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Biodegradation of aromatic compounds and TCE by a filamentous bacteria-dominated consortium

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Abstract

The Michaelis-Menten biodegradation kinetics (k and Ks) of aromatic compounds and trichloroethene (TCE) by an aerobic enrichment culture grown on phenol and dominated by a unique filamentous bacterium were measured. The average k and Ks values for phenol, benzene (B), toluene (T), ethylbenzene (E), o-xylene (oX), p-xylene (pX), naphthalene and TCE in g per g VSS-d and mg L^{-1} were 5.72 and 0.34, 1.20 and 0.51, 2.09 and 0.47, 0.77 and 0.23, 0.61 and 0.16, 0.73 and 0.23, 0.17 and 0.18, and 0.16 and 0.18, respectively. Significant variability in these measured kinetics was noted between tests conducted over the 5-month period during which the fed-batch culture with a 5-day solids retention time was maintained; the coefficient of variation of the k and Ks values ranged from 11–43% and 4–50%, respectively. This variation was significantly greater than the method measurement error on a given date. Degradation of BTEoXpX mixtures could be described by a basic competitive inhibition model. Batch tests during which the culture was fed individual BTEX compounds showed the culture grew poorly on the xylenes and had poor subsequent xylene degradation rates. This work indicates the potential to simultaneously treat a mixture of volatile organic compounds using this consortium, and the ability to predict the mixture biodegradation rates on the basis of the individual compound biodegradation kinetics.

Abbreviations: B = benzene, BTEX = benzene, toluene, ethylbenzene, xylenes; Ci = inhibitor concentration, mg L^{-1}; sCOD = soluble chemical oxygen demand; CV = coefficient of variance; DO = dissolved oxygen; E = ethylbenzene; GC = gas chromatograph; HRT = hydraulic retention time; k = max specific degradation rate, g/g-d; Ki = inhibition constant, mg L^{-1}; Ks = half-saturation concentration, mg L^{-1}; naph = naphthalene; oX = ortho-xylene; PAH = polyaromatic hydrocarbon; pX = para-xylene; rRNA = ribosomal ribonucleic acid; S = substrate concentration, mg L^{-1}; SRT = solids retention time; TCE = trichloroethene; T = toluene; VOC = volatile organic compound; VSS = volatile suspended solids; X = biomass concentration; μ = specific growth rate, d^{-1}; μm = maximum specific growth rate, d^{-1}.

Introduction

Many volatile organic compounds (VOCs) are common contaminants in soil and groundwater. Of these VOCs, benzene, toluene, ethylbenzene and xylenes (BTEX) are widespread and known to be readily degraded by aerobic bacteria (Smith 1990; Zylstra 1995). However, few bacterial cultures, either pure or mixed, have been characterized for the degradation kinetics of each of the BTEX compounds, despite the fact that these compounds frequently occur together in mixtures. Semi-volatile compounds such as naphthalene (naph) may also be found as a co-contaminant with BTEX at fuel spill sites and former manufactured gas plants. There is also the potential for co-contamination of BTEX and chlorinated aliphatic compounds, such

as trichloroethene (TCE), at mixed waste sites. Therefore, the ability of a single bacterial consortium to degrade many of these VOCs is of interest. In contrast to the wide range of aerobic bacteria that can degrade BTEX and naph as growth substrates, TCE must be cometabolically degraded. Substrates commonly used to grow TCE-degrading bacteria include methane, toluene and phenol (Stensel & Bielefeldt 1997). During TCE degradation by methanotrophic bacteria, an intermediate metabolite is produced that can inactivate the bacteria (Fox et al. 1990). This problem has also been observed for some toluene/phenol-degrading bacteria (Wackett & Householder 1989). The use of bacteria which do not suffer intermediate toxicity effects offer significant advantages in treatment applications since a more stable process is possible and less growth substrate is needed.

When multiple compounds are degraded by bacteria, a variety of interactive effects may occur. Machado & Grady (1989) studied the removal of two substitutable substrates by a pure culture. They attempted to describe the interaction by:

$$\mu_1 = \mu m_1/(Ks_1 + S_1 + Ks_1 S_2/Ki) \qquad (1)$$

where μ_1 = specific growth rate on compound 1 (d^{-1}); μm_1= maximum specific growth rate on compound 1 (d^{-1}); Ks_1 = half-saturation concentration of compound 1 (mg L^{-1}); S_1 = concentration of substrate 1 (mg L^{-1}); S_2 = concentration of substrate 2 (mg L^{-1}); Ki = inhibition constant (mg L^{-1}).

For perfectly substitutable substrates that competitively inhibit each others degradation, Ki = Ks_2, the half-saturation concentration of substrate 2 (mg L^{-1}). They also described relationships for augmentative and antagonistic substrates. However, the interactive effects they observed were not describable by the predictive equations. Chang et al. (1993) found competitive inhibition described the interaction of benzene and toluene degradation by a pure culture of *Pseudomonas* strain B1 and xylene and toluene degradation by *Pseudomonas* strain X1. They used a data fit to Eq. 1 to solve for Ki; however, they found that the inhibition constants were equivalent to the measured Ks values of the competing compounds. Oh et al. (1994) studied the interactive effects of benzene and toluene degradation by *Pseudomonas* strain PPO1. Both compounds were used as growth substrates individually, but the interactive effects were not described by the perfectly substitutable competition equation.

A similar equation (Eq. 2) has been used to describe the inhibitory effect of compounds degraded cometabolically on substrate degradation (Strand et al. 1988; Folsom et al. 1990; Broholm et al. 1992; Chang & Alvarez-Cohen 1994), the inhibitory effect of the substrate on degradation of the cometabolite (Strand et al. 1990; Criddle 1993; Broholm et al. 1992; Chang & Alvarez-Cohen 1994), and inhibition between multiple cometabolites (Alvarez-Cohen & McCarty 1991).

$$Ra = \frac{kS}{Ks(1 + Ci/Ki) + S} \qquad (2)$$

where Ra = specific degradation rate of compound A in the presence of a competitive inhibitor (g/g VSS-d); k = maximum specific degradation rate of compound A (mg L^{-1}); S = concentration of compound A (mg L^{-1}); Ks = half-saturation concentration of compound A (mg L^{-1}); Ci = concentration of inhibitor (mg L^{-1}); Ki = inhibition constant for inhibiting compound (mg L^{-1}).

