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Bridget N. Moran
Department of Soil Science
University of Wisconsin-Madison

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ABSTRACT

This study investigated the relative efficacy of selected oxygenase inducers to stimulate trichloroethylene (TCE) biodegradation at psychrophilic temperatures (12°C) encountered in shallow aquifers of the northern United States or mesophilic room temperature (24°C) typical of most laboratory experiments. Experiments were done in flask and batch-exchange column microcosms comparing methane and ammonium as biostimulants. In flask or column microcosms incubated at mesophilic temperatures, lag times preceding methanotrophic TCE biodegradation were shorter than those of the ammonia-oxidizers by 20 to 30 days. Yet, in the long term (>100 days) TCE biodegradation by ammonia-oxidizers and methanotrophs in both microcosm systems was similar in terms of [^{14}C]TCE mineralization levels, TCE biodegradation rates, and TCE biodegradation efficiencies (rates normalized to microbial activity). Incubation at psychrophilic temperatures increased the lag periods and decreased the biodegradation rates and efficiencies for ammonia-oxidizers and methanotrophs in both microcosm types. However, in both the flask and column experiments psychrophilic ammonia-oxidizers were consistently more effective TCE degraders than were psychrophilic methanotrophs. 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 previously believed.

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INTRODUCTION

The possible health hazard associated with exposure to trichloroethylene (TCE) has heightened public concern regarding the clean-up of aquifers contaminated with this chemical (Miller and Guengerich, 1982; Ochsner et al., 1979). 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 (Cherry et al., 1992). 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 (toluene, phenol, benzene, alkylbenzenes, biphenyl), methane, or other alkanes/alkenes (Arciero et al., 1989; Dabrock et al., 1992; Fliermans et al., 1988; Suyama et al., 1996; Tsien et al., 1989; Vannelli et al., 1990; Wackett et al., 1989; Wackett and Gibson, 1988; Wilson and Wilson, 1985). The common feature of these compounds is that they induce the formation of oxygenases that non-specifically oxidize TCE. Pure culture (*in vitro*) tests have shown TCE degradation rates (nmol TCE degraded min^{-1} mg $protein^{-1}$) for resting cells expressing these oxygenases vary substantially and range from up to 580 for a methanotroph (Oldenhuis et al., 1991), to 20 for toluene/phenol-degraders (Sun and Wood, 1996), to ≤ 1 for ammonia-oxidizers (Arciero et al., 1989; Vannelli, 1990).

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 $\mu g\ L^{-1}$) were reduced only 20 to 30% in response to methane injections (Semprini et al., 1990). Enzien et al. (1994) 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, addition of toluene or phenol to soils or aquifers appears to consistently promote high TCE degradation rates (Fan and Scow, 1993; Hopkins et al., 1993). The biochemistry of TCE biodegradation by ammonia-oxidizers has been studied extensively *in vitro* (Hyman et al., 1995; 1988; Rasche et al., 1991). However, to the best of our knowledge, Hopkins et al. (1993) 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 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 U.S. temperatures in shallow aquifers—where *in situ* bioremediation might be applied—are much cooler (i.e., 10 to 13°C). Thus, laboratory tests may be questioned as to whether the results are representative of those that might be obtained during *in situ*

bioremediation of aquifers at lower temperatures. Microbial growth rates and enzymatic transformations may be slower at lower temperatures. This kinetic effect of temperature can be modeled using expressions such as the Arrhenius equation, or estimated by assuming enzyme activities decrease approximately two-fold per 10°C reduction in temperature (i.e., $Q_{10} \approx 2$). But, 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 >15°C, but tolerate 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 has been documented in connection with biogeochemical cycling of carbon, nitrogen, and sulfur (Berthelot et al., 1993; Halm, 1988; Omelchenko et al., 1993; Saad and Conrad, 1993; Stefaniak and Maniewska, 1987). There is comparatively little information, however, on the role of these organisms in xenobiotic chemical biodegradation (Bradley and Chapelle, 1995; Jarvinen et al., 1994), 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. (1993). In the latter study, seven 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 were not elucidated.

