated aliphatic substrate for three situations: (1) the electron-donor concentration varies, while the electron-acceptor concentration remains constant (Fig. 3); (2) the electron-acceptor concentration varies, while the electron-donor concentration remains

Table 1. Kinetic parameters used in model simulations.

Kinetic Parameters	Reference
$k_{RX} = 450$ L/mole enz-s	Gantzer & Wackett (1991)
$k_{d1} = 1.4 \times 10^5$ L/mole enz-s	Schauer et al. (1982)
$k_{d2} = \frac{b}{fX}$; $b = 1.04 \times 10^{-6}\ s^{-1}$	Costello et al. (1991)
$V_{max} = 6.45$ mole $SO_4{}^{2-}$/mole enz-s	Ingvorsen et al. (1984)
$K_{sulfate} = 5.5 \times 10^{-5}$ mole/L	Ingvorsen & Jorgensen (1984)
$k_{a1} = \frac{V_{max}}{K_{sulf}} = 1.2 \times 10^5$ L/mole enz/s	Nethe-Jaenchen & Thauer (1984)
$k_{a2} = 1.4 \times 10^5$ L/mole enz-s	assumed

Table 2. Stoichiometric parameters used in model simulations.

Stoichiometric Parameters	Reference
$f = 2 \times 10^{-10}\ \frac{\text{moles enz}}{\text{mole formate}}$	Dangel et al. (1987)[a]
$\beta = 1\ \frac{\text{mole enz}}{\text{mole formate}}$	$HCOOH + E\text{-}M^{n+2} \rightarrow CO_2 + E\text{-}M^n + 2H^+$
$\delta = 3\ \frac{\text{mole enz}}{\text{mole } SO_4^{2-}}$	$HSO_3{}^- + 3E\text{-}M^n + 6H^+ \rightarrow HS^- + 3H_2O + 3E\text{-}M^{n+2}$
$\gamma = 8.8 \times 10^{-5}\ \frac{\text{moles enz}}{\text{mg biomass}}$	Reaction R4
$\kappa = 1\ \frac{\text{mole enz}}{\text{mole acceptor2}}$	assumed
$g = \frac{1}{3}\ \frac{\text{mole acceptor}}{\text{moles donor}}$	assumed
$[A2] = 9 \times 10^{-5}$ mole/L	$[A2] = [H_2CO_3]$; pH = 7.3, $C_{T,CO3} = 10^{-3}$ M

[a] assuming 1 mole enzyme/mole corrinoid.

constant (Fig. 4); and (3) the concentrations of both primary substrates co-vary, such that the ratio of their concentrations remains constant (Fig. 5). In each case, the curves have a Monod-like shape in which the dependence of the reaction rate on the halogenated-aliphatic substrate concentration is non-linear, changing from a first-order dependence at low substrate concentrations to zero-order at high concentrations. Although all of the curves have the same shape, they show different trends with respect to halogenated substrate concentration. In particular, the first-order and maximum rates vary, as does the transition from the first- to the zero-order region, depending on the relationship between the primary substrate concentrations.

Note that these curves appear to have a shape that differs somewhat from the familair Monod hyperbolic curve. This apparent difference occurs solely because the curves are presented on logarithmic rather than arithmetic axes. This difference is particularly evident in that the plots have no origin (because zero is not defined on a logarithmic scale), but it also affects the slope of the first-order region. On a log-log plot, the slope of the first-order region is the same for all of the curves, because it reflects the ratio of the exponents of the variables, not the magnitude of the pseudo-first-order rate constant, k_{ap}. Instead, the magnitude of k_{ap} is given by the vertical displacement of the curves: As k_{ap} increases, the curves are placed higher on the plot.

The biodegradation kinetics are presented for several concentrations of the primary electron donor and a single electron-acceptor concentration in Fig. 3A. Under these conditions, the apparent maximum rate and the first-order rate increase as the electron donor concentration increases. Whereas $q_{m,ap}$ increases indefinitely as the electron-donor concentration increases, k_{ap} reaches a maximum. Both of these phenomena are caused by changes in the steady-state concentration of the dehalogenase, which becomes more reduced as the electron-donor concentration increases relative to the concentrations of the primary and secondary (i.e., halogenated aliphatic) electron acceptors. The proportion of the dehalogenase that is present in the reduced form is plotted for these reaction conditions in Fig. 3B. The first-order rate saturates,