Folsom et al. (1990) found that the Ki fit to describe the inhibitory effect of TCE on phenol degradation by *Pseudomonas cepacia* G4 was 'less than 25 μM'; this compared to the Ks for TCE determined from single substrate degradation and a Lineweaver-Burk plot of 3 μM. Alvarez-Cohen & McCarty (1991) studied competitive inhibition between degradation of cometabolites chloroform and TCE by resting cells of a mixed culture of methanotrophs; interactive effects were described well by the competitive inhibition equation using the Ks of each compound for its Ki inhibition effect on the other compound. Chang & Alvarez-Cohen (1994) studied the interactive effective of methane on TCE cometabolic degradation, and found very good agreement of the data to the competitive inhibition model using the individually measured methane Ks for the Ki in Eq. 2. Strand et al. (1990) used the independently determined Ks for methane as the Ki in Eq. 2, and then used a non-linear fitting method to solve for the 1,1,1-trichloroethane maximum specific cometabolic degradation rate (k) and half-saturation constant (Ks). Note that all of the above studies were conducted with whole cells rather than purified enzymes; in spite of this, the Michaelis-Menten and enzyme inhibition kinetic expressions common to biochemistry (Bailey & Ollis 1986) were used.

A bacterial consortium dominated by a unique, filamentous organism was previously characterized for its ability to cometabolically degrade TCE and

cis-1,2- dichloroethene without intermediate toxicity effects in batch tests after growth on phenol (Bielefeldt et al. 1995). This research further characterized the aromatic compound (BTEX, phenol and naph) and TCE degrading abilities of this phenol-fed filamentous enrichment, investigated the biodegradation kinetics of a mixture of BTEX compounds, and determined whether, or not, BTEX compounds could serve as growth substrates for the bacteria. This serves to further define the substrate range of the culture and expand its application potential, particularly for bioreactor treatment of liquids or gases containing a range of VOCs. In addition, a general competitive inhibition equation describing degradation of the BTEX mixture on the basis of the individual compound degradation kinetics was found to adequately predict degradation of a five compound BTEX mixture.

Materials and methods

Culture growth

A large filamentous bacterium (A-1, ATCC No. 55581) dominated a mixed enrichment culture grown on phenol in a 2.5-L liquid volume continuously mixed reactor that was operated with a 5-day hydraulic and solids retention time (HRT and SRT). At 2-h intervals, nutrient solution containing trace minerals and 1000 mg L^{-1} phenol was fed to the reactor (Bielefeldt et al. 1995). The culture was maintained under these growth conditions for 6 months, and tested for biokinetics across a 5-month period. The observed yield of the culture was 0.37 g VSS per g phenol, at an average specific loading to the reactor of 0.55 g phenol per g VSS-d. Under microscopic observation at 1000× magnification, the filament appeared to comprise greater than 90% of the total biomass in the culture. The filaments ranged in size from 10–200 μm long × 2 μm wide and contained optically bright internal polyhydroxybutyrate deposits. The filamentous bacterium is gram-negative, variably motile and non- sheathed; 16S rRNA sequencing showed that the bacterium may be a new genus or species (somewhat related to *Comamonas* or *Brachymonas* genera).

Biokinetic tests

The culture was removed from the growth reactor and aerated for approximately 30 min to allow degradation of residual phenol prior to use in batch kinetic tests of phenol, BTEX, naph or TCE degradation. Two batch test methods were used to measure the Michaelis-Menten biodegradation kinetics of the culture: (1) a direct method based on the analysis of liquid samples over time; and (2) an indirect method based on the measurement and interpretation of continuous dissolved oxygen (DO) depletion during degradation of the test compounds (adapted from the method of Ellis et al. 1996a, described in detail in Bielefeldt & Stensel 1998a).

Direct method tests were conducted in 160-mL serum bottles with Mininert septa which were incubated at 20 °C on a constant temperature shaker table. The direct method was used to measure the k of individual compounds and to evaluate the biodegradation of compound mixtures. The initial liquid concentrations of the test compound(s) added to the batch cultures were 2–13 mg L^{-1}. Liquid samples were taken from the bottles over time for gas chromatographic analysis. A linear decline in concentration was observed during the sampling period, and a least-squares fit to the data was conducted in an Excel spreadsheet. The Henry's coefficients of the compounds were used to correct for the amount of VOC partitioned in the bottle headspace, with unitless mass fraction values at 20 °C of 0.20, 0.23, 0.27, 0.25, 0.18, 0.02 and 0.38 for B, T, E, pX. oX, naph and TCE, respectively (Howe et al. 1988; Schwarzenbach et al. 1993). Biomass was measured by volatile suspended solids (VSS) analysis (APHA 1992). In addition, killed controls to which 4% formaldehyde was added were run to confirm that there were no abiotic losses of compounds from the test bottles.

Liquid samples for phenol analysis were acetylated, extracted into hexane, and analyzed on a Perkin-Elmer AutoSystem Gas Chromatograph (GC) with a flame ionization detector (megabore DB-5 column; oven 100 °C for 2 min, ramp 20 °C min^{-1} to 200 °C, hold for 1 min). Liquid samples for BTEX and naph analysis were extracted into pentane and analyzed on the same GC (megabore DB-5 column; oven 50 °C for 2 min, ramp 25 °C min^{-1} to 90 °C, hold for 2.4 min). Liquid TCE samples were extracted into hexane and analyzed on the Perkin-Elmer GC with an electron capture detector (RTX-1 column; oven program 80 °C for 4 min). Liquid concentrations were determined from external standard curves corrected for internal standard (1,2-dibromoethene) response. Detection limits for all compounds ranged from 0.1–0.2 mg/L. The method gave k value reproducibility on a given date of the smaller of within 0.08 g/g-d or 14%.