The objective of the present studies was to determine the relative efficacy of selected oxygenase inducers to stimulate TCE biodegradation at psychrophilic temperatures encountered in a shallow aquifer in north central Wisconsin (12°C) or 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 columns microcosms comparing 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, transferred to sterile flasks and stored on ice during transport. The sediment was 87% sand, 5% silt, and 8% clay and contained 4 g total organic matter kg^{-1}, 0.3 mg NH_4-N kg^{-1}, 2.4 mg kg^{-1}, and 48 mg total N kg^{-1}. The conductivity was < 20 mhos x 10^{-5} cm^{-1}. The sediment was homogenized by passage through a sterile 2-mm sieve, and then stored at 12°C until used.

FLASK MICROCOSMS

For the ammonia-oxidizer microcosms, 10 g of sediment and 50 mL of nitrifier medium (Soriano and Walker, 1968) containing 180 mg NH_4^+ L^{-1} (500 mmoles total) 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 (Henry and Grbic-Galic, 1990), was added to 100 mL serum bottles. Methane (15 mL, 670 mmoles total) 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 mL of a 500 mg L^{-1} TCE (Aldrich Chemical Co., Milwaukee, Wisconsin) solution prepared in sterile ddH_2O; the final TCE concentration was 1.0 mg L^{-1} (380 nmoles total). Three replicates for each treatment were prepared and incubated inverted with shaking at 24 and 12°C. When TCE degradation was detected, the flasks were opened and fresh media added to return the volume to 50 mL; for the ammonia-oxidizers media replenishment also re-adjusted the pH to 7.5. Methane was added to the appropriate microcosms and TCE injected to replenish the 1 mg L^{-1} level removed 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 *n*-pentane, and 50 µL aliquots of the organic layer transferred to 2-ml gas chromatography vials containing limited volume inserts (Alltech Associates, Deerfield, Illinois). 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 (Hanson and Phillips, 1981; Weatherburn, 1967).

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), an electron capture detector (held at 200°C), and using a helium carrier gas flow of 3.0 mL min^{-1}. After injection (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-*/*trans*-dichloroethylene, vinyl chloride) to be separated and measured in the 1 mg L^{-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" Haysep R packed column (Alltech) and 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 using the dimensionless Henry's Law constant.

After TCE degradation was detected, [^{14}C]TCE ([1,2-^{14}C]TCE, 14.2 mCi $mmole^{-1}$, purity >98%; Sigma Chemical, St. Louis, Missouri) was added to quantify TCE mineralization. A solution consisting of 950 mg L^{-1} TCE and 50 mg L^{-1} [^{14}C]TCE 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 TCE concentration of 1 mg L^{-1}. When TCE degradation was again detected, mass balance studies were done as essentially as described by Hickey et al. (1995).

BATCH-EXCHANGE COLUMN MICROCOSMS

Glass columns were constructed based on a design by Lanzarone and McCarty (1990). Exchange solutions were added to the column with a syringe pump and two 100-mL gas-tight syringes (Hamilton, Reno, Nevada). The aquifer material used for the flask microcosms was 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 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 L^{-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 at 12°C; the fluids were exchanged weekly with the appropriate solution prepared in sterile ddH_2O (Table 1). Duplicate 2.5 mL samples were

Table 1. Treatments and incubation conditions for batch-exchange column microcosms.

Column Number	Exchange Solution Amendment[†]	Incubation Treatment (°C)
1	Nutrients[‡]	24
2	Sodium azide (500 mg L^{-1}), nutrients	24
3, 4	Methane (5 mg L^{-1}), nutrients	24
5, 6	Toluene[§] (5 mg L^{-1}), nutrients	24
7, 8	Ammonium[§] (5 mg L^{-1}), nutrients	24
9	Nutrients	12
10	Methane (5 mg L^{-1}), nutrients	12
11	Toluene[§] (5 mg L^{-1}), nutrients	12
12	Ammonium[§] (5 mg L^{-1}), nutrients	12

