Biodegradation (2005) 16: 11–22

Changes in soil microbial community composition induced by cometabolism of toluene and trichloroethylene

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Accepted 30 March 2003

Key words: cometabolism, microbial community, reverse sample genome probing, toluene, trichloroethylene

Abstract

The effects of trichloroethylene (TCE) on microbial community composition were analyzed by reverse sample genome probing. Soil enrichments were incubated in dessicators containing an organic phase of either 1 or 10% (w/w) toluene in vacuum pump oil, delivering constant equilibrium aqueous concentrations of 16 and 143 mg/l, respectively. Increasing the equilibrium aqueous concentration of TCE from 0 to 10 mg/l led to shifts in community composition at 16, but not at 143 mg/l of toluene. In closed system co-degradation studies, TCE at an aqueous concentration of 1 mg/l was effectively degraded by toluene-degrading soil enrichments once the aqueous toluene concentration dropped below 25 mg/l. Little TCE degradation was observed at higher toluene concentrations (50–250 mg/l). The results indicate that TCE changes microbial community composition under conditions where it is being actively metabolized.

Introduction

Trichloroethylene (TCE) and other synthetic chlorinated solvents are common environmental pollutants (Robinson et al. 1998). TCE is toxic and a suspected carcinogen (Pressman et al. 1999), making TCE contamination a problem urgently requiring solutions (Hecht et al. 1995). Biodegradation of TCE can be an attractive option for remediating contaminated sites. Some anaerobic microorganisms use TCE as an electron acceptor in reductive dehalogenation (Lee et al. 1998), although partial TCE reduction can occur, yielding carcinogenic end products (Shim et al. 2001). Aerobic organisms cannot use TCE as a sole carbon and energy source (Hyman et al. 1995). However, cometabolic mineralization of TCE among certain aerobic bacteria is supported by a variety of main substrates such as toluene (Kelly et al. 2000), phenol (Lu et al. 1998), methane (Oldenhuis et al. 1991), propane (Wackett et al. 1989), cumene (Dabrock et al. 1992), 2,4-diphenoxyacetic acid (Harker & Kim 1990), isoprene (Ewers et al. 1990), or ammonia (Yang et al. 1999). Degradation pathways for many of these substrates, including four of five well-characterized toluene degradation pathways, involve oxygenase enzymes (Fang et al. 2000; Parales et al. 2000). Aerobic toluene-degraders thus use oxygen as both an electron acceptor and a substrate for toluene catabolism (Leahy et al. 1996), although nitrate-reducing oxygenase-expressing toluene degraders can also cometabolize TCE (Park et al. 2002). Toluene mono- and dioxygenases have sufficiently relaxed specificity to catalyze the degradation of non-growth substrates such as TCE (Winter et al. 1989), and in some cases TCE induces its own degradation by these enzymes without the main substrate (Lee et al. 2002; Ryoo et al. 2001). Conditions at contaminated sites will dictate the composition of indigenous microbial communities, which in turn dictates activity of toluene catabolic

pathways that may support TCE degradation (Duetz et al. 1994).

Toluene and TCE co-contaminate sites such as railyards, where toluene enters the soil through fuel spills or storage tank leaks, and TCE through accidental release during its use and disposal by maintenance personnel (Hirl 1998; Sung et al. 2003). In other instances of TCE contamination, toluene may be added in order to stimulate cometabolic removal of TCE (McCarty et al. 1998). Here we examined soil contaminated with C5+ hydrocarbons (Shen et al. 1998). C5+ is a byproduct of ethane pyrolysis and consists of primarily benzene, toluene, xylene, styrene, dicyclopentadiene, and naphthalene. The C5+ mixture thus contained toluene but not TCE; however, the presence of toluene-degrading bacteria (Hubert et al. 1999) indicated the community might be capable of TCE cometabolism. It is known that microbial community composition can shift following petroleum spills (Kerry 1990) or TCE contamination (Cox et al. 1994). While much research into TCE co-metabolism has been performed using selected bacterial isolates, monocultures of microorganisms do not normally occur in natural environments. Soil environments harbour complex microbial communities containing thousands of different species (Torsvik et al. 1990), conferring great potential for contaminant degradation by indigenous communities (Downey et al. 1999). Few studies have focused on microbial communities responsible for aerobic TCE cometabolism (El Farhan et al. 1998; Fries et al. 1997b). The ability to identify and monitor such populations would improve understanding and modelling of TCE bioremediation (Hanson et al. 1999). In this work reverse sample genome probing (RSGP) was used to monitor the composition of microbial communities incubated with toluene and TCE with variable exposure time and composition of toluene:TCE mixtures.

