

# Small-Column Microcosm for Assessing Methane-Stimulated Vinyl Chloride Transformation in Aquifer Samples

MARK E. DOLAN\* AND  
PERRY L. MCCARTY†

*Department of Civil Engineering, Stanford University,  
Stanford, California 94305*

A small-column microcosm was designed and operated to evaluate the potential for in-situ vinyl chloride (VC) biotransformation by a methane-stimulated culture. Microcosms consisted of 15-mL test tubes filled with aquifer material and fitted with a fluid exchange system to allow sequential batch testing. Aquifer material was aseptically obtained from two locations at a chloroethene-contaminated Superfund site in St. Joseph, MI. In microcosm tests, influent VC concentrations ranged from 1 to 17 mg/L (15–275  $\mu$ M) with methane concentrations of 0.5–4.0 mg/L (30–250  $\mu$ M) and dissolved oxygen concentrations of about 29 mg/L (900  $\mu$ M). Stimulation of an active methane-utilizing population occurred within 60 days, and VC transformation was observed in all of the methane-fed microcosms. A maximum amount of VC transformation, independent of influent VC concentration, was observed for each methane concentration used. Significant differences in VC transformation ability was found in the aquifer samples from the two locations. Transformation yields ( $T_V$ ) of up to 3.5 mg of VC transformed/mg of methane utilized (0.9 mol of VC/mol of  $CH_4$ ) were found at one location compared to a maximum of 1.0 mg of VC/mg of  $CH_4$  (0.26 mol of VC/mol of  $CH_4$ ) at the other. Up to 16 mg/L VC (250  $\mu$ M) was transformed with an addition of 4 mg/L methane (250  $\mu$ M) with no significant toxic effects observed. The microcosms performed well throughout the study, providing consistent repeatable results.

## Introduction

In-situ bioremediation is receiving increased attention for restoring contaminated soils and groundwater. Methane-oxidizing bacteria (methanotrophs) have been shown to effect aerobic cometabolic degradation of certain chlorinated aliphatic hydrocarbons (CAHs), including vinyl chloride (VC) (1–4). Due to the different CAH transforming abilities of methanotrophic bacteria and greatly differing

environmental conditions of contaminated sites, prediction of primary substrate (methane and oxygen) requirements and efficiency of remediation is difficult. The Committee on In Situ Bioremediation has recommended an evaluation strategy for bioremediation based on converging lines of independent evidence (5). Three types of information are required, one of which is laboratory assays showing that microorganisms from the site samples have the potential to transform the target contaminants under the expected site conditions. Laboratory soil-column microcosms, using aquifer material obtained from the targeted site, have been effectively used to assess the potential for in-situ bioremediation (6–8). Such microcosms have an advantage over aqueous systems or soil slurry reactors in that they have soil to water ratios similar to those found in the field and operate without mechanical mixing, so they tend to simulate aquifer conditions better. However, the columns generally used have been somewhat large, thus requiring large amounts of aseptic (difficult and costly to obtain) aquifer material, as well as requiring much time to operate. The use of smaller test columns would reduce the amount of aquifer material needed and perhaps personnel time for operation, allowing more variables to be tested at one time. In addition, it is easier to prevent extraneous bacterial contamination during preparation and operation of small columns. Because of the potential advantages, a small soil-column microcosm was designed and operated to evaluate its ability to yield repeatable, consistent, and differential results. Here, the potential for in-situ biotransformation of a VC-contaminated aquifer by a methane-stimulated culture was evaluated.