[†]All solutions contained TCE (1 mg L^{-1}).
[‡]Nutrients = KNO_3 (5 mg L^{-1}), KH_2PO_4(1 mg L^{-1}), pH 7.5. Nitrate was omitted from this solution for the ammonia-oxidizer microcosms (columns 7, 8, 12).
[§]Ammonium and toluene concentrations were increased to 50 mg L^{-1} on day 90.

taken from the influent and the exit sampling ports; the latter was done before 25 mL 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. With 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 hours) 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, which was quantified by immediately injecting samples into microcentrifuge tubes and inserting a colorimetric test vial (Chemetrics Inc., Calverton, Virginia). Previous experiments with microcosms known to be anaerobic established this approach as useful for detecting low dissolved oxygen (B.N. Moran, unpublished results). The latter tests verified that aerobic conditions were maintained in the the columns throughout the study.

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 rate of 3% day^{-1} (Figure 1A). The lag time in the 24°C ammonium microcosms was approximately 55 days; the subsequent TCE degradation rates were initially 1% day^{-1} but increased after day 100 to 4% day^{-1}, which was similar to that of the methane systems (Figure 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 incubation (Table 2). The microcosms differed qualitatively, however, in ^{14}C partitioning. In the methane microcosms $^{14}CO_2$ accounted for 56% of the radioactivity and polar metabolites for 24% (Table 2). In the ammonium treatments, 68% of the radioactivity added was recovered as $^{14}CO_2$ and there were no detectable accumulations of polar metabolites. 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.

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 the controls (Figure 2A). TCE biodegradation by methanotrophs was apparent only from the mass balance of the [^{14}C]TCE tracer, which indicated $^{14}CO_2$ accounted for ca. 9% of the added ^{14}C (Table 3). In contrast, TCE biodegradation in the psychrophilic ammonia-oxidizer microcosms was detectable by GC after about a 60 days lag (Figure 2B) with a subsequent TCE biodegradation rate of ca. 0.3% day^{-1}. TCE mineralization in the psychrophilic ammonia-oxidizers treatment was also greater than that of the methanotrophs amounting to ca. 17% of the added radioactivity (Table 2).

A more informative approach for comparing biostimulation performance is to normalize TCE biodegradation to microbial activity. The latter was assayed by measuring metabolic end-product production rates during the 27 day period following the [^{14}C]TCE spike (Tables 4 and 5). Endproducts were used for these comparisons to obviate uncertainties in methane and ammonium losses that might be caused by sorption. In the mesophilic methanotroph microcosms, CO_2 production rates were ca. 1.7-fold greater than those measured at 12°C (Table 4). In the control flasks (inoculated with sediment but lacking methane) CO_2 production was nondetectable. Thus, the ineffectiveness of methane for stimulating psychrophilic TCE biodegradation was not simply attributable to the inability to stimulate psychrophilic methanotrophs.

In the ammonia-oxidizer microcosms, the mesophilic nitrate production rate was ca. 1.4-fold higher than that at 12°C (Table 5). 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 indicated that the activity of nitrite-oxidizers was closely linked to that of ammonia-oxidizers at both temperatures, and as such nitrate production was a

