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Biodegradation of Trichloroethylene in Continuous-Recycle Expanded-Bed Bioreactors

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Experimental bioreactors operated as recirculated closed systems were inoculated with bacterial cultures that utilized methane, propane, and tryptone-yeast extract as aerobic carbon and energy sources and degraded trichloroethylene (TCE). Up to 95% removal of TCE was observed after 5 days of incubation. Uninoculated bioreactors inhibited with 0.5% Formalin and 0.2% sodium azide retained greater than 95% of their TCE after 20 days. Each bioreactor consisted of an expanded-bed column through which the liquid phase was recirculated and a gas recharge column which allowed direct headspace sampling. Pulses of TCE (20 mg/liter) were added to bioreactors, and gas chromatography was used to monitor TCE, propane, methane, and carbon dioxide. Pulsed feeding of methane and propane with air resulted in 1 mol of TCE degraded per 55 mol of substrate utilized. Perturbation studies revealed that pH shifts from 7.2 to 7.5 decreased TCE degradation by 85%. The bioreactors recovered to baseline activities within 1 day after the pH returned to neutrality.

Trichloroethylene (TCE) and other chloroalkenes pose serious pollution problems. Industry in the United States used approximately 90,000 tons of TCE in 1985 (19) and considerably greater amounts in preceding decades. The combination of high usage and disposal methods has resulted in many subsurface aquifers being contaminated with chlorinated ethenes. Sediments beneath some industrial sites are reported to contain chlorocarbons in excess of 1,000 mg kg^{-1} (4, 5, 10). The U.S. Environmental Protection Agency has listed TCE as a priority pollutant, and remediating contaminated aquifers will be a formidable task (4, 10). Processes which result in the destruction of chlorinated ethenes rather than transferring them to other portions of the environment are desirable.

Pure cultures and mixed microbial consortia degrade TCE (4, 5, 9, 12, 16–22). Chlorinated alkenes may be converted anaerobically by reductive dehalogenation to known carcinogens such as vinyl chloride (3). Natural gas stimulated aerobic TCE mineralization in unsaturated soil columns (22) and trickling filter reactors (17). Methanotrophic biomass in soil columns and reactors increased over time, TCE was degraded, and it was hypothesized that methanotrophs catalyzed the mineralization of TCE (14, 17). The oxidases of methanotrophs were thought to be important for the degradation of TCE and other chlorinated organic compounds. Pure cultures of methanotrophs capable of degrading TCE at concentrations of 1 mg $liter^{-1}$ have been obtained (9), and methane monooxygenase appeared to play a key role in TCE oxidation.

Heterotrophic enrichment cultures degraded TCE aerobically when induced with certain aromatic compounds, such as toluene or phenol (12). Toluene dioxygenase has been implicated in the TCE metabolic activities of these organisms and *Pseudomonas putida* F1 (21). Both methanotrophs and these heterotrophs were inhibited at TCE concentrations greater than 10 mg $liter^{-1}$. Heterotrophic consortia obtained from contaminated subsurface sediments degraded TCE at concentrations exceeding 100 mg $liter^{-1}$ with propane, methanol, or yeast extract as the substrate (4, 16).

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The purpose of this study was to examine TCE degradation in continuous-recycle bioreactors containing microbial consortia capable of degrading TCE when fed methane and/or propane. This report describes the performance of the TCE-degrading bioreactors containing resilient consortia operated under various conditions of energy source, pH, and nutrient levels.

MATERIALS AND METHODS

Gases and chemicals. All gases were supplied from MG Industries (Chattanooga, Tenn.). Propane and oxygen were greater than 99.5% pure, and methane was more than 98% pure. All chemicals were of reagent grade and were obtained from Mallinckrodt, Inc. (Paris, Ky.) or Sigma Chemical Co. (St. Louis, Mo.). Glass-distilled solvents and reagents were purchased from J. T. Baker Chemical Co. (Phillipsburg, N.J.). [1,2,-^{14}C]TCE (10 mCi/mmol) was purchased from Dupont, NEN Research Products (Boston, Mass.).

Bacterial cultures. Two separate mixed-culture consortia that degraded TCE were used to inoculate bioreactors. The primary TCE-degrading consortium was isolated from the Savannah River Plant, Aiken, S.C., and degraded TCE at concentrations in excess of 100 mg $liter^{-1}$ (4). A methanotroph isolated from a waste disposal site near Oak Ridge, Tenn., capable of degrading TCE at concentrations of less than 1 mg $liter^{-1}$ (9) was added to both the PM-M and SM-1 cultures. Culture SM-1 contained the Savannah River Plant consortium and the methanotroph. Culture PM-M contained mixtures of propane- and methane-oxidizing bacteria obtained from Ada, Okla. (14), plus the TCE-degrading consortia. Culture SM-1 was amended with 100 mg of yeast extract and Trypticase (BBL Microbiology Systems, Cockeysville, Md.) $liter^{-1}$, 5% methane, and 3% propane (vol/vol, headspace). Culture PM-M was grown solely on methane and propane, 5 and 3%, respectively (vol/vol, headspace). Cultures were transferred monthly and remained stable for 2 years.

