Biodegradation **4**: 59–69, 1993.

Aerobic, phenol-induced TCE degradation in completely mixed, continuous-culture reactors

Charles G. Coyle,[1] Gene F. Parkin & David T. Gibson[2]
University of Iowa, Department of Civil and Environmental Engineering Iowa City, Iowa 52242, USA.; ([1]present address: ED-GE (Coyle), U.S. Army Corps of Engineers, Kansas City District, 601 E. 12th St., Kansas City, MO 64106-2896, USA); [2]Department of Microbiology and Biocatalysis Research Group, University of Iowa, Iowa City, IA 52242, USA

Received 14 January 1992; accepted in revised form 15 June 1992

Key words: trichloroethylene (TCE), biodegradation, phenol, *Pseudomonas*, induction, cometabolism

Abstract

Both *Pseudomonas putida* F1 and a mixed culture were used to study TCE degradation in continuous culture under aerobic, non-methanotrophic conditions. TCE mass balance studies were performed with continuous culture reactors to determine the total percent removed in the reactors, and to quantify the percent removed by air stripping and biodegradation. Adsorption of TCE to biomass was assumed to be negligible. This research demonstrated the feasibility of treating TCE-contaminated water under aerobic, non-methanotrophic conditions with a mixed-culture, continuous-flow system.

Initially glucose and acetate were fed as primary substrates. Pnenol, which has been shown to induce TCE-degrading enzymes, was fed at a much lower concentration (20mg/L). Little degradation of TCE was observed when acetate and glucose were the primary substrates. After omitting glucose and acetate from the feed and increasing the phenol concentration to 50mg/L, TCE biotransformation was observed at a significant level (46%). When the phenol concentration in the feed was increased to 420mg/L, 85% of the incoming TCE was estimated to have been biodegraded. Under the same conditions, phenol utilization by the mixed culture was greater than that of *P. putida* F1, and TCE degradation by the mixed culture (85%) exceeded that of *P. putida* F1 (55%). The estimated percent-of-TCE biodegraded by the mixed culture was consistently greater than 80% when phenol was fed at 420mg/L. Biodegradation of TCE was also observed in mixed-culture, batch experiments.

Introduction

During a U.S. Environmental Protection Agency (EPA) national groundwater survey, 945 drinking water supplies were tested for 29 volatile organic compounds (VOCs). TCE was among the three most commonly found VOCs (Westrick et al. 1984). Under anaerobic conditions, not uncommon to groundwater environments, TCE may be biotransformed to vinyl chloride (Vogel & McCarty 1985). Vinyl chloride is a potent carcinogen and mutagen (Maltoni & Lefemine 1974). Thus, removal of TCE from groundwater is a contemporary problem of considerable interest. The impetus behind the research described herein was to investigate a possible method for removing TCE from contaminated waters.

Where *in-situ* groundwater remediation is not

possible, a pump-and-treat scheme may the best alternative (Mackay & Cherry 1989). The tainted groundwater would be fed into an above-ground reactor system for biological treatment. Removal mechanisms for TCE in these types of biological treatment systems include sorption, biodegradation and stripping. Other studies have shown that TCE does not significantly adsorb onto biomass (Lurker et al. 1982; Namkung & Rittmann 1987). Thus, sorption of TCE by cells was assumed to be negligible for this research project.

Aerobic biodegradation of TCE has been the subject of many studies. Much of the research in this area has focused on pure-culture, batch studies. Toluene monooxygenase (TM) and toluene dioxygenase (TD) enzymes have been shown to catalyze the oxidation of TCE (Shields et al. 1989; Zylstra et al. 1989). In the case of TM and TD, an inducing substrate (often toluene or phenol) is required to induce the enzyme(s) responsible for TCE degradation. Neither enzyme is induced by TCE. *Pseudomonas putida* F1 possesses a TD enzyme system that has been shown to degrade TCE (Zylstra et al. 1989; Nelson et al. 1987). *P. putida* F1 has been induced with toluene or phenol (Nelson et al. 1988; Wackett & Gibson 1988). Studies using radiolabeled TCE and a TM-endowed organism (*P. cepacia* G4) have shown that CO_2 was a product of TCE biodegradation (Nelson et al. 1986). Another study showed that TCE was dechlorinated by *P. putida* F1 after induction (Nelson et al. 1987). Through chemostat studies with *P. cepacia* G4, Folsom and co-workers found that TCE was a competitive inhibitor of phenol degradation and that, when at high enough concentrations, phenol inhibited its own degradation (Folsom & Chapman 1991; Folsom et al. 1990). However, in a full-scale system for treating contaminated groundwater, it may not be practical to maintain a pure culture.

Methanotrophs, aerobic bacteria using methane as their primary carbon and energy source, are also capable of degrading TCE (Wilson & Wilson 1985). Methane monooxygenase enzymes have been shown to catalyze TCE oxidation and are present in most methanotrophs (Oldenhuis et al. 1989). However, the rates of TCE degradation by methanotrophs are slower than those reported for *Pseudomonas* strains (Folsom et al. 1990).

