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Trichloroethylene Biodegradation by Mesophilic and Psychrophilic Ammonia Oxidizers and Methanotrophs in Groundwater Microcosms

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This study investigated the efficiency of methane and ammonium for stimulating trichloroethylene (TCE) biodegradation in groundwater microcosms (flasks and batch exchange columns) at a psychrophilic temperature (12°C) typical of shallow aquifers in the northern United States or a mesophilic temperature (24°C) representative of most laboratory experiments. After 140 days, TCE biodegradation rates by ammonia oxidizers and methanotrophs in mesophilic flask microcosms were similar (8 to 10 nmol day^{-1}), but [^{14}C]TCE mineralization (biodegradation to $^{14}CO_2$) by ammonia oxidizers was significantly greater than that by methanotrophs (63 versus 53%). Under psychrophilic conditions, [^{14}C]TCE mineralization in flask systems by ammonia oxidizers and methanotrophs was reduced to 12 and 5%, respectively. In mesophilic batch exchange columns, average TCE biodegradation rates for methanotrophs (900 nmol $liter^{-1}$ day^{-1}) were not significantly different from those of ammonia oxidizers (775 nmol $liter^{-1}$ day^{-1}). Psychrophilic TCE biodegradation rates in the columns were similar with both biostimulants and averaged 145 nmol $liter^{-1}$ day^{-1}. Methanotroph biostimulation was most adversely affected by low temperatures. At 12°C, the biodegradation efficiencies (TCE degradation normalized to microbial activity) of methanotrophs and ammonia oxidizers decreased by factors of 2.6 and 1.6, respectively, relative to their biodegradation efficiencies at 24°C. Collectively, these experiments demonstrated that in situ bioremediation of TCE is feasible at the psychrophilic temperatures common in surficial aquifers in the northern United States and that for such applications biostimulation of ammonia oxidizers could be more effective than has been previously reported.

The possible health hazard associated with exposure to trichloroethylene (TCE) has heightened public concern regarding the cleanup of aquifers contaminated with this chemical (22, 24). Current remedial technologies are largely based on pump-and-treat methods. However, the behavior of TCE as a dense nonaqueous-phase liquid makes satisfactory removal by this process difficult (5). Bioremediation is an emerging technology that could aid cleanup of TCE-contaminated groundwater by effecting contaminant degradation in situ.

The potential utility of in situ bioremediation lies in the capabilities of indigenous aquifer bacteria to degrade TCE. Aerobically, TCE biodegradation can be mediated by bacteria growing on ammonium, aromatic hydrocarbons (e.g., toluene, phenol, benzene, alkylbenzenes, and biphenyl), methane, or other alkanes or alkenes (1, 7, 10, 34–38, 40). The common feature of these compounds is that they induce the formation of oxygenases that nonspecifically oxidize TCE. Pure culture (in vitro) tests have shown that TCE degradation rates (nanomoles of TCE degraded per minute per milligram of protein) for resting cells expressing these oxygenases vary substantially and range from up to 580 for a methanotroph (25) to 20 for toluene-phenol degraders (33) and to ≤1 for ammonia oxidizers (1, 36).

Because of the high TCE-degrading capacity of methanotrophs measured in vitro, stimulation of these organisms in soils and aquifers has attracted the greatest attention in laboratory microcosm and field tests. However, in such experiments TCE degradation has often been much lower than anticipated based on pure-culture rates. In the first field test of in situ methanotroph biostimulation, groundwater TCE concentrations (51 to 97 μg $liter^{-1}$) were reduced only 20 to 30% in response to methane injections (29). Enzien et al. (8) concluded that the efficiency of stimulating methanotrophs in aerobic column microcosms was so low that the observed cumulative TCE mass loss of 87% was caused largely by anaerobic organisms in anoxic microsites. In contrast, the addition of toluene or phenol to soils or aquifers appears to consistently promote high TCE degradation rates (9, 17). The biochemistry of TCE biodegradation by ammonia oxidizers has been studied extensively in vitro (18, 19, 27). However, to the best of our knowledge, Hopkins et al. (17) reported the only microcosm study evaluating TCE biodegradation by ammonia oxidizers indigenous to an aquifer and concluded that this approach was far less effective than biostimulation of either methanotrophs or aromatic hydrocarbon degraders.