Construction, maintenance, and operation of the bioreactors have been described elsewhere (15). The medium contained the following (per liter): $MgSO_4 \cdot 7H_2O$, 0.055 g; $CaSO_4 \cdot 2H_2O$, 0.054 g; NH_4NO_3, 1.48 g; trace mineral

TABLE 1. Comparison of TCE degradation in bioreactors under conditions of pulsed feeding, continuous feeding, and starvation for 5 days[a]

Expt	Reactor[b]	Propane consumed (mmol)	Methane consumed (mmol)	Carbon dioxide (mmol)	TCE loss (μmol)	% TCE loss	Substrate/TCE loss (μmol/μmol)
Fed day 1, then starved	1	0.05	0.05	<0.01	1.2	3	
	2	0.67	1.10	0.40	31.4	90	54
	3	0.64	1.10	0.42	15.7	45	111
	1	0.04	0.05	<0.01	1.1	3	
	2	0.67	1.01	0.43	29.9	86	55
	3	0.62	1.10	0.51	14.9	43	115
Continuous substrate	1	0.05	0.06	<0.01	1.1	3	
	2	2.10	1.33	1.20	31.4	90	109
	3	1.83	2.15	3.50	29.6	85	135
Starved	1			<0.01	1.0	3	
	2			0.20	2.6	8	
	3			0.75	3.5	10	

[a] Initial TCE concentration of 20 mg liter^{-1} (34.8 μmol reactor^{-1}). Replicate for continuous substrate experiment is shown in Table 2 for comparison.
[b] Reactors: 1, control; 2, culture PM-M; 3, culture SM-1.

solution and vitamin solution (15); 2.0 mM phosphate-bicarbonate buffer solution; and deionized water, 1,000 ml. Culture SM-1 additionally contained 100 mg of yeast extract and Trypticase liter^{-1}. Resazurin (2.0 mg liter^{-1}) was added to monitor the redox of the liquid phase. The pH was 7.2 unless stated otherwise. Crushed glass (70 g of 60 to 80 mesh) served as the substratum. The total liquid-phase volume of each reactor was 230 ml, while the total headspace volume remained at 490 ml. The nominal liquid-phase TCE concentration at the onset of each experiment was 20 mg liter^{-1}. Control reactors contained 0.2% sodium azide and 0.5% Formalin to deter microbial contamination.

Bioreactor experiments. Reactors were inoculated with 1 liter of test cultures, circulated for 8 h, and drained. Reactors did not degrade TCE until several weeks after inoculation. Once test reactors had degraded several TCE additions, various parameters were altered in an effort to maximize the degradation. Pulsed substrate addition studies were performed by adding propane or methane to reactors only at day 0. Continuous substrate experiments were conducted by replenishing daily the energy sources utilized after determining their respective headspace concentrations. Experiments in which no substrate was added to the test reactors were termed starvation. The liquid-phase pH remained at 7.2 throughout time course experiments with the exception of the pH 7.5 experiment. Biomass remained stably attached to the substratum throughout the experiments.

Analytical procedures. Total phospholipids were extracted from frozen and lyophilized bioreactor contents by a modification (2) of the single-phase chloroform-methanol method of Bligh and Dyer (1). Methyl esters of the phospholipid fatty acids (PLFA) were analyzed by capillary gas-liquid chromatography (GC) as previously described (2, 7). Fatty acids are designated by the total number of carbon atoms:number of double bonds followed by the position of the saturation from the aliphatic end. Suffixes c and t indicate *cis* and *trans* geometry, while i represents *iso* branching.

GC was used to measure concentrations of toxicants and substrates. TCE was analyzed with a Hewlett-Packard model 5890 GC equipped with a 50-m Ultra Performance (Ultra 1; Hewlett-Packard) cross-linked methyl silicone capillary column as described previously (4). Vinyl chloride and *cis*- and *trans*-1,2-dichloroethylene were analyzed with a Shimadzu GC-9A GC equipped with a 2.4-m-long, 3.2-mm-diameter Poropak T-packed column and a photoionization detector (HNU Systems, Newton, Mass.). The following conditions were used: oven temperature, 150°C; injector temperature, 160°C; and detector temperature, 230°C. Methane and CO_2 were assayed with a Shimadzu GC-8A GC equipped with a 2.7-m-long, 3.2-mm-diameter Carbosieve 8000-packed column with a thermal conductivity detector. Radioactive CO_2 was measured with a gas proportional counter as described previously (4, 16). Propane was analyzed with a Shimadzu GC-9A GC equipped with a 2.4-m-long, 3.2-mm-diameter Poropak N-packed column and a flame ionization detector.

