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Evaluation of a model for the effects of substrate interactions on the kinetics of reductive dehalogenation

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Abstract

The effects of primary electron-donor and electron-acceptor substrates on the kinetics of TCA biodegradation in sulfate-reducing and methanogenic biofilm reactors are presented. Of the common anaerobic electron-donor substrates that were tested, only formate stimulated the TCA biodegradation rate in both reactors. In the sulfate-reducing reactor, glucose also stimulated the reaction rate. The effects of formate and sulfate on TCA biodegradation kinetics were analyzed using a model for primary substrate effects on reductive dehalogenation. Although some differences between the model and the data are evident, the observed responses of the TCA degradation rate to formate and sulfate were consistent with the model. Formate stimulated the TCA degradation rate in both reactors over the entire range of TCA concentrations that were studied (from 50 μg TCA/L to 100 mg TCA/L). The largest effects occurred at high TCA concentrations, where the dehalogenation kinetics were zero order. Sulfate inhibited the first-order TCA degradation rate in the sulfate-reducing reactor, but not in the methanogenic reactor. Molybdate, which is a selective inhibitor of sulfate reduction, stimulated the TCA removal rate in the sulfate-reducing reactor, but had no effect in the methanogenic reactor.

Introduction

Biodegradation of halogenated aliphatic hydrocarbons occurs through three major pathways: hydrolysis, oxidation, and reductive dehalogenation. Of these available pathways, the most important mechanism for biotransformation of highly halogenated aliphatic compounds in anaerobic environments is reductive dehalogenation, in which carbon-halogen bond cleavage is accompanied by a two electron reduction of the halogenated substrate. Although theoretically possible in some cases, the non-reductive mechanisms are much less likely. Oxidative dehalogenation reactions are catalyzed by oxygenases that require molecular oxygen as a co-reactant (Vogel et al. 1987; Ensley 1991). Therefore, they cannot occur under anaerobic conditions, where O_2 is absent. Hydrolytic dehalogenation reactions involve enzyme- or glutathione-mediated nucleophilic substitution reactions in which water is the ultimate nucleophile. These reactions probably proceed through a bimolecular mechanism in which the nucleophile attacks the halogenated carbon atom before the carbon-halogen bond is broken (Goldman et al. 1968; Goldman 1972; Janssen et al. 1994). Bimolecular nucleophilic substitution reactions are strongly influenced by the accessibility of the halogen-substituted carbon atom to nucleophilic attack (Morrison & Boyd 1973; Wackett et al. 1992; Janssen & Witholt 1992). Access is blocked by bulky halogen and methyl substituents in highly halogenated substrates like carbon tetrachoride (CCl_4) and 1,1,1-trichloroethane (TCA), and these compounds are not substrates for any of the well-characterized hydrolytic dehalogenases (Janssen et al. 1985; Kohler-Staub & Leisinger 1985; Janssen & Witholt 1992).

The anaerobic biodegradation of TCA has been studied in many different systems, ranging from mud- and muck-containing microcosms (Parsons & Lage 1985) to pure cultures (Egli et al. 1987; Egli et al. 1988; Gälli & McCarty 1989a, b). An important prod-

uct of the anaerobic biotransformation of TCA is 1,1-dichloroethane (DCA), which is the expected product from a reductive dehalogenation mechanism that is sometimes referred to as 'hydrogenolysis', in which a carbon-halogen bond is replaced by a carbon-hydrogen bond (Vogel et al. 1987). This is the most commonly recognized pathway for reductive dehalogenation, and ethane is the expected ultimate product of TCA metabolism by this route. Anaerobic dechlorination of TCA and other halogenated aliphatic substrates, however, often results in products that cannot be formed by hydrogenolysis (Bouwer & McCarty 1983; Vogel & McCarty 1987; Egli et al. 1988; Egli et al. 1990; Bouwer & Wright 1988; Gälli & McCarty 1989a; Criddle et al. 1990; Mikesell & Boyd 1990; Stromeyer et al. 1992). Frequently, these oxygen-substituted compounds are the major products of the anaerobic biotransformation of halogenated aliphatic hydrocarbons. Although pathways that proceed through a combination of reductive (hydrogenolytic) and non-reductive (hydrolytic) dehalogenations have been proposed to explain these products (Vogel & McCarty 1985; Vogel & McCarty 1987), it now appears likely that they result directly from a non-hydrogenolytic reductive dehalogenation mechanism that is thought to involve intermediate formation of carbenes (Krone et al. 1991). The existence of this alternate pathway was convincingly demonstrated using an electrolytic model system to reductively dechlorinate CCl_4 and TCA (Criddle & McCarty 1991). Electrolytic dechlorination of these substrates proceeded with input of two electrons per molecule, but the hydrogenolysis products accounted for only a very small proportion of the substrate that was transformed (only 5% of the dehalogenated TCA was converted to DCA). The remainder were completely dechlorinated, oxygen-containing products, such as carbon monoxide, formate, and acetate.

Although a broad spectrum of products can be formed, all reductive dehalogenation reactions involve electron transfer to the halogenated substrate (Wade & Castro 1973; Vogel et al. 1987; Criddle & McCarty 1991; Krone et al. 1991). Therefore, the reaction rate can be affected by the concentrations of primary electron-donor and -acceptor substrates, which control the intracellular availability of electrons. A model based on the interactions among electron donor oxidation, primary electron acceptor reduction, and reductive dehalogenation reactions has been presented previously (Wrenn & Rittmann, in press). In this paper, we evaluate the predictions of that model using biodegradation of TCA in methanogenic and sulfate-reducing biofilm reactors.

The model for substrate interactions during reductive dehalogenation was evaluated by examining the effects of exogenous primary electron-donor and -acceptor substrates on the apparent kinetic parameters for TCA biodegradation in anaerobic biofilm reactors. We measured the disappearance of TCA, rather than the formation of DCA, because a large variety of reductive dehalogenation products can be formed, and DCA is frequently a minor product (Vogel & McCarty 1987; Gälli and McCarty 1989a; Egli et al. 1988; Criddle & McCarty 1991) that is subject to further metabolism, especially in mixed cultures (Parsons & Lage 1985; Vogel & McCarty 1987). Also, the concentrations of TCA or the exogenous electron-donor and -acceptor substrates might affect the distribution of products formed from TCA and the rates of their subsequent metabolism. Thus, the rate of TCA disappearance provides the most direct test of our model.

The model was evaluated by determining the effects of formate and sulfate on the first-order kinetics of TCA biodegradation. The effects of electron donors and acceptors on the first-order kinetics of reductive dehalogenation are of interest, because the maximum contaminant levels for halogenated aliphatics, which may be used to define treatment goals, are in the parts-per-billion range (Vogel et al. 1987), and the biodegradation kinetics for these substrates are often first-order in this concentration range (Lage et al. 1986; Lam & Vilker 1987; Bouwer & Wright 1988; Gälli & McCarty 1989b). Also, a detailed evaluation of the model is more accessible in the first-order region, because kinetics can be analyzed using a single TCA concentration. Finally, the first-order region is free of the inhibitory effects of TCA on the kinetics of primary substrate metabolism (Wrenn 1992), which were not considered during model development (Wrenn & Rittmann, in press).

The effect of formate also was evaluated for TCA concentrations higher than first order, because the model makes important predictions regarding primary substrate effects on the apparent Monod parameters, $q_{m,ap}$ and K_{ap} (Wrenn & Rittmann, in press), and these parameters can be estimated only by measuring biodegradation rates over a wide range of TCA concentrations. Also, practical scenarios may have contaminant concentrations that are relatively high and target effluent concentrations that are low. In such cases, non-linear kinetic equations must be used to design effective and efficient biological treatment.