Temperature is a key variable affecting the efficiency of in situ bioremediation. Most laboratory studies on TCE biodegradation have been done at mesophilic laboratory temperatures (i.e., 20 to 30°C). Yet in the northern United States temperatures in shallow aquifers—where in situ bioremediation might be applied—are much cooler (i.e., 10 to 13°C). Thus, it is questionable whether the results of laboratory tests are representative of those that might be obtained during in situ bioremediation of aquifers at lower temperatures. Microbial growth rates and enzymatic transformations are likely to be slower at lower temperatures. This kinetic effect of temperature can be modeled by using expressions such as the Arrhenius equation or estimated by assuming that enzyme activities decrease approximately twofold per 10°C reduction in temper-

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ature (i.e., $Q_{10} \approx 2$). But what is essentially unknown is the effect of low temperature on selecting for psychrophilic (i.e., growth optima at ca. 5 to 15°C) or psychrotrophic (i.e., growth optima at >15°C but tolerating low temperatures) microbial populations with TCE-degrading abilities that differ from those of mesophilic organisms (i.e., those with growth optima at ca. 20 to 40°C) enriched for and studied under laboratory temperatures.

The occurrence and activity of psychrophilic microbial populations have been documented in connection with biogeochemical cycling of carbon, nitrogen, and sulfur (2, 11, 26, 28, 32). There is comparatively little information, however, on the role of these organisms in xenobiotic chemical biodegradation (3, 20), and to the best of our knowledge, the only reported investigation on TCE degradation by organisms acclimated to low temperatures is that of Broholm et al. (4). In that study, 7 of 10 mixed methanotrophic cultures isolated at 10°C failed to attack TCE and three degraded TCE poorly; the reason(s) for this poor biodegradative performance was not elucidated.

The objective of the present study was to determine the relative efficacies of selected oxygenase inducers to stimulate TCE biodegradation at psychrophilic temperatures encountered in a shallow aquifer in north-central Wisconsin (12°C) or at mesophilic room temperature (24°C). The term "psychrophile" is used here to describe mixed populations possibly composed of organisms that are truly psychrophilic or psychrotrophic-psychrotolerant. Studies were done in flask and batch exchange column microcosms and compared methane and ammonium as biostimulants; toluene supplementation was also evaluated in the batch exchange columns.

MATERIALS AND METHODS

Sediment characterization and microcosm setup. Aquifer sediment samples were obtained from 3.1 to 4.6 m below the former location of a TCE storage tank in north-central Wisconsin, stored on ice during transport, and transferred to sterile flasks. The sediment was 87% sand, 5% silt, and 8% clay and contained 4 g of total organic matter kg^{-1}, 0.3 mg of NH_4-N kg^{-1}, 2.4 mg of NO_3^--N kg^{-1}, and 48 mg of total N kg^{-1}. The sediment was homogenized by passage through a sterile 2-mm-mesh-size sieve and then stored at 12°C until use.

Flask microcosms. For the ammonia oxidizer microcosms, 10 g of sediment and 50 ml of nitrifier medium (31) containing 180 mg of NH_4^+ $liter^{-1}$ (total, 500 μmol) was added to 100-ml (nominal volume) serum bottles. Nitrifier medium was also inoculated with 10 g of autoclaved sediment as a negative control. For methanotroph microcosms, 10 g of sediment and 50 ml of methanotroph medium (14) were added to 100-ml serum bottles. Methane (15 ml; total, 670 μmol) was then injected into the bottle. Nonsterile control microcosms containing sediment and methanotroph medium but lacking methane were also established. The experiment was started by injecting 100 μl of a 500-mg $liter^{-1}$ TCE (Aldrich Chemical Co., Milwaukee, Wis.) solution prepared in sterile double-distilled water (ddH_2O) to give a final dissolved TCE concentration of 1.0 mg $liter^{-1}$ (total, 380 μmol). Three replicates for each treatment were prepared and incubated inverted in the dark with shaking at 24 and 12°C. When TCE degradation was detected, the flasks were opened and fresh medium was added to return the volume to 50 ml; for the ammonia oxidizers, medium replenishment also readjusted the pH to 7.5. Methane was added to the appropriate microcosms, and TCE was injected to replenish the 1 mg $liter^{-1}$ level disturbed by sampling and biodegradation.