Materials and methods

Biochemical reagents

[α-^{32}P] dCTP (10 mCi/ml; 3000 Ci/mmol) was purchased from ICN, while enzymes and bacteriophage λ DNA (0.5 mg/ml) were obtained from Pharmacia. Reagent grade chemicals were obtained from BDH, Fisher, or Sigma. Vacuum pump oil 19, a 100% paraffinic oil with a density of 0.85 g/cm^3, was obtained from VWR Scientific.

Soil samples and toluene-degrading bacterial standards

Soil samples A0.5, A2, Bl, C2 and El were obtained from a soil pile contaminated with C5+ for bioremediation research at a polyethylene plant near Joffre, Alberta (Stehmeier et al. 1999). A composite of these samples (Greene et al. 2000) was also used. Bacterial strains, isolated by enrichment on toluene by Hubert et al. (1999), were maintained as glycerol stocks at −70 °C.

Culture medium and regulation of toluene and TCE concentrations

Minimal salts medium (MSM) was prepared as described previously (Shen et al. 1998). Soil cultures were incubated at room temperature (20 ± 2 °C) in 11.4 l glass dessicators containing a crystallizing dish with a mixture of toluene and/or TCE, and vacuum pump oil (vpo). Dilution in vpo (selected for its negligible vapour pressure and low cost) provided a continuous supply of toluene and TCE to incubated cultures at fixed vapour concentrations (Greene et al. 2000; Hubert et al. 1999), which created the equilibrium aqueous concentrations given in Table 1.

Toluene and TCE degradation in serum bottles

MSM (50 ml) was added to 120-ml serum bottles, which were sealed with butyl rubber stoppers and injected with 15 μl of toluene or a toluene:TCE mixture (250:1 w/w), creating aqueous concentrations of toluene and TCE of 250 and 1 mg/1, respectively. In the dessicator system similar aqueous concentrations would be achieved by incubation with 17% (w/w) toluene and 0.1% (w/w) of TCE in vpo (Table 1). Following overnight incubation, duplicate serum bottles were inoculated with soil enrichments or toluene-degrading strains. The enrichments were obtained by incubating 5 g soil in 10 ml MSM in open capped tubes in dessicators with an organic phase of 0, 1, 10 or 100% (w/w) toluene for 2 weeks, or 10% (w/w) toluene for 6 weeks (Table 1). Toluene and TCE concentrations in the serum bottles were determined by injecting 10 μl of headspace gas

Table 1. Estimated equilibrium aqueous phase concentrations of toluene (C_{tol}) and TCE (C_{TCE}) in dessicators containing an organic phase of toluene and/or TCE and/or vpo, as indicated

Experiment	% Toluene(w/w)	C_{tol}(mg/l)[a]	% TCE(w/w)	C_{TCE} (mg/l)[b]	Organic phase total (g)
Figure 1[c]	0	0	0	0	150[d]
Figure 1	1	16	0	0	150
Figure 1	10	143	0	0	150
Figure 1	100	540	0	0	15[e]
Figure 2	10	143	0	0	100
Figure 2	10	143	0.04	0.4	100
Figure 2	10	143	0.2	2.0	100
Figure 2	10	143	1	10	100
Figure 2	1	16	0	0	150
Figure 2	1	16	0.04	0.4	150
Figure 2	1	16	0.2	2.0	150
Figure 2	1	16	1	10	150
Figure 5	1	16	0	0	150
Figure 5	1	16	1	10	150
Figure 5	0	0	1	10	150

[a] Hubert et al. (1999).
[b] Using a value of 1000 mg/l for the solubility of TCE in water (Perry & Green 1997).
[c] For pre-incubation of soil enrichments.
[d] Organic phase was vpo only.
[e] Organic phase was toluene only.

into a Hewlett-Packard HP5190 gas chromatograph equipped with a flame ionization detector and a 30 m × 0.2 mm 0.5 μm RTX-5 column (Restek, Brockville, ON), using He as the carrier gas (flow rate 1.23 ml/min) and injector and detector temperatures of 210 and 250 °C, respectively. The oven temperature was 60 °C for 2 min, increasing by 10 °C/min to a final temperature of 250 °C. Air (20 μl) was injected into the bottles at each sampling. Bottles were incubated upside down on a shaker at 30 °C. Prior to sampling, bottles were shaken to remove particulates from the inside of the septa and allowed to sit for at least 1 h for equilibration of headspace gases. At the completion of some serum bottle incubation experiments total DNA was isolated for community composition analysis.

