Kinetic and Inhibition Studies for the Aerobic Cometabolism of 1,1,1-Trichloroethane, 1,1-Dichloroethylene, and 1,1-Dichloroethane by a Butane-Grown Mixed Culture

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Received 15 November 2001; accepted 30 April 2002

DOI: 10.1002/bit.10397

Abstract: Batch kinetic and inhibition studies were performed for the aerobic cometabolism of 1,1,1-trichloroethane (1,1,1-TCA), 1,1-dichloroethylene (1,1-DCE), and 1,1-dichloroethane (1,1-DCA) by a butane-grown mixed culture. These chlorinated aliphatic hydrocarbons (CAHs) are often found together as cocontaminants in groundwater. The maximum degradation rates (k_{max}) and half-saturation coefficients (K_s) were determined in single compound kinetic tests. The highest k_{max} was obtained for butane (2.6 μmol/mg TSS/h) followed by 1,1-DCE (1.3 μmol/mg TSS/h), 1,1-DCA (0.49 μmol/mg TSS/h), and 1,1,1-TCA (0.19 μmol/mg TSS/h), while the order of K_s from the highest to lowest was 1,1-DCA (19 μ*M*), butane (19 μ*M*), 1,1,1-TCA (12 μ*M*) and 1,1-DCE (1.5 μ*M*). The inhibition types were determined using direct linear plots, while inhibition coefficients (K_{ic} and K_{iu}) were estimated by nonlinear least squares regression (NLSR) fits to the kinetic model of the identified inhibition type. Two different inhibition types were observed among the compounds. Competitive inhibition among CAHs was indicated from direct linear plots, and the CAHs also competitively inhibited butane utilization. 1,1-DCE was a stronger inhibitor than the other CAHs. Mixed inhibition of 1,1,1-TCA, 1,1-DCA, and 1,1-DCE transformations by butane was observed. Thus, both competitive and mixed inhibitions are important in cometabolism of CAHs by this butane culture. For competitive inhibition between CAHs, the ratio of the K_s values was a reasonable indicator of competitive inhibition observed. Butane was a strong inhibitor of CAH transformation, having a much lower inhibition coefficient than the K_s value of butane, while the CAHs were weak inhibitors of butane utilization. Model simulations of reactor systems where both the growth substrate and the CAHs are present indicate that reactor performance is significantly affected by inhibition type and inhibition coefficients. Thus, determining inhibition type and measuring inhibition coefficients is important in designing CAH treatment systems. © 2002 Wiley Periodicals, Inc. *Biotechnol Bioeng* **80:** 498–508, 2002.

Keywords: aerobic cometabolism of CAH mixtures; competitive and mixed inhibition; direct linear plot; butane-grown mixed culture

INTRODUCTION

Inhibition kinetic studies for aerobic cometabolism of chlorinated aliphatic hydrocarbon (CAH) mixtures are important, since growth substrate must be added to stimulate the required microorganisms, and there is competition among CAHs and the growth substrate and among the CAHs for enzyme active sites. This inhibition can negatively affect the performance of cometabolic treatment, and thus, the ability to model this inhibition is required in designing treatment processes.

Numerous kinetic and inhibition studies for aerobic CAH cometabolism have been performed to model aerobic CAH cometabolism. Most studies have assumed competitive inhibition among CAHs and CAHs and growth substrate (Alvarez-Cohen and McCarty, 1991; Anderson and McCarty, 1996; Broholm et al., 1992; Chang and Alvarez-Cohen, 1995; Speitel et al., 1993; Strand et al., 1990), based on the hypothesis that growth substrate and CAH must bind and compete for the same enzyme active site. This assumption permitted the competitive inhibition coefficient [K_{ic}: an equilibrium constant when an inhibitor (I) binds to a free enzyme (E) to form an enzyme · inhibitor complex (E · I)] to be set equal to the half-saturation coefficient (K_s). Although this assumption seems reasonable, few systematic studies have been carried out to compare the inhibition patterns and kinetic constants of various CAHs on the growth substrate and, conversely, the inhibition patterns and kinetic constants of the growth substrate on these CAHs.

Correspondence to: L. Semprini
Contract grant sponsor: U.S. Environmental Protection Agency
Contract grant number: R-815738

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A methodology for determining the inhibition type and for estimating kinetic and inhibition parameters for 1,1,1-trichloroethane (1,1,1-TCA) transformation by a butane-grown mixed culture was described in detail by Kim et al. (2002). In that study, the kinetic diversity of the butane monooxygenase activity in the mixed culture was investigated using enzyme inactivation studies as described by Silverman (1988). The loss in butane uptake activity as a function of time of exposure to acetylene indicated fairly homogenous monooxygenase activity of the butane-grown mixed culture. Thus, Kim et al. (2002) applied inhibition kinetics developed for homogenous enzyme systems to this mixed culture.

In the previous study (Kim et al., 2002), inhibition types were determined using direct linear plots as described by Cornish-Bowden and Eisenthal (1978) and Eisenthal and Cornish-Bowden (1974). The direct linear plots indicated competitive inhibition of 1,1,1-TCA on butane degradation and mixed inhibition of 1,1,1-TCA transformation by butane. Initial estimates of maximum degradation rates (k_{max}), K_s, K_{ic}, and uncompetitive inhibition coefficient [K_{iu}: an equilibrium constant when an inhibitor binds to an enzyme · substrate complex (E · S) to form an enzyme · substrate · inhibitor complex (E · S · I)] were obtained from linear regression of the linearized forms. Methods for estimating kinetic parameters using the linear plots are described in detail by Kim et al. (2002). Values determined from linear regression were used as initial guesses for nonlinear least squares regression (NLSR) analysis to yield a best-fit model to the data. The inhibition model identified by direct linear plots was found to fit to the experimental rate data well, and the initial guesses of all the kinetic parameters determined from linear plots were in the range of the values estimated from NLSR analysis (Kim et al., 2002).

CAH mixtures, rather than a single CAH, usually contaminate groundwater. One of the CAH mixtures of concern is a combination of 1,1,1-TCA, 1,1-dichloroethylene (1,1-DCE), and 1,1-dichloroethane (1,1-DCA), which are often found together as cocontaminants in groundwater. 1,1-DCE and 1,1-DCA are produced abiotically and biologically from 1,1,1-TCA (Vogel and McCarty, 1987), and use of 1,1,1-TCA as a solvent has resulted in subsurface contamination. These CAHs are difficult to remediate through aerobic cometabolism, since few microorganisms have been known to effectively transform these CAHs. Phenol-, and toluene-oxidizing microorganisms effectively transform chlorinated ethenes [e.g., trichloroethylene (TCE)], however, chlorinated methanes [e.g., chloroform (CF)] and ethanes (e.g.,1,1-DCA and 1,1,1-TCA) are not effectively transformed (Chang and Alvarez-Cohen, 1995; Hopkins and McCarty, 1995). Methanotrophs expressing soluble methane monooxygenase (sMMO) effectively transformed 1,1,1-TCA, while those expressing particulate methane monooxygenase (pMMO) are not effective (Chang and Alvarez-Cohen, 1996; Oldenhuis et al., 1991). 1,1-DCE has been a problematic CAH to transform via aerobic cometabolism due to its high transformation product toxicity (Anderson and McCarty, 1996; Dolan and McCarty, 1995; Hopkins and McCarty, 1995). Thus, finding an aerobic system for effective transformation of 1,1,1-TCA, 1,1-DCA, and 1,1-DCE was of interest.