To assay TCE degradation, aqueous samples (0.5 ml) were periodically taken from the flasks with a gas-tight syringe and extracted with 0.5 ml of *n*-pentane, and 50-μl aliquots of the organic layer were transferred to 2-ml gas chromatography vials containing limited-volume inserts (Alltech Associates, Deerfield, Ill.). Additional 0.5-ml aliquots were taken for determination of pH and nitrogen speciation (ammonium, nitrite, and nitrate); the latter was done by colorimetric methods (12, 39). Samples (1.5 ml) were periodically taken for analysis of dissolved oxygen, which was determined by immediately injecting samples into microcentrifuge tubes and inserting a colorimetric test vial (Chemetrics Inc., Calverton, Va.). Previous experiments with microcosms known to be anaerobic established this approach as useful for detecting low levels of dissolved oxygen (22a). The tests verified that aerobic conditions were maintained in the flasks throughout the study.

TCE was determined by using a Hewlett-Packard 5890A gas chromatograph (GC) equipped with an Rtx-624 capillary column (Alltech), a Hewlett-Packard 7673A autosampler, a split-splitless capillary column injection port (held at 100°C), and an electron capture detector (held at 200°C) at a helium carrier gas flow of 3.0 ml min^{-1}. After injection of *n*-pentane extracts (1 μl), the oven was held at 45°C for 3 min and then ramped to 200°C at 20°C min^{-1}. Standards were used to verify that this GC method allowed products of anaerobic TCE metabolism (*cis*- and/or *trans*-dichloroethylene and vinyl chloride) to be separated and measured in the 1-mg $liter^{-1}$ range. However, these metabolites were not detected in any treatment during the course of either microcosm experiment. Methane and CO_2 were analyzed in 100-μl headspace samples on the GC described above, but with manual injection and thermal conductivity detection. Isothermal (45°C) separation was achieved with a 6 ft by 1/8 in. Haysep R packed column (Alltech) at a helium carrier gas flow rate of 25 ml min^{-1}. The injector and detector were held at 80 and 100°C, respectively. The dissolved methane concentrations were determined by using the dimensionless Henry's law constant.

After TCE degradation was detected, [^{14}C]TCE ([1,2-^{14}C]TCE, 14.2 mCi $mmol^{-1}$, purity >98%; Sigma Chemical, St. Louis, Mo.) was added to quantify TCE mineralization. A solution consisting of 950 mg of TCE $liter^{-1}$ and 50 mg of [^{14}C]TCE $liter^{-1}$ was prepared in sterile ddH_2O. All microcosms were injected with the [^{14}C]TCE-TCE solution to give a total radioactivity level of 0.107 μCi and a TCE concentration of 1 mg $liter^{-1}$. When TCE degradation was again detected, mass balance studies were done essentially as described by Hickey et al. (15). Briefly, the flask headspaces were flushed under a vacuum (1 h, 25 ml min^{-1}) sequentially through an impinger containing purified-grade ethylene glycol monoethyl ether (Fisher Scientific) to trap [^{14}C]TCE and an impinger containing carbon-14 cocktail (a mixture of organic amines in isomeric xylenes; R. J. Harvey Instrument Co., Hillsdale, N.J.) to collect $^{14}CO_2$. The impinger containing carbon-14 cocktail was placed directly in the liquid scintillation counter while a 1-ml aliquot of ethylene glycol was counted in 10 ml of carbon-14 cocktail. The ^{14}C mass balance was completed by counting a 500-μl aliquot of the residual buffer in 10 ml of carbon-14 cocktail. Total TCE degradation rates were determined from GC measurements made between the time that the microcosms were spiked with [^{14}C]TCE and the time when the flasks were sacrificed (27 days). Changes in TCE concentrations measured in the biostimulated flasks between sampling points were corrected for those occurring in the controls, normalized to the incubation interval between the points, and, at the end of the experiment, averaged to give an overall TCE biodegradation rate for each treatment.

Batch exchange column microcosms. Glass columns were constructed based on a design by Lanzarone and McCarty (21). Exchange solutions were added to the column with a syringe pump and two 100-ml gas-tight syringes (Hamilton, Reno, Nev.). The aquifer material used for the flask microcosms was also used for the column beds (200 g). Once the beds were packed, the columns were sealed at the top and wrapped with aluminum foil to prevent the growth of phototrophic organisms. Conservative tracer breakthrough studies were conducted with bromide to determine the volume at which exchange solutions could be detected in the effluent. All columns were equilibrated with a 1-mg $liter^{-1}$ TCE solution prepared in sterile ddH_2O. No nutrients or stimulants were added to the columns during the TCE equilibration period. After 6 months, the influent and effluent TCE concentrations were approximately equal and biostimulation was initiated.