Effect of different toluene and TCE concentrations on the microbial community

Aliquots (l ml) of soil enrichments (5 g of soil in 10 ml of MSM in open capped tubes with 1% (w/w) toluene for 10 days) were transferred to 4 ml of MSM and placed in eight dessicators equilibrated with 1 or 10% (w/w) toluene and 0, 0.04, 0.2 or 1% (w/w) TCE in vpo, respectively. After 15 days of incubation, cultures were removed and total DNA was isolated for community composition analysis. Other soil enrichments were obtained by incubating 5 g of composite soil in 10 ml of MSM in open capped tubes in dessicators with 0 or 1% (w/w) toluene in vpo and 0 or 1% (w/w) of TCE, respectively. Enrichments were removed from the dessicators following 0, 2, 4, 6, 8, 13 and 16 weeks of incubation. Plate counts were performed on each enrichment using one-quarter strength Luria–Bertani agar (Sambrook et al. 1989); the remainder of each enrichment was used for the isolation of total community DNA for community analysis.

Analysis of community composition using RSGP

DNA was isolated from cultures using a modified method of Marmur (1961), as described by Shen et al. (1998). Humic acids were removed using Sepharose 4B spin columns (Jackson et al. 1997). Concentrations of purified DNA were determined fluorimetrically (Voordouw et al. 1992). Labelling

reactions contained 100 ng of total community DNA, 2.5 ng of bacteriophage λ DNA, random hexamers and [α-^{32} P] dCTP as described elsewhere (Hubert et al. 1999). Probes were hybridized with a master filter (a genome array) containing known quantities of denatured genomic DNA from 55 genomically distinct bacterial strains (standards) isolated from the C5+ contaminated site (http://www.bio.ucalgary.ca/divisions/cmmb/voordouw.html; Greene et al. 2000). Following overnight hybridization, the filters were washed, dried, and exposed to BASIII imaging plates, which were subsequently analyzed with a Fuji BAS1000 bioimaging analyzer (Telang et al. 1997). Values for f_x, the weight fraction for each standard DNA x, were calculated as described by Telang et al. (1997). Relative values ($f_{x,\ rel}$) were calculated as $f_{x,\ rel} = (f_x/\Sigma\ f_x) \times 100\%$. Dendrograms of RSGP community profiles were generated with the cluster analysis program SYSTAT 10.2, as described elsewhere (Greene et al. 2000).

Results

Codegradation of toluene and TCE

Enrichments obtained by pre-incubating soil A2 with 0, 1, 10 or 100% (w/w) toluene removed toluene completely within 24 days (Figure 1A).