The indirect test method was used to measure the low half-saturation concentrations (Ks) of the compounds. which were frequently below the liquid concentration detection limits of the gas chromatograph. The batch tests were conducted in a modified 250-mL erlenmeyer flask with a Mininert sampling port and the top adapted to allow air-tight insertion of a Yellow Springs Instruments DO probe (0.01 mg L^{-1} precision). The aerated biomass and nutrient solution mixture were added to the flask, the DO probe was inserted with no headspace remaining, and the endogenous DO uptake rate was measured. Then 1–3 mg L^{-1} of a test compound was added to the flask. The DO concentration was manually recorded at 10–60-s intervals over the test period of 15 min to 1 h, with an average of 86 total DO measurements for each test. The oxygen depletion data were converted to substrate depletion rate by accounting for the endogenous oxygen uptake and using the observed oxygen to substrate consumption ratio. The non-linear curve fitting Marquardt-Levenberg algorithm in SigmaPlot (Jandel 1995) was then used to determine the maximum specific substrate degradation rate, k, and the half-saturation constant, Ks, from the substrate depletion over time data. Replicate tests conducted on the same date yielded k values within 0.08 g/g-d or 12% (the lesser of the 2) and Ks values within 0.06 mg/L. The temperature during the indirect method tests was typically 20–25 °C, and measured k values were corrected for temperature effects to yield approximate 20 °C rates. Both kinetic test methods are described in more detail in Bielefeldt & Stensel (1998a).

Determination of Ks for naph and TCE by evaluating competitive inhibition

Neither the indirect or direct method could be used to measure the Ks values for naph and TCE degradation, since the substrate degradation rate was too slow for the indirect method to allow an accurate analysis of Ks and the liquid detection limits were too high for the direct method to yield a Ks value. Two of the common BTEX degradation enzyme pathways, TOD and TOM, have been shown to transform phenol, naph and TCE (Zylstra 1994; Shields et al. 1994). Competition will occur when any two of these compounds are degraded by the same enzyme, such that both compounds are degraded at a lower rate compared to their single compound degradation rates. This competitive inhibition has frequently been modelled using Eq. 2, which was rearranged to solve for Ki as the unknown, yielding Eq. 3:

$$\mathrm{Ki} = \frac{\mathrm{KsCi}}{(\mathrm{kS/Rs}) - \mathrm{Ks} - \mathrm{S}} \qquad (3)$$

As stated earlier, other researchers have found that Ki is equivalent to the Ks of the inhibiting compound in studies with whole cells (Alvarez-Cohen & McCarty 1991; Chang & Alvarez-Cohen 1994; Chang et al. 1993; Strand et al. 1990). So it was assumed that the filament culture uses the same enzymes in phenol, naph and TCE degradation. With the additional assumption that Ki = Ks, this enables the estimation of Ks for naph or TCE from competition tests with phenol.

To find the Ks of naph or TCE, the degradation kinetics (k and Ks) determined for phenol alone were used with data from direct method batch tests which measured phenol and naph or TCE concentrations with time. From these data the phenol degradation 'rate' (Rs) was determined at different phenol concentrations. Then, Eq. 3 was solved using the measured liquid naph or TCE concentrations corresponding to these Rs rates to determine Ki. This Ki indicates the Ks of the inhibiting compound (either TCE or naph).

Growth on alternate substrates

To determine if the consortium could grow on the various BTEX compounds, a series of short term batch growth tests were conducted. Biomass from the phenol-fed growth reactor and nutrient solution were added to 160-mL serum bottles with Mininert valves, and these bottles were then incubated at 20 °C on a rotary shaker table. Individual growth substrates were added to the bottles at concentrations of 5–16 mg L^{-1} two to three times daily, with duplicate bottles of each feeding condition. Batch-fed substrates were B, T, E, oX, pX or phenol, with a total of 80–145 mg L^{-1} fed to each batch culture after 3–5 days. Unfed controls were incubated with each bottle set in order to compare with the final biomass in the fed cultures and calculate a net growth on the substrate tested.

During the batch growth period, headspace gas samples from the bottles were analyzed for oxygen and CO_2 content (using an EG&G Chandler Engineering CARLE Series 100 AGC Dual-Column Thermal Conductivity Detector with Molecular Sieve 5A and Hayes-Sep Q columns, 60 °C oven, Hewlett Packard 3390 integrator) to confirm that aerobic conditions were maintained and that the cultures remained active.

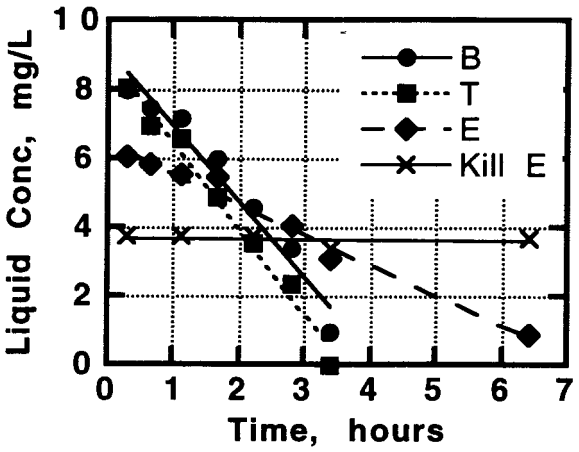

Figure 1. Direct method test results for B, T and E biodegradation an kill control (VSS 32 mg L^{-1}, 20°).

After 3–5 days of growth, the degradation rates of the individual BTEX compounds that were fed to the culture were measured by analyzing liquid samples taken over time. The final VSS concentration in the bottles was measured to determine the biomass growth due to batch substrate feeding.

Results

Aromatics and TCE degradation kinetics

An example plot of direct method biodegradation test results for individual compounds (B, T, E and kill control) with 32 mg L^{-1} VSS is shown in Figure 1. The k values are calculated from the linear decline in compound concentrations with time. Figure 2 shows example results of an indirect method biokinetic test for ethylbenzene using 325 mg L^{-1} VSS: part A shows the measured DO concentration over time, part B shows the DO data with the endogenous DO uptake subtracted out, and part C shows the calculated E concentration over time, The data in part C were evaluated by the nonlinear least-squares fit in SigmaPlot (Jandel 1995) to determine that k = 0.51 ± 0.01 g/g-d and Ks = 0.19 ± 0.01 mg L^{-1}.