In our previous transformation and kinetic studies (Kim et al., 2000; Kim et al., 2002), a butane-grown mixed culture effectively transformed 1,1,1-TCA, 1,1-DCA, and 1,1-DCE. Our butane-grown culture more effectively transformed 1,1,1-TCA on the basis of the amount transformed per unit mass cells than achieved with a methane-grown mixed culture reported by Chang and Alvarez-Cohen (1996). It also had a much higher affinity (much lower K_s) for 1,1,1-TCA compared to *M. trichosporium* OB3b and *M. trichosporium* OB3b PP358 expressing sMMO (Aziz et al., 1999; Oldenhuis et al., 1991). The initial transformation rates of 1,1-DCE were comparable with that achieved by *M. trichosporium* OB3b expressing sMMO (Oldenhuis et al., 1991), while the transformation capacity (T_c) was a factor of 4 to 9 higher than achieved with methanotrophs (Chang and Alvarez-Cohen, 1996).

Our previous methodology (Kim et al. 2002) well described kinetic and inhibition patterns for the cometabolism of one CAH, but CAH mixtures are most commonly encountered as contaminants in aquifer, so the applicability of the method on a variety of CAHs is assessed in this study. A systematic and detailed kinetic and inhibition study of the aerobic cometabolism of 1,1,1-TCA, 1,1-DCE, and 1,1-DCA, by a butane-grown mixed culture was performed here. The objectives of this study were: (1) to determine kinetic parameters and inhibition coefficients, (2) to provide a systematic comparison of three inhibition cases (i.e., inhibition among different CAHs, inhibition of growth substrate on different CAHs, and inhibition of different CAHs on the growth substrate), and (3) to evaluate if two widely used assumptions in aerobic CAH transformation models are valid for this system.

MATERIALS AND METHODS

A Butane-Utilizing Mixed Culture

The butane-utilizing culture was obtained from Hanford soil microcosms (Kim et al., 1997). Our butane enrichment was stained with 4′ 6′-diamidino-2-phenylindole for living cells and propidium iodide for nonliving cells. Observation under a fluorescence microscope showed 1 to 2 μm long rods that form chains of up to two or three microorganisms. Gram stain test indicates that the larger cells (2 μm) are gram-positive and smaller cells (1 μm) are gram-negative.

The culture was batch grown in media and stored in a cryogenic dewar containing liquid nitrogen to ensure a consistent inoculum for all the experiments, and cells were grown and harvested for the batch kinetic tests as described in detail by Kim et al. (2002).

Chemicals

Butane (≥99%) was purchased from AIRCO (Vancouver, WA). 1,1,1-TCA (99.5% anhydrous) and 1,1-DCE (99%)

were purchased from Aldrich Chemical Co. (Milwaukee, WI). 1,1-DCA, (≥99%) was obtained from Acros Organics (Pittsburgh, PA).

Batch Experiments

Batch kinetic test methods described by Kim et al. (2002) were used in this study. A volumetric quantity of the saturated aqueous stock solution of the CAHs of interest was added to crimp-sealed glass vials (26 mL) containing 4.5 mL of autoclaved mineral medium and air-filled headspace (21.5 mL) to achieve desired initial aqueous concentrations. Butane was added volumetrically as a gas. A headspace sample was taken to measure initial concentrations prior to adding the prepared cells, to initiate transformation reactions. After cell addition, the bottles were vigorously hand-shaken for 10 s, and then shaken at 260 rpm on a rotary shaker. Based on the results of mass transfer experiments, there were no mass transfer limitations to the liquid phase over the time scale of the kinetic experiments (Kim, 2000). Headspace concentrations were measured at five equally spaced time intervals over a period 10 to 20 min. The aqueous concentration and total compound mass in each batch reactor was calculated, using the headspace concentrations, the headspace and solution volumes, and published Henry's constants (Gossett, 1987; Mackay and Shiu, 1981). Initial transformation/degradation rates were determined by linear regression of six temporal observations (remaining mass in a batch reactor and time).

To minimize effects of a finite CAH transformation capacity on estimating initial transformation rate, a ratio of the amount CAH transformation to initial cell mass is kept low, compared with the finite capacity. Based on our earlier studies (Kim et al., 2000), the mass of CAH ultimately transformed per cell mass over 30 h were 2.4, 0.33, and 0.92 μmol/mg TSS for 1,1-DCA, 1,1,1-TCA, and 1,1-DCE, respectively. Thus, in these kinetic studies, the mass transformed was limited to 5 to 21% of these values, so that transformation rates would not be affected by the amount of CAH transformed.

For a single substrate kinetic test to determine k_{max} and K_s values, duplicate or triplicate vials were prepared at 10 different concentrations. For inhibition tests the inhibitor was added at five different concentrations and at four different substrate concentrations, requiring 20 reactors for each test. Preliminary inhibition experiments were performed to determine the range of substrate and inhibitor concentrations to use in the studies.

Analysis

The gaseous concentrations of butane and the CAHs of interest were determined by reactor headspace analysis. Calibration curves for all compounds were developed using external standards. The headspace concentrations were determined by injecting gas sample (100 μL) into a HP5890 series gas chromatograph (GC) connected to a photoionization detector (PID) followed by a flame ionization detector (FID) operated at 250°C. Detailed GC operating conditions are described by Kim et al. (2002).

The culture density was determined as total suspended solids (TSS) (American Public Health Association, 1985), using 0.1-μm membrane filter (Micro Separation Inc., Westboro, MA). The OD_{600} of cultures was measured using an HP8453 UV-Visible spectrophotometer.

Determination of Inhibition Types

The direct linear plot reported by Eisenthal and Cornish-Bowden (1974) and Cornish-Bowden and Eisenthal (1978) was used to determine the inhibition type. Details of applying the direct linear plot to a CAH transformation test are described by Kim et al. (2002). The basis for the method is illustrated by rearranging the Michaelis-Menten equation to show the dependence of apparent k_{max} (k_{max}^{app}) on apparent K_s (K_s^{app}):

$$k_{max}^{app} = v + \frac{v}{S_L} K_s^{app} \quad (1)$$

where v is specific substrate degradation rate (μmol/mg TSS/h), k_{max}^{app} and K_s^{app} are the apparent values of k_{max} and K_s in the presence of inhibitor, respectively. Thus, k_{max}^{app} and K_s^{app} are equal to k_{max} and K_s, respectively, in the absence of inhibitor. S_L is the substrate concentration in liquid phase (μM).