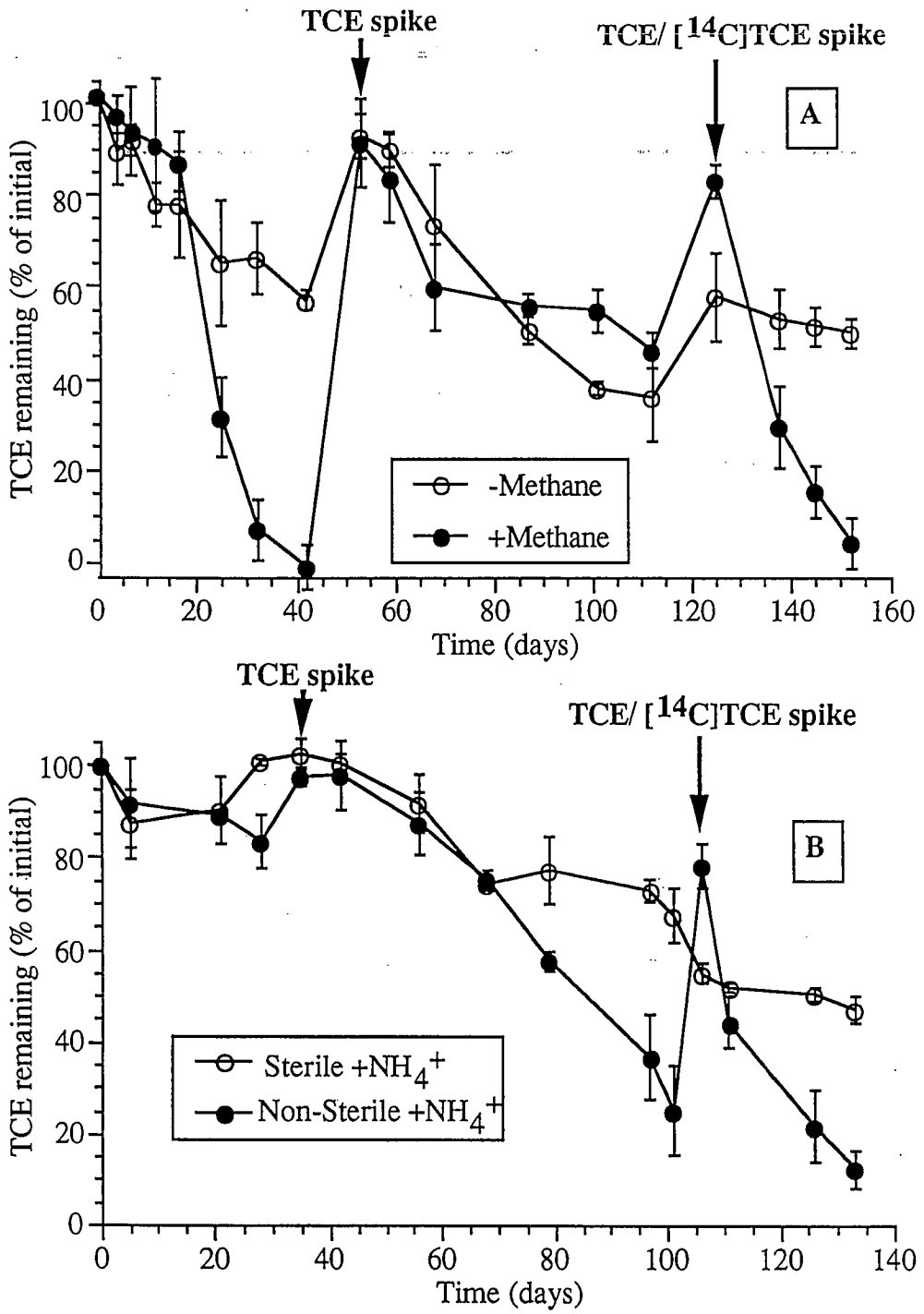

Figure 1. TCE biodegradation in microcosms incubated at 24°C and amended with methane (A) or ammonium (B). Arrows indicate points at which the microcosms were respiked with TCE or a TCE/[^{14}C] TCE mixture.

Table 2. ^{14}C mass balance for flask microcosms incubated at 24°C.

	VOC[†]	Aqueous[‡]	CO_2	
Microcosm	% of initial ^{14}C	% of initial ^{14}C	% of initial ^{14}C	Total Recovery[§]
Methanotrophs				
Biostimulated	19.1 ± 2.4	24.1 ± 3.2	56.0 ± 3.5	99.2 ± 1.9
Control[¶]	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[#]	89.7 ± 1.5	7.3 ± 0.5	4.5 ± 0.2	101.5 ± 1.9

[†] Volatile organic compounds (non-biodegraded TCE).
[‡] Activity in the aqueous phase following headspace flushing (TCE metabolites).
[§] Initial radioactivity was 0.107 μCi added on days 125 and 109 in the methanotroph and ammonia-oxidizer microcosms, respectively (see Figure 1A, B). All values are the means ± standard deviation (n = 6).
[¶] No methane added.
[#] Sterilized sediments amended with ammonium.