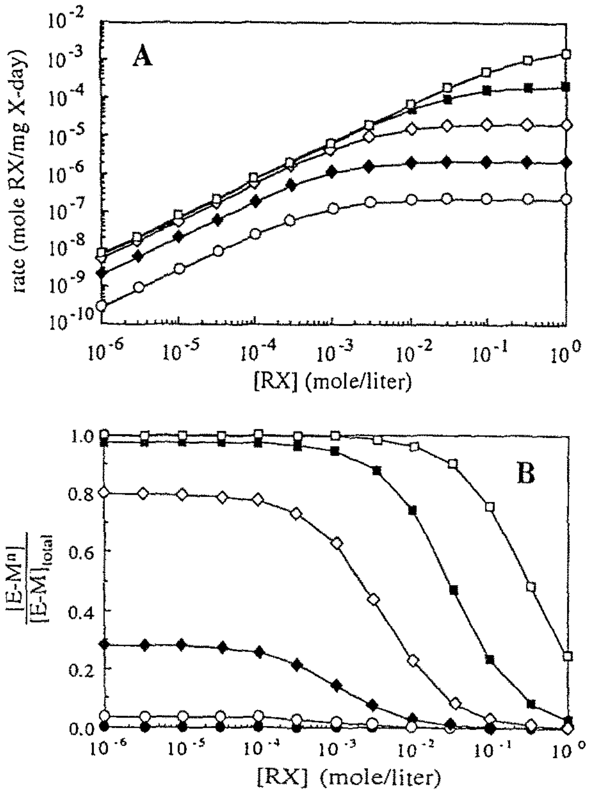

Fig. 3. The effects of varying electron donor concentration on (A) reductive dehalogenation kinetics and (B) the fraction of the reductive dehalogenase that is present in the reduced form, as given by the basic model for substrate interactions. The kinetic and stoichiometric parameters that were used in these simulations are listed in Tables 1 and 2, respectively. The electron-acceptor concentration for all curves is 10^{-6} M. The electron-donor concentrations are: 10^{-7} M (-○-), 10^{-6} M (-◆-), 10^{-5} M (-◇-), 10^{-4} M (-■-), and 10^{-3} M (-□-). When the electron donor is absent, the reductive dehalogenation rate is zero.

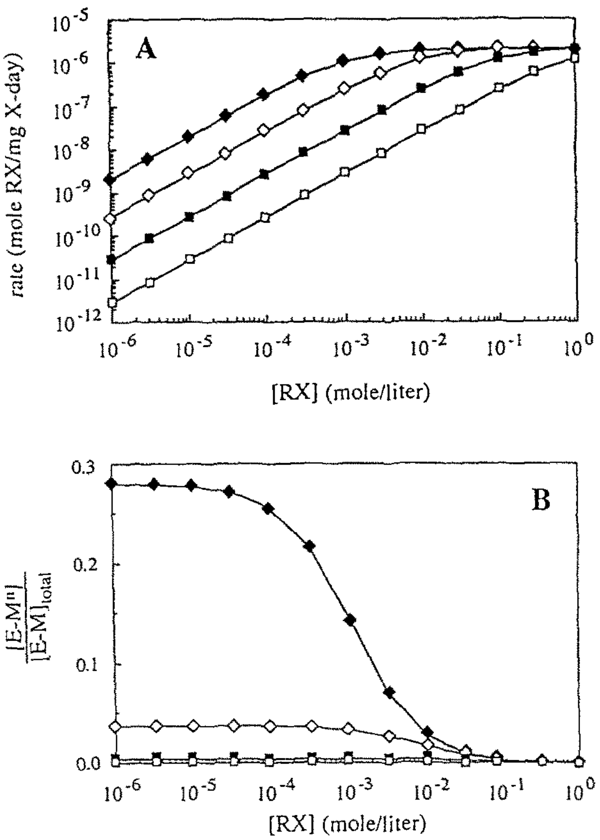

Fig. 4. Effects of varying electron-acceptor concentration on (A) the kinetics of reductive dehalogenation and (B) the fraction of the reductive dehalogenase that is present in the reduced form, as given by the basic model for substrate interactions. The kinetic and stoichiometric parameters that were used in these simulations are listed in Tables 1 and 2, respectively. The electron-donor concentration for all curves is 10^{-6} M. The electron acceptor concentrations are as follows: 10^{-6} M (-◆-)), 10^{-5} M (-◇-), 10^{-4} M (-■-), and 10^{-3} M (-□-).

because the dehalogenase eventually becomes completely reduced, and the reductive dehalogenation rate becomes dependent only on the concentration of the halogenated substrate and the intrinsic rate constant for the dehalogenation reaction. That is, when the concentration of the electron-donor substrate is high relative to the electron acceptor, Eq. 11 reduces to:

$$k_{ap} = fk_{RX} \tag{12}$$

When the halogenated-substrate concentration is high, however, the dehalogenase exists mainly in its oxidized form, and the rate of reductive dehalogenation is limited by the rate of oxidation of the electron donor.