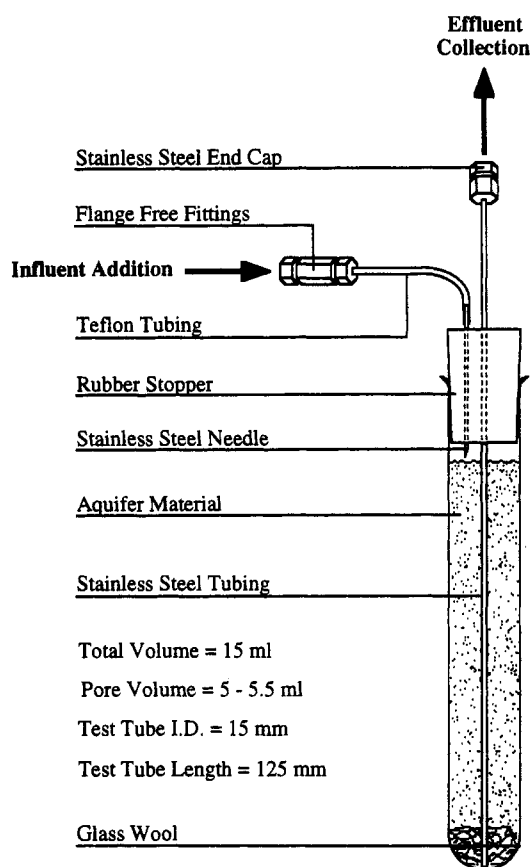
## Materials and Methods

**Microcosm Preparation.** The column microcosms consisted of nominal 15-mL Kimax glass test tubes approximately 125 mm in length with an inside diameter of 15 mm (Figure 1). Each column was sealed with a size 00 rubber stopper fitted with a 20-gauge 1.5 in. long steel hypodermic needle penetrating to the bottom of the stopper. The top of the needle was connected with Teflon tubing to a flange-free fitting, providing the influent port for feedstock solution. A hypodermic stainless steel tube (170 mm by 0.7 mm inside diameter, Small Parts Inc., Miami, FL) was also inserted through the stopper to the bottom of the column to act as the effluent collection tube. A straightened piano wire with the same diameter as the inside of the effluent collection tube (0.68 mm diameter, Small Parts Inc.) was placed inside the effluent tube to prevent aquifer material from being forced into it during column assembly. Fittings at the top of the effluent collection tube allowed samples to be obtained using a 2.5-mL glass-barrel syringe with a luer-lok connector. Fine glass wool was placed in the bottom of the column to prevent clogging of the effluent collection tube by aquifer solids.

Before aquifer solids addition, each test tube with glass wool and each rubber stopper assembly were individually wrapped in aluminum foil. All microcosm components and the equipment used to transfer aquifer material were then autoclaved at 120 °C for 20 min. The aquifer material to be evaluated was obtained aseptically from a Superfund site in St. Joseph, MI (9), and kept in sterilized mason jars at 4 °C until use. The aquifer materials consisted primarily

\* E-mail address: mdolance@leland.stanford.edu; Telephone: (415) 723-0315; Fax: (415) 725-3162.

† Silas H. Palmer Professor of Civil Engineering.



**FIGURE 1.** Column microcosm design.

of a uniform fine sand with low organic carbon content.

Microcosm assembly took place in a laminar-flow hood next to a bunsen burner to prevent contamination from airborne bacteria. The aluminum was removed, and the test tubes were filled with filter-sterilized groundwater to maintain saturated conditions in the column during filling. Next, aquifer material was aseptically spooned into the test tube and allowed to settle through the overflowing groundwater. To eliminate the formation of trapped air or water pockets, the test tube was periodically vibrated and stirred with a glass rod. Each test tube was filled to approximately 2 cm from the top with about 25 g of dry material. The rubber stopper assembly with the piano wire in place was then unwrapped, and the effluent collection tube was forced through the soil into the glass wool at the bottom of the test tube. The influent port was opened to allow the rubber stopper to displace the groundwater at the top of the column, and the rubber stopper was pushed a short way down the effluent collection tube to firmly seal the top of the column. The piano wire was then removed from the tube, and the influent and effluent ports were closed. Finally, the test tubes were wrapped in aluminum foil to prevent possible interference from light and to inhibit growth of photosynthetic organisms and were stored at room temperature (23 °C) in a dark cabinet between feedings.

**Feed Solution Preparation.** Groundwater obtained from an uncontaminated zone at the St. Joseph site (9) was filter-sterilized using 0.2- $\mu$ m membrane filters before being placed in two autoclaved glass bottles. Each bottle was purged with either oxygen or methane gas (99.9% purity, Liquid Carbonics, Chicago, IL) to create gas-saturated solutions. Feed solutions consisted of a mix of the oxygen

and methane-saturated groundwater spiked with either a sodium bromide stock solution (4.75 g/L NaBr, Alltech Assoc. Inc., Deerfield, IL) for the tracer tests or a vinyl chloride stock solution for the methanotrophic degradation tests. The vinyl chloride stock solution was prepared in a gas-tight 50-mL glass-barreled syringe by saturating approximately 35 mL of demineralized water with vinyl chloride gas (99.9% purity, Fluka Chem., Buchs, Switzerland). Seven 4.5-mL amber glass vials with mininert screw-cap closures were filled with the saturated solution and stored at 4 °C until needed.