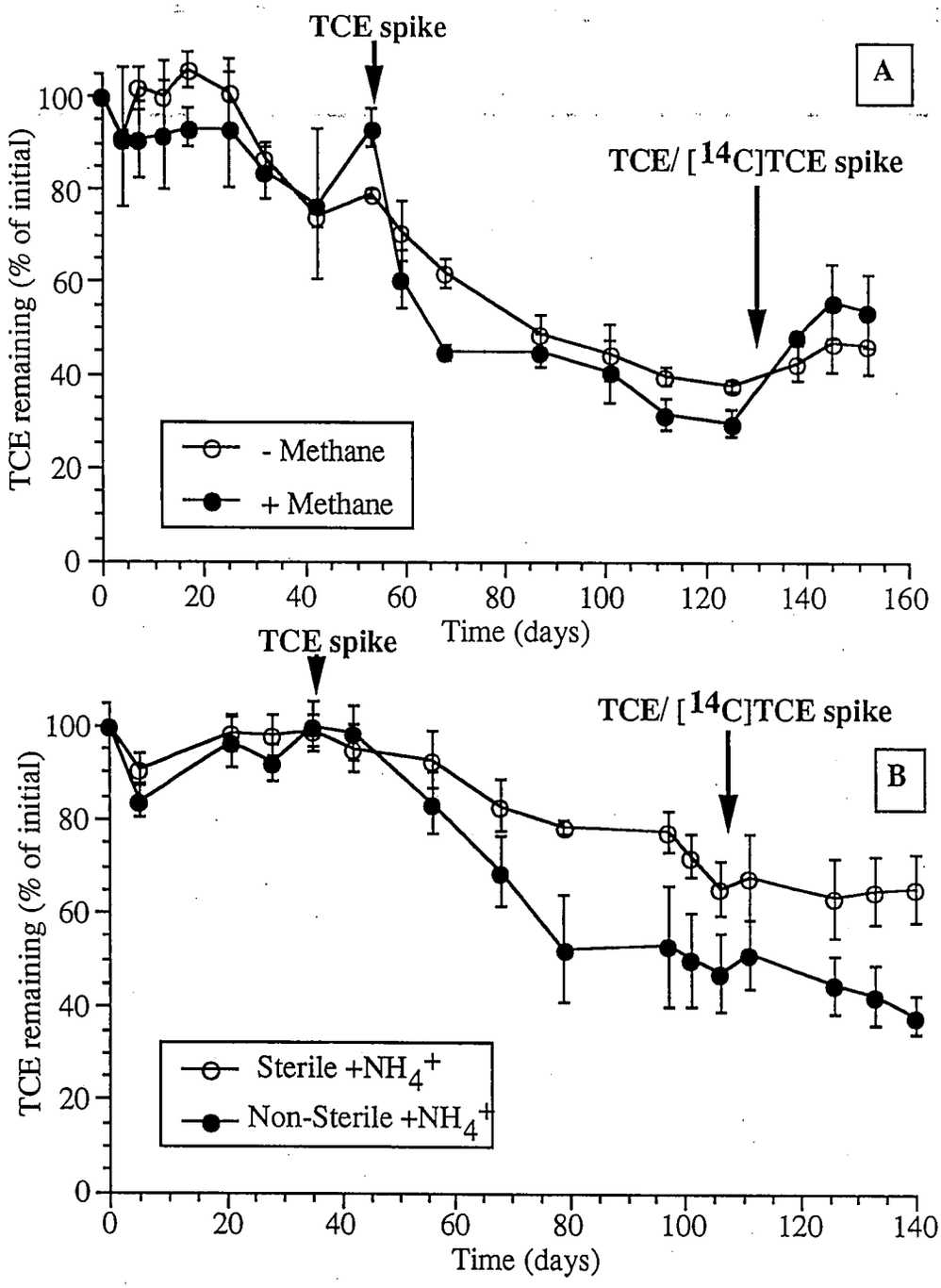

Figure 2. TCE biodegradation in microcosms incubated at 12 °C and amended with methane (A) or ammonium (B). Arrows indicate points at which the microcosms were respiked with TCE or a TCE/[^{14}C] TCE mixture. The amount of TCE added in the latter spike was less than that needed to restore the TCE concentration to 100% of the initial amount.