Concentrations of propane, methane, and CO_2 were determined by relating peak heights of samples to those of prepared gas standard calibration curves. Chlorinated volatile compounds sampled from the columns were compared with standard curves based on peak areas of the respective compounds by using an analytical chromatography software system (model 2600, version 4.0; Nelson Systems, Cupertino, Calif.). The standards had the same headspace/liquid phase ratio as the columns following the principles of Henry's gas laws (6). The limits of detection for sampled compounds were as follows: TCE, 0.1 μg liter^{-1}; *cis*-1,2-dichloroethylene, 10 μg liter^{-1}; *trans*-1,2-dichloroethylene, 10 μg liter^{-1}; vinyl chloride, 10 μg liter^{-1}; propane, CH_4, and CO_2, 0.05% (vol/vol). Detection limits for chlorinated compounds are nominal liquid-phase concentrations.

RESULTS

Preliminary studies indicated that in the absence of added microbial cultures, over 98 ± 2% of the TCE added to the bioreactor systems at nominal liquid concentrations of 20 mg liter^{-1} was still present after 5 days. When experimental reactors were inoculated with test cultures, they did not show any indication of TCE loss until several weeks after inoculation.

Pulsed feeding. Table 1 compares TCE degradation in the bioreactors during three feeding regimens. The continuous substrate regimen consisted of methane plus propane additions each day, while the pulsed feeding regimen consisted of feeding the reactors at day 0 only. The starvation mode included the addition of oxygen and TCE but no energy source after the completion of a pulsed substrate cycle. TCE

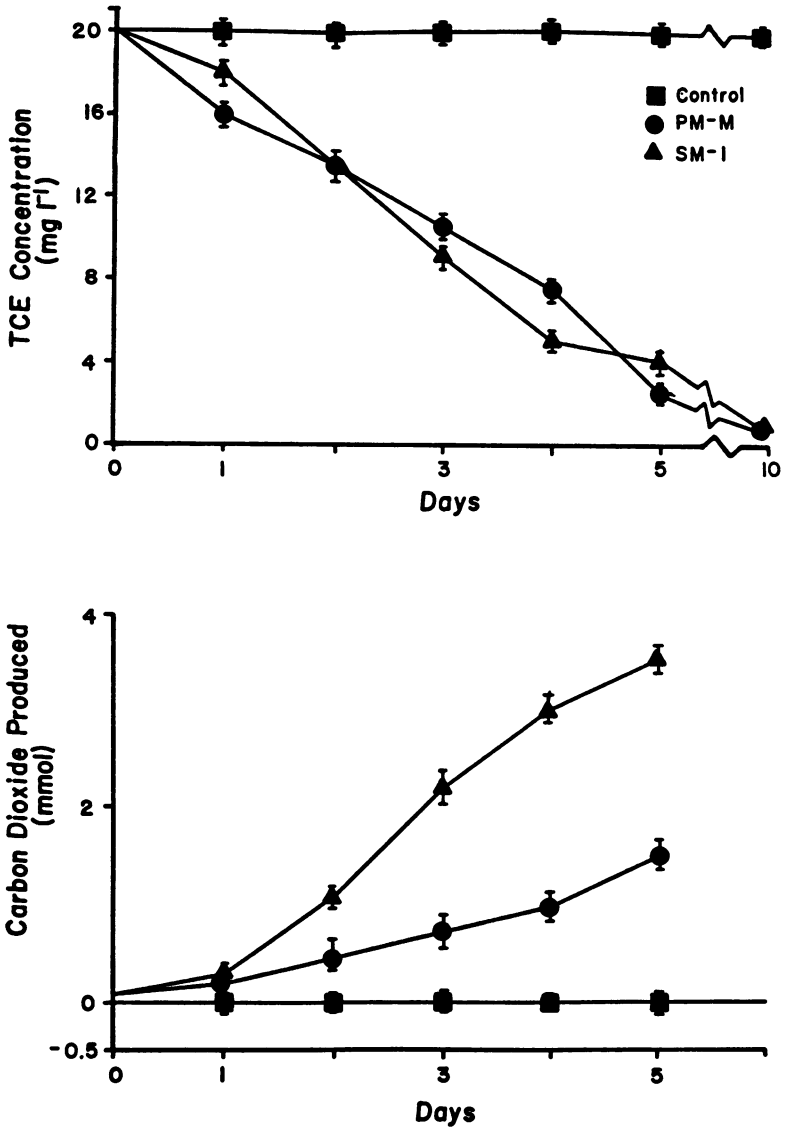

FIG. 1. Utilization of TCE and accumulation of carbon dioxide during experiments supplemented daily with methane plus propane. Experimental reactors contained consortium PM-M or SM-1, and a control reactor contained 0.5% Formalin plus 0.2% sodium azide. Error bars represent the standard deviation of three replicate measurements.