Although this model predicts that the maximum rate of reductive dehalogenation will increase indefinitely with increasing concentrations of the electron-donor substrate, this behavior cannot occur in reality. The model's behavior results from our choice of first-order reaction kinetics as basis functions for the model, but experience with biochemical systems suggests that these reactions will exhibit saturation kinetics. Thus, our assumption of linearity in the rate equations will not hold over the entire range of substrate concentrations. Fortunately, many environmentally important substrate concentrations are within this first-order range. We have developed an analogous model based on Monod kinetics, in which the dehalogenation rate reaches a maximum at high concentrations of the electron donor. Derivation of this alternative model is straightforward, but the resulting dehalogenation rate equation is sufficiently complex that the principles that we wish to illustrate are obscured. Thus, we have chosen to present the simpler first-order model, despite some limitations.

Figure 4A shows how the reaction kinetics are expected to respond to changes in the concentration of the primary electron acceptor when the electron-donor concentration remains constant. For this situation, all of the curves converge to the same maximum rate, but the first-order rate decreases as the concentration of the electron acceptor increases. The maximum rate is not affected by the concentration of the primary electron acceptor, because, at high concentrations of the halogenated substrate, oxidation of the reduced dehalogenase is dominated by the reductive dehalogenation reaction, and the rate is determined by the oxidation rate of the primary electron donor (Fig. 4B). Of course, the concentration of halogenated substrate that produces a maximal dehalogenation rate depends on the concentration of the primary electron acceptor (i.e., K_{ap} is a function of the primary electron-acceptor concentration). The first-order rate decreases as the primary electron-acceptor concentration increases, because reductive dehalogenation and reduction of the primary electron acceptor are competing reactions. Increasing the concentration of the primary electron acceptor causes the dehalogenase to become relatively more oxidized at steady state (Fig. 4B).

The effects of simultaneous changes in both primary substrates – such that the ratio of their concentrations remains constant – are shown in Fig. 5. Under these conditions, all of the curves converge to the same first-order rate, but the apparent maximum rate increases as the concentrations of the primary substrates increase (Fig. 5A). The first-order rate is independent of the concentrations of the primary substrates when their ratio is constant, because, at low concentrations of the halogenated substrate, the steady-state concentration of the reduced dehalogenase depends on the ratio of the primary-substrate concentrations, not on their absolute concentrations (Fig. 5B). That is, when:

$$\alpha k_{RX}[RX] << \beta k_{d1}[DH_{2\beta}] + \delta k_{a1}[A]$$

Eq. 7 can be rewritten as:

$$[E\text{-}M^n]_{ss} = \frac{fX}{1 + \frac{\delta k_{a1}}{\beta k_{d1}} \frac{[A]}{[DH_{2\beta}]}} \qquad (13)$$

Of course, the halogenated substrate concentration at which the transition from first- to zero-order kinetics occurs depends on the absolute concentrations of the primary substrates (Fig. 5A), because the oxidation state of the dehalogenase is affected more quickly by the halogenated substrate when the primary substrate concentrations are low (Fig. 5B).

In summary, the basic model describes the manner in which reductive dehalogenation reactions interact with the energy-generating pathways of bacterial metabolism. The effects of primary substrates on the kinetics of these reactions are mediated by their effects on the intracellular availability of electrons, which is modeled by the oxidation state of the dehalogenase. Electron donors stimulate the reductive dehalogenation rate by causing the steady-state concentration of the reduced dehalogenase to increase. Increasing the concentration of the primary electron acceptor relative to the primary electron donor has the opposite effect: The steady-state concentration of the reduced dehalogenase decreases, inhibiting the rate of the reductive dehalogenation reaction.

The trends predicted by the basic model are qualitatively consistent with many of the effects observed in anaerobic systems. Electron donors stimulate reductive dehalogenation (Fathepure & Boyd 1988; Freedman & Gossett 1989; Krone et al. 1989b; Mikesell & Boyd 1990; DeWeerd et al. 1991; Dolfing & Tiedje 1991a, b), while electron acceptors inhibit the reactions (DeWeerd & Suflita 1990; DeWeerd et al. 1991; Criddle et al. 1990a, b; Gibson & Suflita 1990; Kuhn et al. 1990). The basic model is inadequate in one important point, however. Reductive dehalogenation reactions commonly occur in the absence of exogenous electron-donor substrates (Fathepure & Boyd 1988; Krone et al. 1989b; DeWeerd & Suflita 1990; DeWeerd et al. 1991; Freedman & Gossett 1989; Wrenn & Rittman submitted), but this basic model for substrate interactions during reductive dehalogenation predicts that the reaction rate should be zero when exogenous electron donors are absent. Clearly, another source of electrons must be available to dehalogenating bacteria. In fact, many bacteria store electrons in the form of glycogen or poly-β-hydroxybutyrate for use when exogenous electron donors are absent, and bacterial decay, in which macromolecules such as proteins and lipids are metabolized for energy during starvation, is a common phenomenon. In the next section, the basic model is expanded to include endogenous, as well as exogenous, electron donors.