Table 3. ^{14}C mass balance for flask microcosms incubated at 12°C.

	VOC[†]	Aqueous[‡]	CO_2	
Microcosm	% of initial ^{14}C	% of initial ^{14}C	% of initial ^{14}C	Total Recovery[§]
Methanotrophs				
Biostimulated	77.4 ± 4.3	7.1 ± 1.3	8.9 ± 0.6	93.3 ± 4.3
Control[¶]	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[#]	89.6 ± 1.5	9.8 ± 1.9	5.1 ± 2.4	104.5 ± 2.9

[†] Volatile organic compounds (non-biodegraded TCE).
[‡] Activity in the aqueous phase following headspace flushing (TCE metabolites).
[§] Initial radioactivity was 0.107 μCi added on days 125 and 109 in the methanotroph and ammonia-oxidizer microcosms, respectively (see Figure 1A, B). All values are the means ± standard deviation (n = 6).
[¶] No methane added.
[#] Sterilized sediments amended with ammonium.

Table 4. Comparison of microbial activity, TCE degradation, and TCE degradation efficiencies in methanotroph microcosms.[†]

Temperature and Microcosm Type	Lag Time[‡] (days)	CO_2 Produced[§] (nmol day^{-1})	TCE Degraded[§] (nmol day^{-1})	nmol TCE Degraded/nmol CO_2 Produced
24°C				
Column	70	2,000 (3.0)	109 (7.8)	0.055 (2.8)
Flask	20	12,000 (1.7)	15 (ND)[¶]	0.001 (ND)
12°C				
Column	115	671	14	0.02
Flask	ND	7,000	ND	ND

[†] Values in parentheses are 24°C/12°C ratios for the parameter in the indicated microcosm type.
[‡] Days of incubation preceding detectable TCE degradation.
[§] 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).
[¶] Not determined because at 12°C TCE degradation was non-detectable by GC.

Table 5. Comparison of microbial activity, TCE degradation, and TCE degradation efficiencies in ammonia-oxidizer microcosms.[†]

Temperature and Microcosm Type	Lag Time[‡] (days)	CO_2 Produced[§] (nmol day^{-1})	TCE Degraded[§] (nmol day^{-1})	nmol TCE Degraded/nmol CO_2 Produced
24°C				
Column	90	3,751 (2.6)	87 (4.1)	0.023 (1.6)
Flask	55	1,100 (1.4)	11 (10)	0.01 (10)
12°C				
Column	115	1,452	21	0.014
Flask	60	800	1.1	0.001

[†] Values in parentheses are 24°C/12°C ratios for the parameter in the indicated microcosm type.
[‡] Days of incubation preceding detectable TCE degradation.
[§] 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).

reliable indicator of ammonia-oxidizer activity. The 24°C/12°C ratio of 1.4 for nitrate production indicated somewhat higher psychrophilic ammonia-oxidizer activity than anticipated assuming a Q_{10} ≈ 2. In contrast, psychrophilic TCE degradation rates and efficiencies were 10-fold lower than those under mesophilic conditions (Table 5). This represented a substantial departure from rates expected based on the assumed Q_{10} ≈ 2, and could have reflected differences in the enzyme systems of the organisms stimulated and/or differences in physical/chemical processes (e.g., diffusion). Overall, while the TCE biodegradation efficiency of ammonia-oxidizers was reduced at 12°C, psychrophilic populations of these organisms were nevertheless more effective TCE degraders than were those of psychrophilic methanotrophs.