Figure 1 illustrates the results of a direct linear plot of initial transformation rates (v) measured at four different substrate concentrations (1,1-DCE) and five different inhibitor concentrations (1,1-DCA). Values of v are plotted on the y-axis and the corresponding negative S_L values are

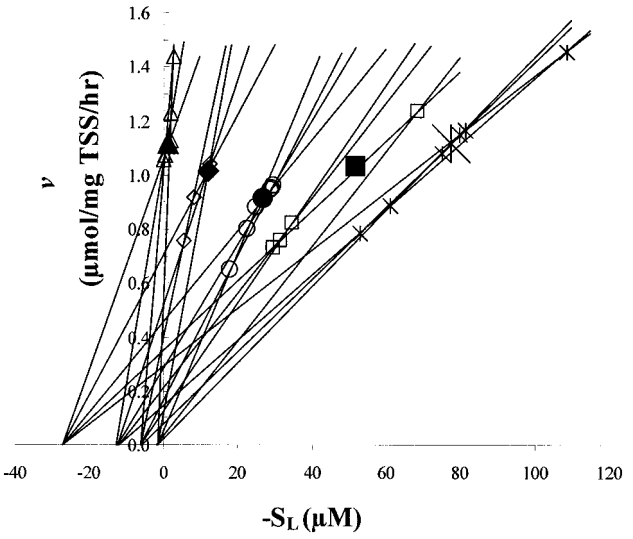

Figure 1. Direct linear plot showing competitive inhibition of 1,1-DCA on 1,1-DCE transformation. The inhibitor (1,1-DCA) was added at five different concentrations [0 (▲), 153 (♦), 387 (●), 608 (■), and 887 (✳) μM] and at four different substrate (1,1-DCE) concentrations (S_L: 1.6, 6.1, 12 and 27 μM) requiring 20 batch reactor tests.

plotted on x-axis (Fig. 1). A line is drawn and extrapolated between the v and negative S_L. The coordinates of the intersection on lines (smaller symbols) define a unique pair of k_{max}^{app} and K_s^{app} values that satisfy the sets of observations. The medians of intersections (larger symbol) provide best estimates of k_{max}^{app} and K_s^{app}. The shift in the direction of best estimate point of k_{max}^{app} and K_s^{app} at each value of inhibitor concentration (I_L) is an indicator of the inhibition type. For competitive inhibition, the shift is to the right; for uncompetitive inhibition, the shift is towards the origin; for mixed inhibition, the shift is between these extremes; and for the special case of mixed inhibition (noncompetitive inhibition), the shift is vertically down. Thus, direct linear plot shown in Figure 1 indicates competitive inhibition of 1,1-DCA on 1,1-DCE transformation.

Determination of k_{max}, K_s, K_{ic}, and K_{iu}

For single compound batch kinetic studies, k_{max} and K_s were determined by fitting the data to Michaelis-Menten equation including a mass balance between the air and aqueous phase by NLSR analysis (Kim et al., 2002). A statistical package of S-PLUS (MathSoft Inc., Cambridge, MA) was used for the NLSR fitting. In the inhibition study, k_{max}, K_s, K_{ic}, and K_{iu} were determined by NLSR analysis for the inhibition model determined by the direct linear plot method. The linearized inhibition forms reported by Kim et al. (2002) were used to obtain the initial guesses of all kinetic parameters for the NLSR fitting. The NLSR method and more detailed discussion of the methodology used here are described by Kim et al. (2002).

RESULTS

k_{max} and K_s for Butane, 1,1-DCE, 1,1-DCA, and 1,1,1-TCA

Results of the single compound tests and NLSR fits to determine k_{max} and K_s are provided in Figure 2. Results for 1,1,1-TCA and butane are provided by Kim et al. (2002). The measured k_{max} and K_s along with their 95% confidence intervals and the pseudo-first-order rate coefficient ($k_1 = k_{max}/K_s$) are summarized in Table I. The order of k_{max} from the highest to lowest was butane, 1,1-DCE, 1,1-DCA, and 1,1,1-TCA, while the order of K_s from the highest to the lowest was 1,1-DCA, butane, 1,1,1-TCA and 1,1-DCE. The K_s for 1,1-DCE was one order of magnitude lower than those for the other compounds, indicating that 1,1-DCE has a higher binding affinity to the monooxygenase enzyme than the other compounds. The k_1 value for 1,1-DCE was also greater than those for the other compounds, indicating very rapid transformation of this compound would be achieved at low concentrations.

Inhibition Types and Inhibition Coefficients

Butane Inhibition of CAH Transformation

The direct linear plot showing the inhibition of butane on 1,1-DCE transformation is presented in Figure 3A. The

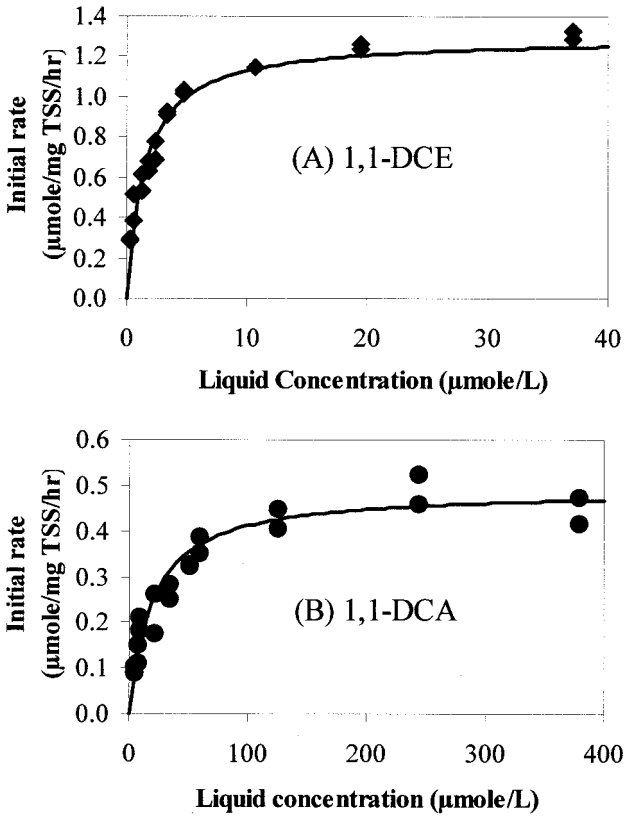

Figure 2. Initial degradation rates at various initial concentrations of 1,1-DCE (A) and 1,1-DCA (B) in single compound studies. Liquid concentrations and total compound mass in each batch reactor were calculated using measured headspace concentrations, headspace and solution volumes, and published Henry's constants. The curves represent the best fits using NLSR of Michaelis-Menten equation including a mass balance between the air and aqueous phase presented by Kim et al. (2002).