Eight columns were incubated at 24°C, and four columns were incubated at 12°C; the fluids were exchanged weekly with a nutrient solution (5 mg of KNO_3 $liter^{-1}$, 1 mg of KH_2PO_4 $liter^{-1}$ [pH 7.5]) containing TCE (1 mg $liter^{-1}$) and one of the biostimulants (methane, ammonium, or toluene) at 5 mg $liter^{-1}$. Nitrate was omitted from the nutrient solution applied to the ammonia oxidizer columns. Control columns were exchanged with either the nutrient solution alone or with nutrient solution containing sodium azide (500 mg $liter^{-1}$) to account for TCE losses attributable to nonspecific microbial activity or abiotic processes, respectively. Duplicates of the biostimulated columns and of one of each of the control columns were incubated at 24°C. Single columns of each of the biostimulant treatments and the nutrient-amended control were maintained at 12°C.

Duplicate 2.5-ml samples were taken from the influent and the exit sampling ports; the latter was done before 25 ml had eluted (initial effluent) and after >120 ml had passed (final effluent). TCE in the column water samples was determined as described above. In the ammonia oxidizer columns, 1.5-ml samples were taken for analysis of pH and nitrogen speciation (nitrite, nitrate, and ammonium) by colorimetric methods as described above. For the methanotroph columns, 5-ml samples were removed for methane and CO_2 determinations. These were injected into 8-ml (total volume) serum bottles, crimp sealed with Teflon-lined rubber septa, and incubated inverted overnight (>20 h) at 37°C in preparation for methane and CO_2 analysis by GC as described above. Samples (1.5 ml) were taken from all columns for analysis of dissolved oxygen as described above. These tests verified that aerobic conditions were maintained in the columns throughout the study. To determine TCE biodegradation rates, changes in TCE concentrations between sampling points were normalized to the incubation time interval between the points.

RESULTS AND DISCUSSION

Flask microcosms. In the mesophilic methane-amended microcosms, there was a lag of approximately 20 days before TCE degradation began; TCE biodegradation then proceeded at a

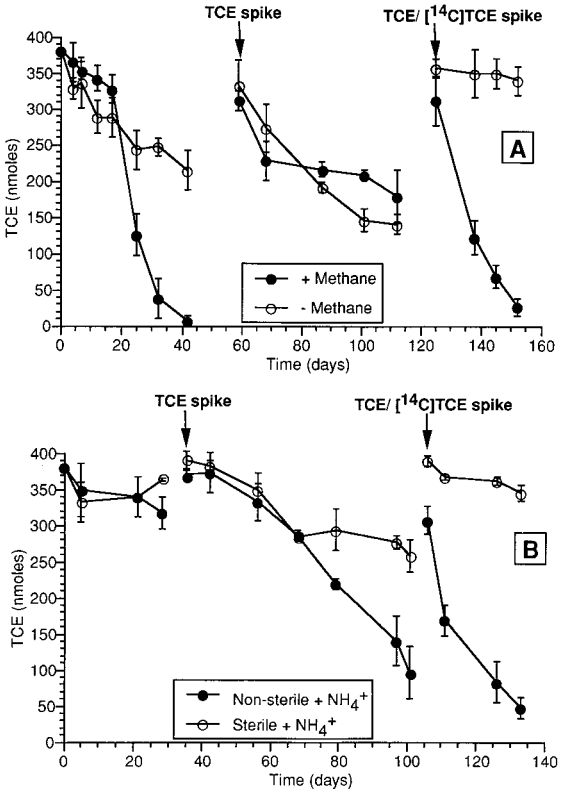

FIG. 1. TCE biodegradation in flask microcosms incubated at 24°C and amended with either methane (A) or ammonium (B). Arrows indicate points at which the microcosms were respiked with TCE or a TCE-[^{14}C]TCE mixture.