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 (Figure 3). Following 140 days of biostimulation, TCE biodegradation rates were on average greater with methanotrophs (900 nmol L^{-1} day^{-1}) than ammonia-oxidizers (775 nmol $L^{-1}day^{-1}$). 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 these experiments essentially as a positive control because results from our preliminary tests with organisms isolated from these sediments were consistent with that of others (Fan and Scow, 1993; Hopkins and McCarty, 1995; Hopkins et al., 1993; Mu and Scow, 1994) in suggesting that aromatic hydrocarbon-degraders functioned more reliably as effective TCE degraders (B.N. Moran, unpublished results). The lag period preceding biostimulation of toluene-degraders was similar to that of the methanotrophs and after 140 days TCE biodegradation rates averaged ca. 950 nmol L^{-1} day^{-1} (Figure 3). It should be noted that stimulation of microbial activity, as evidenced by CO_2 or NO_3- production, was detected 10 days before biostimulant concentrations were increased. Thus, the latter change did not affect the length of the lag period preceding TCE biodegradation. Psychrophilic TCE biodegradation was detectable in response to all biostimulants after about a 110 day lag period (Figure 4). Following 140 days of biostimulation, TCE biodegradation rates were not significantly different and averaged ca. 145 nmol L^{-1} day^{-1}. There was no loss of TCE in either the nonstimulated 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 nonspecific heterotrophic activity or abiotic loss.

As with the flask microcosms, microbial activity measurements were obtained to normalize TCE biodegradation rates between treatments, and focused on comparing methanotrophs and ammonia-oxidizers. In the methanotroph columns, methane consumption paralleled CO_2 production (data not shown) while in the ammonium-amended columns nitrate formation was never accompanied by detectable nitrite accumulations, which indicated physiological linkage of ammonia- and nitrite-oxidizers. Comparing the normalized TCE degradation rates showed that at 24°C methanotrophs were about twice as efficient as ammonia-oxidizers (i.e., 0.055 vs. 0.023 nmol TCE degraded per nmol product formed; Tables 4 and 5), but at 12°C this relationship was reversed. It is worth considering that if TCE biodegradation were normalized to biomass production, ammonia-oxidizers would likely appear even more efficient because their growth yields are expected to be almost an order of magnitude lower than those of methanotrophic bacteria (Heijnen, 1992).

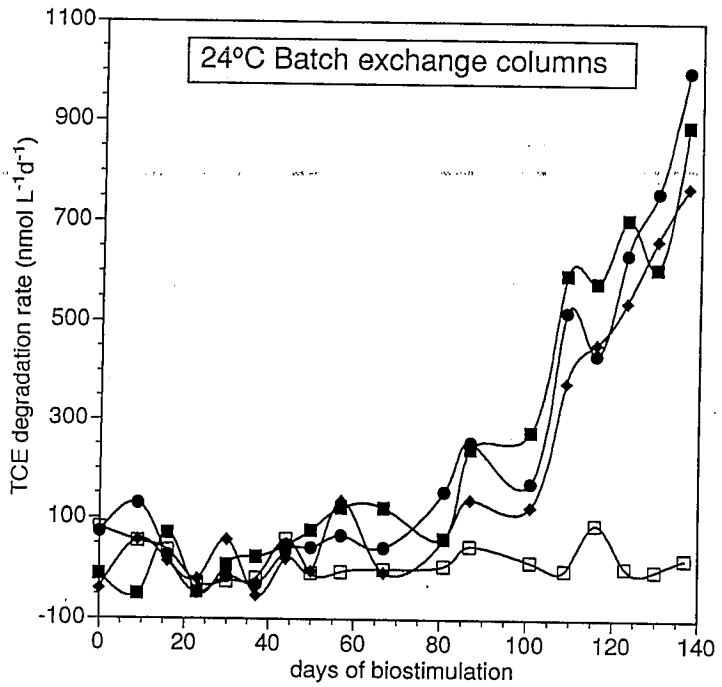

Figure 3. TCE biodegradation rates in batch exchange columns incubated at 24°C and amended with amonium (♦), methane (■), toluene (●), or the control columns (□). Data points represent duplicate determinations made on replicate columns. Control columns were supplemented with nutrients only or nutrients and sodium azide. Error bars are omitted for clarity; variability in determinations between and within treatments was generally ±10%.