Materials and methods

Chemicals

Octane (99+%), methanol (99.97%), 1,1,1-trichloroethane (99%), formic acid (96%), propionic acid (99%), and sodium molybdate dihydrate (99%) were obtained from Aldrich Chemical Co., Milwaukee, Wisconsin. Sodium acetate (99.7%) and D-glucose were obtained from Mallinckrodt Chemical, St. Louis, Missouri.

Culture conditions

Sulfate-reducing and methanogenic consortia were grown in completely mixed, fixed-bed biofilm reactors similar to those described previously (Rittmann et al. 1986). These reactors were constructed from glass columns (2.5 cm in diameter and approximately 7.5 cm in length) that were packed with glass beads (3 mm diameter) and inoculated with anaerobic groundwater. Uniform distribution of biomass throughout the reactors was promoted by using a high recycle flow rate (80 l/day) relative to the overall flow rate through the system (1 l/day) and by reversing the direction of flow on a weekly basis.

Methanogens and sulfate reducers are strict anaerobes that are very sensitive to inhibition by traces of O_2. Oxygen contamination of the systems by diffusion of O_2 through the reactor tubing was minimized by using the most oxygen-impermeable tubing that was available: Flexible PVC tubing (1.52 mm I.D.; Gilson Medical Electronics) was used in the feed pump, Norprene (Size 15; Cole-Parmer) was used in the recycle pump, and the transfer tubing was 1/8" (O.D.) Teflon encased within 1/4" (O.D.) × 1/8" (I.D.) Norprene. The feed pump was a Gilson Minipuls 2 peristaltic pump, and the recycle pump was a Masterflex variable speed peristaltic pump (Cole-Parmer).

The basal nutrient medium was common to both reactors and had the following composition (in mg/l): glucose (10), NH_4Cl (58), KH_2PO_4 (82), K_2HPO_4 (69), $NaHCO_3$ (8.4), $CaCl_2 \cdot 2H_2O$ (2.0), $MnCl_2 \cdot 4H_2O$ (0.2), $FeCl_2 \cdot 4H_2O$ (0.1), and resazurin (0.4). Vitamins were added from a stock solution (1.25 ml/l) that contained the following (in mg/l): biotin (2), folic acid (2), pyridoxine-HCl (10), riboflavin (5), thiamine (5), nicotinic acid (5), pantothenic acid (5), vitamin B_{12} (0.1), *p*-aminobenzoic acid (5), and thioctic acid (5). Medium that was fed to the methanogenic reactor also contained $MgCl_2 \cdot 6H_2O$ (17 mg/l) and $Na_2MoO_4 \cdot 2H_2O$ (0.1 mg/l), and the medium that was supplied to the sulfate-reducing reactor contained $MgSO_4 \cdot 7H_2O$ (25 mg/l) and Na_2SO_4 (11 mg/l).

Dissolved oxygen was removed by bubbling the hot media extensively with N_2 after they had been sterilized by autoclaving. The media were reduced by addition of $Na_2S \cdot 9H_2O$ to give a final concentration of 24 mg/l. They were stored under N_2 at all times to prevent recontamination by atmospheric O_2.

Analytical methods

The concentrations of TCA in reactor influent and effluent samples were measured after extraction into octane. Because TCA is highly volatile, the extractions were performed in sealed serum bottles that were completely filled to exclude a gas phase (Henderson et al. 1976). Trichloroethene (TCE), which was used as an internal standard, was added to filled serum bottles by using a Hamilton syringe to inject 5 μL of a methanolic solution (100 mg TCE/l methanol) through the septa. After mixing the spiked samples, octane was added by injection through the septa of inverted serum bottles, displacing a portion of the aqueous sample. The extraction efficiencies of TCA and TCE into octane under these conditions were essentially 100%.

The concentrations of TCA and TCE in octane following extraction were measured by gas chromatography using a Hewlett-Packard HP-5890 gas chromatograph equipped with an electron capture detector. TCA and TCE were separated on a 6′ × 1/4" glass column packed with 80/100 Carbopak B coated with 1% SP-1000 (Supelco, Inc., Bellefonte, PA). The column was operated isothermally at 190° C. The injector port and detector temperatures were 230° C. The carrier gas was 5% methane in argon, and the flow rate was 30 ml/minute. Under these conditions, the retention times were 1.8 minutes for TCA and 2.5 minutes for TCE.

Measurement of TCA biodegradation

The effects of electron-donor and -acceptor substrates on the kinetics of TCA biodegradation were evaluated in intact biofilm reactors to avoid disruption of microbial interactions that could affect the survival of TCA-degrading organisms. The experiments were kept as short as possible to preclude any changes in the compositions of the microbial consortia. The TCA biodegradation rates were measured in once-through – rather than complete-mix – biofilm reactors, because

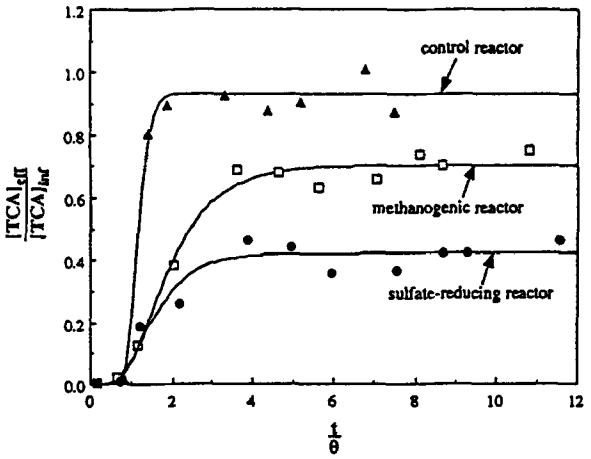

Fig. 1. Breakthrough curves for TCA in the methanogenic and sulfate-reducing biofilm reactors. The effluent concentrations are plotted as a function of dimensionless time (i.e., the ratio of actual time, t, to the reactor detention time, θ). Steady-state was achieved after approximately five hydraulic detention times, and effluent samples were collected between about 6 (20 hours) and 10 (32 hours) detention times.

significant abiotic losses of TCA were observed when the recycle pump was operating. These abiotic losses appeared to result from sorption of TCA to the tubing that was used in the recycle peristaltic pumps. Similar phenomena have been observed in other laboratories (Cseh et al. 1989; Stanley et al. 1989). Recovery of TCA from control reactors, which were identical to the biofilm reactors except that they lacked biomass, averaged 96% when they were operated in once-through configurations.

A syringe pump (Sage Instruments) and 50-ml Hamilton Gas-Tight syringes (Supelco, Inc.) were used to feed media supplemented with TCA to the reactors during these short-term biodegradation experiments. The flow rate used during these experiments was approximately 4 ml/hr. After the systems had reached steady state with respect to TCA concentration, influent and effluent samples were collected in ground-glass syringes to minimize volatilization losses. Steady state was achieved in approximately 20 hours (approximately six hydraulic detention times), and these experiments typically lasted no more than 32 hours (approximately ten hydraulic detention times). The reactors were returned to complete-mix operation immediately after completion of each experiment.

Typical breakthrough curves for TCA in methanogenic and sulfate-reducing biofilm reactors are shown in Fig. 1. The lines plotted through these data represent the best fits of a one-dimensional reactive-solute-transport model (van Genuchten 1982). These curves show no evidence of a lag preceding the start of TCA degradation. (This model assumes a constant, first-order reaction rate during transport.) Any changes in the numbers or activity of the TCA degraders in the systems would be reflected in the steady-state effluent TCA concentrations, but they are relatively constant over the sampling interval for a typical experiment. Thus, these measurements of the TCA degradation rates provide accurate estimates of the activity of the existing microbial populations in the reactors, and they were not affected by changes in the composition of the microbial populations or by physiological changes in the TCA-degrading bacteria.