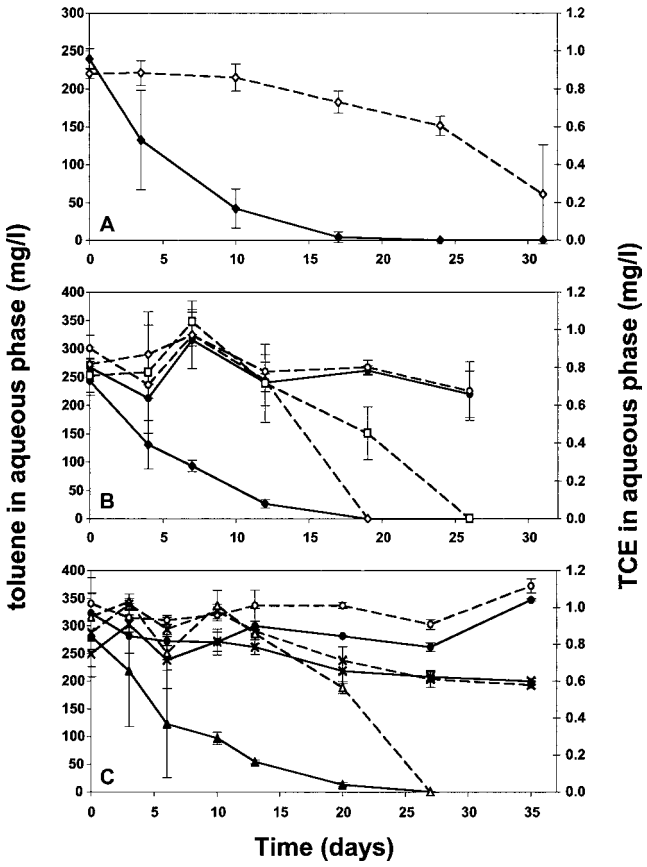

Figure 1. Degradation of toluene (solid lines) and TCE (dashed lines) by enrichment and pure cultures of toluene-degrading bacteria in closed serum bottles, containing MSM (4 ml), enrichment or pure culture (1 ml), and a 250:1 toluene:TCE mixture (15 μl). (A) Enrichments from soil A2 were pre-incubated for 2 weeks with 100, 10, 1 or 0% toluene. The values for toluene (♦) and TCE (◇) concentrations are averages of eight experiments (two for each pre-incubation condition). (B) Soil enrichments pre-incubated with 10% toluene for 6 weeks. Toluene concentrations are averages of six experiments (♦; two each for soils A0.5, A2 and El). TCE concentrations are averages of four (◇; two each for soils A2 and El) or two experiments (□; two for soil A0.5). Averages for duplicate incubations of an uninoculated control are also shown (●, ○). (C) Pure cultures of *Pseudomonas* sp. strains. The values for toluene and TCE concentration are averages of four (▲, △; two each for strains Cstd5 and Cstd7) or two experiments (×; two for strain Cstd1). Averages for duplicate incubations of an uninoculated control are also shown (●,○).

Because no significant differences in degradation kinetics were noted, averaged data for eight serum bottles (two for each pre-incubation condition) are shown. TCE degradation kinetics were also very similar in all eight serum bottles up to 24 days, where 0.75 ± 0.06 mg/l of TCE remained. From 24 to 31 days kinetics were more variable. On average 0.24 ± 0.24 mg/1 of TCE remained at 3l days with all TCE being removed from 2 of 8 serum bottles. Hence TCE concentrations decreased more rapidly once toluene was completely removed. Toluene removal in the absence of TCE had a similar time course as in Figure 1A (data not shown), indicating that TCE at 1 mg/1 did not affect the rate of toluene degradation at 250 mg/1.

Enrichments from soils A2, A0.5 and El, pre-incubated with 10% (w/w) toluene for 6 weeks, gave similar toluene degradation kinetics (Figure 1B) to those for the enrichments in Figure 1A. However, TCE degradation was faster and was complete after 19 (A2, E1) or 26 days (A0.5). Hence increasing the length of pre-incubation with toluene from 2 to 6 weeks appeared to stimulate TCE degradation.

Toluene-degrading *Pseudomonas* strains Cstd5 and Cstd7 (represented by standards 38 and 40 in Figure 2) removed toluene and TCE completely with similar kinetics, whereas strain Cstd1 (represented by standard 36 in Figure 2) showed only slight toluene and TCE degradation relative to the uninoculated control (Figure 1C). These results agree with previous findings that Cstd5 and Cstd7 grow in relatively high toluene concentrations (Hubert et al. 1999), as used in Figure 1C, whereas Cstd1 grows optimally at lower toluene concentration and thus performed poorly in this study.

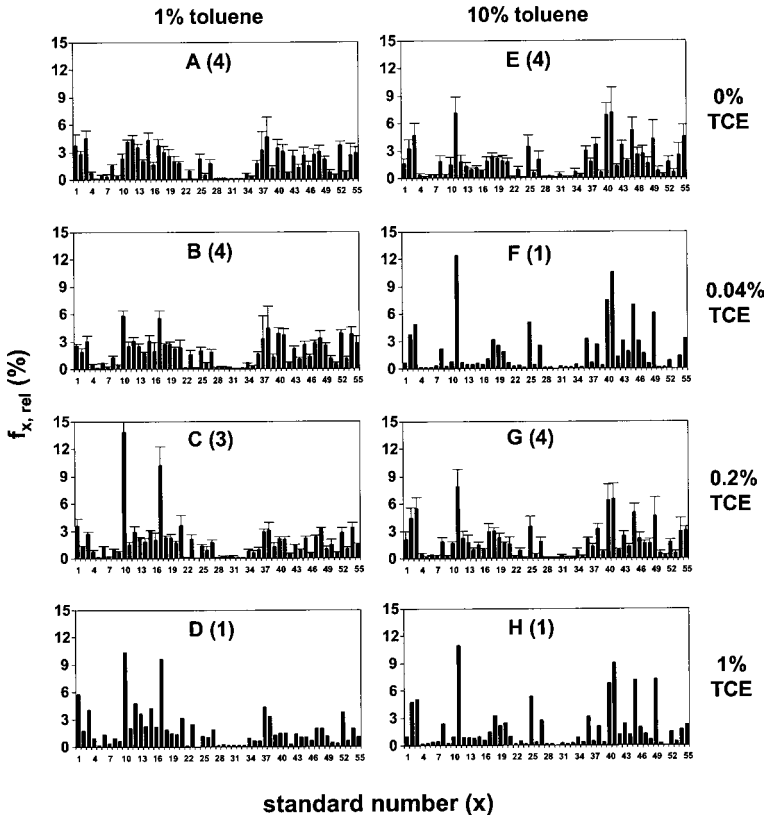

Figure 2. Community compositions determined by RSGP for A0.5 soil enrichments incubated in dessicators with an organic phase consisting of vpo and toluene (% w/w) and TCE (% w/w) as indicated. Average data are presented when the number of hybridizations with extracted community DNA was 3 or 4, as indicated.