points of intersection, shown as smaller symbols, give the estimate of k_{max}^{app} and K_s^{app} from each combination of inhibitor and substrate concentrations. The larger symbols are the medians of the individual values of k_{max}^{app} and K_s^{app} at the various I_L concentrations (butane). Note that axis titles in Figure 3A are different from those in Figure 1, since only best estimate values for k_{max}^{app} and K_s^{app} are plotted. As shown in Figure 3A, k_{max}^{app} and K_s^{app} of 1,1-DCE shifted with increasing inhibitor (butane) concentrations in the direction of decreasing k_{max}^{app} and increasing K_s^{app}, indicating mixed inhibition of butane on 1,1-DCE transformation. The kinetic parameters (k_{max}, K_s, K_{iu}, and K_{ic}) were estimated by two linearized plots for the mixed inhibition using Eqs. (2) and (3).

$$\frac{K_s^{app}}{k_{max}^{app}} = \frac{K_s}{k_{max}} + \frac{K_s}{k_{max}K_{ic}} I_L \quad (2)$$

$$\frac{1}{k_{max}^{app}} = \frac{1}{k_{max}} + \frac{1}{k_{max}K_{iu}} I_L \quad (3)$$

Excellent fits to the linearized forms were obtained (R^2 = 0.97 and 0.99), and the k_{max} and K_s values for 1,1-DCE of

Table I. Determined inhibition types and k_{max}, K_s, K_{ic}, and K_{iu} values with 95% confidence intervals that are separately estimated from single-compound rate studies, and linear plots and NLSR analysis using rate data in the presence of inhibitors.

Inhibitor	Parameter	Method	Substrate			
			Butane	1,1-DCE	1,1-DCA	1,1,1-TCA
—	k_{max} (μmol/mg TSS/h)	Single compound	2.6 ± 0.14	1.3 ± 0.09	0.49 ± 0.03	0.19 ± 0.01
—		Linearized[a]	2.2 ± 0.18	1.2 ± 0.21	0.49 ± 0.04	0.22 ± 0.02
—		NLSR[a]	2.2 ± 0.28	1.2 ± 0.02	0.50 ± 0.07	0.20 ± 0.001
—	K_s (μ*M*)	Single compound	19 ± 3.3	1.5 ± 0.39	19 ± 5.0	12 ± 2.8
—		Linearized[a]	9.7 ± 2.3	1.7 ± 4.2	20 ± 8.1	15 ± 3.0
—		NLSR[a]	11 ± 2.6	1.6 ± 0.60	17 ± 3.3	16 ± 3.2
—	K_1 (L/mg TSS/h)	Single compound	0.14	0.87	0.03	0.02
Butane	Inhibition type	Direct linear plot	—	Mixed	Mixed	Mixed
	K_{ic} (μ*M*)	Linearized	—	0.23	1.8	0.52
		NLSR	—	0.33 ± 0.07	2.8 ± 1.6	0.28 ± 0.13
	K_{iu} (μ*M*)	Linearized	—	4.6	3.3	0.36
		NLSR	—	6.9 ± 1.6	3.8 ± 0.88	0.51 ± 0.09
1,1-DCE	Inhibition type	Direct linear plot	Com	—	Com	Com
	K_{ic} (μ*M*)	Linearized	13	—	8.6	0.95
		NLSR	8.7 ± 2.3	—	3.6 ± 1.5	1.1 ± 0.30
1,1-DCA	Inhibition type	Direct linear plot	Com	Com	—	Com
	K_{ic} (μ*M*)	Linearized	253	−16.3	—	9.6
		NLSR	403 ± 51	18 ± 4.9	—	16 ± 4.8
1,1,1-TCA	Inhibition type	Direct linear plot	Com	Com	Com	—
	K_{ic} (μ*M*)	Linearized	350	81	12	—
		NLSR	313 ± 88	17 ± 4.0	9.8 ± 2.2	—

[a]k_{max} and K_s values obtained from linearized plot and NLSR were the average of three values obtained from three inhibition studies. For example, k_{max} and K_s values for 1,1-DCE are averages of three values obtained from butane, 1,1-DCA and 1,1,1-TCA inhibition tests.

1.37 μmol/mg TSS/h, and 0.84 μ*M,* respectively, agreed well with the values obtained in the single compound tests. K_{ic} and K_{iu} for butane were, 0.23 μ*M,* and 4.6 μ*M,* respectively, with an excellent agreement of NLSR analysis to the mixed inhibition model as shown in Figure 3C. Both inhibition coefficient values for butane were much smaller than K_s for butane.

The inhibition by butane of 1,1-DCA and 1,1,1-TCA transformation was also evaluated. In both tests best estimate point ($k_{\max}^{app}$,K_s^{app}) shifted with increasing inhibitor (butane) concentrations in the direction of significantly decreasing $k_{\max}^{app}$ and slightly increasing K_s^{app}. This shift can be interpreted as mixed or potentially as noncompetitive inhibition. Because the inhibition model is more inclusive than the noncompetitive inhibition model, we analyzed the results as mixed inhibition of butane on 1,1,1-TCA and 1,1-DCA transformation. The kinetic parameters obtained from linearized plots agreed well with the values obtained from the single compound tests and NLSR analyses (Table I). Again, the K_s value for butane was much greater than both K_{ic} and K_{iu} values for butane in both tests. The K_{ic} and K_{iu} values for butane on 1,1,1-TCA and 1,1-DCA transformations were very similar, indicating butane has very similar affinity on free enzyme (E) and enzyme · 1,1-DCA (or 1,1,1-TCA) (E · S). However, the K_{iu} value for butane on 1,1-DCE transformation was a factor of 20 higher than K_{ic} for butane on 1,1-DCE transformation, indicating butane more competitively inhibits 1,1-DCE transformation than noncompetitively. Both inhibition coefficients (K_{ic} and K_{iu}) for butane are smaller than K_{ic} for CAHs, showing butane more strongly inhibits CAH transformations.

Inhibition Among CAHs

Inhibition types among CAHs were then investigated. In all cases direct linear plots indicate competitive inhibition among CAHs. Inhibition coefficient values obtained from linearized plots were generally in good agreement with those estimated by NLSR analysis (Table I). In the case of 1,1,1-TCA inhibition on 1,1-DCE inhibition, the K_{ic} value from the linear plot was a factor of 4.5 higher than that obtained from NLSR analysis. A negative value of K_{ic} for 1,1-DCA was also obtained from the linearized plot for the case of 1,1-DCA inhibition on 1,1-DCE due to a negative y intercept value of 1.4 μ*M* (K_s for 1,1-DCE). As presented in Table I, the K_s value for 1,1-DCE is low and close to 0 (1.5 ± 0.39 μ*M*). Thus, linear regression on data having small errors may result in a slightly negative value for the y intercept. In this case, the K_{ic} value was changed by trial and error until NLSR converged and yielded positive values for kinetic parameters. The RSE values obtained from NLSR analysis among CAHs were low, ranging from 0.006 to 0.025, and the 95% confidence intervals were narrow. These results suggest that the data was well fit by the competitive inhibition model.