Data collection and analysis for first-order TCA biodegradation kinetics

The effects of formate and sulfate concentrations on the first-order kinetics of TCA biodegradation were determined by measuring TCA removal in the presence of several different concentrations of these substrates. The influent TCA concentration in these experiments was approximately 100 μg/L, which is within the first-order region for these systems (Table 2). The effects of formate and sulfate were evaluated separately by varying the influent concentration of one substrate while the other was held constant.

The data were analyzed using a first-order biofilm model. The steady-state flux of substrate, $J(M_sL^{-2}T^{-1})$, into a biofilm of constant thickness and density is given by (Gantzer et al. 1988):

$$J = k_f S \tag{1}$$

where $S(M_sL^{-3})$ is the bulk liquid substrate concentration and $k_f(LT^{-1})$ is the first-order biofilm flux coefficient. For a fully penetrated biofilm, k_f is a linear function of the intrinsic first-order biodegradation rate coefficient, $k(L^3M_x^{-1}T^{-1})$:

$$k_f = L_f X_f k \tag{2}$$

where $L_f(L)$ is the biofilm thickness and $X_f(M_xL^{-3})$ is the concentration of catalytically active biomass in the biofilm.

A biofilm is fully penetrated when substrate consumption within it is slow relative to its rate of transport to and within the biofilm. This relationship can be characterized using the effectiveness factor, η, which is defined to be the ratio of the rate of substrate con-

sumption by a biofilm to the rate that would be observed in the absence of mass transport limitation (Atkinson & Davies 1974; Rittmann & McCarty 1981; Suidan et al. 1987). A fully-penetrated biofilm has an effectiveness factor equal to one. For the substrates and conditions used in these experiments, the minimum effectiveness factors, η_{min} (which would be observed when the substrate concentration at the biofilm surface approached zero and the first-order biodegradation coefficients were maximal), were very nearly one: for TCA, η_{min} was 0.998; for formate, it was 0.994; and η_{min} for sulfate was 0.977 (Wrenn 1992). Thus, for the purposes of this data analysis, it is reasonable to assume that the biofilms were fully penetrated for all substrates.

The apparent first-order biofilm flux coefficient, $k_{f,ap}$, can be determined from a mass balance on the once-through reactor. If dispersion in the bulk liquid can be neglected, $k_{f,ap}$ is given by:

$$k_{f,ap} = \frac{1}{a\theta} \ln\left(\frac{S_{inf}}{S_{eff}}\right) \quad (3)$$

where a (L^{-1}) is the specific surface area in the reactor and θ (T) is the empty-bed detention time. When dispersion is important, Eq. 3 is only an approximation of the true apparent first-order biofilm flux coefficient. The accuracy of this approximation depends on the importance of dispersion as a transport mechanism and on the rate of substrate removal in the system. For the conditions encountered in these experiments, Eq. 3 underestimated the true $k_{f,ap}$, but the error was never greater than 6% and usually was less than 1% (Wrenn 1992). Therefore, apparent first-order TCA biodegradation rate coefficients, k_{ap}, were computed using Eqs. 2 and 3.

The effect of formate on the first-order kinetics of TCA biodegradation was studied by determining k_{ap} in the presence of several concentrations of formate. Sulfate was present at zero (methanogenic reactor) or 0.1 mM (sulfate-reducing reactor) during these experiments. The concentrations of biomass and any second primary electron acceptor were assumed to be constant throughout this series of experiments. Under these conditions, the general model (Wrenn & Rittmann, in press) for the effect of the primary electron-donor concentration on reductive dehalogenation kinetics takes the form:

$$k_{ap} = \frac{k_{min} + k_{max}\frac{[formate]}{C_{av}}}{1 + \frac{[formate]}{C_{av}}} \quad (4)$$

where k_{max} is the maximum first-order biodegradation rate coefficient for TCA (which occurs when the formate concentration is very large), k_{min} is the minimum rate coefficient for TCA degradation (which occurs when the formate concentration equals zero), and C_{av} is the formate concentration at which k_{ap} is the average of k_{min} and k_{max}. These parameters are functions of the intrinsic kinetic and stoichiometric parameters for reductive dehalogenation, oxidation of the primary electron donors, and reduction of the primary electron acceptors, and of the concentrations of the primary electron acceptors and the biomass density in the biofilm. Although k_{max}, k_{min}, and C_{av} depend on the various kinetic and stoichiometric parameters and the primary substrate concentrations in somewhat different manners for the methanogenic and sulfate-reducing reactors (because of the dual role – as electron donor and acceptor – that is assumed for formate in the methanogenic reactor), they can be derived directly from the equations presented previously (Wrenn & Rittmann, in press).

The effect of sulfate on TCA biodegradation kinetics was examined by determining k_{ap} at several concentrations of sulfate. The formate concentration was either 1 or 2 mM. If sulfate is used as a primary electron acceptor by the TCA degraders, it should inhibit the rate of TCA biodegradation (Wrenn & Rittmann, in press). Under the conditions of these experiments (constant concentrations of primary electron donor, alternative electron acceptor, and biomass), the general model has the following form:

$$k_{ap} = \frac{C_1}{C_2 + [sulfate]} \quad (5)$$

where C_1 and C_2 are functions of the concentrations of the primary electron donors and the intrinsic kinetic and stoichiometric parameters for the model that has been described previously (Wrenn & Rittmann, in press).

Data collection and analysis for estimation of Monod kinetic parameters for TCA biodegradation

The effect of formate concentration on the kinetics of TCA biodegradation was determined by measuring the flux of TCA, $J_{TCA}(M_{TCA}L^{-2}T^{-1})$, in the methanogenic and sulfate-reducing biofilm reactors for influent TCA concentrations ranging from approximately 50 μg/L to 100 mg/L. The influent formate concentrations in these experiments ranged from zero to 4 mM. Sulfate was present at either zero (methanogenic reactor) or

0.1 mM (sulfate-reducing reactor). The TCA flux was computed from the reactor mass balance (Rittmann et al. 1986):

$$J_{TCA} = \frac{[TCA]_{inf} - [TCA]_{eff}}{a\theta} \quad (6)$$

Kinetic parameters for TCA biodegradation were estimated by equating the mass-balance flux, J_{TCA}, to an average local flux, $\bar{J}$, for a once-through biofilm reactor:

$$\bar{J} = \eta X_f L_f q_{m,ap} \frac{[TCA]_{1-m}}{K_{ap} + [TCA]_{1-m}} \quad (7)$$

where $q_{m,ap}$ ($M_{TCA}M_x^{-1}T^{-1}$) is the apparent maximum specific rate of TCA biodegradation, $K_{ap}(M_{TCA}L^{-3})$ is the apparent half-saturation concentration, and $[TCA]_{1-m}(M_{TCA}L^{-3})$ is the log-mean TCA concentration (Rittmann et al. 1986):

$$[TCA]_{1-m} = \frac{[TCA]_{inf} - [TCA]_{eff}}{\ln\left(\frac{[TCA]_{inf}}{[TCA]_{eff}}\right)} \quad (8)$$

For the purposes of this analysis, the effectiveness factor, η, was assumed to be equal to one, because the biofilm was always fully penetrated for TCA.

The accuracy of this analysis depends on the validity of the assumption that the mass-balance flux, J_{TCA}, is equal to the average local flux, $\bar{J}_{TCA}$, for the conditions of these experiments. There are three special cases for which these two fluxes are exactly equal: (1) when biodegradation follows zero-order kinetics, regardless of the degree of dispersion in the reactor; (2) when biodegradation kinetics are first-order and the reactor is plug flow; and (3) when the reactor is plug flow, the biofilm is fully penetrated, and external mass transfer resistance is negligible. The most important factor affecting the accuracy of this assumption is dispersion, and the greatest errors occur when biodegradation kinetics are first order. For the conditions of these experiments, the maximum error introduced by assuming that J_{TCA} and $\bar{J}_{TCA}$ are equal is approximately 4% (Wrenn 1992).