Except for a few batch studies, no reports of aerobic, non-methanotrophic, TCE degradation by mixed cultures were found in the literature. It was felt that continuous culture experiments employing mixed cultures would more accurately simulate a process that could be used to remove TCE from contaminated waters. The goal of this research was to investigate TCE degradation by a phenol-induced, mixed culture in continuous culture reactors under aerobic, non-methanotrophic conditions. For comparison, a culture of *P. putida* F1 was also monitored. The focus of the project was to conduct mass balance studies, so that TCE removal could be partitioned into air-stripped and biodegraded portions.

The literature indicated that aerobic, biological treatment of TCE-contaminated water would require an inducing substrate. It was not known if TCE degradation would be observed if the inducing substrate (phenol in this research) was provided in combination with non-inducing substrates (acetate and glucose in this research). Since phenol was considered to be a toxic pollutant and more expensive than many non-inducing substrates, the concentration of phenol in the feed solution was initially kept much lower than that of acetate or glucose.

Materials

Bioreactors

Completely-mixed, continuous-culture reactors were designed to minimize adsorption of TCE to tubing and reactor surfaces, and to maintain a steady dissolved-oxygen concentration, a pure culture, and constant biomass (volatile suspended solids) levels. The reactor system is shown in Fig. 1.

Filter flasks, with side arms at the bases of the flasks, were used as reactors. The end of a screw-cap, glass test tube was attached to the side of the flask and used for a sample port. The reactors were placed on top of magnetic stir plates to provide

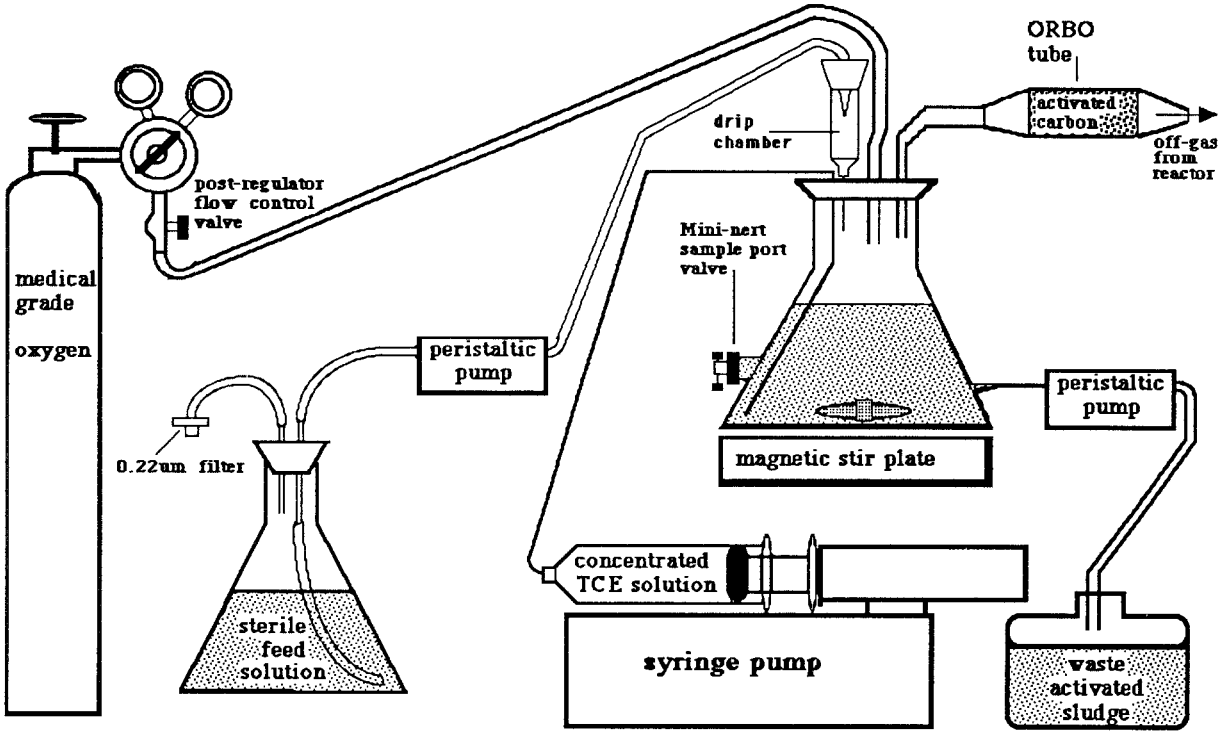

Fig. 1. Experimental reactor system. (From syringe to reactor sample port and/or ORBO tube, all TCE containing gases and solutions came into contact with only steel, glass or teflon surfaces to minimize sorption of TCE to the system).

mixing. Flasks containing freshly prepared feed solution were always autoclaved prior to use. Before entering the reactor, the feed solution passed through a drip chamber to prevent contamination from migrating upstream into the feed-line tubing. Peristaltic pumps transported media from the feed flask to the reactor, and from the reactor to the waste-sludge container. A volume of ca. 1 liter was maintained within the reactors. The combined flow of feed and TCE solution into the reactors remained at ca. 8.3 ml/hr (200 ml/day). The hydraulic retention time equaled the solids retention time; both were maintained at ca. 5 days.