Inhibition coefficients for 1,1-DCE were much smaller than those for 1,1-DCA and 1,1,1-TCA. These comparisons suggest that 1,1-DCE is a stronger inhibitor than the other two CAHs, as expected due to its low K_s value.

CAH Inhibition on Butane

Inhibition studies of the CAHs on butane degradation were also performed. All CAHs competitively inhibited butane

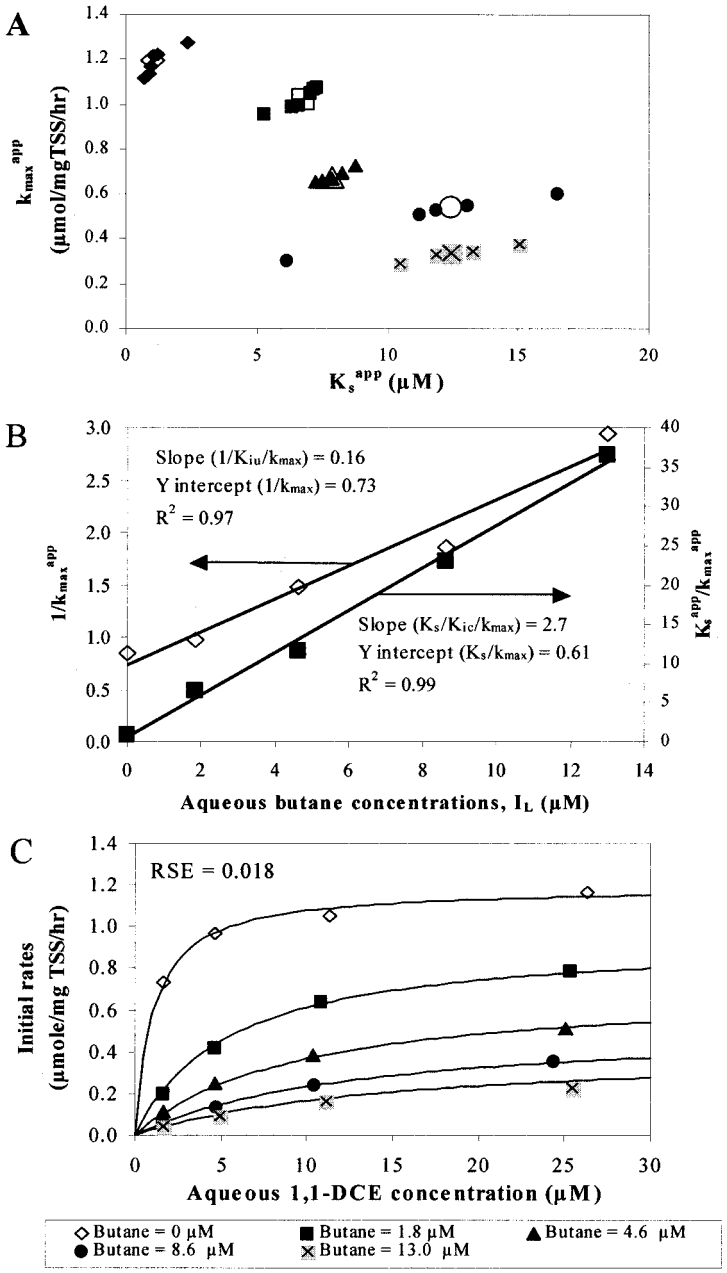

Figure 3. Direct linear plot showing mixed inhibition of butane on 1,1-DCE transformation (A), plots of $1/k_{max}^{app}$ or K_s^{app}/k_{max}^{app} vs. I_L to graphically evaluate k_{max}, K_s, K_{ic}, and K_{iu} (B), and the NLSR best fit of the data to the mixed inhibition equation (C).

degradation. For all cases of CAH competitive inhibition on butane degradation, K_{ic} values obtained from linearized plots were comparable with those obtained from NLSR analysis, and the R^2 values obtained from linearized plots ranged from 0.89 to 0.94. K_{ic} value for 1,1-DCE was much smaller than those for 1,1-DCA and 1,1,1-TCA, indicating that 1,1-DCE more strongly inhibited butane degradation than the other CAHs, consistent with its lower K_s value than the other CAHs.

Comparison of K_s With K_{ic}

K_s values of individual compounds are often used to estimate competitive inhibition coefficients using the ratio of K_s values. To evaluate if the K_s value is a good indicator of competitive inhibition, the ratios of K_s of substrate over K_s of inhibitor, and K_s of substrate over K_{ic} of inhibitor, were compared (Fig. 4). Here log values of the ratios are plotted to cover the wide range of values reported here. If the ratios of K_s values are good predictors of competitive inhibition, the ratio values should give a line having a slope of 1 and equal absolute values of x- and y-intercepts. The ratio values for CAHs inhibition on butane did not yield a slope of 1, or equal absolute values of x- and y-intercepts (dashed line), indicating the K_s values were not good predictors of the observed inhibition. The inhibition observed was weaker than predicted by their K_s values. The ratio values

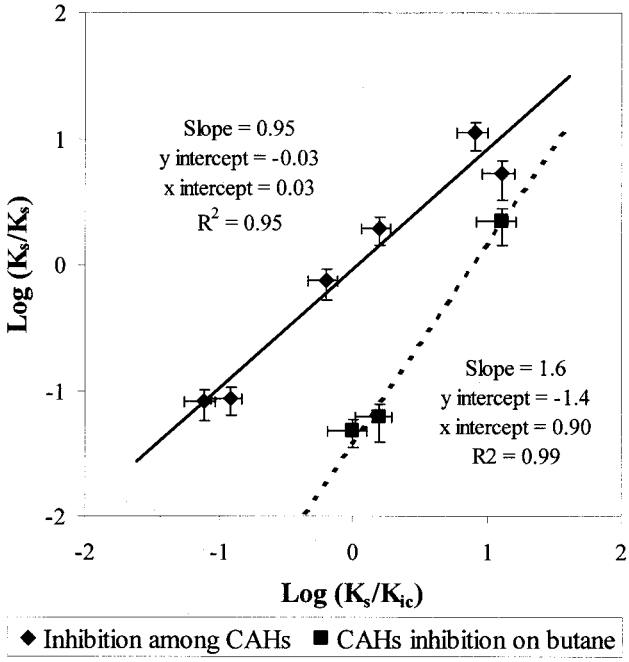

Figure 4. Comparison of the ratio of K_s of the substrate over K_s of the inhibitor and the K_s of substrate over estimated K_{ic} of the inhibitor. The data were obtained from competitive inhibition among the CAHs (◆) and from competitive inhibition of CAHs on butane degradation (■). If the ratios of K_s values are good predictors of competitive inhibition, the ratio values should give a line having a slope of 1 and equal absolute values of x- and y intercepts. Lines represent linear regression fits to the data.

for inhibition among CAHs yielded a slope of 0.95 and equal x- and y-intercept absolute values of 0.03. The results indicate that the ratios of K_s values of CAHs are good indicators for competitive inhibition among CAHs. However, for the case of CAH inhibition on the growth substrate degradation, the ratios of K_s values would predict stronger inhibition of CAHs on butane utilization than was observed.