Parameter estimation

The parameters for the models were estimated by minimizing the sum-of-squared relative errors, SS_{rel}:

$$SS_{rel} = \sum\left(\frac{r_i - \bar{r}}{\bar{r}}\right)^2 \quad (9)$$

where r_i is the measured estimate of the reaction rate (e.g., J or k_{ap}) and $\bar{r}$ is the rate given by the model. It is preferable to minimize the sum-of-squared relative errors rather than the sum-of-squared absolute errors when there is a large difference between the highest and lowest observed rates and when the relative, rather than the absolute, errors are constant (Cornish-Bowden 1979; Sáez & Rittmann 1992). Equation 9 was used to estimate the model parameters, because the relative errors (i.e., the coefficients of variance) in these data were always approximately constant, and a wide range of fluxes (spanning two orders of magnitude) were observed when the apparent Monod parameters (Eq. 7) were estimated.

It is simple to estimate the apparent Monod parameters for Eq. 7, because the derivatives of SS_{rel} with respect to $q_{m,ap}$ and K_{ap} can be calculated exactly. Thus, closed-form solutions for these parameters exist (Cornish-Bowden 1979). The Monod parameters are given by:

$$q_{m,ap} = \frac{1}{X_f L_f} \frac{\sum\left(\frac{J_i}{[TCA]_{1-m,i}}\right)^2 \sum J_i^2 - \left(\sum \frac{J_i^2}{[TCA]_{1-m,i}}\right)^2}{\sum\left(\frac{J_i}{[TCA]_{1-m,i}}\right)^2 \sum J_i - \sum \frac{J_i^2}{[TCA]_{1-m,i}} \sum \frac{J_i}{[TCA]_{1-m,i}}} \quad (10)$$

$$K_{ap} = \frac{\sum J_i^2 \sum \frac{J_i}{[TCA]_{1-m,i}} - \sum \frac{J_i^2}{[TCA]_{1-m,i}} \sum J_i}{\sum\left(\frac{J_i}{[TCA]_{1-m,i}}\right)^2 \sum J_i - \sum \frac{J_i^2}{[TCA]_{1-m,i}} \sum \frac{J_i}{[TCA]_{1-m,i}}} \quad (11)$$

Similarly, the parameters C_1 and C_2 for Eq. 5, which describes the effect of sulfate on the first-order rate to TCA degradation, can be obtained explicitly. The best estimates for these parameters are given by:

$$C_1 = \frac{\sum k_{ap,i}^2 \sum (k_{ap,i}[SO_4^{2-}]_i)^2 - (\sum k_{ap,i}^2 [SO_4^{2-}]_i)^2}{\sum k_{ap,i}^2 \sum (k_{ap,i}[SO_4^{2-}]_i) - \sum k_{ap,i}^2 [SO_4^{2-}]_i \sum k_{ap,i}} \quad (12)$$

$$C_2 = \frac{\sum k_{ap,i} \sum (k_{ap,i}[SO_4^{2-}]_i)^2 - \sum k_{ap,i}[SO_4^{2-}]_i \sum k_{ap,i}^2 [SO_4^{2-}]_i}{\sum k_{ap,i}^2 \sum k_{ap,i}[SO_4^{2-}]_i - \sum k_{ap,i}^2 [SO_4^{2-}]_i \sum k_{ap,i}} \quad (13)$$

Explicit solutions do not exist for the best estimates k_{max}, k_{min}, and C_{av} of Eq. 4, and therefore, they must be estimated numerically. Microsoft Excel Solver was used to minimize Eq. 9 to obtain estimates for these parameters.

Results

Stimulation of TCA biodegradation by electron-donor substrates

The most effective electron-donor substrate for testing the primary substrates model was chosen by evaluating the ability of several common anaerobic electron donors to stimulate TCA biodegradation in the methanogenic and sulfate-reducing biofilm reactors. Most of these substrates are intermediates that can be formed during anaerobic carbohydrate metabolism. The influent concentration for each substrate was chosen based on the normal glucose mass loading rate (10 mg/day), the flow rate used for the TCA biodegradation experiments (4 ml/hr), and the stoichiometric ratio for a reaction by which the substrate can be produced from glucose by fermentation. An influent glucose concentration of 100 mg/l was required to maintain the normal glucose loading rate at the reduced flow rate that was used in these experiments, and all other electron-donor concentrations were based on this value. Stoichiometric ratios of 1 mole of propionate, 2 moles of acetate, and 2 moles of formate per mole of glucose were assumed. Methanol, which is not formed during anaerobic glucose metabolism, was added at a molar concentration equal to those used for formate and acetate. The stoichiometric ratios that were used were intended to approximate the fraction of glucose-derived electrons that flowed to each of the target microbial groups under the standard glucose loading rate. Therefore, the loading rates of electron equivalents for the different electron donors were unequal, but if the target group could degrade TCA, the observed stimulations would be roughly equal to that observed when glucose was the electron donor.

Figure 2 shows the extent of TCA removal that was observed in the methanogenic and sulfate-reducing biofilm reactors in the presence of each electron donor and in the absence of all exogenous electron donors. The influent TCA concentration used in these experiments was approximately 1 mg/l. Formate stimulated TCA biodegradation in both reactors, and glucose

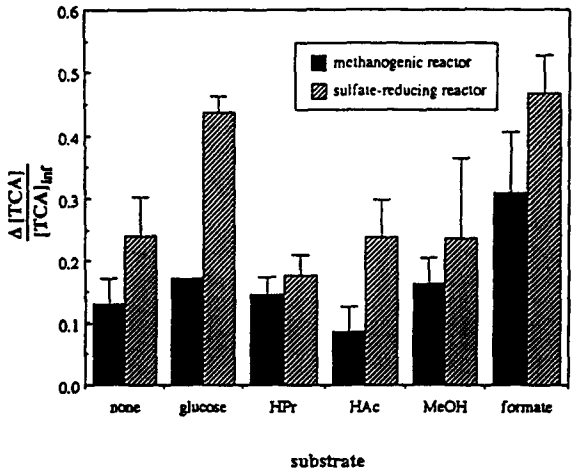

Fig. 2. Effect of several electron-donor substrates on the removal of TCA in methanogenic and sulfate-reducing biofilm reactors. Abbreviations for substrates: HPr, propionate; HAc, acetate; MeOH, methanol.

stimulated its removal in the sulfate-reducing reactor. These effects were significant at the 95% confidence level. None of the other electron-donor substrates significantly affected the extent of TCA removal in either reactor. Because formate stimulated TCA degradation in both reactors, it was used as the electron-donor substrate during testing of the primary substrates model.

Primary substrate effects on first-order TCA biodegradation kinetics

The apparent first-order rate coefficients for TCA degradation, k_{ap} (Eqs. 2 and 3), that were measured in the methanogenic and sulfate-reducing biofilm reactors at formate concentrations ranging between zero and 8 mM are shown in Fig. 3. The best fits of Equation 4 are also plotted.

The general model for substrate interactions during reductive dehalogenation (Eq. 4) captures the major trends exhibited in the data: TCA degradation proceeded in the absence of an exogenous electron donor, the first-order reaction rate increased in both reactors as the formate concentration increased, and the stimulatory effect saturated at higher formate concentrations. A subtle, but systematic deviation from the model occurred when the formate concentration was low. Specifically, a threshold formate concentration exists, below which the reaction rate was indepen-

Table 2. Apparent kinetic parameters for TCA biodegradation.