Tables 1 summarizes the biodegradation kinetics, k and Ks, of the filament enrichment measured by the direct and indirect methods for each compound. Standard deviations of the average kinetic values and the number of test dates are shown in parenthesis. Statistical analysis using a student's t-test showed that differences in the k-values measured by the direct and indirect methods were not significant at a 0.05 level;

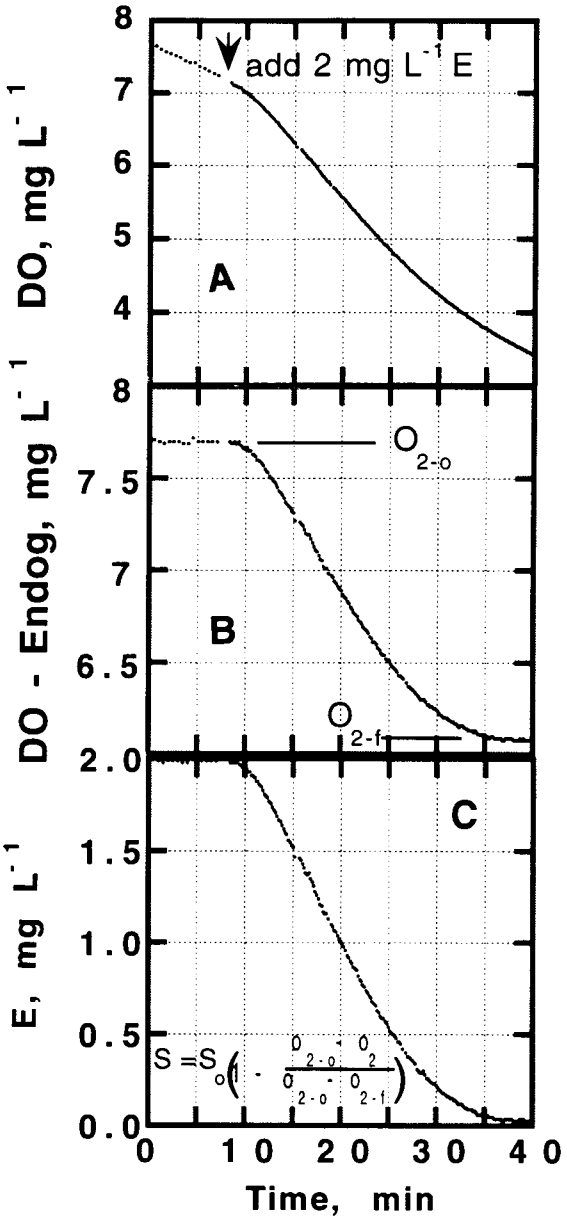

Figure 2. Example of indirect method biokinetic test results for E (VSS 325 mg L^{-1}, 23°).

the p-values shown in the last column of the table are the probability that it is incorrect to state that the means are different. The overall average k values measured by both methods were the highest for phenol (5.7 g/g-d), followed by T (2.1 g/g-d) and B (1.2 g/g-d), slower for E, oX and pX (0.6–0.8 g/g-d), and lowest for naph and TCE (0.2 g/g-d). The Ks concentrations of E, oX, pX and naph were similar (0.16–0.23 mg

Table 1. Summary of biokinetic coefficients determined for phenol-fed filament culture over a 5-month period

Compound	Direct method k (g/g VSS-d)	Indirect method k (g/g VSS-d)	Ks (mg L^{-1})	t-test for diff in dir vs. indir k, p-value
Phenol	6.12 (0.49; 2)[a]	5.33 (0.55; 2)	0.25 (0.14; 4)	0.27
B	1.19 (0.36; 3)	1.21 (0.72; 2)	0.51 (0.02; 2)	0.97
T	2.15 (0.47; 3)	2.00 (0.06; 2)	0.47 (0.04; 2	0.70
E	0.89 (0.26; 3)	0.59 (0.1; 2)	0.23 (0.05; 2)	0.22
oX	0.75 (0.22; 3)	0.41 (0.19; 2)	0.16 (0.08; 2)	0.18
pX	0.72 (0.27; 3)	0.80 (0.13; 2)	0.23 (0.07; 2)	0.65
Naph	0.17 (0.08; 3)	NM	0.18[b] (0.01; 2)	NM
TCE	0.16 (0.05; 8)	NM	0.18[b] (0.11; 2)	NM

[a] Standard deviation; number of test dates.
[b] Estimated from competitive inhibition test.
NM = not measured.

L^{-1}), with somewhat higher values for phenol (0.34 mg L^{-1}), and highest for B and T (0.51, 0.47 mg L^{-1}).

The significant variability in the biokinetic coefficients evident in Table 1 is related to the fact that biokinetics were measured on multiple test dates over a 5-month period. The coefficient of variance (CV), which is the percentage of the standard deviation relative to the average, ranged from 11–43% for the k values (when direct and indirect results were grouped together) and 4–50% for the Ks values. By microscopic observation, the predominance of filaments in the culture did not appear to change significantly during the 5-month period of reactor operation, so large-scale changes in the mixed culture do not seem to account for this kinetic variability.

Naphthalene and TCE Ks values from competition tests

An example of the batch competition tests in which the presence of naphthalene or TCE decreased the phenol degradation rate is shown in Figure 3. The measured degradation rates of phenol and the 'inhibiting' compound for each of the competition tests are given in Table 2; test set A is for the data shown in Figure 3. To find the Ks for naph or TCE, Eq. 2 was applied (where Ki = the inhibitor Ks) using the measured liquid concentrations of the two competing compounds, the measured k and Ks values for phenol alone, and the measured phenol degradation rate in the mixture. The calculated Ks values from the two test sets were 0.18 mg L^{-1} for naph, and 0.10 and 0.25 mg L^{-1} for TCE.