was readily degraded in pulsed experiments or experiments fed daily, whereas little degradation was observed during long periods of starvation. Reactor 2, containing the PM-M consortium, degraded over 86% of the TCE with pulsed feeding. Reactor 3, with the SM-1 consortium, showed over 43% loss after 5 days in pulsed feeding experiments. Reactor 1, the inhibited control, showed insignificant loss of analytes. The liquid-phase pH remained at 7.2 in all reactors throughout the time course experiments. Methane and propane consumption was similar in both test bioreactors and in repeat experiments of pulsed feeding. Comparing moles of substrate consumed per mole of TCE degraded is one method of evaluating efficiencies of bioremediation. As shown in Table 1, reactor 2 required only 55 μmol of substrate per μmol of TCE degraded during pulsed feeding versus greater than 100 μmol of substrate for reactor 3 or either reactor during experiments fed daily. These results suggested that pulsed feeding could enhance the efficiency of TCE degradation. However, if the starvation period was too long, as in the additional 5-day starvation experiments, little additional TCE was degraded (Table 1).

Continuous substrate. Bioreactors fed daily with continuous substrate availability showed 90 and 85% removal of the initial TCE added to reactors 2 and 3, respectively, after 5 days (Table 1; Fig. 1). The rate of TCE loss was linear for 5 days, reducing the TCE concentration from 20 to less than 4 mg liter^{-1}. From days 5 to 10, TCE concentrations dropped from 4 to 0.5 to 1.0 mg liter^{-1}. Carbon dioxide accumulation was greater in the reactor containing SM-1. Similar to the linear degradation of TCE, there was near-linear accumulation of carbon dioxide from both reactors during the experiments fed daily (Fig. 1).

pH shift. In an attempt to decrease the 0.1-atm (10.1-kPa) headspace pressure resulting from carbon dioxide production, experiments were initiated at an elevated pH. When the medium pH was elevated from 7.2 to 7.5, only 15% of the

TABLE 2. Comparison of TCE degradation in bioreactors under conditions of continuous substrate, elevated pH, and increased nutrients[a] for 5 days

Expt	Reactor[b]	Propane consumed (mmol)	Methane consumed (mmol)	Carbon dioxide (mmol)	TCE loss (μmol)	% TCE loss	Substrate/TCE loss (μmol/μmol)
Continuous substrate	1	0.1	0.1	<0.01	1.1	3	
	2	2.2	1.3	1.47	32.3	93	109
	3	1.7	1.7	3.92	28.2	81	139
pH 7.5	1	<0.1	<0.1	<0.01	1.2	3	
	2	0.5	0.4	0.22	5.2	15	170
	3	0.3	0.4	0.77	6.1	18	115
2× nutrients[c]	1	<0.1	0.1	<0.01	1.2	3	
	2	2.1	1.8	2.10	26.1	75	146
	3	2.1	1.0	3.40	20.0	58	158
	1	<0.1	0.1	<0.01	1.0	3	
	2	2.2	1.7	2.42	25.3	73	154
	3	2.0	1.1	3.21	19.4	56	157

[a] Initial TCE concentration of 20 mg liter^{-1} (34.8 μmol reactor^{-1}).
[b] Reactors: 1, control; 2, culture PM-M; 3, culture SM-1.
[c] 2× nutrients, Doubling of micronutrients, vitamins, phosphates, and nitrates in medium.

initial TCE concentration was degraded (Table 2). The amount of methane and propane utilized as well as the amount of carbon dioxide produced also declined sharply. This demonstrated that the microbial activities within the bioreactors were sensitive to a small increase in pH. When the medium was returned to pH 7.2, the bioreactors resumed previous activities in less than 1 day. Results of the continuous substrate experiment shown in Table 2 were a repeat of the experiment in Table 1 performed after the pH shift experiment. There was very little difference in the amounts of substrates consumed, carbon dioxide produced, or TCE degraded (Tables 1 and 2). These results suggested that the TCE-degrading activities of the consortia were sensitive to elevated pH but that the consortia were resilient in that baseline activities were rapidly achieved after removal of the pH stress.