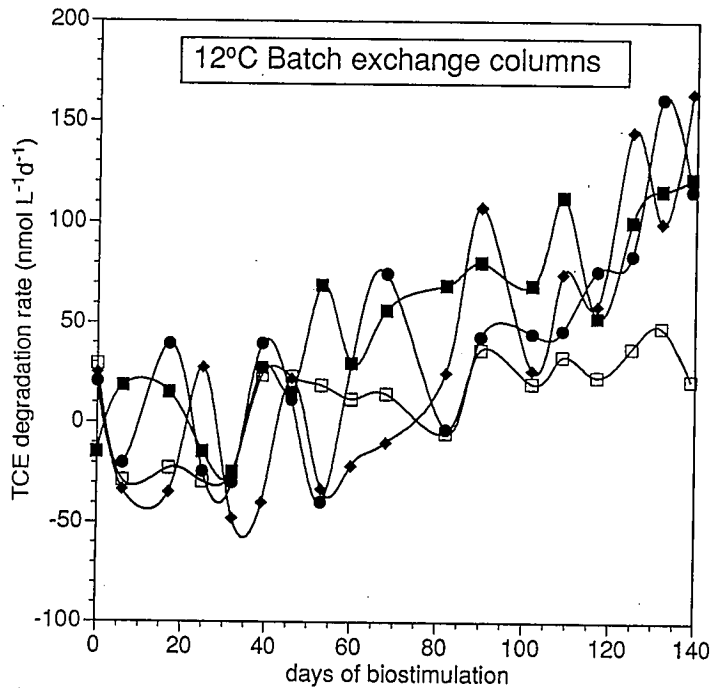

Figure 4. TCE biodegradation rates in batch exchange columns incubated at 12°C and amended with amonium (♦), methane (■), toluene(●), or nutrients only (□).

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°C/12°C ratios for microbial activity and normalized TCE degradation rates were 3- and 2.8-fold greater, respectively, at 24°C than at 12°C (Table 4). Mesophilic ammonia-oxidizer activity was 2.6 times greater than that of the psychrophiles and the 24°C/12°C ratio for normalized TCE degradation was 1.6 (Table 5). All of these ratios were thus in the range where an assumed $Q_{10} \approx 2$ was probably adequate to extrapolate between temperatures. These results were consistent with the flask experiments in suggesting that psychrophilic ammonia-oxidizers were more effective TCE degraders than were psychrophilic methanotrophs.

FLASK VS. BATCH-EXCHANGE COLUMN MICROCOSM COMPARISON

The combination of microcosms were used to first 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 (Cohen et al., 1995; Hopkins and McCarty, 1995; Siegrist and McCarty, 1987). The primary advantage of the flask microcosms was the simplicity of establishment and 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 enhancing 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 flask mixing that likely enhanced growth substrate availability. In contrast, the closed nature of the flasks probably adversely affected ammonia-oxidizer activity in 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 as the of the effluent pH was 7.5 for the duration of the experiment. Third, TCE degradation rates were lower in the flasks than the columns, which could have reflected increased competitive inhibition effected 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 U.S. While qualitative differences between mesophilic 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. Biostimulation of ammonia-oxidizers resulted in greater levels of TCE-biodegradation than has been previously reported, and under psychrophilic conditions TCE biodegradation by ammonia-oxidizers was superior to that of methanotrophs. Collectively, these findings suggest *in situ* biostimulation of ammonia-oxidizers could be a viable alternative for remediating TCE-contaminated aquifers. Ammonium is inexpensive, 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 nitifiers could be employed to biodegrade TCE *in situ* without incurring further environmental damage.

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