BTEX mixture degradation

The biodegradation of BTEoXpX mixtures was tested with the filament enrichment culture on five test dates using the direct method. On the same date as the mixture test, the k values of the individual compounds were measured by either the direct or indirect method; the Ks values were measured within 2 weeks by the indirect method. The initial concentration of each compound in the mixture ranged from 1.4–15.6 mg L^{-1}, with the total BTEX concentration between 14–43 mg L^{-1}; the percentage of B, T, E, oX and pX relative to the total mixture concentration were 25, 25, 25, 12.5 and 12.5%, respectively. During degradation of compound mixtures, all the compounds were degraded simultaneously but at a rate significantly slower than the degradation rates of the compounds alone.

Table 2. Competition tests with phenol and naphthalene or TCE

Test set	Test condition	Init conc phenol (mg L^{-1})	Phenol deg rate (g/g-d)	Initial inhibitor conc (mg L^{-1})	Meas inhibitor deg rate (g/g-d)	Calc Ks inhibitor (mg L^{-1})
A	Phenol alone	10.3	6.5			
	Naph + phenol	10.9	4.9	4.0	0.11	0.18
	TCE + phenol	9.8	3.2	5.0	0.05	0.10
B	Phenol alone	10.5	5.8			
	Naph + phenol	12.7	4.3	5.0	negligible	0.18
	TCE + phenol	12.3	4.5	4.5	0.07	0.25

Phenol Ks 0.19 and 0.15 mg L^{-1} for test sets A and B, respectively.

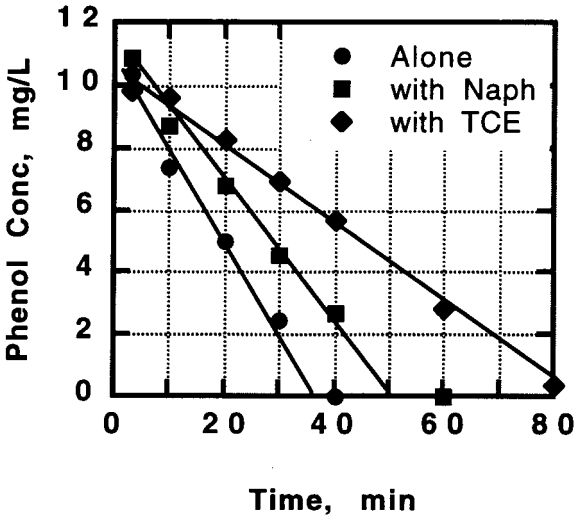

Figure 3. Phenol degradation in batch tests slowed by the presence of naph or TCE (initial naph and TCE concentrations were 11 and 10 mg L^{-1}, respectively; VSS 63 mg L^{-1}; temperature 20°).

Assuming that simple substrate competitive inhibition predominated the mixture effects, Eq. 4 was used to predict the effect of multiple BTEX compounds in a mixture on the biodegradation rate of one of the compounds (as derived from Yoon et al. 1977).

$$\frac{dS_1}{dt} = \frac{k_1 S_1 X}{Ks_1(1 + S_2/Ks_2 + S_3/Ks_3 + \cdots) + S_1} \quad (4)$$

where dS_1/dt = degradation rate of compound 1 in the mixture (mg/L-d); k_1 = maximum specific substrate degradation rate for compound 1 (g/g VSS-d); Ks_1 = half-saturation concentration for compound 1 (mg L^{-1}); X = biomass concentration (mg VSS L^{-1}); S_1= concentration of compound A (mg L^{-1}); S_2, S_3, . . . = concentration of other competing compounds 2, 3, etc. (mg L^{-1}); Ks_2, Ks_3, . . . = half-saturation concentration for the competing compounds 2, 3, etc. (mg L^{-1}).

Using the concentration of the compounds measured in the direct method mixture tests and the measured individual compound biokinetics (k and Ks), Eq. 4 was solved in an Excel spreadsheet to predict the initial degradation rates of the individual compounds in a mixture of BTEoXpX. A comparison of the predicted initial degradation rates of each compound to the measured degradation rates in the mixture is shown in Figure 4a.

The data shows that Eq. 4 gave fairly accurate biodegradation rate predictions since the data falls near the line which represents exact predictions. For B and T, the model always slightly under-predicted the observed initial compound degradation rate, with predicted degradation rates between 63–98 and 72–96% of the measured rates, respectively. For E, oX and pX, the model did not consistently over- or under-predict the measured biodegradation rate (predictions 82–138, 85–175 and 38–114% of the measured rates, respectively). These errors are not excessively large when one takes into account the uncertainty in the measured kinetics (k and Ks) on any given date. When this uncertainty is incorporated into the model, predictions improve to 92–108, 89–102, 97–120, 83–133 and 46–100% within the measured initial rates for B, T, E, oX and pX, respectively. The improved fits achieved when measured k and Ks values were varied within the measurement uncertainty is shown in Figure 4b. Note that since the best fit to all five compounds with a given set of kinetics is desired, while the overall model fit improved, the model fit for some individual

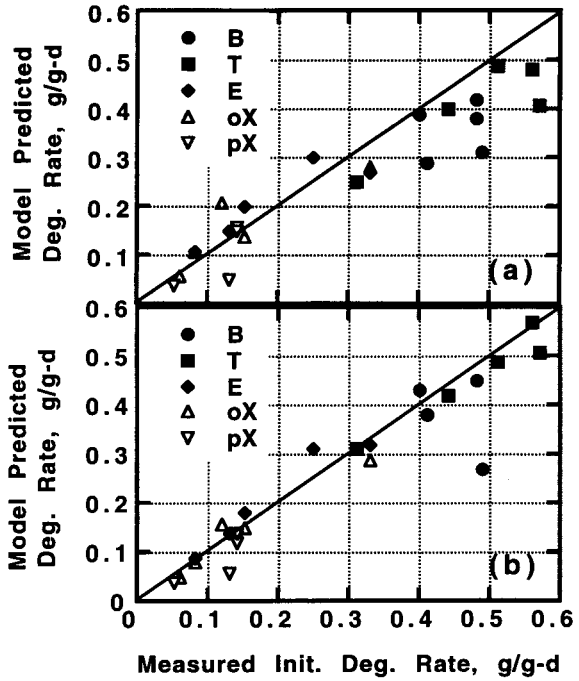

Figure 4. Comparison of the initial specific degradation rates of a mixture of BTEX compounds predicted by the competitive inhibition model to the measured degradation rates: (a) using measured individual compound biokinetics; (b) using individual compound biokinetics within the confidence interval of the measured values

compounds got worse (such as the measured initial B degradation rate of 0.49 g/g-d was predicted as 0.31 g/g-d in Figure 4a but as 0.27 g/g-d in Figure 4b; but the total error in the initial degradation rates of the other four compounds decreased from 51 to 33%, or from 0.15 g/g-d total error to 0.09 g/g-d from Figure 4a and 4b).