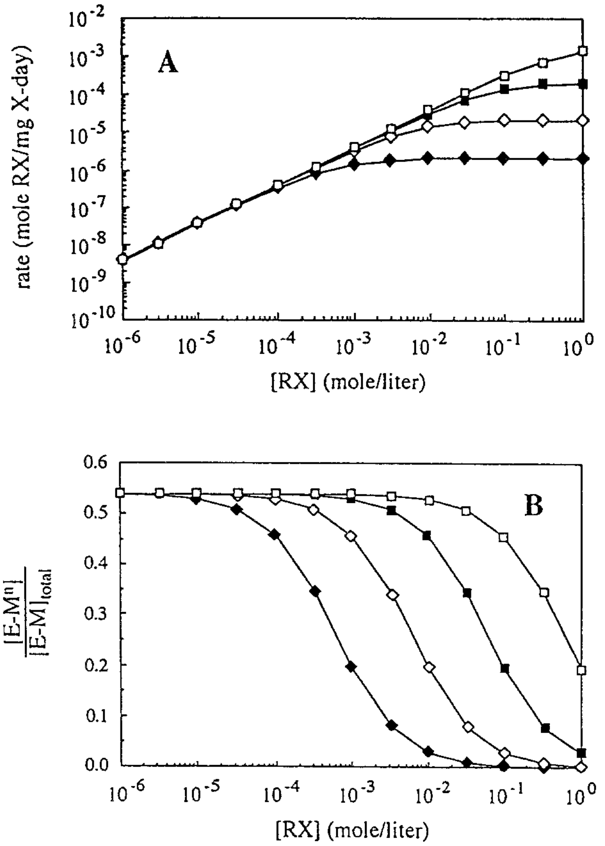

Fig. 5. Reductive dehalogenation kinetics predicted by the basic model for substrate interactions at several electron-donor concentrations when the ratio of primary substrate concentrations is held constant (g = 1/3). The kinetic and stoichiometric parameters that were used in these simulations are listed in Tables 1 and 2, respectively. The electron-donor concentrations are: 10^{-6} M (–◆–), 10^{-5} M (–◇–), 10^{-4} M (–■–), and 10^{-3} M (–□–).

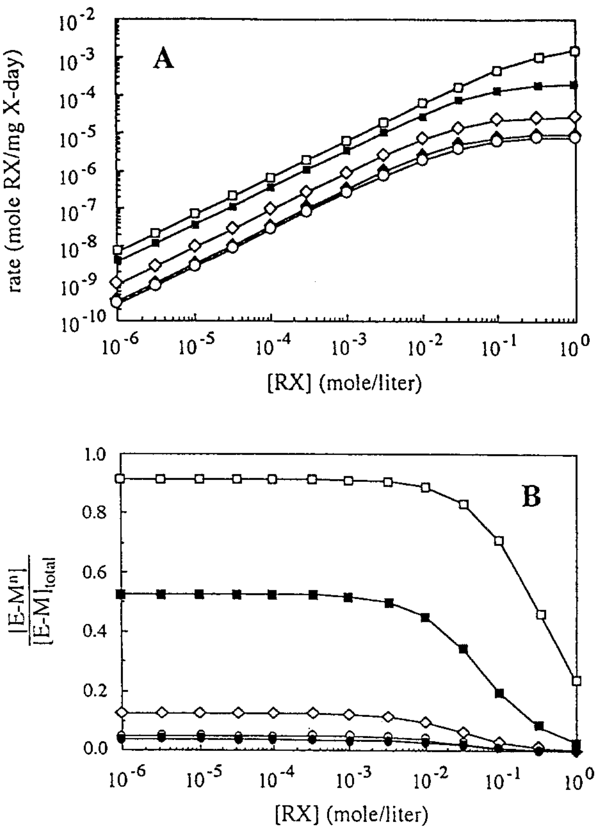

Fig. 6. Reductive dehalogenation kinetics predicted by the general model for substrate interactions at several electron-donor concentrations when the electron-acceptor concentration is held constant. The kinetic and stoichiometric parameters that were used in these simulations are listed in Tables 1 and 2, respectively. The primary electron-acceptor concentration for all curves is 10^{-6} M. The exogenous electron-donor concentrations are: zero (–○–), 10^{-6} M (–◆–), 10^{-5} M (–◇–), 10^{-4} M (–■–), and 10^{-3} M (–□–).