Influence of increasing TCE concentration on microbial community composition

Aliquots (1 ml) of soil enrichments (5 g in 10 ml MSM with 1% (w/w) toluene for 10 days) were transferred to 4 ml MSM and incubated in dessicators with 1 or 10% (w/w) toluene and 0, 0.04, 0.2 or 1% TCE for 15 days. Microbial community composition was then analyzed by RSGP. For soil A0.5 these represent averages of 3–4 hybridizations of the same community DNA to different master filters (Figure 2A–C, E and G) or the results of single hybridizations (Figure 2D, F and H). In cases of multiple hybridizations the standard deviations from average $f_{x,\,rel}$ values are shown. The average community composition for incubation with 1% (w/w) toluene was distinct from that with 10% (w/w) toluene (Figure 2A and E). In the latter, *Pseudomonas* spp. standards 11, 40 (=Cstd7) and 41 were dominant, whereas the community with 1% (w/w) toluene was more evenly distributed. The relationship between the eight community compositions determined for soil A0.5 is shown in Figure 3. When the compositions of 3–4 single replicates for the same community DNA sample were compared in a dendrogram as in Figure 3, they were found to span a maximal inter-replicate Euclidean distance (IRED) of 0.007 (four replicates for A0.5-1/0), 0.008 (four replicates for A0.5-1/0.04), 0.010 (three replicates for A0.5-1/0.2), 0.008 (four replicates for A0.5-10/0) and 0.008 (four replicates for A0.5-10/0.2). Hence when community compositions are compared by the DNA hybridization method used here, changes in community composition are only significant when the Euclidean distance span exceeds the IRED of 0.008. This value has been indicated in Figure 3 (↔). The community compositions observed with 10% (w/w) toluene, treeing together in the bottom part of the dendrogram, barely exceed the IRED indicating little effect of TCE concentration on community composition. Indeed, the community profiles are very similar with *Pseudomonas* spp. standards 11, 40 and 41 being main community components throughout (Figure 2E–H). In contrast the community compositions observed with 1% (w/w) toluene, which tree at the top of the dendrogram, clearly exceed the IRED of 0.008 (Figure 3). This corresponds to a distinct change of community composition with increasing TCE concentration in which standards 10 and 17 (*Flavobacterium* sp. and genus not determined,

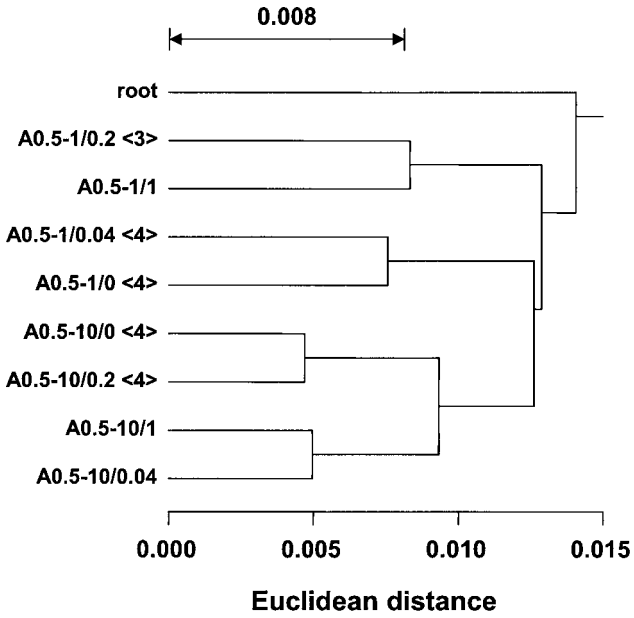

Figure 3. Dendrogram for the community compositions indicated in Figure 2. The tree is rooted relative to a community in which all $f_{x,\,rel}$ values are equal. The codes indicate sequentially: soil type (A0.5), concentration (% w/w) of toluene in the organic phase (either 1 or 10), concentration (% w/w) of TCE in the organic phase (either 0, 0.04, 0.2 or 1), and the number of replicates if not 1 (⟨3⟩ or ⟨4⟩). An inter-replicate Euclidean distance (IRED) of 0.008 is indicated, as explained in the text.

respectively) become major community components (Figure 2A–D).