On the basis of Figures 4a, b, it appears that the competitive inhibition model (Eq. 4) based on individual compound biokinetics fit the data well, which implies that competitive inhibition is the predominant interactive effect in the BTEoXpX mixture for this culture. The more common approach used to evaluate competitive inhibition kinetics is to plot the inverse of the degradation rate of a compound against the inverse of its concentration at varying concentrations of inhibitor (Chang et al. 1993; Bailey & Ollis 1986). However, it was difficult to determine these values with accuracy due to the fact that the concentrations of both the substrate and inhibitor(s) were simultaneously decreasing. This made getting the substrate degradation rate at different substrate concentrations and different discrete inhibitor concentrations quite

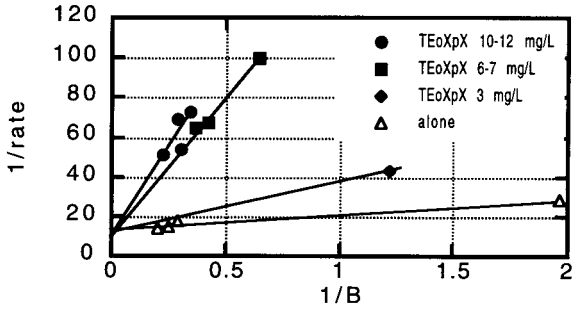

Figure 5. The degradation rate of B versus its concentration in mixture tests with varying concentration of inhibitors (total T, E, oX and pX concentration) present used to illustrate competitive inhibition.

difficult. The results of a rough analysis with B degradation inhibited by the combination of TEoXpX is shown in Figure 5. The slope of the lines increases with increasing inhibitor concentrations (where the inhibitor concentration was the sum of T+E+oX+pX) while the intercept is quite similar. (For TEoXpX 10–12 mg/L the slope is 166.4 and intercept is 14.0; TEoXpX 6–8 mg/L the slope is 132.9 and intercept is 13.8; line shown with 3 mg/L inhibitor has a slope of 24 and intercept of 14; without inhibitor the slope is 7.5 and intercept is 14.0). This shows the result expected when competitive inhibition predominates the mixture effects (the intercept on the plot does not change with slope increasing at higher inhibitor concentrations).

Short-term growth on BTEX

Short-term growth tests were conducted to determine if the filament enrichment could use the BTEX compounds as growth substrates, and to determine the biodegradation rates after growth on BTEX. Batch bottles containing the filament culture were fed an average of 30 mg/L-d of B, T, E, oX, pX or phenol as growth substrates over a 3–5-day period. To compare the increase in biomass due to feeding the different substrates, the net growth was calculated from the difference in VSS concentration in the fed and unfed bottles:

Net growth, g/g

$$= \frac{(\text{mg L}^{-1}\text{ VSS in substrate-fed bottle}) - (\text{mg L}^{-1}\text{ VSS in unfed bottle})}{\text{mg L}^{-1}\text{ substrate degraded}} \quad (5)$$

Table 3 shows that the net growth on phenol, B and T were similar, ranging from 0.90–0.95 g VSS per

Table 3. Growth on phenol or individual BTEX compounds in batch-fed bottles and resulting changes in BTEX degradation rates after 3–5 days (20 °C)

Growth substrate	Days/fed before tests	Net growth (g VSS per g substrate)	Ratio of post-batch growth k to pre-batch growth k
Phenol	3, 5	0.90 ± 0.04	Not tested
B	3, 5	0.95 ± 0.23	0.79–0.91
T	3, 5	0.95 ± 0.30	1.98–2.18
E	4, 5	0.62 ± 0.05	1.20–1.13
oX	3, 5	0.23 ± 0.04	0.19–0.26
pX	4, 5	0.32 ± 0.27	0.28–0.58

g substrate, with net growth on the other compounds only 0.62 g VSS per g E, 0.23 g VSS per g oX and 0.32 g VSS per g pX. In addition, a brown color formed in the liquid of the pX-fed culture, which was not observed in any of the other cultures. The metabolite responsible for the brown color did not appear on the gas chromatogram and was not identified.

The biodegradation rates of individual BTEX compounds were measured prior to alternative substrate growth, and again after 3, 4 and/or 5 days of growth on respective substrates. Table 3 shows that variable responses in degradation ability occurred after growth on the individual BTEX compounds. Toluene feeding increased the T degradation rate of the culture by approximately 2×, ethylbenzene feeding did not significantly change the E degradation rates, benzene feeding decreased the subsequent B degradation rate by 10–20% (which is not significant compared to the measurement uncertainty and daily variance), and o-xylene and p-xylene feeding significantly decreased the subsequent xylene degradation rates. The significant decrease in xylene degradation rates could be due to poor induction of BTEX-degrading enzymes by the xylenes in comparison to BTE and phenol, or the inhibition of xylene degradation by accumulated metabolites. The increased T degradation rate could have been due to the T feeding promoting greater enzyme induction compared to the phenol-induced growth in the continuous reactor. Alternatively, another bacteria in the mixed culture that had a greater T degradation rate than the filament bacteria may have grown during the 5 days of batch feeding in the bottle. However, the filament appeared predominant in all bottles at the end of the batch growth period, making a large population shift seem unlikely.