General model

In the general model for substrate interactions during reductive dehalogenation, the steady-state mass balance on the reduced dehalogenase is:

$$\frac{d[E-M^n]}{dt} = \beta r_{d1} + \gamma r_{d2} - \delta r_{a1} - \kappa r_{a2} - \alpha r_{RX} = 0 \quad (14)$$

where γ is the stoichiometric coefficient that relates dehalogenase reduction to oxidation of the endogenous electron donor, r_{d2} is the rate of oxidation of the endogenous electron donor and r_{a2} is the rate at which a second electron acceptor is reduced. The rates of these reactions are assumed to follow mixed-second-order kinetics:

$$r_{d2} = k_{d2}[E\text{-}M^{n+2}]X = bX \quad (15)$$

$$r_{a2} = k_{a2}[E\text{-}M^n][A2] \quad (16)$$

where k_{d2} and k_{a2} are mixed-second-order rate coefficients for oxidation of the endogenous electron donor and reduction of the second electron acceptors, respectively, and b is the first-order biomass decay coefficient.

The steady-state concentration of the reduced dehalogenase results from substitution of equations 2, 3, 4, 15, and 16 into equation 14:

$$[\mathrm{E\text{-}M^n}]_{ss} = \frac{(\beta k_{di}[DH_{2\beta}]+\gamma k_{d2}X)fX}{\beta k_{d1}[DH_{2\beta}]+\gamma k_{d2}X+\delta k_{a1}[A]+\kappa k_{a2}[A2]+\alpha k_{RX}[RX]} \quad (17)$$

Substitution of Eq. 17 into Eq. 2 gives a Monod-like rate expression (i.e., Eq. 8) for which the apparent Monod kinetic parameters are:

$$q_{m,ap} = f\frac{\beta}{\alpha}k_{d1}[DH_{2\beta}] + f\frac{\gamma}{\alpha}k_{d2}X \quad (18)$$

$$K_{ap} = \frac{\beta k_{d1}}{\alpha k_{RX}}[DH_{2\beta}] + \frac{\gamma k_{d2}}{\alpha k_{RX}}X + \frac{\delta f k_{a1}}{\alpha k_{RX}}[A] + \frac{\kappa K_{a2}}{\alpha k_{RX}}[A2] \quad (19)$$

The apparent pseudo-first-order rate coefficient is:

$$k_{ap} = \frac{f f k_{RX}(\beta k_{d1}[DH_{2\beta}] + \gamma k_{d2}X)}{\beta k_{d1}[DH_{2\beta}] + \gamma K_{d2}X + \delta k_{a1}[A] + \kappa k_{a2}[A2]} \quad (20)$$

For the special case in which the concentrations of the exogenous electron donor and acceptor co-vary, the apparent pseudo-first-order rate coefficient is:

$$k'_{ap} = \frac{f k_{RX}(\beta k_{d1}[DH_{2\beta}] + \gamma k_{d2}X)}{(\beta k_{d1} + \delta g k_{a1})[DH_{2\beta}] + \gamma k_{d2}X + \kappa k_{a2}[A2]} \quad (21)$$

where:

$$g = \frac{[A]}{[DH_{2\beta}]} = \text{constant} \quad (22)$$

This special case provides the practical motivation for including the second electron acceptor in the general model. If the second electron acceptor is not included, the model predicts a response that is contrary to experience. The reason for the contrary prediction is the following. When a single electron acceptor is considered and the concentrations of the primary substrates co-vary such that the ratio of their concentrations remains constant, the apparent pseudo-first-order rate coefficient is given by:

$$k'_{ap} = \frac{f k_{RX}(\beta k_{d1}[DH_{2\beta}] + \gamma k_{d2}X)}{(\beta k_{d1} + \delta g k_{a1})[DH_{2\beta}] + \kappa k_{d2}X} \quad (23)$$

Equation (23) approaches two limiting values. When exogenous electron donors are absent, the pseudo-first-order rate coefficient is:

$$k'_{ap,o} = f k_{RX} \quad (24)$$

When the rate of reduction of the dehalogenase by electrons from the exogenous donor is much larger than the rate at which the enzyme is reduced by electrons from the endogenous donor (i.e., $\beta k_{d1}[DH_{2\beta}] >> \gamma k_{d2}X$), the apparent first-order rate coefficient is:

$$k'_{ap,\infty} = \frac{\beta k_{d1}}{\beta k_{d1} + \delta g k_{a1}} f k_{RX} \quad (25)$$