Mineral supplements. It was hypothesized that mineral nutrients could have limited microbial biomass and activities within the bioreactors. Doubling the mineral concentrations in the reactor medium in terms of phosphates, nitrates, vitamins, and trace minerals did not increase TCE degradation when maintained at continuous substrate levels of propane and methane (Table 2). The increase in mineral nutrients resulted in 20% less TCE removal than in both continuous substrate experiments (Tables 1 and 2). However, the bioreactors responded differently to the increased nutrients. Reactor 2 showed a 25% increase in methane consumption, while reactor 3 displayed a 50% decrease in methane consumption. Total carbon dioxide production increased in reactor 2 but not in reactor 3.

Methane versus propane. When methane alone was added to the reactors as an energy source (3%, vol/vol), the total TCE degraded decreased by approximately 60% in both systems compared with experiments in which both methane and propane were maintained at 5 and 3% (vol/vol), respectively (Table 3). Although methane facilitated TCE degradation, there was a slower degradation rate and the linear rate of degradation ceased at approximately 10 mg of TCE liter^{-1}

TABLE 3. Comparison of TCE degradation in bioreactors under conditions of continuous methane or continuous propane substrate for 5 days[a]

Expt[b]	Reactor[c]	Propane consumed (mmol)	Methane consumed (mmol)	Carbon dioxide (mmol)	TCE loss (μmol)	% TCE loss	Substrate/TCE loss (μmol/μmol)
Propane	1	<0.1		<0.01	1.1	3	
	2	2.4		1.80	30.5	88	78
	3	2.8		2.70	27.9	80	101
	1	0.1		<0.01	1.1	3	
	2	2.6		2.10	31.7	91	82
	3	2.7		2.59	26.1	75	102
Methane	1		<0.1	<0.01	1.1	3	
	2		1.6	0.36	12.2	35	133
	3		1.7	1.10	10.5	30	158
	1		<0.1	<0.01	1.1	3	
	2		1.7	0.41	12.0	34	138
	3		1.7	1.32	10.2	29	167

[a] Initial TCE concentration of 20 mg liter^{-1} (34.8 μmol reactor^{-1}).
[b] Propane maintained at 3% (vol/vol) in headspace. Methane maintained at 5% (vol/vol) in headspace.
[c] Reactors: 1, control; 2, culture PM-M; 3, culture SM-1.