**Column Operation.** Microcosm pore fluids were exchanged by forcing fluid into the microcosm influent port at a flow rate of about 2.5 mL/min using a syringe pump (Sage Instruments, Division of Orion Research Inc., Cambridge, MA) with two 100-mL glass-barrel gas-tight syringes and adjustable Teflon-lined plungers (Spectrum, Houston, TX). Bromide tracer tests were conducted on six microcosms by exchanging microcosm fluid with 10 mM sodium bromide in groundwater solution. Each microcosm effluent sample was collected with a 1-mL glass-barrel syringe and transferred to a 2-mL glass vial and sealed with a Teflon-lined screw cap. All of the microcosm effluent was collected, and sample volumes were determined by before and after weighing of the sample vials. Average sample size was approximately 1 mL with smaller samples taken at the expected initial breakthrough point between 3 and 6 mL introduced.

During methane-stimulated VC transformation tests, the microcosms were operated in sequential batch mode with exchanges of microcosm fluid occurring intermittently. Microcosm effluent was collected for analysis in a manner to prevent loss of volatile components. As part of each exchange, 3.25 mL of a 2 mM sodium bromide solution was first injected into the microcosm. Then the normal exchange fluid was added. During each exchange, the first 0.75 mL of effluent was wasted because it was not considered representative of the soil pore water. The next 2.5 mL of effluent was saved as the initial sample and was considered to represent conditions in the soil pore water just prior to the exchange. The optimal initial sample size of 2.5 mL was determined from bromide tracer tests, which indicated it would not be contaminated with the fresh exchange water. A final effluent sample of 2.5 mL was taken after 20 mL of feed solution had passed through the microcosm and was considered to represent the initial microcosm pore water condition for the next incubation period. The 2.5-mL samples, collected with a luer-lok glass-barrel syringe, were slowly injected with the needle end below the water surface to completely fill a 2-mL glass vial, which was then sealed with a Teflon-lined septa and open-holed screw cap to eliminate headspace and to ensure maximum containment of volatile components.

**Analytical Procedures.** Each effluent sample was analyzed within 48 h of the exchange for some or all of the following components: VC, DO, bromide, and methane. In general, 5–50  $\mu$ L was first removed through the 2-mL sample vial septa to analyze for VC. If DO analyses were required, 300  $\mu$ L was then removed through the septa, followed by removal of 100  $\mu$ L for bromide analysis. The remainder of the effluent sample was used for methane analysis. Here, partial vacuum was created in a 9 mL serum vial sealed with a rubber sleeve stopper by removing air from the vial with a 20-mL glass-barrel syringe. The remaining fluid in the 2-mL sample vial was then transferred

through a double-ended needle into the serum vial with the volume of sample transferred determined by before and after weighing of the serum vial. After equilibration, the headspace of the serum vial was analyzed for methane.

**VC Analysis.** VC concentration was determined by adding the 5–50  $\mu\text{L}$  aqueous sample, after dilution in 4 mL of demineralized degassed water to a Tekmar Model 4000 purge and trap system. This consisted of an ALS autosampler connected to a Hewlett Packard 5890A gas chromatograph (GC) equipped with a 70-m J&W Scientific DB-624 megabore column and a Tracor electrolytic conductivity detector with data integration using a Series 900 Perkin Elmer Nelson analytical interface. Five samples with volumes between 1 and 250  $\mu\text{L}$  were taken from an aqueous VC standard solution and used to create a standard curve for the Tekmar purge and trap system. The procedure used to calibrate the standard solution is described in the following.

First, VC calibration standards were prepared by injecting 50, 100, 150, and 200  $\mu\text{L}$  of VC-saturated stock solution (approximately 2 g/L) into four respective 62-mL bottles each containing 30 mL of demineralized water. The bottles were shaken at 20 °C for about 20 min to reach gas/saturation equilibrium. Using a Hamilton gas-tight syringe, 300  $\mu\text{L}$  of headspace gas was withdrawn from each bottle and analyzed on a Hewlett-Packard 5890 GC equipped with a 30-m J&W Scientific DB-624 megabore column using an OI Corporation Model 4420 electrolytic conductivity detector. Headspace response was compared to that of a canister of NBS traceable vinyl chloride in nitrogen gas obtained from Scotty II Analyzed Gases ( $1027 \pm 10$  ppm) and using a Henry's constant of 0.92 at 20 °C (10). Aqueous VC concentrations were calculated for the four bottles, and the standard with headspace concentration closest to the 1027 ppm gas standard was used as the VC standard solution to develop the standard curve for the purge and trap system.