A Hamilton syringe pump continuously injected TCE (dissolved in water) into the reactors. A narrow teflon tube, that extended to the bottom of the reactor, carried the TCE solution to the culture. The end of the teflon tube was submerged to minimize volatilization of TCE. A glass tube conducted pure oxygen into the reactor headspace. Another glass tube carried off-gas out of the reactor. The reactors were covered with aluminum foil to prevent the growth of photosynthetic organisms.

Media and cultures

The feed solution included basic nutrient salts (NH_4Cl, $MgSO_4$, $ScCl_2{\cdot}2H_2O$, $FeCl_3{\cdot}4H_2O$, $MnSO_4{\cdot}H_2O$ and $ZnSO_4{\cdot}7H_2O$), carbon source(s) and a phosphate buffering system (K_2HPO_4 and KH_2PO_4) (see Table 1). Initially, sodium acetate and glucose were the primary substrates. Subsequently, acetate and glucose were omitted and phenol became the sole carbon source.

The mixed-culture reactor was seeded with landfill leachate and samples from the trickling filter and final clarifier of the Iowa City Wastewater Treatment Plant. The pure culture reactor was seeded with an isolated colony of *P. putida* F1 obtained from Dr. David Gibson's Laboratory.

Experimental

The reactors were continually monitored to assess the health of the cultures, to measure the extent of substrate utilization, and to perform mass-balance experiments on TCE. All experiments were conducted at ca. 25°C. Within the reactors, the levels of glucose, acetate, pH, dissolved oxygen, volatile suspended solids, optical density, phenol, and TCE were measured. When mass balance experiments were performed, volatilized TCE (in reactor off-gas) and aqueous TCE concentrations (in syringes and reactors) were measured.

Measurement of acetate, glucose and phenol

Acetate was measured by direct injection into a packed-column gas chromatograph equipped with a flame-ionization detector. The acetate detection limit was between 2 and 5 mg/L. The relative standard devation (100× standard deviation/mean) for nine acetate measurements at 50 mg/L was 4.9. A colorimetric enzyme assay, purchased from Sigma Chemical Company (Kit No. 115-A), was used to measure glucose. After reacting with the enzyme, the absorbance of the samples at 520 nm was measured with a spectrophotometer. The glucose detection limit was 5 mg/L. Acetate and glucose concentrations were determined by comparison to standards. A colorimetric test kit (manufactured by CHEMetrics) was used to measure phenol. Phenol concentrations were determined by color comparison to standards. The detection limit for phenol was 0.5 mg/L. Periodically, the concentrations of acetate and/or phenol were measured before and after autoclaving the feed solution. No significant difference was detected in the concentration (s) of substrate(s) before and after autoclaving.

Table 1. Composition of nutrient media.

Constituent	Concentration (mg/L)
C_6H_5OH (phenol)	20 initially; 420 after omitting acetate and glucose
$C_6H_{12}O_6$ (glucose)	470 (when present)
CH_3COOH (acetate)	530 (when present)
K_2HPO_4	4397*
KH_2PO_4	1100*
NH_4Cl	400
$MgSO_4$	36.7
$CaCl_2 \cdot 2H_2O$	7.5
$FeCl_3 \cdot 4H_2O$	9.4
$MnSO_4 \cdot H_2O$	3.0
$ZnSO_4 \cdot 7H_2O$	2.5

*The concentration of the buffer was occasionally adjusted to maintain a neutral pH In the reactors.

Measurement of pH, dissolved oxygen, optical density and volatile suspended solids (VSS)

The pH, dissolved oxygen and optical density (absorbance at 600 nm) were frequently measured with a pH meter (Beckman Psi 72), dissolved oxygen probe (YSI model 58) and spectrophotometer (Milton Roy Spectronic 601), respectively. During mass-balance experiments, pH and dissolved-oxygen levels in the mixed-culture reactor ranged from 6.9 to 7.2 and 12 to 20 mg/L, respectively. Optical density and VSS were measured to assess biomass concentrations. VSS levels were measured according to *Standard Methods* (1989). Duplicate, 25-ml reactor samples were used to determine VSS levels.

Measurement of TCE in reactor off-gas

A modification of a National Institute for Occupational Safety and Health (NIOSH) method for measuring airborne contaminants was used to measure TCE in reactor off-gas (U.S. Dept. of H.E.W. 1987). NIOSH-approved glass tubes (trade name, ORBO; purchased from Supelco) containing coconut-shell-derived activated carbon were attached to the off-gas line to capture volatilized TCE. The activated carbon was extracted with carbon disulfide, diluted into iso-octane, and the sample was then injected into a gas chromatograph (GC) capillary column. The Hewlett-Packard 5890 GC was equipped with a DB5 (J&W Scientific) capillary

column. At the inlet, the sample was split at a 40/1 ratio with a septum purge of 3 ml/min. The flow rate of the carrier gas, helium, was 1.5 ml/min. Detection was by a ^{63}Ni electron-capture detector. The makeup gas was argon/methane (95%/5%). The GC was programmed as follows: 3 min at 30° C, increasing to 70° C at 5° C/min and a final hold of 1 min at 70° C. TCE concentrations were determined by comparing the peak areas of samples to those of standards.