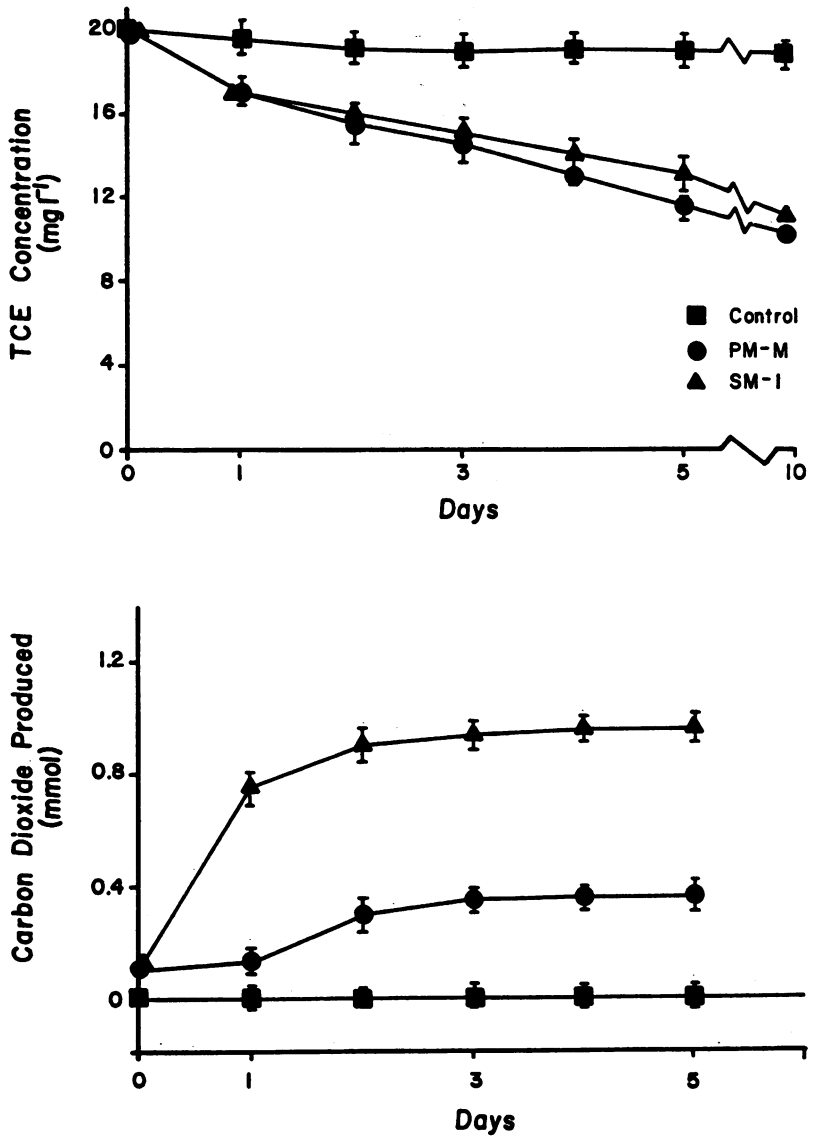

FIG. 2. Utilization of TCE and accumulation of carbon dioxide during experiments fed methane daily. Experimental reactors contained consortium PM-M or SM-1, and a control reactor contained 0.5% Formalin plus 0.2% sodium azide. Error bars represent the standard deviation of three replicate measurements.

versus 3 mg liter^{-1} in the methane plus propane experiments (Fig. 1 and 2). The amount of TCE degraded and the rate of degradation were less than in the methane plus propane experiments. Carbon dioxide accumulation was also less in experiments with methane as the energy source even though methane was not depleted. Carbon dioxide accumulation was not linear over time, and 80% of the total accumulation was observed after 2 days even though less than 30% of the TCE had been degraded. Methane-fed reactors were also less efficient degraders of TCE because of the requirement for 135 to 160 mol of substrate per mol of TCE removed.

When propane was provided as the sole energy source (Table 3), the total TCE loss was very similar to that observed when methane plus propane were supplied daily. Propane-fed reactors were the most efficient of the experiments fed daily, requiring only 80 to 100 mol of substrate per mol of TCE degraded (Table 3). This value was intermediate between pulsed experiments and experiments fed daily with methane plus propane energy sources. The increased efficiency per mole of substrate could in part be due to the increased energy value of propane versus methane. Carbon dioxide accumulation in propane-fed experiments was also similar to that observed with methane-plus-propane-fed experiments. More TCE was degraded per day with propane as an energy source than with methane (Fig. 2 and 3). Carbon dioxide production increased each day in both test reactors during propane experiments (Fig. 2 and 3). During all experiments, less carbon dioxide was recovered from the reactor containing the PM-M culture. In propane and methane plus propane experiments, carbon dioxide production was nearly linear over 5 days (Fig. 1 to 3), and the linear accumulation of carbon dioxide corresponded to linear rates of TCE utilization. In contrast, methane-fed experiments accumulated less carbon dioxide and it increased very little after day 2. Oxygen in the gas phase of the reactors should have been sufficient for complete oxidation of greater than 3 mmol of