**Bromide Analysis.** Bromide concentrations were determined by dilution of a 100- $\mu\text{L}$  sample in 5 mL of DI water, filtration through a 0.2- $\mu\text{m}$  membrane filter, and analysis of filtrate on a Dionex Series 4000i ion chromatograph equipped with an HPIC AS4A Dionex ion pak column and a conductivity detector using a bicarbonate solution as eluent. Calibration standards of gravimetrically prepared solutions of sodium bromide in demineralized water were used to create a standard curve.

**Methane Analysis.** The serum vials containing the methane samples were inverted and shaken vigorously for approximately 1 min, then a 300- $\mu\text{L}$  headspace sample was removed with a Hamilton gas-tight syringe and analyzed for methane on a Hewlett Packard 5730A GC using a 5 ft by 1/8 in. Supelco 60/80 Carbosieve packed column at 120 °C with a flame ionization detector. A two point standard curve (10 and 100 ppm) developed using methane in helium gas standards (Alltech Assoc. Inc.) was used to determine the methane content of the headspace samples. The methane concentration of the aqueous sample was calculated using known aqueous and headspace volumes and considering all of the methane to be in the headspace of the 9-mL serum vials after equilibration.

**Dissolved Oxygen Analysis.** Dissolved oxygen (DO) concentrations were determined by dilution of a 300- $\mu\text{L}$  sample in 300  $\mu\text{L}$  of air-saturated DI water and analysis on a Hansatech Model CB1-D mini-DO probe. A standard curve was developed using standards of DI water purged for 20 min with either nitrogen or oxygen.

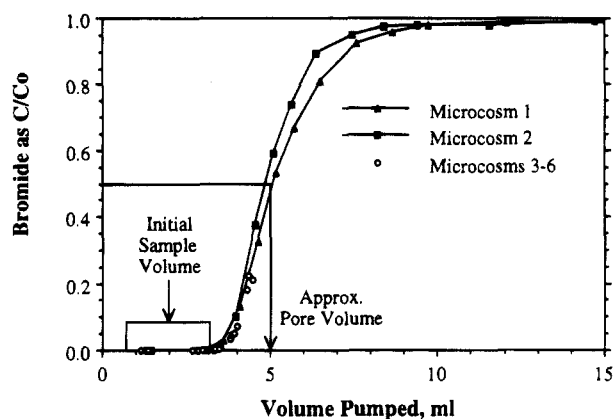


FIGURE 2. Bromide tracer breakthrough results.

TABLE 1

### Efficiency of Microcosm Exchange Method

	VC	DO	methane
av influent concn (mg/L)	4.24	26.8	3.39
influent SD (mg/L)	0.10	1.14	0.12
av $C/C_0$ <sup>a</sup>	1.01	1.01	1.01
$C/C_0$ SD	0.038	0.042	0.058
no. of microcosms	10	10	7

<sup>a</sup>  $C/C_0$  is the concentration in initial effluent sample from second exchange divided by the concentration in final effluent sample from first exchange.

## Results

### Column Flow Characteristics and Sampling Efficiency.

Breakthrough curves from bromide tracer tests for two microcosms (Figure 2) show sharp bromide fronts beginning at about 3.5 mL and reaching influent concentrations after about 10–15 mL was introduced. Both microcosms were shown in this way to have pore volumes of approximately 5 mL and similar flow characteristics. An additional four microcosms with somewhat more frequent sampling produced similar results with bromide breakthrough occurring after 3.25–3.5 mL was added, and all exhibited similar initial breakthrough slopes. From these results, the decision was made to use, as the initial microcosm effluent sample, 2.5 mL of fluid collected after an initial 0.75 mL was wasted.