Known quantities of TCE that were within the range of TCE quantities stripped during mass balance experiments were injected onto activated carbon in fresh ORBO™ tubes to determine the desorption efficiency. Following carbon disulfide extraction and iso-octane dilution, 90% ± 5% of the TCE was recovered. Thus, a correction factor was applied to all measurements of TCE in the reactor off-gas.

Measurement of aqueous-phase TCE

TCE concentrations in the syringes that delivered TCE to the reactors, and in the reactors themselves, were measured during mass balance experiments. Initially, a headspace assay was utilized; subsequently, liquid/liquid extractions were employed to measure aqueous-phase TCE. It was found that the concentrations of TCE in standards prepared by liquid/liquid extraction were more stable than those of headspace standards.

Headspace samples were analysed via a Hewlett-Packard Series II 5890 GC. The GC was equipped with a VOCOL capillary column purchased from Supelco. The make-up gas, nitrogen, flowed at 30 ml/min. Helium, the carrier gas, flowed at 10 ml/min. The column head pressure was kept at 6 PSI. The injector and detector were set at 100° C and 250° C, respectively. The GC was equipped with a flame-ionization detector and programmed to run at 80° C for 8 min. TCE concentrations were determined by comparing the GC response of samples to that of standards.

An EPA method for extraction of trihalomethanes was used to perform liquid/liquid extractions (Lynch 1990; U.S.E.P.A. 1987). Aqueous samples were extracted with iso-octane prior to GC analysis. The GC settings were the same as those described for measurement of TCE in reactor off-gas.

Standards for measuring aqueous TCE concentrations were prepared using TCE, methanol and/or Fisher HPLC grade iso-octane. To minimize volatilization, standards were sealed in small, screw-cap vials with teflon-lined caps and a minimal amount of headspace. The standards were stored at −15° C. New standards were prepared at least every 6 weeks, or if the GC response had significantly changed since the previous run. The standards always exhibited a linear response from 0.09 mg/L (detection limit) to 3.8 mg/L. The relative standard deviation for 10 TCE measurements at 1.29 mg/L was 1.62. Control-reactor and syringe samples were diluted prior to analysis.

The average recovery of TCE from aqueous solutions was 83% ± 3%. Since the same extraction procedure was used for standards and samples, no correction factor was necessary. An aliquot from the mixed-culture reactor (sans TCE) was used to perform a sample matrix, spike experiment. Mixed-culture and distilled-water samples of the same, known TCE concentration were prepared and subjected to the same filtration and extraction procedure. A significant difference between the TCE concentrations of the two sample types was not detected.

Biotic reactor samples were filtered with 0.45 µm filters prior to extraction. Aqueous TCE solutions of known concentration, were prepared to determine if TCE was sorbed during filtration. The TCE concentration (0.14 mg/L) was representative of concentrations observed in the reactors. Sub-samples were filtered before the extraction. The average recovery of TCE, after filtration was 66% ± 9%. Thus, a correction factor was applied to all biotic reactor samples.

Mass-balance experiments

Mass-balance experiments allowed TCE removal to be divided into air-stripped and biodegraded fractions. Parameters measured during mass-balance studies included flow rate of feed solution,

flow rate of TCE solution (from syringe), TCE concentration in both the syringe and the reactor, and mass of TCE adsorbed onto activated carbon (TCE from reactor off-gas). Most mass-balance experiments were conducted over a period of six to eight hours. Before starting a mass-balance study, the volume of TCE solution in the syringe and the time was noted. The level of culture within the reactor was also marked and monitored during the experiment. The experiment began when the ORBO™ tube was attached to the off-gas line of the reactor. After initiating an experiment, small samples were immediately withdrawn from the syringe and the reactor to measure aqueous-phase TCE concentrations. The flow rate of feed into the reactor was then measured. Lastly, the flow rate of oxygen into the reactor was measured. During the middle and at the end of the monitoring period the above measurements were repeated. The monitoring period ended when the ORBO™ tube was removed from the off-gas line. After noting the volume left in the syringe and the time, the flow rate of the TCE solution (syringe flow rate) was calculated. Following a mass-balance study, three values for each measured parameter (except TCE from reactor off-gas) were obtained. Before performing mass-balance calculations, the three values were averaged. Sample mass balance calculations are presented in Table 4.

The most important QA/QC measures were the control-reactor (CR) mass-balance experiments. Each mass-balance experiment included a pure- or mixed-culture reactor, and the CR. Both reactors were operated under virtually identical conditions, except that the CR received water instead of feed solution. For both reactors, the monitoring period began and ended at the same time. Samples were always withdrawn from both reactors and both syringes at the same time. If TCE entering the CR could be accounted for, then the fate of TCE in the biotic reactor (stripped or biodegraded) could be quantitatively estimated.