The relationships between community compositions for all soil enrichments incubated for 15 days at different toluene and TCE concentrations are presented in Figure 4. With 1% (w/w) toluene, changes exceeding the IRED of 0.008 are observed for enrichments from soils A0.5, A2, B1 and C2, but not for enrichments from soil El. With 10% (w/w) toluene the 20 community compositions fall within (18) or barely exceed (2) the IRED indicating little effect of TCE under these conditions. Hence community composition appears to be influenced by TCE at low but not at high toluene concentration (equilibrium aqueous concentrations of 16 and 143 mg/l, respectively).

Changes in microbial community composition with time

Multiple samples of composite soil (5 g in 10 ml MSM) were placed in dessicators equilibrated with an organic phase of 1% toluene, 1% toluene and 1% TCE, or 1% TCE in vpo for up to 16 weeks. Total culturable heterotrophs increased 900- and 500-fold during incubation with toluene, or

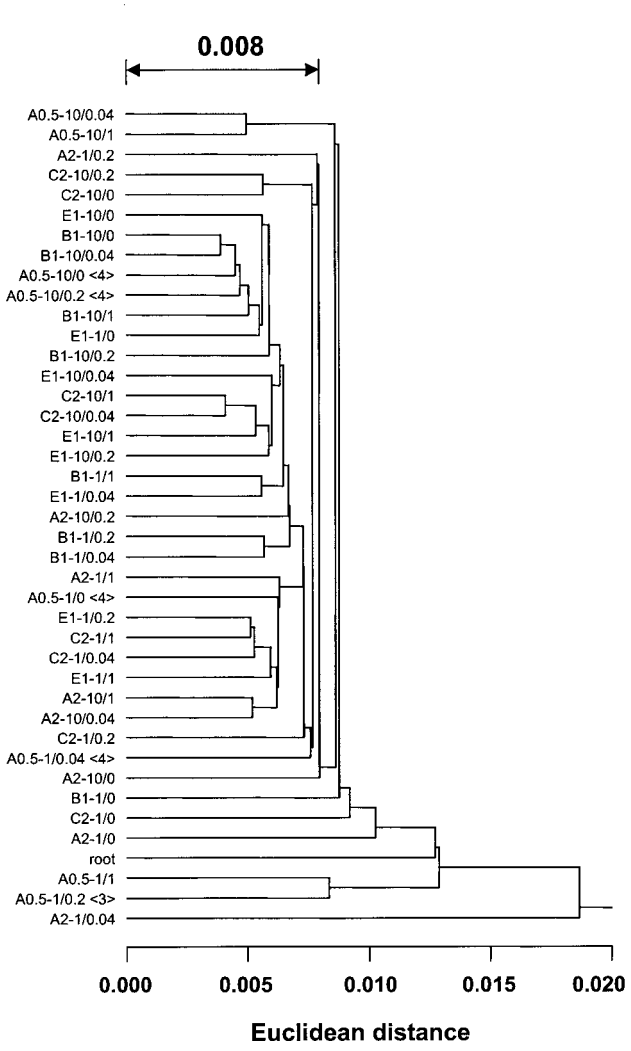

Figure 4. Dendrogram for all community compositions obtained following incubation as in Figure 2. Compositions for enrichments from soils A0.5 (the same as in Figure 3), A2, B1, C2 and El are compared. The codes, root and IRED of 0.008 are as explained in the legend to Figure 3.

toluene and TCE, respectively, but only 3-fold during incubation with TCE as the carbon and energy source (Figure 5). Rapid growth from 0 to 4 weeks was followed by a slower increase in cell numbers from 4 to 16 weeks. Fourteen community compositions were determined for incubations with 1% (w/w) toluene or 1% (w/w) toluene and 1% (w/w) TCE for each of the time points indicated in Figure 5. Four of these were averages of four replicates, whereas the remainder were derived from single hybridizations. A dendrogram of these compositions indicated that only those for incubation with toluene and TCE for 2 and 8 weeks exceeded the IRED of 0.008. Compositions at 13 and 16 weeks treed close together, indicating that differences in community composition during the slow phase of growth tended to be less than during exponential growth at 2 weeks (results not shown).