Ten inactive microcosms were exchanged in order to evaluate the accuracy and reliability of the microcosm exchange method. The microcosms, filled with aquifer material obtained from the St. Joseph site, were exchanged using groundwater containing VC, dissolved oxygen (DO), and in seven of the microcosms, methane. Final effluent samples were collected and analyzed for VC, DO, and methane. Within 48 h from the first exchange, each microcosm was again exchanged with initial effluent samples taken and analyzed for remaining VC, DO, and methane. The average influent concentrations and the amount of each compound remaining at the second exchange are shown in Table 1. Methane, VC, and DO results show on average the amount measured in the initial effluent of the second exchange to be within 1% of the amount measured in the final effluent of the first exchange with a standard deviation of less than 6%. Here, it was correctly assumed that no significant biological activity had yet developed in the microcosms.

**Methane Enrichment and VC Transformation.** Sixteen microcosms were assembled using aquifer material taken

from just below the groundwater table at the St. Joseph, MI site near the center of the known VC plume (9). One set of eight microcosms was filled with aquifer material taken from 10.8 m below ground level (39 cm below the water table), 0.9 m south of observation well 18 (OW18). The other set of eight microcosms was filled with aquifer material taken from about 90 m away, 10.5 m below ground level (33 cm below the water table), 3.3 m south-southeast of observation well 29 (OW29). With each set of microcosms, the groundwater used for exchange in two microcosms contained no methane (controls), two contained 0.5 mg/L (31  $\mu$ M) methane, two contained 1.5 mg/L (95  $\mu$ M) methane, and the final two contained about 4.0 mg/L (250  $\mu$ M) methane. VC concentration in groundwater exchanged in all microcosms ranged from near 1 mg/L (16  $\mu$ M) for the first few exchanges up to 17 mg/L (275  $\mu$ M) for the final exchange. With all of the microcosms, the groundwater exchange fluid contained 26–30 mg/L (800–940  $\mu$ M) dissolved oxygen (DO).

Previous work with St. Joseph aquifer material resulted in methane consuming potential being exhibited within 35–45 days of initial incubation with methane (data not shown). The soil microcosms initially fed 1 mg/L VC were allowed to incubate for 60 days in order to enrich a methane-utilizing culture. Effluent analyses indicated that in 10 of the 12 methane-enriched microcosms all of the methane was consumed, while in two (the OW18 microcosms fed 3.9 mg/L methane) all but 0.6 mg/L methane was consumed. There were significant solution VC losses (from 38 to 45%) in the control microcosms over the incubation period, but all 12 methane-containing microcosms exhibited greater VC removals, although the two microcosms that had methane remaining after incubation had less VC depletion than the other methane-enriched microcosms. Following three subsequent exchanges with influent VC concentrations ranging from 0.70 to 0.97 mg/L (11–16  $\mu$ M) and after incubation periods of 9–17 days, similar results were produced, except that methane was completely utilized in all of the 12 methane-enriched microcosms, and there were less solution VC losses in the control microcosms (approximately 18%).

To estimate the extent of VC transformation related to methane stimulation, influent VC concentrations for the methane-enriched microcosms were adjusted to reflect VC losses in the control microcosms (this assumes similar losses occurred in the methane-containing microcosms and results in a conservative estimate of methane-stimulated VC transformation). On this basis, the extent of VC transformation due to methane enrichment for the second through fourth incubation periods is shown in Figure 3. An increase in VC transformation with increasing methane utilization is clearly indicated for both sets of microcosms. There was about 35% transformation of VC in the microcosms receiving 0.5 mg/L (31  $\mu$ M) methane, from 60 to 75% transformation in the microcosms fed 1.5 mg/L (95  $\mu$ M) methane, and greater than 90% transformation in the microcosms using 4–5 mg/L (250–300  $\mu$ M) methane. Similar transformation efficiencies were attained for each group of columns over the three exchange periods with no significant difference in results between the 17-day and 9-day incubation periods.

For exchanges 5–10, influent VC concentrations were raised to approximately 3–4 mg/L (50–64  $\mu$ M). In exchanges 5 and 6, the group of microcosms fed 0.5 mg/L (31  $\mu$ M) methane transformed only 10% of the influent VC,

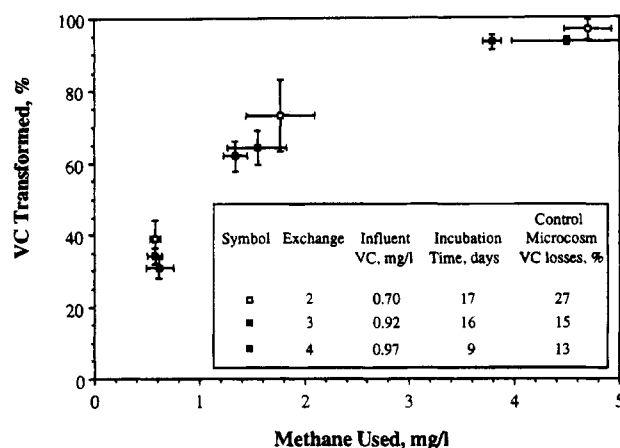


FIGURE 3. Methane-stimulated vinyl chloride transformation in column microcosms (each point is the average of four microcosms with 95% confidence intervals).