Batch experiments

Batch experiments were performed to confirm that the mixed culture was biodegrading TCE. The experiments were conducted at 25°C using glass serum vials and teflon-faced, silicone septa, sealed with aluminum crimp-top closures. The total volume in each vial was 122 ml. After centrifuging, cells were resuspended in a phosphate buffer solution. 25 ml of the suspension was dispensed into the vial along with TCE. A control vial was run in parallel with the vial containing the mixed culture. The control contained 25 ml of the phosphate buffer and the same initial TCE concentration but no culture. Headspace samples were analyzed via a Hewlett-Packard Series II 5890 GC. The GC was equipped with a VOCOL capillary column purchased from Supelco. The make-up gas, nitrogen flowed at 30 mg/min. Helium flowed at 10 ml/min. The column head pressure was kept at 6 PSI. The

Table 2. Control-reactor, mass-balance data.

Date of experiment	Reactor aqueous [TCE], range & average (mg/L)	Syringe aqueous [TCE], range & average (mg/L)	Length of monitoring period (hr)	Total % removal of TCE	% Stripped	% Not accounted for
8/17	2.0–2.1, 2.1	660–840, 750	6.07	89	83	6
8/22	3.8–7.5, 5.4	430–660, 562	13.70	88	74	14
8/27	5.4–5.5, 5.4	350–520, 495	11.47	87	63	24
9/3	2.9–5.9, 4.4	250–410, 329	7.12	85	94	–9
Aqueous TCE concentrations for the above experiments were measured by headspace; for the below experiments they were measured by liquid/liquid extractions.						
9/13	2.7–3.1, 2.9	150–280, 224	7.33	86	73	13
10/1	1.1–1.3, 1.2	175–175, 175	6.08	92	75	17
10/3	1.0–1.1, 1.0	125–150, 138	6.08	91	96	–5
10/16	2.0–2.1, 2.1	200–220, 210	6.07	89	81	8

Table 3. Sample mass-balance data.

Experiment[b]	Length of monitoring period (hr)	Feed flow rate (ml/hr)	Syringe flow rate (ml/hr)	Average aqueous [TCE] in reactor (mg/L)	Average aqueous [TCE] in syringe (mg/L)	TCE stripped (mg)
Control	6.07	7.1	0.7	2.1 (2.0–2.1)[a]	210 (200–220)	0.72
Mixed culture[c]	6.07	8.0	0.7	0.1 (0.1–0.1)	210 (200–220)	0.06

[a]Numbers in parenthesis represent the range of TCE concentrations measured during the monitoring period.
[b]The control and mixed culture experiments were performed simultaneously.
[c]Phenol, at 420 mg/L, as sole carbon source.

following settings were used for headspace assays: injector 100°C, detector 25°C. The GC was equipped with a flame-ionization detector and was programmed to run at 80°C for 8min.

Results and discussion

Mass-balance data

Control-reactor, mass-balance experiments were performed concomitantly with biotic-reactor, mass-balance experiments. Factors that may have contributed to the variation in the '% not accounted for' values from control-reactor experiments included length of the monitoring period (shorter monitoring perids generally yielded better results), variations in aqueous TCE concentrations within the reactor during the monitoring period, and experimental errors. Also, some TCE may have diffused through the narrow-guage teflon tubing between the syringe and the reactor. Lastly, TCE concentrations within the syringes were greater than those of the reactors (see Table 2 and 3). Thus, the precision of the syringe-TCE measurements was less than that of reactor-TCE measurements, because the syringe samples were diluted prior to extraction and analysis. Variations in the measured syringe-TCE concentrations may have contributed to variations in the '% not accounted for' values from control-reactor experiments. The liquid/liquid extraction, control-reactor experiments all came to within 17% of accounting for all the TCE (see Table 2). The averate '% not accounted for' for all control-reactor experiments was 8.5%.

Typical data from actual mass-balance experiments, control- and mixed-culture reactors, are presented in Table 3. Sample control- and mixed-culture, mass-balance calculations are shown in Table 4, using the data from Table 3. To calculate the 'estimated % biodegraded', the average '% not accounted for' value for all the control reactor

Table 4. Sample mass-balance calculations.

Parameter calculated	Control reactor	Mixed culture reactor
Total volume flowing into reactor	7.1 ml/hr (feed) + 0.7 ml/hr (syringe) = 7.8 ml/hr	8.0 ml/hr (feed) + 0.7 ml/hr (syringe) = 8.7 ml/hr
Mass of TCE entering reactor	210 mg/L (0.0007 L/hr) = 0.147 mg/hr	210 mg/L (0.0007 L/hr) = 0.147 mg/hr
Total mass of TCE that entered the reactor	0.147 mg/hr (6.07 hr) = 0.892 mg	0.147 mg/hr (6.07 hr) = 0.892 mg
Influent TCE concentration	0.147 mg/hr /0.0078 L/hr = 18.8 mg/L	0.147 mg/hr /0.0087 L/hr = 16.9 mg/L
Percent of TCE that remained in the aqueous phase	100 (2.1 mg/L/ 18.8 mg/L) = 11.2%	100 (0.1 mg/L/ 16.9 mg/L) = 0.6%
Total percent removal of TCE	100 – 11.2 = 88.8%	100 – 0.6 = 99.4%
Percent of TCE stripped	100 (0.72 mg/ 0.892) = 80.7%	100 (0.06 mg/ 0.892) = 6.7%
Percent unaccounted for	100 – (11.2 + 80.7) = 8.1%	100 – (0.6 + 6.7) = 92.7%
Estimated percent biodegraded		92.7 – 8.1 = 84.6%

Table 5. Summary of mass-balance data.