	[formate] (mM)	$q_{m,ap}$ (μg TCA/mg biomass COD-hr)	K_{ap} (μg TCA/L)	k_{ap} (L/mg biomass COD-hr)
methanogenic	0	7.3×10^{-3}	6,400	1.2×10^{-6}
reactor	0.5	8.7×10^{-3}	8,100	1.1×10^{-6}
	1	1.5×10^{-2}	7,900	1.9×10^{-6}
	2	4.6×10^{-2}	24,000	2.0×10^{-6}
	4	4.7×10^{-2}	25,000	1.9×10^{-6}
sulfate-reducing	0	5.3×10^{-3}	2,800	1.9×10^{-6}
reactor	0.5	5.2×10^{-3}	3,200	1.6×10^{-6}
	1	9.4×10^{-3}	2,800	3.3×10^{-6}
	2	5.3×10^{-2}	15,000	3.6×10^{-6}
	4	4.8×10^{-2}	12,000	4.1×10^{-6}

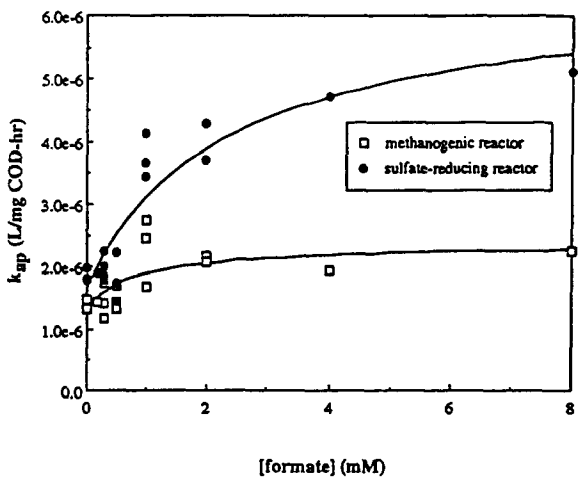

Fig. 3. Effect of formate concentration on the first-order rate coefficients for TCA biodegradation in methanogenic and sulfate-reducing biofilm reactors. The curves are the best fit of Eq. 4 to the data from each reactor.

dent of formate concentration. In both reactors, this threshold concentration is approximately 0.5 mM, and the first-order TCA degradation rate increased abruptly between 0.5 and 1 mM formate. Above 1 mM formate, the two systems responded differently to additional formate. In the methanogenic reactor, further changes in the reaction rate did not occur with addition of more formate, but the TCA degradation rate continued to increase as the formate concentration increased in the sulfate-reducing reactor (Fig. 3).

Table 1. Effect of molybdate on the first-order rate of TCA biodegradation.

[Molybdate] (mM)	k_{ap} (L/mg biomass COD-hr)	
	methanogenic reactor	sulfate-reducing reactor
0	$(2.1 \pm 0.44) \times 10^{-6}$	$(3.3 \pm 0.26) \times 10^{-6}$
10	$(2.1 \pm 0.01) \times 10^{-6}$	$(4.2 \pm 0.37) \times 10^{-6}$

The relationships between the apparent first-order rate coefficients for TCA biodegradation in the methanogenic and sulfate-reducing reactors and the influent sulfate concentration are shown in Fig. 4. Sulfate had no effect in the methanogenic reactor (Fig. 4A), but it strongly inhibited TCA degradation in the sulfate-reducing reactor (Fig. 4B). The TCA degradation rates observed in the sulfate-reducing reactor were higher in the presence of 2 mM than 1 mM formate, but the rates observed in the methanogenic reactor were the same at both formate concentrations. These results are consistent with those shown in Fig. 3 and with our model for substrate interactions during reductive dehalogenation (Wrenn & Rittmann, in press).

If sulfate inhibited TCA biodegradation by competing for electrons, then inhibition of sulfate reduction should stimulate TCA biodegradation in the sulfate-reducing reactor. Molybdate is a selective inhibitor that blocks the first step (formation of adenylphosphosulfonate from sulfate and ATP) in the assimilatory and dissimilatory metabolic pathways for sulfate

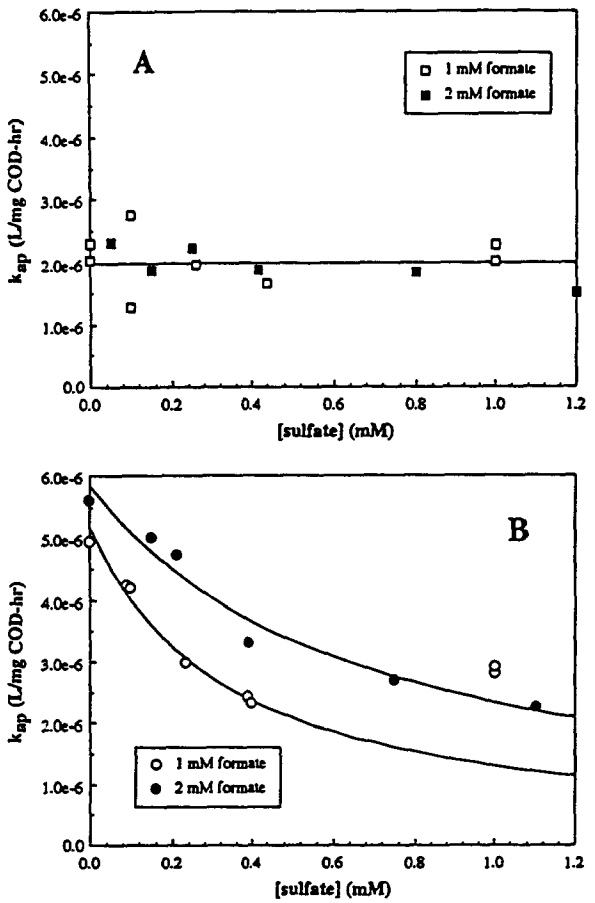

Fig. 4. Effect of sulfate on the first-order rate coefficients for TCA biodegradation in (A) methanogenic and (B) sulfate-reducing biofilm reactors. Determinations were made in the presence of 1 mM and 2 mM formate. For the sulfate-reducing reactor, the curves are the best fit of Eq. 5.

reduction (Oremland & Capone 1988). The apparent first-order rate coefficients for TCA degradation in the methanogenic and sulfate-reducing reactors in the presence of 1 mM formate, 0.1 mM sulfate, and either zero or 10 mM molybdate (which was sufficient to completely inhibit sulfate reduction in both reactors) are presented in Table 1. Molybdate stimulated the TCA removal rate in the sulfate-reducing reactor, but had no effect on the reaction rate in the methanogenic reactor. The effect of molybdate on the rate of TCA degradation in the sulfate-reducing reactor is significant at the 95% confidence level. These results are consistent with the effects of sulfate in these reactors (Fig. 4).

Although molybdate stimulated TCA degradation in the sulfate-reducing biofilm reactor used here, it has previously been shown to inhibit reductive dechlorination of 3-chlorobenzoate by the sulfate-reducing, dehalogenating organism *Desulfomonile tiedjei* (DeWeerd et al. 1991). The differences between our system and *D. tiedjei* probably reflect the energy requirements for dehalogenation, because molybdate depletes the ATP pool of sulfate reducers. The stimulation that we observed suggests that ATP is not required for TCA degradation in our system, but it might be required for transport or activation of 3-chlorobenzoate by *D. tiedjei*. Whereas TCA is an uncharged, lipophilic molecule that can easily enter microbial cells by passive diffusion, 3-chlorobenzoate is a weak acid that is negatively charged at neutral pH. An energy-dependent transport system, similar to that found in the coryneform bacterium NTB-1 for uptake of 4-chlorobenzoate (Groenewegen et al. 1990), might be involved in the accumulation of 3-chlorobenzoate by *D. tiedjei*.