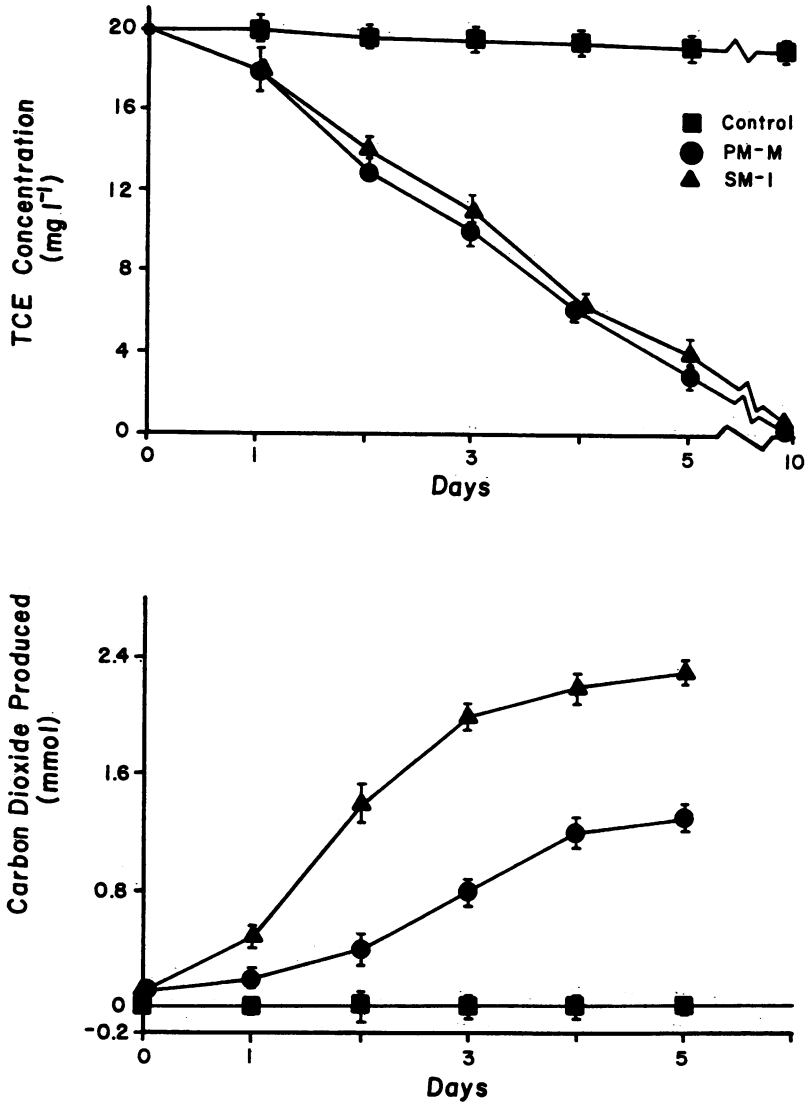

FIG. 3. Utilization of TCE and accumulation of carbon dioxide during experiments fed propane daily. Experimental reactors contained consortium PM-M or SM-1, and a control reactor contained 0.5% Formalin plus 0.2% sodium azide. Error bars represent the standard deviation of three replicate measurements.

propane or 10 mmol of methane. Resazurin, a redox indicator added to the medium, never became reduced, suggesting that oxygen was not limiting. Reasons for poor methane utilization after day 2 in both reactors during two experiments with methane as the energy source were not understood, but the result was poor TCE degradation.

PLFA. PLFA profiles of the microorganisms residing within the reactors are shown in Table 4. The presence of type II methanotrophs was demonstrated by 18:1ω8 and 18:1ω7t fatty acids (13, 14). Actinomycete-type microorganisms were evidenced by high levels of cy19:0 and 10me18:0 with evidence of a small eucaryotic community shown by 20-carbon polyenoic fatty acids (2). Both bioreactors exhibited diverse microflora corresponding to approximately 20 mg (dry weight) of cell mass or 2×10^9 cells g of substratum^{-1}.

TCE intermediates. No volatile intermediates of TCE degradation were detected in either of the test reactors during any of the experiments performed. Samples were withdrawn from the reactors to which [1,2-^{14}C]TCE was added. Greater than 60% of the [1,2-^{14}C]TCE was converted to $^{14}CO_2$ within 3 days as determined by GC-gas proportional counting procedures (4, 16). Volatile intermediates from the sample tests were not detected by GC or radioisotope techniques. The control reactor, however, typically contained 0.5 mg of *trans*-1,2-dichloroethylene liter^{-1} and 20 μg of vinyl chloride liter^{-1} after a 5-day time course experiment.

Another measure of efficiency was the amount of TCE consumed per gram of all cell biomass each day. During propane or methane plus propane continuous substrate experiments, both bioreactors averaged TCE losses of 3.5 mg liter^{-1} or 0.8 mg of TCE degraded day^{-1} for 5 days (Fig. 1 and 3; Table 4). Maximum biomass utilization efficiencies during pulsed feeding experiments suggested that each gram of biomass was capable of degrading 80 mg of TCE day^{-1}, or approximately 8 to 10% of its weight equivalent. These results demonstrated consistent, resilient, and highly active

TABLE 4. PLFA profiles of bioreactor contents

PLFAME[a]	mol%	
	Reactor PM	Reactor SM
i15:0	2.0	1.4
15:0	2.2	2.2
16:0	0.7	0.7
16:1ω7c	2.9	4.3
16:1ω7t	1.5	1.4
16:0	7.0	8.4
10me16:0	1.1	0.9
i17:1	0.4	0.5
i17:0	1.4	0.9
a17:0[b]	1.8	2.8
cy17:0	3.2	3.1
17:0	1.5	2.3
18:0[b]	7.1	8.8
18:1ω9c	3.8	4.1
18:1ω8c/t	15.1	16.2
18:1ω7c	13.1	11.3
18:1ω7t	0.6	0.8
18:0	1.5	1.5
10me18:0	4.6	4.5
br19:1	0.8	1.2
19:1ω12c	0.7	1.1
cy19:0	23.4	17.9
20:4ω6	0.7	0.5
20:0	1.2	1.3
Total PLFAME (pmol g [dry wt]$^{-1}$)	36,000	23,200
Cells g (dry wt)$^{-1}$[c]	2.2×10^9	1.4×10^9
Cell mass (mg [dry wt])	25	16

[a] Analyzed as PFLA methyl esters.
[b] Questionable peak identification.
[c] Assuming 100 μmol of PLFAME g (dry weight) of cells^{-1}, 6×10^{12} bacteria g^{-1} with 70 g of substratum reactor^{-1}.

TCE-degradative capabilities from mixed microbial consortia in bioreactors.