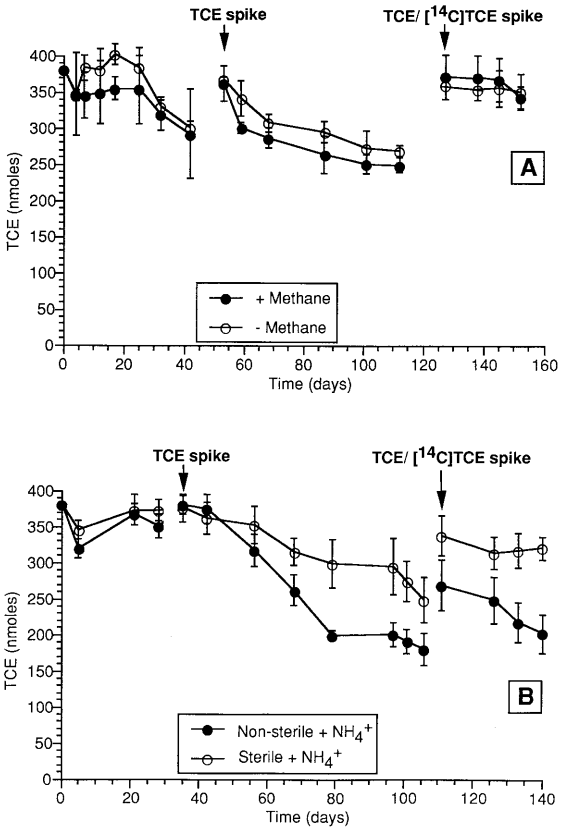

FIG. 2. TCE biodegradation in flask microcosms incubated at 12°C and amended with either methane (A) or ammonium (B). Arrows indicate points at which the microcosms were respiked with TCE or a TCE-[^{14}C]TCE mixture.

rate of 10 nmol day^{-1} (Fig. 1A). The lag time in the 24°C ammonium microcosms was approximately 55 days; the subsequent TCE degradation rates were initially low but increased after day 100 to 8 nmol day^{-1}, which was not significantly different from that of the methane systems (Fig. 1B). Similar levels of TCE biodegradation in the 24°C methane and ammonium microcosms were also indicated by the ^{14}C balance: 18 to 19% of the [^{14}C]TCE added remained after 27 days of incubation (Table 1). The microcosms differed qualitatively, however, in ^{14}C partitioning. In the methane microcosms, $^{14}CO_2$ accounted for 53% of the radioactivity and polar metabolites accounted for for 16% (percentages are corrected for controls) (Table 1). In the ammonium treatments, 63% of the radioactivity added was recovered as $^{14}CO_2$ (percentage corrected for control) and there were no detectable accumulations of polar metabolites (Table 1). The latter result suggested that the ammonia oxidizers and/or commensal nitrite oxidizers were more effective at degrading TCE metabolites than were the methanotrophs and any commensal heterotrophic populations.

TABLE 1. ^{14}C mass balance for flask microcosms incubated at 24°C

Microcosm	% of initial ^{14}C			
	VOC[a]	Aqueous[b]	CO_2	Total recovery[c]
Methanotrophs				
Biostimulated	19.1 ± 2.4	24.1 ± 3.2	56.0 ± 3.5	99.2 ± 1.9
Control[d]	81.3 ± 2.4	7.7 ± 1.3	3.3 ± 0.5	92.2 ± 2.2
Ammonia oxidizers				
Biostimulated	17.9 ± 4.9	7.1 ± 0.5	67.9 ± 5.6	92.9 ± 0.7
Control[e]	89.7 ± 1.5	7.3 ± 0.5	4.5 ± 0.2	101.5 ± 1.9

[a] VOC, volatile organic compounds (nonbiodegraded TCE).
[b] Activity in the aqueous phase following headspace flushing (TCE metabolites).
[c] Initial radioactivity was 0.107 μCi added on days 125 and 109 in the methanotroph and ammonia oxidizer microcosms, respectively (see Fig. 1). All values are means ± standard deviations (n = 6).
[d] No methane added.
[e] Sterilized sediments amended with ammonium.

In the psychrophilic methanotroph microcosms, TCE degradation was difficult to detect by GC and changes in TCE concentrations in the biostimulated treatments were generally not significantly different from those in the controls (Fig. 2A). TCE biodegradation by methanotrophs was apparent only from the mass balance of the [^{14}C]TCE tracer, which indicated that $^{14}CO_2$ accounted for ca. 5% of the added ^{14}C (control-corrected percentage) (Table 2). In contrast, TCE biodegradation in the psychrophilic ammonia oxidizer microcosms was detectable by GC after a lag of about 60 days (Fig. 2B), with a subsequent TCE biodegradation rate of 2 nmol day^{-1}. TCE mineralization in the psychrophilic ammonia oxidizer treatment was also greater than that of the methanotrophs, amounting to ca. 12% of the added radioactivity (control-corrected percentage) (Table 2).