Reactor	Carbon sources		Total % removal of TCE	% Stripped	% Not accounted for	Estimated % biodegraded[a]
	Glucose and acetate	Phenol (mg/L)				
Abiotic control[b]	–	–	88	80	8	–
Mixed culture	present[c]	20	86	64	22	14
Mixed culture	absent	50	92	38	54	46
Mixed culture	absent	420	99	6	93	85
Mixed culture	absent	420	99	5	94	86
Mixed culture	absent	420	99	6	93	85
P. putida F1 (pure culture)	absent	420	96	41	55	47

[a] 'Estimated % biodegraded' was calculated by subtracting the control '% not accounted for' value (8) from the mixed culture '% not accounted for' values.

[b] Average values from eight control reactor mass balance experiments.

[c] Glucose and acetate concentrations were 470mg/L and 530mg/L, respectively.

mass balance experiments (8.5%) was substracted from the '% not accounted for' value from the biotic-reactor, mass-balance experiment.

During the first phase of the research, acetate and glucose were fed as the primary carbon sources (at 530 and 470mg/L, respectively). Phenol, at 20mg/L, was also included in the feed. Only very low levels of apparent TCE degradation were observed when glucose and acetate were the primary substates (see Table 5). It is possible that glucose and/or acetate may have been repressing the synthesis of the toluene oxygenase enzymes or selecting for microbes not endowed with toluene oxygenase genes. The concentrations of acetate, glucose, and phenol in the biotic reactors were below detection limits each time they were measured. It was found that the cultures could tolerate aqueous phase TCE concentrations of at least 8mg/L with nearly complete utilization of the acetate, glucose, and phenol. Judging by substrate utilization, TCE did not appear to be toxic to the cultures when acetate and glucose were the primary carbon sources.

During the last phase of the experiments glucose and acetate were omitted from the feed, and phenol was the sole carbon source. After omitting acetate and glucose and increasing the phenol concentration to 50mg/L, the estimated percent-of-TCE biodegraded by the mixed culture increased from 14 to 46% (Table 5). After the phenol concentration in the feed was increased from 50 to 420mg/L, the estimated percent-of-TCE biodegraded in the mixed culture reactor increased from 46 to 85%, respectively (Table 5).

Although these experiments did not prove that induction of toluene oxygenase enzymes was responsible for TCE biodegradation, there was strong circumstantial evidence that this was the case. No evidence was available in the literature to indicate that acetate or glucose can induce toluene oxygenase activity. Batch studies have shown that TCE degradation, observed when phenol was present, did not occur when phenol was absent (Nelson et al. 1986; Nelson et al. 1987). Increasing the phenol concentration (from 20 to 50mg/L, and then to 420mg/L) most likely selected for higher

Table 6. Reaction-rate-constant data for TCE removal in the mixed-culture reactor.

Date of experiment	S_0 (mg/L)	% Stripped	S_0, corrected (mg/L)	S (mg/L)	θ (day)	X (mg/L)	k (L/mg·VSS·d)
9/22	18.5	6.1	15.9	0.2	5.6	120	0.12
10/3	11.8	4.9	10.3	0.09	5.3	150	0.14
10/16	17.1	6.5	14.6	0.1	5.2	142	0.20

concentrations of organisms expressing toluene oxygenase enzymes. Since the total chemical oxygen demand (COD) available in the feed solution was actually greater when glucose, acetate, and the small amount of phenol (20mg/L) were present (1,114mg COD/L) than when phenol was present alone (420mg/L of phenol represents 1,001mg COD/L), it is not likely that differences in availability of electron donors were responsible for differences in levels of TCE degradation. Also VSS levels were similar under both feed formulations. Thus, the results of this research suggest that an inducing compound may be required as the primary substrate to obtain high levels of TCE degradation.

The following observations were made when the feed solutions of the mixed and pure cultures were identical (phenol, at 420mg/L, as the sole carbon source). Phenol utilization by the mixed culture was greater than that of the pure culture. Phenol was below detection limits in the mixed-culture reactor, while in pure-culture reactor the phenol concentration ranged from 20 to 30mg/L. The estimated percent-of-TCE biodegraded by the mixed culture (85%) was substantially greater than that of the pure culture (47%). The steady-state level of volatile suspended solids in the pure-culture reactor (ca. 80mg/L) was less than that of the mixed-culture reactor (ca. 150mg/L).

Performance of the mixed-culture reactor

When phenol was the sole carbon source and being fed at 420mg/L, essentially all the phenol was utilized, approximately 85% of the TCE entering the reactor was estimated to have been biodegraded, about 6% of the TCE was being stripped, and effluent TCE concentrations ranged from 0.09 to 0.2mg/L. During the last three mixed culture mass balance experiments, the level of VSS remained at about 150mg/L. Using data from Table 3, biodegradation of 85% of the introduced TCE represented 27mg phenol utilized per mg TCE degraded (37 moles of phenol per mole of TCE degraded). This corresponds to 20mg TCE biodegraded/g VSS-day).