By definition, all of the stoichiometric and kinetic coefficients are positive. Therefore:

$$\frac{\beta k_{d1}}{\beta k_{d1} + \delta g k_{a1}} < 1$$

and: $k'_{ap,o} > k'_{ap,\infty}$ Thus, this model predicts that the rate of reductive dehalogenation will decrease upon addition of primary substrates that function simultaneously as electron donors and acceptors. This surprising behavior is predicted, because, in the absence of exogenous primary substrates, the halogenated substrate is the only electron acceptor in the system, and all of the electrons derived from endogenous metabolism must be transferred to the halogenated substrate. When the exogenous primary substrate is added, its electron acceptor function enables it to compete with the halogenated substrate for electrons derived from biomass oxidation. If additional electron acceptors are not considered in the model, the dehalogenase becomes more oxidized upon addition of substrates that are both electron donors and acceptors. Since this is contrary to experience (Fathepure & Boyd 1988; Krone et al. 1989b; Freedman & Gossett 1989; Wrenn & Rittmann submitted), the model was modified to eliminate this behavior.

Overall, the basic and general models behave similarly with respect to the relationship between reductive dehalogenation rate and the concentration of the halogenated substrate (i.e., they both exhibit Monod kinetics). This is especially true at high concentrations of the primary electron donor, where the two models behave identically. Also, the basic and general models make qualitatively similar predictions for the situation in which the primary electron-acceptor concentration varies and the exogenous electron-donor concentration remains constant.

The differences between these two models occur for two important situations. The first is illustrated in Fig. 6, which presents the response of the reductive dehalogenation kinetics to changes in the exogenous electron donor concentration when the primary electron acceptor concentration is held constant. The kinetic curves cluster when the exogenous electron-donor concentration is low (Fig. 6A), because biomass oxidation allows the dehalogenase to remain partially reduced in the absence of an exogenous electron donor (Fig. 6B). Thus, the kinetic coefficients approach constant, nonzero values as the exogenous electron-donor concentration approaches zero. This allows reductive dehalogenation to proceed when exogenous electron donors are absent. The basic model requires the reaction to stop under these conditions.

The general and basic models also predict different types of behavior when the concentrations of the primary electron-donor and -acceptor substrates co-vary. In the basic model, the first-order rate of reductive dehalogenation remains constant as the electron-donor concentration increases, as long as the ratio of the electron-donor and -acceptor concentrations is constant (Fig. 5A), but the first-order rate increases for this situation in the general model (Fig. 7A). In fact, the response of the first-order dehalogenation rate to increasing electron-donor concentrations is qualitatively similar for this case, where the ratio of exogenous primary substrate concentrations remains constant, and for the case where the concentrations of the exogenous electron-donor and -acceptor substrates vary independently. Because additional electron-donor and -acceptor substrates are considered in the general model, the total concentrations of electron-donor and -acceptor substrates (endogenous plus exogenous substrates) vary independently regardless of how the relationship between the exogenous primary substrate concentrations is handled. Thus, the special case for which the ratio of the exogenous substrate concentration remains constant for all absolute concentrations of these substrates has no importance.

Although the general model incorporates additional electron sources and sinks, the more important modification is the incorporation of an endogenous source of electrons, which enables reductive dehalogenation to proceed in the absence of exogenous electron-donor substrates. Many observations of microbially catalyzed reductive dehalogenation reactions are consistent with this modification (Fathepure & Boyd 1988; Krone et al. 1989b; DeWeerd & Suflita 1990; DeWeerd et al. 1991; Freedman & Gossett 1989; Wrenn & Rittmann submitted). Furthermore, addition of this term and one for reduction of a second electron acceptor causes the general model to predict that primary substrates will affect the first-order dehalogenation rate even when the ratio of the concentrations of the electron donor and acceptor remains constant. The basic model predicts no effect of primary substrate concentration under these conditions (Fig. 5), which occur when the same substrate serves as both electron donor and acceptor (such as during methanogenesis from acetate, formate, and methanol), but electron donors of this type stimulate reductive dechlorination reactions at chlorinated substrate concentrations that are likely to be in the first-order range (Fathepure & Boyd 1988; Freedman & Gossett 1989). Thus, the general model for substrate interactions during reductive dehalogenation provides a more complete description of the observed effects of primary substrates than does the basic model.