DISCUSSION

The combined method of both direct linear plots to identify the inhibition type and NLSR analysis to estimate the kinetic parameters (using graphically estimated kinetic parameters as initial guesses) as described by Kim et al. (2002) was applied to study inhibition among three CAHs (i.e., 1,1-DCE, 1,1,1-TCA and 1,1-DCA), butane inhibition on each of the CAHs transformation, and inhibition of the CAHs on butane degradation. Results presented here show that different inhibition types exist between the CAHs and butane. Competitive inhibition was observed among the CAHs, and the CAHs inhibition of butane utilization. Butane, however, was a mixed inhibitor on CAH transformation. Thus, competitive inhibition and mixed inhibition are both important in cometabolism of CAHs with this culture. Two inhibition types were also observed in the cometabolism of aliphatic hydrocarbons and CAHs by *N. europaea* (Keener and Arp, 1993). They reported noncompetitive inhibition (defined as mixed inhibition in this study) of chloromethane and chloroethane and competitive inhibition of methane and ethylene on NH_4^+- dependent NO_2^- production by *N. europaea*. Thus, the types of inhibition mechanisms observed may differ with different microorganisms, growth substrates, and CAHs.

Competitive inhibition results from substrate and inhibitor binding to the same enzyme site, while mixed inhibition occurs when there is a separate binding site for the inhibitor. The notion of multiple binding sites of soluble methane monooxygenase was reported by George et al. (1996). They identified three potential active sites of soluble methane monooxygenase. The binding site for the short-chain-length substrates methane, propene and pyridine, and the suicide substrate ethylene was located approximately 3 Å from the binuclear iron center. Longer chain-length substrates bound to further sites and a conformational change in the structure of the hydroxylase was thought to be required for interaction of these sites with the diiron center. Thus, mixed inhibition of butane on CAHs transformation may indicate separate binding sites for butane and the CAHs. Other mechanisms, however, cannot be ruled out since the measurements were done with whole cells and not with purified enzymes. Thus, substrate transport to the enzyme and other cell dynamic processes may have an influence on the inhibition observed.

Many studies have assumed that the K_{ic} value was equal to the independently measured K_s value of the inhibitor (Alvarez-Cohen and McCarty, 1991; Anderson and McCarty, 1996; Broholm et al., 1992; Chang and Alvarez-Cohen, 1995, 1996; Speitel et al., 1993; Strand et al., 1990). This assumption has also been used to model CAH transformation in the presence of growth substrate or other CAHs. Although the models have successfully simulated aerobic CAH cometabolism, several studies with pure and mixed cultures have estimated K_{ic}, and found deviations from the K_s value (Chang and Criddle 1997; Landa et al., 1994). Chang and Criddle (1997) found that for a mixed methanotrophic culture the K_{ic} for methane inhibition of trichloroethylene (TCE) transformation was about 60 times lower than K_s of methane, while the K_{ic} for TCE inhibition of methane degradation was a factor of 5 higher than the K_s for TCE. Landa et al. (1994) reported similar inhibition results for TCE transformation using *Burkholderia* (*Pseudomonas*) *cepacia* G4 grown on toluene. The K_{ic} for toluene inhibition on TCE transformation was 5 times lower than the K_s of toluene, while K_{ic} for TCE was a factor of 5 higher than the K_s of TCE. Both studies showed that the growth substrate was a stronger inhibitor than TCE, even though the K_s of the growth substrate was greater than that of CAH.

Presented here are results where both the K_s and K_{ic} were determined. Among CAHs, the K_{ic} of the CAH was comparable to the K_s of the inhibiting substrate, suggesting that K_s values for the CAH are reasonable indicators for the competitive inhibition observed (Fig. 4). However, for CAH inhibition on butane utilization, the K_{ic} of CAH was a factor of 6 to 25 greater than the K_s of the inhibiting substrate,

Table II. Kinetic parameters, reactor operating parameters, and model equations used for the completely mixed reactor simulations.

Parameters	Case I	Case II
Butane inhibition on 1,1-DCE	Mixed	Competitive
1,1-DCE inhibition on butane	Competitive	Competitive
$k_{max,butane}$ (μmol/mg TSS/h)	2.2	2.2
$k_{max,DCE}$ (μmol/mg Tss/h)	1.2	1.2
$K_{s,butane}$ (μM)	11	11
$K_{s,DCE}$ (μM)	1.6	1.6
$K_{ic,butane}$ (μM)[a]	0.33	11 ($=K_{s,butane}$)
$K_{ic,DCE}$ (μM)[b]	8.7	1.6 ($=K_{s,DCE}$)
$K_{iu,butane}$ (μM)	6.9	—
$T_{C,DCE}$ (μmol/mg TSS)	0.92	0.92
Cell yield, Y (mg TSS/μmol)	0.046	0.046
X_0 (mg TSS/L)[c]	3000	3000
Reactor volume, V (L)	10	10
Flow rate, Q (L/h)	0.2	0.2
Butane degradation[d]	$\frac{dS}{dt}=\frac{QS_{in}}{V}-\frac{QS}{V}-\frac{k_{max,butane}SX}{K_{s,butane}\left(1+\frac{C}{K_{ic,DCE}}\right)+S}$	$\frac{dS}{dt}=\frac{QS_{in}}{V}-\frac{QS}{V}-\frac{k_{max,butane}SX}{K_{s,butane}\left(1+\frac{C}{K_{s,DCE}}\right)+S}$
1,1-DCE transformation[e]	$\frac{dC}{dt}=\frac{QC_{in}}{V}-\frac{QC}{V}-\frac{\frac{k_{max,DCE}}{\left(1+\frac{S}{K_{iu,butane}}\right)}CX}{\frac{K_{s,DCE}\left(1+\frac{S}{K_{ic,butane}}\right)}{\left(1+\frac{S}{K_{iu,butane}}\right)}+C}$	$\frac{dC}{dt}=\frac{QC_{in}}{V}-\frac{QC}{V}-\frac{k_{max,DCE}CX}{K_{s,DCE}\left(1+\frac{S}{K_{s,butane}}\right)+C}$
Cell growth and toxicity	$\frac{dX}{dt}=Y\left(\frac{dS}{dt}\right)-\frac{QX}{V}-\frac{1}{T_C}\left(\frac{dC}{dt}\right)$	$\frac{dX}{dt}=Y\left(\frac{dS}{dt}\right)-\frac{QX}{V}-\frac{1}{T_C}\left(\frac{dC}{dt}\right)$

[a] K_{ic} for butane.
[b] K_{ic} for 1,1-DCE.
[c] X_0 indicates an initial cell concentration in a reactor (mg TSS/L).
[d] S_{in} and S indicate influent and effluent butane concentrations, respectively (μM).
[e] C_{in} and C indicate influent and effluent 1,1-DCE concentrations, respectively (μM).

indicating weaker inhibition, similar to the observations of Chang and Criddle (1997) and Landa et al. (1994). Consequently, the assumption ($K_{ic} = K_s$) is valid for inhibition among CAHs (i.e., model for resting cell CAH transformation), but not always valid, especially for CAH inhibition on the growth substrate degradation and growth substrate inhibition on CAH transformation (i.e., model for concurrent degradation of growth substrate and CAH transformation).