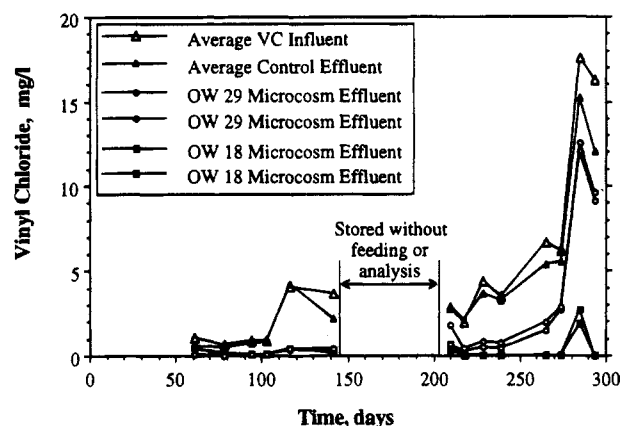


FIGURE 4. Vinyl chloride influent and effluent concentrations in microcosms fed 4 mg/L methane over 14 exchanges.

while those receiving 1.5 and 4 mg/L (95 and 250  $\mu$ M) methane transformed about 50% and roughly 88%, respectively. Following exchange 6, all of the microcosms were stored without feeding or analysis for 58 days. When microcosm exchanges were resumed, the transformation efficiencies dropped with the first exchange (2%, 11%, and 71% VC transformation with 0.5, 1.5, and 4 mg/L methane, respectively), but returned to previous values during the next exchange, and then improved further with subsequent exchanges. By exchange 10, VC transformations of 29%, 59%, and 92% were achieved in microcosms fed 0.5, 1.5, and 4 mg/L (31, 95, and 250  $\mu$ M) methane, respectively, with VC influent concentrations of about 3.5 mg/L (56  $\mu$ M).

However, during these exchanges a noticeable difference in the VC transformation efficiencies between OW18 and OW29 microcosms developed. This difference was most significant in the microcosms fed 1.5 mg/L (95  $\mu$ M) methane, OW18 microcosms transformed up to 80% of the influent VC while OW29 microcosms transformed only 39%. As the influent VC concentration was raised to 6–7 mg/L (95–110  $\mu$ M) for the next two exchanges and up to 17 mg/L (275  $\mu$ M) for the final two exchanges, the difference in OW18 and OW29 microcosm results became more pronounced for the microcosms fed 4 mg/L (250  $\mu$ M) methane, as shown in Figure 4. OW18 microcosms were able to transform up to three times as much VC as the OW29 microcosms.

The microcosm results show an increase in VC transformation as the influent VC concentration increased (Figure 5). However, for each methane concentration fed,

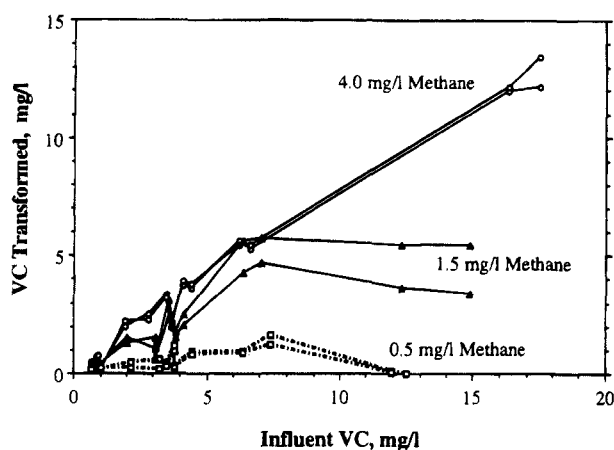


FIGURE 5. VC transformed as a function of influent VC concentration for OW18 microcosms.