Discussion

Biodegradation kinetics of aromatics and TCE

Although various researchers have reported the ability of cultures to degrade the multiple aromatic compounds studied in this work, none have reported biodegradation kinetics for the same broad range of compounds studied here. The widest range of compounds was tested with a single mixed culture by Deeb and Alvarez-Cohen (1994), but naph and phenol degradation by their culture was not tested; kinetics were not reported. Biodegradation kinetics of these compounds have been reported for various cultures; however, caution must be taken when interpreting literature reported biokinetics since the growth conditions of a culture can affect the measured kinetics (Grady et al. 1996; Templeton & Grady 1988). Specifically, Templeton & Grady (1988) found that a decreasing SRT increased the k and decreased the Ks of phenol for a pure culture grown on phenol. The biokinetic testing methods can also affect the kinetics of the culture; tests conducted at low initial substrate concentrations tend to reflect the 'extant' or existing kinetics of the culture due to its growth condition while 'intrinsic' kinetics reflective of the maximum induced state of the culture are conducted at high initial substrate concentrations (Grady et al. 1996). The discussion below compares the measured biokinetics of the filamentous enrichment culture to the reported kinetics for other cultures, with information provided to help determine the 'extant' or 'intrinsic', nature of these kinetics. Where possible, kinetics measured under conditions most similar to those in this work have been included in the discussion.

The average k and Ks values measured for the phenol-fed filament enrichment at 20 °C (5.7 g/g-d and 0.34 mg L^{-1}) compare to reported values for other phenol-fed mixed and pure cultures of 4.3–37 g/g-d and 0.03–1.42 mg L^{-1} at 23–26 °C (Brown et al. 1990; Ellis et al. 1996a, b; Folsom et al. 1990; Saez & Rittmann 1993). *Pseudomonas cepacia* G4 had reported k and Ks values of 31.5 g/g-d and 0.8 mg L^{-1}, respectively, when the culture was grown at a 10–20 h SRT and kinetics tested at 26 °C with initial phenol concentrations of 1–50 mg L^{-1} (Folsom et al. 1990). *Pseudomonas putida* PpG4 grown at a 40-h SRT had measured phenol degradation kinetics of k = 8.5 g/g-d and Ks 1.22 mg L^{-1} in kinetic tests at 23–26 °C and initial phenol concentrations of 50–250 mg L^{-1}. Two mixed cultures tested by Brown et al. (1990) had

k values of 14.2 and 7.7 g/g-d and Ks of 1.01 and 1.42 mg L^{-1}. These kinetics were measured at 25 °C with an initial phenol concentration of 42 mg/L in 3 and 4 tests, respectively, with coefficients of variation between the replicate measures of 4 and 33% for k and 61 and 36% for Ks. Cultures tested were selected during growth at a 2-d SRT and then taken after batch growth to early plateau phase on 42 mg L^{-1} phenol. Due to the high initial substrate concentrations tested, the previous kinetics are likely 'intrinsic' for the respective cultures. The mixed culture tested for phenol kinetics by Ellis et al. (1994) was grown at a 6-d SRT, tested with initial phenol concentrations of less than 2 mg L^{-1} at 25 °C, and was found to have an extant k of 4.9 g/g-d and Ks of 0.07 mg L^{-1} (with coefficients of variation of 8 and 10%, respectively). This Ellis culture was grown and tested under conditions most similar to that in our work, and has a similar k value.

Biodegradation kinetics of naph with other cultures at 20 °C have been reported. Guerin & Boyd (1992) measured degradation kinetics for two pure cultures by non-linear fits to batch rate tests with stationary cells at 0.02–0.55 mg/L initial naph. The Ks values were 0.09 and 0.21 mg L^{-1}, which is similar to the Ks value of the filament enrichment culture (0.18 mg L^{-1}). The reported naph k values for these cultures were 2–4 g/g-d (Guerin & Boyd 1992), which are significantly higher than rates reported for mixed cultures grown at a 20–60-d SRT of 0.09–1.12 g/g-d (Tittle 1994; Kader 1995; Liu 1994). The mixed culture kinetic tests were conducted with initial naph concentrations of 4–35 mg/L. The naph k of the filament culture from this research (0.17 g/g-d) is in the lower range of the rates reported for other mixed cultures. However, in this work the filament culture was not grown on naphthalene, unlike the other mixed and pure culture work reported that was conducted with cultures grown on naphthalene or polyaromatic hydrocarbon (PAH) compound mixtures. Cultures which were acclimated to growth on naphthalene would be likely to have higher naphthalene degradation rates than unacclimated cultures. For example, the enrichments of Tittle (1994) which were grown only on naph had a k value of 0.84 versus 0.09 g/g-d for a culture from the same seed source that was grown on a PAH mixture (which included both naphthalene and higher ring PAHs).

TCE degradation by the filament enrichment culture was previously characterized (Bielefeldt 1994; Bielefeldt et al. 1995). In this work, the TCE k at 20 °C was determined on eight different test dates over a 7-week period using the direct method with initial liquid TCE concentrations between 2.5–7 mg L^{-1}. The average measured TCE k was 0.16 g/g-d with a standard deviation of 0.05, which is comparable to the previously reported k of 0.18 g/g-d with 0.03 standard deviation (Bielefeldt 1994). The TCE biokinetic values of mixed phenol and toluene-fed cultures (grown in CSTRs at 3- and 5-day SRTs, respectively, and measured at 20 °C) reported by Chang & Alvarez-Cohen (1994) were 0.21 and 0.17 g/g-d, respectively; quite similar to the filament enrichment. However, the TCE Ks for the filament enrichment is significantly lower than that reported by Chang & Alvarez-Cohen (1994) of 2.04 and 8.64 mg L^{-1}, respectively. The TCE degradation k values of *P. cepacia* G4 reported by Folsom et al. (1 990) and Landa et al. (1994) of 0.38–0.76 and 0.94 g/g-d, respectively, were measured with cultures grown at a 10–20 h SRT, and temperatures of 26 and 28 °C and, therefore, should not be directly compared to the filament culture kinetics at a 5-SRT and 20 °C. The Ks concentration of the filament enrichment (0.10–0.25 mg L^{-1}) is lower than the reported values for *P. cepacia* of 0.39 and 0.79 mg L^{-1} (Folsom et al. 1990; Landa et al. 1994). Therefore, similar extant TCE k values have been reported when phenol or T-fed cultures were grown under similar conditions.