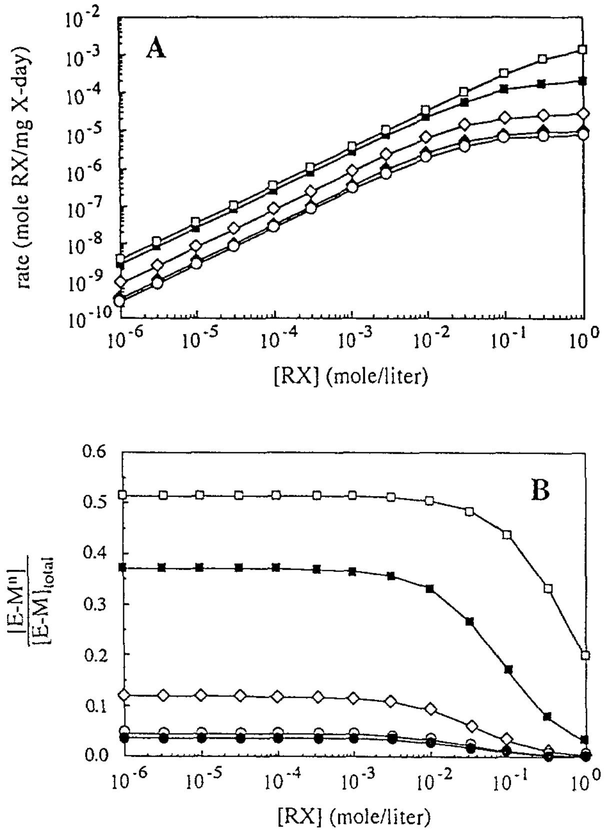

Fig. 7. Reductive dehalogenation kinetics predicted by the general model for substrate interactions at several electron-donor concentrations when the ratio of primary substrate concentrations is held constant (g = 1/3). The kinetic and stoichiometric parameters that were used in these simulations are listed in Tables 1 and 2, respectively. The exogenous electron-donor concentrations are: zero (-○-), 10^{-6} M (-◆-), 10^{-5} M (-◇-), 10^{-4} M (-■-), and 10^{-3} M (-□-).

Discussion

The general model for substrate interactions during reductive dehalogenation makes three very important predictions regarding the kinetics of reductive dehalogenation reactions. First, it states that exogenous electron-donor substrates will stimulate the reaction rate by providing the electrons needed to return the oxidized dehalogenase to its reactive (reduced) form. Second, electron-acceptor substrates, which compete with the halogenated substrate for electrons derived from oxidation of the electron donor, inhibit the rate of reductive dehalogenation. Finally, in the absence of exogenous electron donors, electrons derived from endogenous metabolism can support the dehalogenation reaction. The observations that drove development of this model are not new, however, and some of these concepts have been included in previous models of reductive dehalogenation kinetics. The major innovation of this model is that its behavior arises from fundamental considerations of the reaction mechanism, whereas previous models have been largely empirical.

In particular, the roles of endogenous metabolism and exogenous energy substrates in reductive dehalogenation have been considered in previous models. Gälli & McCarty (1989), for example, modeled the role of endogenous electrons by incorporating a first-order decay term for dehalogenase activity into the rate equation for TCA degradation. This term is an integrated form of a biomass decay rate expression similar to Equation 15. In the Gälli & McCarty model, endogenous electrons affect only the maximum reaction rate, whereas in the model presented here, both apparent Monod coefficients are affected. Although this is an important conceptual difference between these models, the difference is likely to be difficult to detect in practice. Criddle (1993), in his model for cometabolic reactions, considered endogenous metabolism as one factor that contributes to the biomass transformation capacity. Other factors that affect the transformation capacity include the effects of toxic reaction products, depletion of cofactors, and protein turnover. Criddle's model, which is much more general than the one described here, also includes terms for stimulation of the reaction rate by growth and energy substrates. Unlike our model, there is not a tight coupling between the cometabolic and energy-yielding reactions in Criddle's model. This is appropriate, however, because his model attempts to simulate the behavior of a wide variety of cometabolic reactions of which reductive dehalogenation is only one specific example.

Neither of these previous models specifically considers the effects of electron-acceptor substrates. Although there is ample evidence to suggest that electron acceptors can inhibit reductive dehalogenation reactions (DeWeerd & Suflita 1990; DeWeerd et al. 1991; Criddle et al. 1990a, b; Gibson & Suflita 1990; Kuhn et al. 1990), previous models have not distinguished between the effects of electron donors and acceptors, both of which are growth and energy substrates. Our model shows that these substrates affect the reaction rate in fundamentally different ways.

Finally, we should note that, although our model was developed specifically for reductive dehalogenation reactions, it can apply to other biodegradation reactions in which the rate-limiting step depends on the input of electrons. For example, appropriate electron donors stimulated the reductive cleavage of the ether linkage in phenoxyacetate and several clorophenoxyacetates by a phenoxyacetate-degrading enrichment culture obtained from a methanogenic aquifer (Gibson & Suflita 1993). In principle, many aspects of this model can be applied to oxidations catalyzed by monooxygenases. For example, oxidation of trichloroethene by methane monooxygenase is stimulated by addition of electron-donating substrates, such as formate (Alvarez-Cohen & McCarty 1991), and, in the absence of exogenous electron donors, to be dependent on intracellular electron donors, such as poly-β-hydroxybutyrate (Henry & Grbić-Galić 1991). Also, cometabolic biotransformation of 4-chlorophenol by *Pseudomonas putida* PpG4 was dependent upon the presence of the growth substrate, phenol, for a sufficient supply of endogenous electrons (Saéz & Rittmann 1993). These phenomena are very similar to those described for reductive dehalogenation reactions, and the monooxygenases that catalyze these reactions cycle between oxidation states in a manner that is analogous to the oscillations described for the reductive dehalogenase in this model. Therefore, our model should have broad utility.