Discussion

In contaminated soils, volatile organic compounds partition into three phases: they (i) volatilize in soil gas, (ii) dissolve in soil solution and (iii) adsorb to soil colloids (Fan & Scow 1993). Bioremediation typically occurs in water films surrounding soil particles where microbes can access liquid phase contaminants. Phase partitioning can make quantifying bioavailable concentrations extremely difficult, and TCE concentrations may be overestimated because of this (Barth et al. 2002; Folsom et al. 1990). These problems are overcome with the dessicator approach presented here. The toluene/TCE/vpo mixture establishes constant equilibrium vapour phase concentrations that maintain constant bioavailable aqueous concentrations, regardless of sorption onto soil particles. Futamata et al. (2003) recently showed that complete co-metabolic removal of TCE required constant re-addition of phenol; supplying phenol in a single dose at the beginning of the experiments resulted in much less TCE removal. Constant concentrations of bioavailable volatile organics are ensured by dilution in vpo. This system is ideal for evaluating the effect of exposure to specific concentrations of hydrocarbons on community composition (Greene et al. 2000).

However, degradation rates cannot be evaluated under these conditions as toluene and/or TCE degraded in the aqueous phase are constantly replenished from the vapour phase. Hence the experiments shown in Figure 1 did not involve incubation at constant, buffered organic compound concentrations. Instead a range of aqueous toluene (from 250 to 0 mg/l) and TCE (from 10 to 0 mg/l) concentrations was sampled (Figure 1). The results indicate that co-metabolic degradation of TCE did not start until most of the toluene had been removed, as is shown in Figure 6, where corresponding aqueous toluene and TCE concentrations are plotted for all experiments in Figure 1 displaying co-metabolism. The resulting curve indicates little TCE degradation until the aqueous toluene concentration falls below approximately 25 mg/1 (Figure 6).

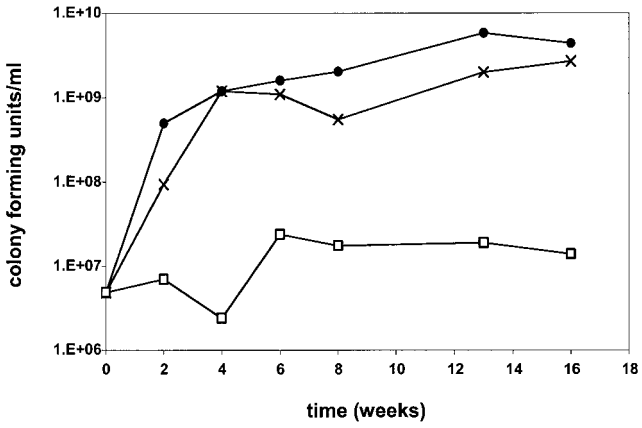

Figure 5. Growth of soil bacteria in cultures equilibrated with 1% (w/w) toluene (●), 1% (w/w) toluene and 1% (w/w) TCE (×), or 1% (w/w) TCE (□) in vpo. The number of total culturable heterotrophs (CFU/ml) was determined by plating periodically, as indicated.

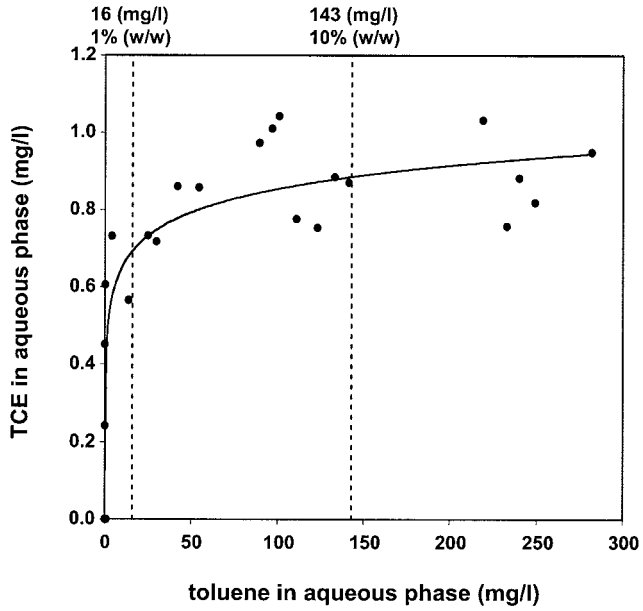

Figure 6. Relationship between aqueous toluene and TCE concentrations during co-metabolism. Values for aqueous toluene and TCE concentrations in Figure 1 have been plotted, except those for the uninoculated controls and for Cstd1. The vertical dotted lines represent equilibrium aqueous toluene concentrations established in dessicators with an organic phase of 1 or 10% (w/w) toluene in vpo.