Effect of formate concentration on the monod kinetic parameters for TCA biodegradation

The measured flux of TCA (Eq. 6) in the anaerobic biofilm reactors is plotted as a function of the log-mean TCA concentration in Fig. 5 for several different influent formate concentrations. The curves that are plotted in this figure are the best fits of Eq. (7) to the TCA flux data for each formate concentration. The best-fit parameters, $q_{m,ap}$ and K_{ap}, were estimated independently for each data set, and they are presented in Table 2. Because of the wide range of substrate concentrations that were used, these figures are presented as log-log plots. In this format, all curves have the same slope in the first-order region, and differences in the first-order rate appear as vertical displacements of the curves.

Formate stimulated the TCA biodegradation rate in both reactors over the entire range of TCA concentrations that were tested, and the effect was particularly apparent at high TCA concentrations. Between 0.5 and 2 mM formate, the apparent maximum specific rate of TCA degradation, $q_{m,ap}$, increased nearly 10-fold in the sulfate reducing reactor and more than 6-fold in the methanogenic reactor. Above and below this range, however, the maximum rates were independent of formate concentration. Although the maximum rates achievable were approximately the same in both reactors at all formate concentrations, the half-saturation concentrations were always lower in the sulfate-reducing reactor. Thus, the dehalogenating bac-

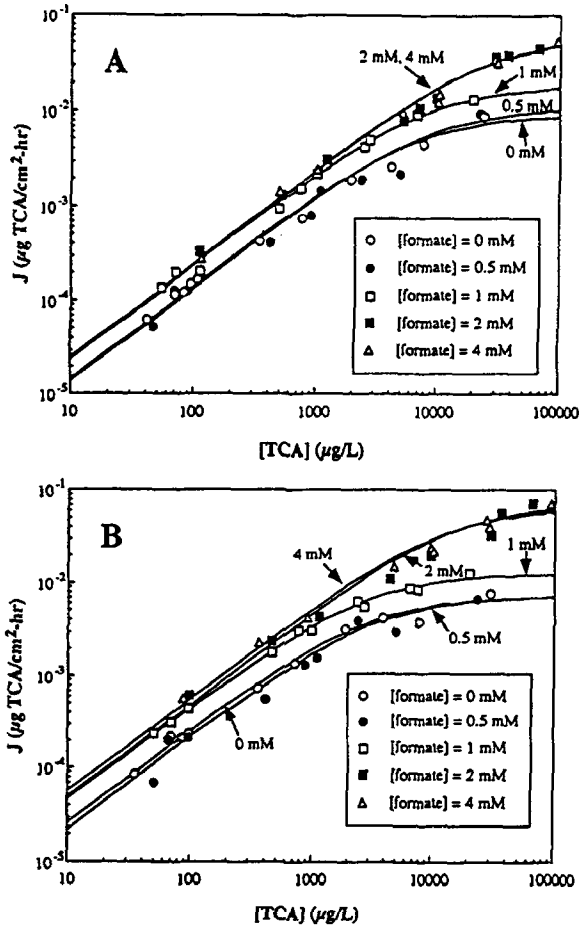

Fig. 5. Effect of formate concentration on the flux of TCA in (A) methanogenic and (B) sulfate-reducing biofilm reactors. TCA degradation rates were measured in the presence of 0 mM, 0.5 mM, 1 mM, 2 mM, and 4 mM formate. Curves are the best fit of Eq. 7 to the measured fluxes, and the best-fit parameters are given in Table 2.

teria in that system had a higher affinity for TCA than did the TCA degraders in the methanogenic reactor. The apparent half-saturation concentrations were unaffected by formate concentration up to 1 mM formate, but then increased dramatically between 1 and 2 mM formate. Below 1 mM formate, the half-saturation concentrations for both reactors were within the range of previously reported values for TCA (Gälli & McCarty 1989b) and TCE (Lage et al. 1986; Lage et al. 1987). The effect of formate in the first-order region is similar to what was observed when first-order kinetics were examined in isolation, and the values that were calculated from the data in Fig. 5 are similar to those obtained from the single concentration estimates (Fig. 3). Thus, the experimental methods and parameter estimation procedures that were used produced consistent and reliable results.

Discussion

The main goal of the research reported in this paper was to evaluate a model that describes the interactions that occur among electron-donor and -acceptor substrates and halogenated aliphatics during reductive dehalogenation reactions. Short-term experiments were performed in intact biofilm reactors to insure that the results were not complicated by changes in the composition or population density of the microbial consortia. The model that was tested is based on the hypothesis that the rate of reductive dehalogenation reactions is determined in part by the intracellular availability of electrons and that the availability of these electrons is determined by the relative rates of electron-donor oxidation and electron-acceptor reduction reactions (Wrenn & Rittmann, in press). Two forms of this model have been developed: The basic model considers only the effects of exogenously supplied primary electron-donor and -acceptor substrates. A more general model considers that electrons can also be made available by oxidation of endogenous electron donors and that additional electron-acceptor substrates (not explicitly supplied by the experimenter) may be available to the dehalogenating organisms (Wrenn & Rittmann, in press).

The results reported here support the conceptual foundation of the primary substrates model. For example, the availability of reducing equivalents affected the dehalogenation rate in the expected manner. Appropriate electron-donor substrates stimulated the rate of TCA degradation in both anaerobic biofilm reactors, but sulfate inhibited the reaction rate only in the sulfate-reducing reactor. Also, formate stimulated the TCA degradation rate in both reactors over a wide range of TCA concentrations. As predicted by the model, its effects were most pronounced at high TCA concentrations, where the reaction kinetics were zero-order with respect to TCA. Finally, Monod kinetics provide a reasonable description of the relationship between TCA concentration and its dehalogenation rate for all formate concentrations that were tested, suggesting that the effects of exogenous electron donors and acceptors can be adequately modeled by considering the kinetic parameters to be functions of the primary substrate concentrations.

Although the major trends exhibited by the interactions between formate and TCA in these anaerobic biofilm reactors are captured by the primary substrates model, some of the details are incorrect. For example, the model predicts that the apparent Monod kinetic parameters, $q_{m,ap}$ and K_{ap}, are linear functions of the formate concentration. Although both increased with increasing formate, the trends are not linear. Saturation of the electron-donor effect occurred at about 2 mM formate for both parameters, suggesting that an intrinsic limit to the TCA degradation rate had been reached. At this point, the maximum reaction rate might have been limited by the catalytic turnover rate of the dehalogenase, not by its supply of electrons. Thus, our assumption of first-order kinetics for the reductive dehalogenation reaction was probably inappropriate (Wrenn & Rittmann, in press). A more accurate model would employ Monod-type saturation kinetics for this reaction.

The data reported here and elsewhere (Fathepure & Boyd 1988a; Krone et al. 1989; DeWeerd & Suflita 1990; DeWeerd et al. 1991; Freedman & Gosset 1989; Gibson & Suflita 1990) clearly demonstrate that an endogenous source of electrons must be considered when modeling the kinetics of reductive dehalogenation reactions. In the first-order region, however, our general model predicts a smooth increase in the reaction rate as the concentration of the exogenous electron donor increases (Wrenn & Rittmann, in press). Instead, we observed a threshold in the response of the TCA degradation rate to formate concentration (Fig. 3). The reason for the existence of this threshold is not known. One possible explanation is that it reflects competition for reducing equivalents between the TCA degraders and a group of high-affinity, low-maximum utilization rate formate consumers. Alternatively, it could reflect interaction between the rates of oxidation of endogenous and exogenous electron donors, such that the oxidation rate for the endogenous electron donor decreases as the concentration of the exogenous electron donor increases (Grady et al. 1989; Dang et al. 1989). Our model assumes that endogenous electron donors are oxidized at a rate that is independent of the exogenous electron-donor concentration (Wrenn & Rittmann, submitted).