Biokinetic variability

Ellis et al. (1996b) reported on the variability of biokinetic parameters of a pure culture, and reported CVs for measured phenol degradation kinetics over an 11-month period of growth reactor operation of 29% for k and 80% for Ks. Chang et al. (1993) reported CVs of 13–39% and 8–67% for BTX k and Ks values, respectively, although it was unclear whether these values were between 'replicate' tests on the same day or multiple tests over time. Bielefeldt & Stensel (1998a) found CVs of 27–44 and 43–100% for the k and Ks of individual BTEX compound degradation by a mixed BTEX-fed culture from a steady-state 5-d SRT reactor over a 9-month test period. The 'uncertainty range' of the biokinetic coefficient values of BTEX and phenol measured in this work for the filament culture are consistent with previous findings.

Biodegradation kinetics of mixtures

Three other papers have reported model predictions of measured BTEX compound interactions in mixtures. Chang et al. (1993) used two pure strains of *Pseudomonas* bacteria, B1 and X1, and measured the

maximum specific growth rate (μm), Ks, and yield (mg cells per mg substrate) for the individual B, T or pX compounds. Competitive inhibition was used to model the interactions of the two compounds degraded as growth substrates by each strain of bacteria. The competitive inhibition model was the same as that used in this work, Eq. 2, with k = μm/yield. The Ki values fit to the data for B and T (3.10 ± 0.12 and 1.71 mg L^{-1}, respectively) were similar to the measured Ks values (3.17 ± 0.82 and 1.96 ± 0.91 mg L^{-1}). The kinetic model in Eq. 4 was also used by Bielefeldt & Stensel (1998b) to describe the interaction of 2–5 BTEoXpX compounds degraded by mixed cultures acclimated to growth on BTEoXpX and B, and grown at 5- and 20-d SRTs. The model-predicted initial degradation rates of the individual compounds were most accurate for the 20-d SRT BTEX-fed culture and the 5-d SRT B-fed culture. For the 5-d SRT BTEX-fed culture the model significantly under-predicted the E degradation rates. The model fit achieved with the filament culture equaled or exceeded the accuracy of the model fit for the BTEX cultures, possibly due to the predominance of the single filamentous bacterium in the enrichment. Oh et al. (1994) studied the biodegradation of 2-compound mixtures of B, T and pX by a pure and mixed culture of bacteria; the model used to fit their data was significantly different, with an inhibition parameter that was fit to the data and could not be determined from the individual compound kinetics.

Short-term growth on BTEX compounds

The poor net growth observed on the xylene compounds may be due to incomplete degradation resulting in the formation of metabolites. In work using the filament culture to continuously treat BTEoX-contaminated gases in a suspended-growth reactor, metabolite accumulation during treatment of high BTEoX concentrations was noted by an increase in the soluble chemical oxygen demand (sCOD) in the reactor liquid (Bielefeldt & Stensel 1998c). When liquid containing these metabolite compounds was used in batch tests, this sCOD was found to inhibit B and T degradation by the filament culture (Bielefeldt & Stensel 1998c). When the gas treatment reactor continuously degraded 0.86 g/d ToXpX over 5 days (0.28 g pX d^{-1} or 888 mg d^{-1} as COD), the sCOD formation rate (300 mg d^{-1}) was significantly faster than when the reactor degraded 1.03 g/d ToX (130 mg d^{-1}) (data not shown; Bielefeldt 1996). Normalizing for sCOD metabolite contribution from the ToX, this gives a net sCOD formation rate of 226 mg d^{-1} due to pX (or 25% of the pX COD loading rate). This indicates that pX degradation by the filament culture produced significant amounts of metabolite compounds. In addition, the observed biomass yield in the gas treatment reactor was 0.34 g VSS per g ToXpX and 0.35 g VSS per g ToX (Bielefeldt 1996); these values are similar to the net growth observed in the batch bottles for oX and pX of 0.23 and 0.32 g/g, respectively.

Summary and conclusions

A mixed culture dominated by a unique filamentous bacterium was found to have the ability to degrade a wide range of aromatic compounds, including phenol, BTEoXpX and naph, in addition to cometabolically degrading TCE. The Michaelis-Menten biodegradation kinetics of the phenol-grown culture were determined for each of the test compounds. The average maximum specific degradation rate (in g/g-d) of phenol was the fastest (5.7), followed by T (2.7), B (1.5), E (0.8), pX (0.7), oX (0.6), naph (0.17) and TCE (0.16). Using an oxygen uptake method, the measured half-saturation coefficients of B, T, E, oX, pX and phenol were found to range from 0.16–0.51 mg L^{-1}. A competition test with phenol was used to estimate the Ks values for TCE and naph, which both averaged 0.18 mg L^{-1}. There was a large degree of variation in the measured biokinetic coefficients over the 5-month testing period, which appears to be attributable to natural biokinetic variability that has also been observed by other researchers. The measured biokinetics of the culture are similar to reported extant kinetics with other cultures that were grown under similar conditions.

Biodegradation tests with a mixture of B, T, E, oX and pX were conducted, and it was found that a competitive inhibition equation for multiple compounds was able to accurately predict the degradation rates of individual compounds in the mixture on the basis of the individual compound biokinetics. This model might be useful for predicting mixture degradation for other bacterial cultures and substrates. Short-term (3–5 days) growth of the culture on individual BTEX compounds either increased (T), caused little change (B, E), or decreased (oX, pX) the subsequent degradation rates of those compounds. Specifically, the culture appeared to have a poor ability to grow on oX or pX. The ability of the mixed culture to degrade a mixture of compounds under a range of growth conditions

may be important for a variety of waste treatment applications.

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