A more informative approach for comparing biostimulation performance is to normalize TCE biodegradation to microbial activity. The activity was assayed by measuring metabolic end product production rates (CO_2 production for methanotrophs and nitrate formation for ammonia oxidizers) during the 27-

TABLE 2. ^{14}C mass balance for flask microcosms incubated at 12°C

Microcosm	% of initial ^{14}C			
	VOC[a]	Aqueous[b]	CO_2	Total recovery[c]
Methanotrophs				
Biostimulated	77.4 ± 4.3	7.1 ± 1.3	8.9 ± 0.6	93.3 ± 4.3
Control[d]	81.7 ± 2.5	6.8 ± 1.6	3.9 ± 0.5	92.5 ± 0.7
Ammonia oxidizers				
Biostimulated	77.4 ± 6.8	6.8 ± 0.3	17.2 ± 3.1	101.3 ± 4.1
Control[e]	89.6 ± 1.5	9.8 ± 1.9	5.1 ± 2.4	104.5 ± 2.9

[a] VOC, volatile organic compounds (nonbiodegraded TCE).
[b] Activity in the aqueous phase following headspace flushing (TCE metabolites).
[c] Initial radioactivity was 0.107 μCi added on days 125 and 109 in the methanotroph and ammonia oxidizer microcosms, respectively (see Fig. 1). All values are means ± standard deviations ($n = 6$).
[d] No methane added.
[e] Sterilized sediments amended with ammonium.

day period following the [^{14}C]TCE spike (Tables 3 and 4). End products were used for these comparisons to obviate uncertainties in methane and ammonium losses that might be caused by sorption. In the psychrophilic methanotroph microcosms, CO_2 production rates were only 1.7-fold lower than those measured at 24°C (Table 3), and in the control flasks (inoculated with sediment but lacking methane) CO_2 production was undetectable. These analyses established that background microbial activity levels were negligible (and thus, TCE biodegradation could be attributed to stimulation of methanotrophs) and that the ineffectiveness of methane for stimulating psychrophilic TCE biodegradation was not attributable simply to the inability to stimulate psychrophilic methanotrophs.

In the ammonia oxidizer microcosms, the mesophilic nitrate production rate was 1.4-fold higher than that at 12°C (Table 4). There was no detectable formation of nitrate in the controls (ammonium-amended, sterilized sediments), and nitrite was not detected in the biostimulated or control flasks during the study. The latter fact indicated that the activity of nitrite oxidizers was closely linked to that of ammonia oxidizers at both temperatures, and thus, nitrate production was a reliable indicator of ammonia oxidizer activity. The 24/12°C ratio of 1.4 for

TABLE 3. Comparison of microbial activity, TCE degradation, and TCE degradation efficiencies in methanotroph microcosms[a]

Temp and microcosm type	Lag time[b] (days)	CO_2 produced[c] (nmol day^{-1})	TCE degraded[c] (nmol day^{-1})	Efficiency (nmol TCE degraded/ nmol CO_2 produced)
24°C				
Column	70	2,000 (3.0)	109 (7.8)	5.5×10^{-2} (2.6)
Flask	20	12,000 (1.7)	10 (ND[d])	8.3×10^{-4} (ND)
12°C				
Column	115	671	14	2.1×10^{-2}
Flask	ND	7,000	ND	ND

[a] Values in parentheses are 24/12°C ratios for the parameter in the indicated microcosm type.
[b] Days of incubation preceding detectable TCE degradation.
[c] Measured in the flask microcosms during the 27-day period following addition of the [^{14}C]TCE and in the columns during the last three exchanges (21 days total). TCE biodegradation rates were determined as explained in Materials and Methods.
[d] ND, not determined because at 12°C TCE degradation was undetectable by GC.

TABLE 4. Comparison of microbial activity, TCE degradation, and TCE degradation efficiencies in ammonia oxidizer microcosms[a]

Temp and microcosm type	Lag time[b] (days)	NO_3^- produced[c] (nmol day^{-1})	TCE degraded[c] (nmol day^{-1})	Efficiency (nmol TCE degraded/ nmol NO_3^- produced)
24°C				
Column	90	3,751 (2.6)	87 (4.1)	2.3×10^{-2} (1.6)
Flask	55	1,100 (1.4)	8 (4.0)	7.3×10^{-3} (3.0)
12°C				
Column	115	1,452	21	1.4×10^{-2}
Flask	60	800	2	2.4×10^{-3}

[a] Values in parentheses are 24/12°C ratios for the parameter in the indicated microcosm type.
[b] Days of incubation preceding detectable TCE degradation.
[c] Measured in the flask microcosms during the 27-day period following addition of the [^{14}C]TCE and in the columns during the last three exchanges (21 days total). TCE biodegradation rates were determined as explained in Materials and Methods.

nitrate production (Table 4) indicated somewhat higher psychrophilic ammonia oxidizer activity than anticipated assuming a Q_{10} of ~2. In contrast, psychrophilic TCE degradation rates were fourfold lower than those under mesophilic conditions (Table 4). This represented a substantial departure from the rates expected based on the assumed Q_{10} of ~2 and could have reflected differences in the enzyme systems of the organisms stimulated and/or differences in physical or chemical processes (e.g., diffusion).