Despite some flaws, our model describes the major interactions that occur among electron-donor, -acceptor, and halogenated aliphatic substrates during reductive dehalogenation. Because we have made explicit assumptions regarding the nature of these interactions, our data can be used to analyze the ecology of reductive dehalogenation in the anaerobic biofilm reactors that we studied. An advantage of studying dehalogenation ecology in intact consortia is that we avoid artifacts that might arise during selective enrichment and isolation of the dehalogenating organisms. Although there are some recent examples to the contrary (Scholz-Murumatsu et al. 1990; Holliger et al. 1993), reductive dehalogenation of halogenated aliphatics does not usually support growth of the organisms that catalyze this reaction. Thus, selective enrichment of the dehalogenating bacteria from systems such as these can be difficult. The most common approach is to obtain representatives of the major metabolic groups (e.g., H_2-oxidizing or acetoclastic methanogens, glucose fermenters, etc.) and test them for dehalogenating activity (Egli et al. 1987; Egli et al. 1988; Fathepure & Boyd 1988a; Gälli & McCarty 1989a; Criddle et al. 1990). Traditional enrichment procedures select for fast-growing organisms that might not be representative of the organisms that are active in situ. These biofilm reactors were design to select for slower growing, oligotrophic bacteria that are expected to be important in contaminated aquifers (Rittmann et al. 1986). By using intact consortia, we increased the likelihood that we studied the activity of the ecologically important dehalogenating bacteria in our systems.

Our data can be used to evaluate the dehalogenation ecology of our reactors at several levels. The first level is the identification of electron-donor substrates that stimulated TCA biodegradation in our reactors. Substrates that affect the reaction rate must be metabolized by the dehalogenating bacteria or they must be converted by other organisms into substrates that are metabolized by the dehalogenaters. We tested several electron-donor substrates for their ability to stimulate TCA biodegradation in our anaerobic biofilm reactors. Each of these substrates targeted a group of microorganisms expected to be important in the anaerobic mineralization of glucose.

Glucose was used to stimulate the carbohydrate-fermenting bacteria, but the electrons derived from glucose can be made available to all other trophic groups in the consortia by well-characterized metabolic interactions (Wolin 1982; Zeikus 1983; Oremland 1988). Thus, the glucose-mediated stimulation of TCA biodegradation in the sulfate-reducing reactor is not surprising and does not imply that carbohydrate fermenters catalyzed dechlorination reactions in that system, because electron availability was increased to all of the metabolic groups when glucose was present. It is surprising that a similar effect was not observed in

the methanogenic reactor, but that might have been due to the inhibitory effect of TCA on the methanogenic population in that system (Wrenn 1992). Inhibition of the methanogens could have disrupted the normal flow of carbon and electrons through the consortium so that the substrate used by the dechlorinating bacteria was not produced. Sulfate reduction was not affected by TCA.

Propionate is consumed by H_2-producing acetogens, but it had no effect on the rate of TCA biodegradation in either reactor. These organisms, which grow slowly, are thought to constitute a small proportion of the total biomass in anaerobic carbohydrate-degrading consortia (Mosey 1983; Zeikus 1980). The low glucose loading rate for our reactors probably makes them even less important, because the carbohydrate fermenters probably can oxidize glucose directly to acetate and H_2 without production of more reduced fermentation products.

Acetoclastic methanogens and acetate-oxidizing sulfidogens are important members of anaerobic carbohydrate-degrading consortia. These organisms are probably not involved in reductive dechlorination, however, because acetate had no effect on the TCA biodegradation rate in either reactor. Methanol, which is metabolized by *Methanosarcina* spp. but not by *Methanothrix* spp (Jones et al. 1987), also had no effect in either reactor. Because methanol is a poor substrate for sulfate-reducing bacteria (Widdel 1988) its failure to elicit a response in that system is not surprising. Methanol also is metabolized by some H_2-oxidizing acetogens (Zeikus et al. 1985; Dolfing 1988), however, and its failure to stimulate TCA degradation suggests that these organisms did not catalyze reductive dehalogenation in our reactors.

Formate was the only electron-donor substrate that stimulated TCA removal in both reactors, and it probably was used directly by the dechlorinating bacteria. Many common methanogens, acetogens, and sulfidogens can grow on formate (Jones et al. 1987; Dolfing 1988; Widdel 1988), and representatives of all three of these groups are known to catalyze reductive dehalogenation reactions (Egli et al. 1987; Egli et al. 1988; Fathepure & Boyd 1988a, b; Krone et al. 1989; Mikesell & Boyd 1990). Methanogens and sulfidogens, in particular, were present in these anaerobic glucose-degrading consortia, and acetogens can survive in these systems as carbohydrate degraders.

Because formate is a relatively simple substrate that can be oxidized by a wide variety of microorganisms, it is difficult to draw any definitive conclusions regarding the ecology of reductive dechlorination in these biofilm reactors based only on stimulation of TCA degradation by formate. In some systems, the dominant dechlorinaters appear to be bacteria that grow on products formed by the growth and decay of other organisms (Gälli & McCarty 1989a; Semprini et al. 1992). If organisms such as these – which exist outside of the main pathway for anaerobic glucose degradation – can oxidize formate, they can be responsible for TCA biodegradation in our reactors.

An additional level of interpretation is possible, however, because our model predicts that electron-acceptor substrates that are used directly by the dehalogenating bacteria will inhibit reductive dehalogenation (Wrenn & Rittmann, in press). Therefore, inhibition of the TCA biodegradation rate by sulfate in the sulfate-reducing reactor suggests that sulfidogenic bacteria catalyzed reductive dechlorination in that system. Although sulfate reducers were present in the methanogenic reactor (Wrenn 1992), sulfate had no effect on the TCA degradation rate in that system. Also, molybdate, which completely inhibited sulfate reduction in both reactors, stimulated the rate of TCA biodegradation in the sulfate-reducing reactor, but it had no effect on the reaction rate in the methanogenic reactor. Interpreted in light of our model, these results suggest that sulfidogens catalyzed TCA dechlorination in the sulfate-reducing reactor, but they were not involved in the methanogenic reactor.

In the basic model for substrate interactions during reductive dehalogenation (Wrenn & Rittmann, in press), the apparent first-order rate coefficient for the dehalogenation reaction is a function of the ratio of the concentrations of the primary electron-donor ($[DH_{2\beta}]$) and -acceptor ([A]) substrates and the ratio of the intrinsic first-order kinetic and stoichiometric coefficients for reactions that involve these substrates ($C_{r/o}$):

$$k_{ap} = \frac{k_{max}}{1 + C_{r/o}\frac{[A]}{[DH_{2\beta}]}} \qquad (14)$$

When the same substrate serves as electron donor and acceptor simultaneously, as it does when methanogens and acetogens grow on formate, the ratio of the electron-acceptor to electron-donor concentrations is constant for all primary substrate concentrations, and k_{ap} for reductive dehalogenation is expected to be constant. This is precisely what occurred in the methanogenic reactor at formate concentrations greater than 0.5 mM. Even though the maximum specific rate of TCA degradation, $q_{m,ap}$, increased by a

factor of three between 1 and 2 mM formate (Table 2), there was no change in k_{ap}. The apparent first-order rate coefficient for TCA degradation was unaffected by formate concentration between 1 and 8 mM in the methanogenic reactor (Fig. 3), but k_{ap} increased with increasing formate over this same range in the sulfate-reducing reactor, where the electron-donor and -acceptor concentrations varied independently. Thus, in the methanogenic reactor, our results for formate concentrations greater than 0.5 mM are consistent with the basic form of our model, suggesting that the dechlorinating bacteria used formate as the electron donor and acceptor.