Model simulations were performed to show how both inhibition types and kinetic parameters are important in designing in situ or ex situ CAH treatment processes. For these model systems, a completely mixed reactor, all liquid system with no recycle of cells, was chosen. The model equations are provided in Table II, along with the model parameters. Non-steady-state reactor equations are presented for butane degradation, 1,1-DCE transformation, and cell growth. The model is based on equations described by Chang and Alvarez-Cohen (1995, 1996). Transformation capacity model (Alvarez-Cohen and McCarty, 1991; Chang and Alvarez-Cohen, 1995, 1996) was adapted to model CAH transformation toxicity on cells. The three equations were solved in Stella (High Performance Systems, Inc., Hanover, NH) by a simultaneous numerical integration using a fourth-order- Runge-Kutta method.

Two different simulations were performed to compare 1,1-DCE transformation efficiencies in a complete-mixed reactor continuously fed dissolved butane and 1,1-DCE. In Case I, kinetic parameters and inhibition types determined in this study were used for the simulation, and in Case II, the simulation was performed with the same input data as Case I, except assuming competitive inhibition and $K_{ic} = K_s$ (Table II).

Steady-state removal efficiencies of butane, 1,1-DCE and the cell concentration were obtained at different influent concentrations of butane ($0 - 350\ \mu M$) and 1,1-DCE (0.5, 2, and 8 μM) (Fig. 5). The simulations show that butane and 1,1-DCE removal efficiencies at different influent butane concentrations are significantly different. In Case II with inhibition based on the K_s value, removal efficiencies for both 1,1-DCE and butane dramatically increase from 0% to

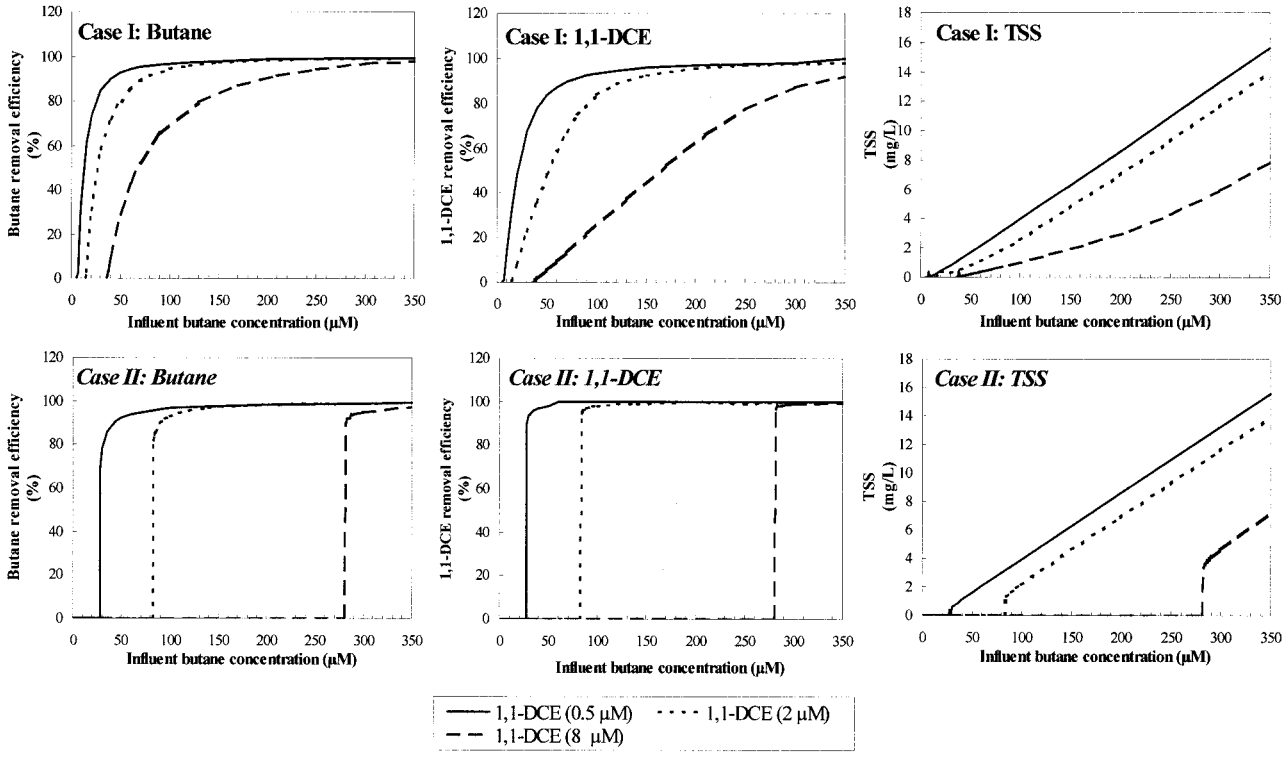

Figure 5. Simulation of butane and 1,1-DCE removal efficiencies and TSS at steady-state in a complete-mixing reactor obtained from two different simulations. Case I: a simulation using inhibition types and inhibition coefficients determined in this study; and Case II: a simulation using the same input as Case I, except assuming competitive inhibition and $K_{ic} = K_s$.

more than 90% removal efficiency, once influent butane concentrations are enough to maintain cell growth in the reactor (see Case II: TSS in Fig. 5). In Case I with the inhibition models and parameters determined in this study, removal efficiencies for both compounds and TSS gradually increases with increasing influent butane concentrations, and the removal and cell growth start at lower influent butane levels, compared to those in Case II. However, to achieve 1,1-DCE removal efficiencies of greater than 85%, higher influent butane concentrations are required. These comparisons demonstrate that for reactor systems where growth substrate and CAHs are both present, determining inhibition type and measuring kinetic parameters are important.

The butane-grown mixed culture studied here is highly suitable for treating CAH mixtures based on its broad substrate range (Kim et al., 2000). The butane culture has higher k_{max} and lower K_s for 1,1-DCE than 1,1-DCA and 1,1,1-TCA. These results are consistent with our previous studies (Kim et al., 2000), in that, 1,1-DCE was transformed more rapidly than the chlorinated ethanes. The K_s values for 1,1-DCA and 1,1,1-TCA were similar, and the k_{max} value for 1,1-DCA was a factor of 2.5 higher than 1,1,1-TCA. In our previous studies (Kim et al., 2000), the initial transformation rate of chlorinated ethanes decreased with increased chlorine substitution. This trend likely reflects differences in k_{max} among chlorinated ethanes.