Data from the last three mixed-culture, mass-balance experiments were used to estimate reaction-rate constants (see Table 6). A reaction-rate constant (k) for TCE removal can be estimated by assuming pseudo-first-order kinetics for TCE biodegradation:

$$r_{bio} = kSX$$

where: r_{bio} = TCE removal rate, S = aqueous TCE concentration in the reactor, and X = VSS.

For a continually stirred, tank reactor at steady state: $k = \frac{S_0 - S}{S\theta X}$

where: S_0 = influent TCE concentration and θ = hydraulic retention time.

To ensure that k would reflect only biodegradation, the value of S_o was corrected to exclude unaccounted-for (assumed to be 8% of S_o) and stripped TCE. The reactor was assumed to be at steady state during the last two mass balance experiments, because more than three solids retention times had elapsed (since increasing phenol to 420mg/L) and the VSS level had stabilized. The average k value, from the last two mass balance experiments, was 0.17 L/mg·VSS·d. To more accurately determine k, TCE degradation would have to be studied over a range of θ values.

Batch experiments

Aliquots from the mixed-culture reactor were used in batch studies. The following concentrations are reported as if all TCE were present in the aqueous phase, unless otherwise noted. Equal volumes of TCE-in-methanol-standards were dispensed into control and biotic vials so that the initial TCE concentrations were ca. 2.4mg/L. (The initial, aqueous-phase TCE concentration in the vials was calculated to be 0.94mg/L, assuming a dimensionless Henry's constant of 0.4 (Folsom et al. 1990).) In the biotic vial, the TCE concentration dropped to ca. 0.2mg/L in 7 hours (see Fig. 2). In the experiments that were run for longer periods, TCE was depleted

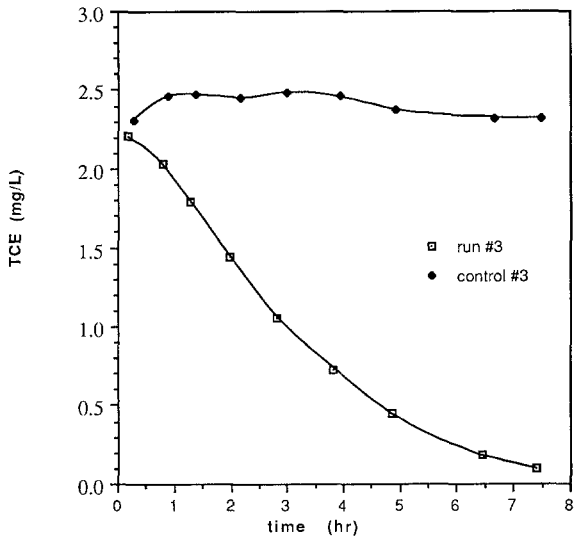

Fig. 2. Mixed-culture, TCE degradation in batch experiment. (Concentrations are reported as if all TCE were present in the liquid phase.)

to below detection limits. In the control vial, the TCE concentration decreased to 2.3 mg/L during the 7 hours. Data from these batch studies provided additional evidence to support the observation that biotransformation of TCE was occurring in the mixed-culture reactor.

Summary and conclusions

Summary

This research demonstrated the feasibility of using a mixed culture to treat TCE-contaminated water under aerobic, non-methanotrophic conditions. For comparison, a pure culture of *P. putida* F1 (with a known capability for degrading TCE) was also maintained and monitored. Mass-balance studies were performed so that TCE removal could be partitioned into air-stripped and biodegraded portions.

Initially, the pure culture and the mixed culture were fed acetate and glucose as primary substrates, at 530 and 470 mg/L, respectively. Phenol was present at 20 mg/L. Only very low levels of apparent TCE degradation (zero to 21%) were observed with this substrate formulation. Nearly complete utilization of acetate, glucose and phenol was observed when aqueous-phase TCE concentrations were as high as 8 mg/L.

After omitting glucose and acetate, and increasing the phenol concentration to 50 mg/L, 46% of the incoming TCE was estimated to have been biodegraded by the mixed culture. After increasing the phenol concentration in the feed solution to 420 mg/L, 85% of the incoming TCE was estimated to have been biodegraded by the mixed culture. Under the same conditions (phenol, at 420 mg/L, as the sole carbon source), the estimated percent-of-TCE-biodegraded by the mixed culture (85%) exceeded that of *P. putida* F1 (47%). Batch studies supported the tenet that biodegradation of TCE was occurring in the mixed-culture reactor.

The process described herein could be used to remove TCE from contaminated groundwater without transferring substantial quantities of TCE into the air. More experiments are needed to determine if equivalent or increased levels of TCE biodegradation can be realized through increased VSS levels, shorter hydraulic retention times, and/or varied solids retention times. Also studies should be performed to address the effects of phenol concentration on TCE degradation, the extent to which phenol adsorbs onto waste sludge, if lower effluent-TCE concentrations can be attained, and if mixed cultures are capable of completely mineralizing TCE. Lastly phenol and TCE are believed to be substrates for the same enzyme (or enzymes) (Folsom et al. 1990). Research into minimizing competition between TCE and the inducing substrate, through intermittent feeding of phenol, is warranted.