The basic model for substrate interactions also describes the combined effects of sulfate and formate on the TCA degradation rate in the sulfate-reducing reactor for formate concentrations greater than 0.5 mM. Although the TCA removal rate is higher at 2 mM than at 1 mM formate for most sulfate concentrations (Fig. 4B), the basic form of our model predicts that the rate is really determined by the ratio of the sulfate and formate concentrations (Eq. 14). When the data are plotted this way, the biodegradation rates for both formate concentrations fall on the same curve (Fig. 6), providing additional support for our hypothesis that the TCA degraders in the sulfate-reducing reactor are formate-oxidizing sulfidogens. These data do not follow Eq. 14 exactly, however, because the effect of sulfate saturated when the ratio of sulfate to formate concentrations was approximately 0.4. Since complete oxidation of formate with sulfate reduction requires a concentration ratio of only 0.25, saturation of the sulfate effect is probably due to exhaustion of the exogenous electron donor.

Our hypothesis that the metabolism rate for endogenous substrates was not constant is supported by the observation that both reactors behaved as if the dechlorinating bacteria metabolized only the exogenously supplied electron-donating and -accepting substrates when the formate concentration was greater than 0.5 mM. Further refinement of this model will require a better understanding of the relative contributions of endogenous and exogenous metabolism to the reductive dehalogenation rate.

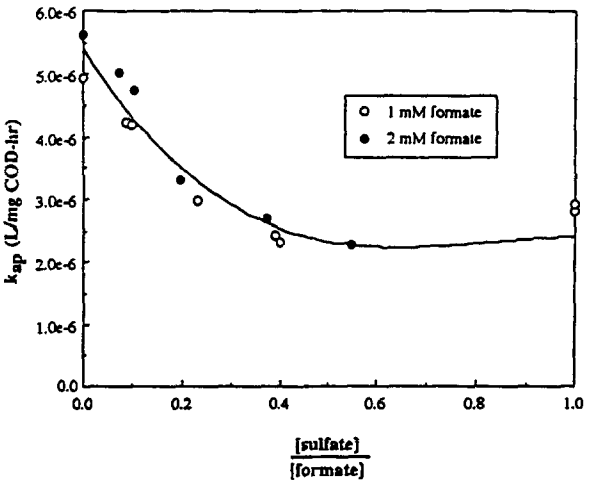

Fig. 6. Effect of the ratio of sulfate to formate concentrations on the first-order rate coefficients for TCA biodegradation in the sulfate-reducing reactor. Reaction rates were measured in the presence of 1 mM and 2 mM formate.

Conclusions

The results presented here show clearly that the rate of TCA biodegradation in anaerobic biofilm reactors can be affected by the concentrations of exogenous primary electron-donor and electron-acceptor substrates. Electron-donor substrates, such as formate and glucose, stimulated TCA degradation, and electron-acceptors, such as sulfate, inhibited the reaction. Formate affected TCA degradation by altering the Monod kinetic coefficients, $q_{m,ap}$ and K_{ap}, both of which increased as the formate concentration increased. When the TCA degradation rate was measured in the presence of a constant amount of formate, the Monod equation provides a good description of the reaction kinetics.

Much of the data that we have presented is consistent with the hypothesis that formate-oxidizing methanogens or acetogens catalyzed TCA degradation in the methanogenic reactor, while formate-oxidizing sulfate reducers were responsible in the sulfate-reducing reactor. Formate is the only substrate that stimulated TCA degradation in both reactors, and in anaerobic ecosystems, its potential catabolic pathways are limited. Although glucose also stimulated the reaction in the sulfate-reducing reactor, its fermentation products feed into many anaerobic metabolic pathways, and formate and H_2 are among its potential products. The hypothesis that sulfidogens were the active organisms in the sulfate-reducing reactor is supported by the strong inhibition of TCA degradation that was exerted by sulfate and by molybdate's stimulation of the reaction rate. Since neither of these compounds affected the TCA degradation rate in the

methanogenic reactor, their effects could not have been due to non-specific interactions with the dehalogenating bacteria. Finally, the first-order effects of formate in the methanogenic reactor are consistent with the use of formate as an electron donor and an electron acceptor by the TCA degraders in that system. Methanogens and acetogens share this metabolic characteristic.

Although the substrate interactions that occurred during anaerobic biodegradation of TCA in these biofilm reactors appear to be more complex than is considered in either the basic or general formulations of the primary-substrates model, the major trends predicted by the model are largely correct. Further refinement of the model can provide additional insight into the metabolic interactions that occur among primary substrates and halogenated aliphatics, facilitating the design of effective and efficient biological dehalogenation processes. The current model provides guidance for optimization of these processes, and it can be used as a tool for studying the microbial ecology of reductive dehalogenation in intact natural and engineered systems.

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Parameter definitions

J	= steady-state flux of substrate into a biofilm ($M_s L^{-2} T^{-1}$)
$\tilde{J}$	= average local flux for substrate in a once-through biofilm reactor ($M_s L^{-2} T^{-1}$)
S	= bulk-liquid substrate concentration ($M_s L^{-3}$)
S_{inf}	= influent substrate concentration ($M_s L^{-3}$)
S_{eff}	= effluent substrate concentration ($M_s L^{-3}$)
k_f	= first-order biofilm flux coefficient (LT^{-1})
$k_{f,ap}$	= apparent first-order biofilm flux coefficient (LT^{-1})
k	= intrinsic first-order biodegradation rate coefficient ($L^3 M_x^{-1} T^{-1}$)
k_{ap}	= apparent first-order biodegradation rate coefficient ($L^3 M_x^{-1} T^{-1}$)
k_{max}	= maximum first-order biodegradation rate coefficient for TCA ($L^3 M_x^{-1} T^{-1}$)
k_{min}	= minimum first-order biodegradation rate coefficient for TCA ($L^3 M_x^{-1} T^{-1}$)
L_f	= biofilm thickness (L)
X_f	= biomass concentration in the biofilm ($M_x L^{-3}$)
a	= specific surface area in the reactor (L^{-1})
θ	= empty-bed detention time (T)
C_{av}	= formate concentration at which k_{ap} is half-way between k_{min} and k_{max} ($N_{formate} L^{-3}$)
C_1	= coefficient that describes inhibition of TCA biodegradation by sulfate ($N_{sulfate} M_x^{-1} T^{-1}$)
C_2	= concentration of sulfate that reduces the TCA biodegradation rate to half of its maximal rate ($N_{sulfate} L^{-3}$)
$q_{m,ap}$	= apparent maximum specific rate of TCA biodegradation ($M_{TCA} M_x^{-1} T^{-1}$)
K_{ap}	= apparent half-saturation concentration for TCA ($M_{TCA} L^{-3}$)
$[TCA]_{l-m}$	= log-mean TCA concentration ($M_{TCA} L^{-3}$)
$[TCA]_{inf}$	= influent TCA concentration ($M_{TCA} L^{-3}$)
$[TCA]_{eff}$	= effluent TCA concentration ($M_{TCA} L^{-3}$)
η	= biofilm effectiveness factor (dimensionless)
$C_{r/o}$	= ratio of intrinsic kinetic and stoichiometric coefficients for reduction of the primary electron acceptor and oxidation of the primary electron donor (dimensionless)
Fundamental units:	
M_i	= mass of substance *i*
N_i	= number of moles of substance *i*
L	= length
T	= time