The butane culture has the ability to transform 1,1,1-TCA and 1,1-DCE as effectively as, or better than, other microorganisms, based on comparison of kinetic parameters (k_{max}, K_s, and k_1). Table III presents comparison of kinetic parameters for 1,1-DCE and 1,1,1-TCA obtained in this study, with *M. trichosporium* OB3b producing sMMO and pMMO (Oldenhuis et al., 1991; van Hylckama Vlieg et al., 1996), *Nitrosomonas europaea* (Ely et al., 1997) and other mixed cultures. For the butane culture, the K_s for 1,1-DCE is a factor of 3 to >20 lower, and the $k_{max}$for 1,1-DCE is a factor of 3 to 4 greater than those reported for *M. trichosporium* OB3b producing sMMO and *Nitrosomonas europaea*. The k_{max} for 1,1-DCE with this butane culture is a factor of 2 lower than that reported for *M. trichosporium* OB3b PP358 producing sMMO, while K_s for 1,1-DCE is a factor of >20 lower. For the butane culture, the T_c for 1,1-DCE is a factor of 1.5 greater than that for *M. trichosporium* OB3b PP358 producing sMMO, and a factor of 10 to 20 greater than that for *Nitrosomonas europaea*. Thus, T_c for 1,1-DCE is favorable for the butane culture. The K_s for 1,1,1-TCA measured here was a factor of 20 lower than achieved with *M. trichosporium* OB3b, however, k_{max} is an order of magnitude lower. These comparisons indicate that this butane culture has much lower K_s values and comparable or greater k_{max} values for both 1,1-DCE and 1,1,1-TCA than other microorganisms. To the best of our knowledge there are no previous reports of 1,1-DCA transformation kinetics through aerobic cometabolism, thus no comparison is made here.

Table III. Comparison of kinetic parameters for 1,1-DCE and 1,1,1-TCA transformation.

Compound	Microorganisms	Growth substrate	Additional substrate	Temp. (C)	k_{max} (μmol/mg TSS/h)[a]	K_S (μ*M*)	k_1 (L/mg TSS/h)[a]	T_c (μmol/mg TSS)	Reference
1,1-DCE	*M. trichosporium* OB3b, sMMO	Methane	Formate	30	0.36	5.1	0.07	—	Oldenhuis et al., 1991
	M. trichosporium OB3b PP358, sMMO	Methane	Formate	23	>3.2	>35	0.10	0.36	Aziz et al., 1999
	M. trichosporium OB3b, pMMO	Methane	Formate	30	—	—	<0.002	—	van Hylckama Vlieg et al., 1996
	Nitrosomonas europaea	Ammonia	Ammonia	22	0.43	9.2	0.05	0.023–0.04	Ely et al., 1997
	Butane-grown mixed culture	Butane	None	20	1.3	1.5	0.87	0.52	This study
1,1,1-TCA	*M. trichosporium* OB3b, sMMO	Methane	Formate	30	1.4	214	0.007	—	Oldenhuis et al., 1991
	Mixed culture	Methane	Methane	—	—	—	0.0001	—	Stramd et al., 1990
	Mixed culture	Propane	None	20	—	—	0.003	—	Keenan et al., 1994
	Butane-grown mixed culture	Butane	None	20	0.19	12	0.02	—	This study

[a]Cell mass is reported in mg TSS; unit conversions assume TSS is 50% protein.

With respect to in situ cometabolism of CAH, the contaminant concentrations are often much lower than the K_s value. Thus, the pseudo-first-order rate, k_1 (k_{max}/ K_s) is an important parameter. The k_1 for 1,1-DCE measured here is a factor of 9 to 18 greater than that of *M. trichosporium* OB3b producing sMMO and *Nitrosomonas europaea,* and 2 orders of magnitude greater than achieved with pMMO (Table III). The k_1 for 1,1,1-TCA obtained with our culture is a factor of 2 to 200 greater than those observed in other studies. Thus, the butane-grown culture studied here has potential advantages for bioremediation of 1,1-DCE and 1,1,1-TCA. Based on the inhibition types and coefficients that were observed for three CAHs, it is of interest to predict the transformation expected when mixtures of the three CAHs are present. Assuming the same concentrations of the CAHs, and butane is present at a higher concentration as a growth substrate, butane would be most rapidly degraded followed by 1,1-DCE, 1,1-DCA, and 1,1,1-TCA. The reasons for the sequence transformation are: (1) the lower inhibition coefficients for butane (i.e., strong inhibition on the transformation of the other compounds), (2) the highest k_{max} value for butane, (3) the greater k_{max} value for 1,1-DCE than the other CAHs, (4) the lower K_s (or K_{ic}) value for 1,1-DCE than the other CAHs, and (5) the greater k_{max} of 1,1-DCA than 1,1,1-TCA.

Future studies should evaluate the transformation of broad mixtures of 1,1-DCE, 1,1,1-TCA and 1,1-DCA and butane to see if the inhibition models and parameters can be extended to more complicated systems. It is also of interest to determine whether the models can be applied when growth on butane is occurring in the presence of contaminant mixtures, which would be more representative of conditions in treatment systems. Reactor studies, such as those simulated here, need to be performed and compared with model predictions.

The research was supported by research grant from the R2D2 program of the U.S. Environmental Protection Agency-sponsored Western Region Hazardous Substance Research Center under agreement R-815738. This article has not been reviewed by the agency, and no official endorsement should be inferred. We acknowledge the help of Dr. James M. Tiedje for discussions on the interpretation of these data.

NOMENCLATURE

C_{in}	influent 1,1-DCE concentration	(μ*M*)
C	effluent 1,1-DCE concentrations	(μ*M*)
I_L	inhibitor concentrations in liquid phase	(μ*M*)
k_1	pseudo first order rate constant	(L/mg TSS/h)
k_{max}^{app}	apparent maximum degradation/ transformation rates	(μmol/mg TSS/hr)
K_s^{app}	apparent half-saturation coefficient	(μ*M*)
K_{ic}	competitive inhibition coefficient	(μ*M*)
K_{iu}	uncompetitive inhibition coefficient	(μ*M*)
k_{max}	maximum degradation/transformation rates	(μmol/mg TSS/h)
K_s	half-saturation coefficient	(μ*M*)
Q	flow rate	(L/h)
S	effluent butane concentration	(μ*M*)
S_{in}	influent butane concentration	(μ*M*)
S_L	substrate concentrations in liquid phase	(μ*M*)
T_c	transformation capacity	(μmol/mg TSS)
v	specific degradation rates	(μmol/mg TSS/h)
V	reactor volume	(L)
X_0	initial cell concentration in a reactor	(mg TSS/L)
Y	cell yield	(mg TSS/μmol)

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