Hence, sequential toluene and TCE degradation was observed both for soil enrichments and pure cultures in this study, except for Cstdl which appeared to degrade both compounds poorly, but simultaneously (Figure 1C). Both sequential (Futamata et al. 2003; Mu & Scow 1994; Winter et al. 1989) and simultaneous (Fuller et al. 1995; Guo et al. 2001) toluene/TCE cometabolism have been reported. Mu & Scow (1994) observed sequential removal of 20 μg/ml toluene then 1 μg/ml TCE by indigenous soil populations. Toluene removal was slower and TCE was not degraded when 20 μg/ml of both compounds were present. The lower toluene degradation activity was attributed to competitive inhibition when both substrates were present in equal amounts, highlighting the importance of relative toluene and TCE concentrations for cometabolism.

Incubation at constant concentrations of organics indicated a strong influence of toluene concentration on community composition (Figure 2A and E), as observed previously (Hubert et al. 1999). No significant effects of TCE on community composition were seen at high toluene concentration (143 mg/l in the aqueous phase), whereas a distinct response was observed at low toluene concentration (16 mg/l in the aqueous phase) as shown in Figures 2–4. In view of the data in Figures 1 and 6, a likely explanation for this result is that TCE is being metabolized at the lower, but not at the higher toluene concentration. Assuming that co-metabolism at low toluene concentration gives rise to toxic degradation products, community composition would be expected to shift to favor strains able to cope with this toxicity. Wackett & Householder (1989) demonstrated that TCE is toxic only to organisms that degrade it, using mutants lacking toluene dioxygenase that grew faster in the presence TCE than wild type cells co-metabolically degrading these compounds. Degradation-mediated toxicity is attributed to breakdown products (e.g. TCE epoxides) attacking cellular components including oxygenase enzymes (Smith & McCarty 1997; Sun & Wood 1997) and is proportional to the extent of TCE co-metabolism (Alvarez-Cohen & McCarty 1991). Interestingly, standards 10 and 17 (*Flavobacterium* sp. and genus not determined, respectively), which are favored at low toluene concentration in the presence of TCE

(Figure 2B–D), do not grow with toluene as the sole carbon and energy source (Greene et al. 2000). Assuming that these strains use oxygenated toluene derivatives excreted by other consortium members, they would suffer less from TCE metabolism-mediated toxicity because they lack the TCE-metabolizing oxygenases. Similar effects on community composition were found when monitoring TCE co-metabolism with 0.2 or 2.0 mM phenol as the main substrate (Futamata et al. 2003). DGGE analysis showed that increased phenol concentration caused a community shift from γ to β proteobacteria. TCE degradation at the lower phenol concentration resulted in a third distinct community structure (Futamata et al. 2003), much like the community shifts in the presence of TCE at low aqueous toluene concentration observed here (Figures 2–4).

Communities incubated for up to 16 weeks with toluene or toluene and TCE both experienced large increases in total culturable heterotrophs. Both enrichments showed similar growth during log phase (Figure 5), consistent with findings by others that the presence of TCE does not affect growth curves of toluene grown-cultures (Fries et al. 1997a; Fuller et al. 1997). Because toluene and TCE were constantly replenished from the vpo reservoir, transition into stationary phase must have been due to another limiting nutrient or accumulated hydrocarbon toxicity. Despite early TCE-dependent differences, the two populations converged to a common profile at 13–16 weeks (results not shown). This convergence may be caused by a slower rate of toluene metabolism under these stationary phase conditions, giving rise to less TCE degradation-mediated toxicity. Munakata-Marr et al. (1997) found that TCE co-metabolism by indigenous populations declined during longer incubations, while others have observed populations unable to degrade TCE being selected over time (Ely et al. 1997; Fries et al. 1997a, b).

In summary it appears that TCE co-metabolism is stimulated by low toluene concentration. Strains capable of co-metabolizing TCE with toluene (i.e Cstd5 and Cstd7, standards 38 and 40, respectively) do not appear as major community components under these conditions (Figure 2C and D). Instead, strains incapable of toluene oxidation (and hence presumably of TCE metabolism) are favored in some cases.

Acknowledgements

This work was supported by a Strategic Grant from the Natural Sciences and Engineering Research Council of Canada (NSERC) and by the Dupont Educational Aid Program. The authors would like to thank Dr. Martin Odom for his interest and support. Casey Hubert was supported by scholarship funding from the Government of Alberta, the Alberta Ingenuity Fund and NSERC. The authors would like to thank Dr. Anne Greene for assistance with molecular techniques.

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