Conclusion

The models that have been developed in this paper provide quantitative descriptions of the effects of primary-donor and -acceptor substrates on the kinetics of reductive dehalogenation reactions. These models are based on a conceptual model that considers the fundamental characteristics of reductive dehalogenation reactions and the metabolic interactions that control the intracellular availability of electrons. The basic model serves as a simple illustration of the most important effects of primary substrates, and the general model incorporates additional interactions in an attempt to more accurately describe the complexities of real microbial systems.

The effects of primary substrates on dehalogenation kinetics are modeled by using a rate equation that involves kinetic parameters that are functions of the concentrations of these substrate, and, therefore, they behave similarly to traditional rate coefficients in most respects. If dehalogenation kinetics are measured in the presence of constant concentrations of primary substrates, the parameters will appear to be constant, and Monod kinetics will provide an adequate description of the results. This model can be tested by determining the Monod kinetic parameters for reductive dehalogenation in the presence of different concentrations of the primary substrates. We have evaluated the predictions of this model by determining the kinetic parameters for biodegradation of 1,1,1-trichloroethane in two anaerobic biofilm reactors (Wrenn & Rittmann submitted).

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Definitions for model parameters

RX	halogenated aliphatic substrate
$E\text{-}M^{n}$	reduced dehalogenase
$E\text{-}M^{n+2}$	oxidized dehalogenase
$[E\text{-}M^{n}]$	steady-state concentration of the reduced dehalogenase (moles of reduced dehalogenase per unit volume)
$[E\text{-}M^{n+2}]$	steady-state concentration of the oxidized dehalogenase (moles of reduced dehalogenase per unit volume)
$DH_{2\beta}$	primary exogenous electron-donor substrate
A	primary exogenous electron-acceptor substrate
A2	second primary exogenous electron-acceptor substrate
X	biomass concentration (biomass per unit volume)
f	fraction of biomass that is comprised of the dehalogenase (moles of dehalogenase per unit biomass)
α	stoichiometric coefficient for the reductive dehalogenation reaction (moles of dehalogenase oxidized per mole of halogenated substrate reduced)
β	stoichiometric coefficient for oxidation of the primary electron donor (moles of dehalogenase reduced per mole of donor oxidized)
γ	stoichiometric coefficient for oxidation of the endogenous electron donor (moles of dehalogenase reduced per unit biomass oxidized)
δ	stoichiometric coefficient for reduction of the primary electron acceptor (moles of dehalogenase oxidized per mole of acceptor reduced)
κ	stoichiometric coefficient for reduction of the second electron acceptor (moles of dehalogenase oxidized per mole of acceptor reduced)
r_{RX}	rate of the reductive dehalogenation reaction (moles of halogenated substrate reduced per unit volume per unit time)
r_{d1}	rate of oxidation of the primary exogenous electron donor (moles of donor oxidized per unit volume per unit time)
r_{d2}	rate of oxidation of the endogenous electron donor (biomass oxidized per unit volume per unit time)
r_{a1}	rate of reduction of the primary exogenous electron acceptor (moles of acceptor reduced per unit volume per unit time)
r_{a2}	rate of reduction of the second primary electron acceptor (moles of acceptor reduced per unit volume per unit time)
k_{RX}	mixed second-order rate coefficient for the reductive dehalogenation reaction (volume per mole dehalogenase per unit time)
k_{d1}	mixed-second-order rate coefficient for oxidation of the primary electron donor (volume per mole dehalogenase per unit time)
k_{d2}	mixed-second-order rate coefficient for oxidation of the endogenous electron donor (volume per mole dehalogenase per unit time)
b	first-order biomass decay coefficient (biomass oxidized per unit biomass per unit time)
k_{a1}	mixed-second-order rate coefficient for reduction of the primary electron acceptor (volume per mole dehalogenase per unit time)
k_{a2}	mixed-second-order rate coefficient for reduction of the second primary electron acceptor (volume per mole dehalogenase per unit time)
$q_{m,ap}$	apparent maximum specific rate of reductive dehalogenation (moles of RX per unit biomass per unit time)
K_{ap}	apparent half-saturation concentration for the halogenated aliphatic substrate (moles of RX per unit volume)
k_{ap}	apparent pseudo-first-order rate coefficient for reductive dehalogenation (volume per unit biomass per unit time)

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