DISCUSSION

These experiments demonstrated that bioreactors containing aerobic mixed culture consortia are capable of degrading over 90% of the TCE present in a 20-mg liter^{-1} solution utilizing propane or methane plus propane as the energy sources. Control reactors contained 98 ± 2% of the initial TCE added after 5 days of operation.

When propane was provided as a substrate in the bioreactors, the extent and rates of TCE degradation were similar to those of methane plus propane substrate mixtures. However, when methane was the only substrate available, the total amount of TCE degraded decreased by approximately 60% in both bioreactors. This suggests that the consortia use propane more efficiently as a growth substrate or that propane does not compete as effectively as methane with TCE-transforming enzymes. When the bioreactors were starved (no substrate added), TCE degradation decreased significantly. In methane-stimulated soil columns, Nichols et al. (14) reported that methanotrophic TCE degradation correlated with high storage polymer/PLFA ratios indicative of nutrient stress. TCE degradation under stressful conditions is in accord with the results presented by other researchers (4, 9).

Pulsed substrate additions (e.g., fed and then starved conditions) showed that the bioreactor inoculated with culture PM-M had a substrate/TCE degradation ratio of approximately 55 while degrading over 85% of the initial nominal liquid-phase TCE concentration of 20 mg liter^{-1} within 5 days. In experiments with pulsed and daily substrate replenishment, the threshold TCE concentration was approximately 0.5 mg liter^{-1}. This limit of TCE degradation was observed if experiments were allowed to proceed for 8 to 10 days. It is not known why the initial TCE concentration was not degraded to levels below 0.2 mg liter^{-1}, considering that cell suspensions and growth cultures degraded 99.9% of the TCE present at liquid-phase concentrations of 20 to 50 mg liter^{-1} (6). Similar lower thresholds for biological methanotrophic degradation of TCE in trickling filter reactors have recently been reported (17).

Differences in microbial populations present in the bioreactors may reflect the substrates available and the inocula. The TCE removal efficiency was higher in the reactor inoculated with culture PM-M under all conditions with the exception of elevated pH. Culture PM-M was represented by a more diverse microbial community than was culture SM-1 at the time of reactor inoculation. This suggests that a diverse community structure is desirable for stability of TCE degradation under various conditions of operation. After experimentation was completed and phospholipid profiles were determined, there was evidence that microeucaryotes had colonized the bioreactors. It is speculated that bioreactors fed contaminated waters will be colonized by microeucaryotes, including protozoa capable of grazing on methanotrophs (S. M. Pfiffner et al., unpublished data of this laboratory).

Anaerobic TCE degradation results in formation of vinyl chloride, which is mutagenic and recalcitrant to degradation (3, 20). Volatile TCE metabolite formation, including vinyl chloride, was not observed at our limits of detection. Resazurin was used as a redox indicator in these experiments to monitor the aerobic status of the liquid phase in the bioreactors. The medium in the bioreactors remained oxidized through 5-day experimental runs. When experiments were performed for periods longer than 1 week, the medium would turn pink (somewhat reduced) unless additional oxygen was added to the headspace of the experimental reactors. This indicates that the test consortia were capable of reducing the redox potential of the liquid phase.

Several researchers have observed chlorinated alkene biodegradation in methane- or natural gas-enriched mixed cultures obtained from various environmental sources (5, 8, 9, 17, 22). Our results suggest that propane is a suitable substrate for promoting TCE degradation and that mixed microbial consortia offer greater stability and promise than methanotrophic pure cultures. Combinations of propane and methane in bioreactors containing methanotrophs and mixed heterotrophic populations may be advantageous for optimization of bioremediation of chlorinated hydrocarbons.

Four unexpected findings resulted from these studies which warrant summarizing: resiliency, substrate efficiency, biomass efficiency, and colonization of bioreactors. Microbial activities directed toward TCE degradation were very sensitive to elevated pH, although the consortia proved resilient and capable of regaining degradative activities promptly after returning to neutral pH. Another resilient characteristic was stable TCE degradation over 6 months. These bioreactors were capable of sustaining TCE degradation for months at utilization rates of 50 to 150 mol of substrate mol of TCE degraded^{-1}. These reactors also

exhibited the capacity to degrade 15 to 80 mg of TCE g (dry weight) of biomass^{-1} each day, which was greater than the 10 to 20 mg g^{-1} from our cell suspension studies. The biomasses within these reactors mineralized nearly 10% of their dry weight equivalents of TCE each day. We also speculate that colonization of the reactors by bacteria-grazing protozoa is a likely event during treatment of contaminated wastes. Finally, previous studies and the work presented here indicate the potential for bioremediating environments contaminated with chlorinated ethenes.

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