Conclusions

The following conclusions were drawn from this research:

(1) TCE did not appear to substantially inhibit acetate, glucose or phenol utilization by mixed cultures at aqueous TCE concentrations up to at least 8 mg/L.
(2) Little, apparent TCE degradation by the mixed culture was observed when phenol was present

at 20mg/L and the primary carbon sources were acetate and glucose.

(3) When phenol was present as the primary carbon source, *P. putida* F1 and the mixed culture were capable of degrading TCE under aerobic, non-methanotrophic conditions in continuous culture.

(4) The estimated percent-of-TCE biodegraded by the mixed culture increased when the feed solution was modified by removing acetate and glucose, and increasing the phenol concentratio from 20 to 420mg/L.

(5) In continuous culture and under the same conditions, the estimated percent-of-TCE biodegraded by the mixed culture (85%) substantially exceeded that of the pure culture (55%).

Acknowledgements

This work was funded by the Region 7 & 9 Hazardous Substance Research Center of the U.S. Environmental Protection Agency (EPA) at Kansas State University. C.G.C. received support from the EPA and in part from grant AFSOR-88-6225 from the U.S. Air Force Office of Scientific Research. Special thanks to Dr. M. St.Clair for his comments during the preparation of this manuscript.

References

Folsom BR & Chapman PJ (1991) Performance characterization of a model bioreactor for the biodegradation of trichloroethylene by *Pseudomonas cepacia* G4. Appl. Environ. Microbiol. 57: 1602–1608

Folsom BR, Chapman PJ & Pritchard PH (1990) Phenol and trichloroethylene degradation byy *Pseudomonas cepacia* G4: kinetics and interactions between substrates. Appl. Environ. Microbiol 56: 1279–1285

Lurker PA, Clark CS & Elis VJ (1982) Atmostpheric release of chlorinated organic compounds from the activated sludge process. J. Water Pollut. Control Fed. 54: 1566–1573

Lynch NA (1990) Transformations of pesticides and halogenated hydrocarbons in the subsurface environment. Ph.D. thesis, University of Iowa, Iowa City, Iowa

Mackay DM & Cherry JA (1989) Groundwater contamination: pump-and-treat remediation. Environ. Sci. Technol. 23: 630–636

Maltoni C & Lefemine G (1974) Carcinogenicity bioassays of vinyl chloride. Environ. Res. 7: 387–396

Namkung EN & Rittmann BE (1987) Estimating votatile organic compound emissions from publicly owned treatment works. J. Water Pollut. Control Fed. 59: 670–678

Nelson MJK, Montgomery SO & Pritchard PH (1988) Trichloroethylene metabolism by microorganisms that degrade aromatic compounds. Appl. Environ. Microbiol. 54: 604–606

Nelson MJK, Montgomery SO, Mahaffey WR & Pritchard PH (1987) Biodegradation of trichloroethylene and involvement of an aromatic biodegradative pathway. Appl. Environ. Microbiol. 53: 949–954

Nelson MJK, Montgomery SO, O'Neil EJ & Pritchard PH (1986) Aerobic metabolism of trichloroethylene by a bacterial isolate. Appl. Environ. Microbiol. 52: 383–384

Oldenhuis R, Vink RLJM, Janssen DB & Witholt B (1989) Degradation of chlorinated aliphatic hydrocarbons by *Methylosinus trichosporium* OB3b expressing soluble methane monooxygenase. Appl. Environ. Microbiol. 55: 2819–2826

Shields MS, Montgomery SO, Chapman PJ, Cuskey SM & Pritchard PH (1989) Novel pathway of toluene catabolism in the trichloroethylene-degrading bacterium G4. Appl. Environ. Microbiol. 55: 1624–1629

Standard Methods for the Examination of Water and Wastewater (1989) APHA, AWWA, WPCF, 17th edition, Washington, DC

U.S. Department of Health, Education, and Welfare (1987) NIOSH Manual of Analytical Methods, 3rd ed., Method: 1022

U.S. Environmental Protection Agency (1987) Title 40, Code of Federal Regulations, Ch. 1, Pt 141, Subpart C. App, C, pp 559

Vogel TM & McCarty PL (1985) Biotransformation of tetrachloroethylene to trichloroethylene, dichloroethylene, vinyl chloride and carbon dioxide under methanogenic conditions. Appl. Environ. Microbiol. 49: 1080–1083

Wackett LP & Gibson DT (1988) Degradation of trichloroethylene by toluene dioxygenase in whole-cell studies with *Pseudomonas putida* F1. Appl. Environ. Microbiol. 54: 1703–1708

Westrick JJ, Mello JM & Thomas RF (1984) The groundwater supply survey. J. Am. Water Works Assoc. 76: 52–59

Wilson JT & Wilson BH (1985) Biotransformation of trichloroethylene in soil. Appl. Environ. Microbiol. 49: 242–243

Zylstra GJ, Wackett LP & Gibson DT (1989) Trichloroethylene degradation by *Escherichia coli* containing the cloned *Pseudomonas putida* F1 toluene dioxygenase genes. Appl. Environ. Microbiol. 55: 3162–3166