Batch exchange column microcosms. At mesophilic temperatures, TCE biodegradation was detectable in the methanotroph and ammonia oxidizer columns after ca. 70 and 90 days, respectively (Fig. 3). Following 140 days of biostimulation,

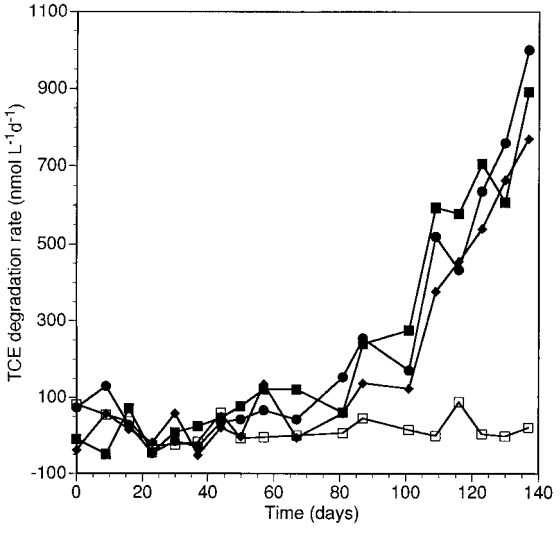

FIG. 3. TCE biodegradation rates in batch exchange columns incubated at 24°C and amended with ammonium (◆), methane (■), or toluene (●) or the control columns (□). All columns were preequilibrated with TCE for 6 months before applications of biostimulant and control treatments were initiated on day zero. For the biostimulants, data points are averages of duplicate determinations made on replicate columns. Control data points are the averages of duplicate determinations from a column supplemented with nutrients only and one supplemented with nutrients and sodium azide; there were no significant differences between these columns in the TCE measurements. Error bars are omitted for clarity; variability in TCE determinations between and within treatments was generally ± 10%.

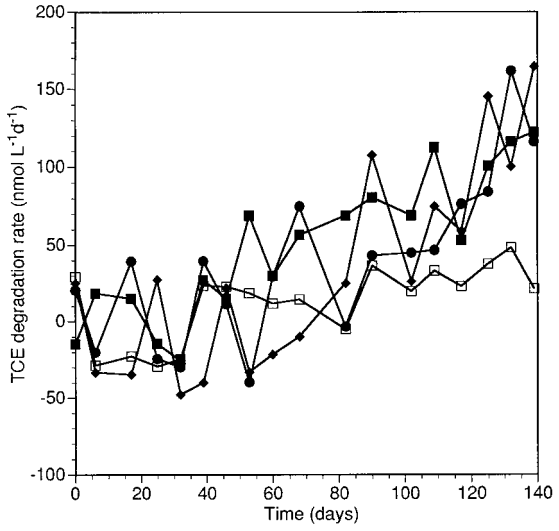

FIG. 4. TCE biodegradation rates in batch exchange columns incubated at 12°C and amended with ammonium (◆), methane (■), toluene (●), or nutrients only (□). All columns were preequilibrated with TCE for 6 months before applications of biostimulant and control treatments were initiated on day zero. Data points are averages of duplicate determinations made on single columns for each treatment. Error bars are omitted for clarity; variability in TCE determinations between and within treatments was generally ± 10%.

TCE biodegradation rates were on average greater for methanotrophs (900 nmol liter^{-1} day^{-1}) than ammonia oxidizers (775 nmol liter^{-1} day^{-1}). However, given that the variability in TCE determinations within and between treatments (columns) averaged ± 10%, these rates were not significantly different. Toluene was included in the experiments essentially as a positive control because results from our preliminary tests with organisms isolated from these sediments were consistent with those of others (9, 16, 17, 23) in suggesting that aromatic hydrocarbon degraders functioned more reliably as effective TCE degraders (22a). The lag period preceding biostimulation of toluene degraders was similar to that of the methanotrophs, and after 140 days, TCE biodegradation rates averaged 950 nmol liter^{-1} day^{-1} (Fig. 3). Psychrophilic TCE biodegradation was detectable in response to all biostimulants after about a 110-day lag period (Fig. 4). Following 140 days of biostimulation, TCE biodegradation rates were not significantly different and averaged 145 nmol liter^{-1} day^{-1}. There was no loss of TCE in either the unstimulated treatment or the sodium azide-inhibited control column. Thus, the TCE removal observed in the biostimulated columns was attributable to biodegradation by organisms of the targeted physiological groups and not to nonspecific heterotrophic activity or abiotic loss.

As with the flask microcosms, microbial activity measurements were obtained to normalize TCE biodegradation rates between treatments, and they focused on comparing methanotrophs and ammonia oxidizers. In the methanotroph columns, methane consumption paralleled CO_2 production (data not shown). In the ammonium-amended columns, nitrate formation was never accompanied by detectable nitrite accumulations, which indicated physiological linkage of ammonia and nitrite oxidizers. Comparison of the normalized TCE degradation rates showed that at both temperatures methanotrophs were about twice as efficient as ammonia oxidizers (i.e., 5.5×10^{-2} versus 2.3×10^{-2} nmol of TCE degraded per nmol of product formed [Tables 3 and 4]). It should be noted, however, that if TCE biodegradation were normalized to biomass production ammonia oxidizers would likely appear more efficient because their growth yields are expected to be almost an order of magnitude lower than those of methanotrophic bacteria (13).

The other comparison of interest was how microbial activity and TCE degradation varied as a function of temperature within physiological groups. In the methanotroph columns, 24/12°C ratios for microbial activity and normalized TCE degradation efficiency were 3- and 2.6-fold greater, respectively, at 24 than at 12°C (Table 3). Mesophilic ammonia oxidizer activity was 2.6 times greater than that of the psychrophiles, and the 24/12°C ratio for TCE degradation efficiency was 1.6 (Table 4). All of these ratios were thus in the range where an assumed Q_{10} of ~2 was probably adequate to extrapolate between temperatures.

Comparison of flask and batch exchange column microcosms. The combination of flask and batch exchange microcosms was used first to evaluate TCE biodegradation under optimal conditions (flasks) and then under those considered more appropriate for simulating an aquifer environment, where mass transfer processes may limit microbial growth and contaminant degradation (6, 16, 30). The primary advantages of the flask microcosms were the simplicity of establishment and the ease with which [^{14}C]TCE could be used to construct mass balances, the latter allowing evaluation of TCE fate beyond parent compound depletion. Yet, assuming results from column experiments gave better estimates of biostimulant performance in situ, the flask microcosms were inaccurate in three respects. First, the lag periods preceding TCE biodegradation were shorter in the flasks, perhaps reflecting the effects of mixing on the enhancement of TCE and/or nutrient mass transfer rates. Second, microbial activity levels were overestimated for methanotrophs but underestimated for ammonia oxidizers. For the methanotrophs this effect could be accounted for by the greater amount of methane present in the flasks and by flask mixing that likely enhanced growth substrate availability. In contrast, the closed nature of the flasks probably adversely affected ammonia oxidizer activity by preventing the dissipation of acidity generated by nitrification: in the flasks during the last 27 days the pH decreased from 7.5 to as low as 5.8, whereas weekly exchanges prevented acidity accumulation in the columns and therefore the effluent pH was 7.5 for the duration of the experiment. Third, TCE degradation rates were lower in the flasks than in the columns, which could have reflected increased competitive inhibition caused by the presence of greater levels of growth substrates.

Conclusions. These experiments indicated that in situ bioremediation of TCE-contaminated aquifers is feasible at psychrophilic temperatures common in the northern United States. While qualitative differences between meso- and psychrophilic organisms undoubtedly exist, our results suggested that a Q_{10} of 2 was adequate to extrapolate activities of ammonia oxidizers and methanotrophs between temperatures. To the best of our knowledge, this is the first report of ammonia oxidizer biostimulation producing levels of TCE biodegradation comparable to those of methanotrophs. Possibly, this could suggest that the ammonia oxidizer type cultures studied in vitro are not representative of the dominant populations biostimulated in situ; this hypothesis is supported by recent direct phylogenetic analysis of ammonia oxidizer populations in soil and marine sediments (32a). Collectively, these findings suggest that in situ biostimulation of ammonia oxidizers could be a viable alternative for remediating TCE-contaminated aquifers. Ammonium is inexpensive and nontoxic and has a higher aqueous solubility than methane, making introduction into, and distribution throughout, an aquifer easier. Perhaps the greatest limitation of this technology is the accumulation of

nitrate in groundwater. However, nitrification can be predicted and with proper management nitrifiers could be utilized to biodegrade TCE in situ without incurring further environmental damage.

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