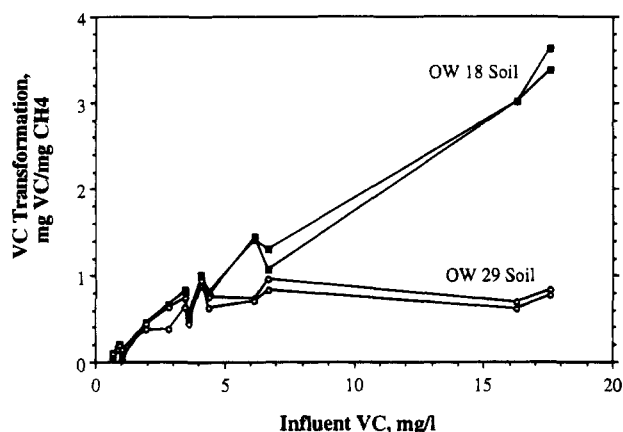


FIGURE 6. VC transformation yield as a function of influent VC concentration for microcosms fed 4 mg/L methane.

there appeared to be a maximum amount of VC transformation attainable regardless of further influent VC increases. When VC transformation is calculated as a transformation yield ( $T_y$ ), expressed as mg of VC transformed/mg of methane utilized (Figure 6),  $T_y$  values for OW18 and OW29 microcosms are very similar up to a certain influent VC concentration. Past this concentration, OW29 microcosms produce a constant  $T_y$ , while with the OW18 microcosms,  $T_y$  continued to increase as influent VC concentrations were increased to the highest VC concentration of 17 mg/L (275  $\mu$ M) used in this study. OW29 microcosms produced a maximum  $T_y$  of about 1.0 mg of VC/mg of  $CH_4$  (0.26 mol of VC/mol of  $CH_4$ ) and OW18 microcosms achieved  $T_y$  of up to 3.5 mg of VC/mg of  $CH_4$  (0.90 mol of VC/mol of  $CH_4$ ).

## Discussion

Microcosm flow characteristics were similar for the six microcosms tested. Initial bromide breakthrough occurred after between 3.25 and 4.0 mL fluid was added for all six microcosms, indicating consistent flow patterns and little short-circuiting of influent flow along the column sides or the effluent collection tube. Bromide analyses of initial effluent samples for the first four methane- and VC-containing exchanges showed less than 0.5% influence from the subsequent bromide-containing feed solution (data not shown), indicating good sample integrity.

A test exchange procedure using 10 microcosms exchanged twice in 48 h demonstrated the reliability of the microcosm exchange method. The amount of methane,

VC, and DO measured in the final effluent of the first exchange and the initial effluent of the second exchange was within 1% of each other on the average with a standard deviation of less than 6% for the group. This indicated that sample acquisition and analysis were repeatable and fairly precise and that the microcosms operated as designed.

Stimulation of an active methane-utilizing population in the soil microcosms was achieved within the first 60 days of incubation. The two OW18 microcosms with methane remaining and less VC transformation after the first exchange period may have experienced oxygen limitation. Since methanated and oxygenated water were mixed to create the feed solution, feeds having a high methane concentration also contain less oxygen. In all subsequent exchanges, these two microcosms oxidized all of the methane and exhibited VC removals similar to other microcosms with the same influent methane concentrations.

Although there were large solution VC losses (40%) in the control microcosms during the initial 60-day incubation period, subsequent exchanges with shorter incubation times resulted in significantly less losses (10–15%). In a separate study using these microcosms (11), a 7-day exchange produced a chloride released to VC removed ratio of 0.83 (95% confidence interval of  $\pm 0.07$ ) in methane-fed microcosms indicating that transformation was responsible for most of the VC removal during short incubations. Sorption effects probably accounted for most of the higher control-microcosm losses during the first exchange period. Longer incubation periods also tended to result in higher VC losses in the control microcosms, perhaps due to slow volatile losses, long-term sorption into the soil or the butyl rubber stopper, or even slow heterotrophic VC degradation (12–17).

The extent of sorption of VC to the aquifer material, in any event, was relatively small. Had sorption been greater, then mass balances may have been more difficult to obtain since significant sorption could have occurred while passing the 20 mL of exchange solution through the microcosm. This effect, although small in the current case, would need to be addressed more particularly for cases where stronger sorption occurs.

The first four exchanges yielded consistent VC transformation efficiencies, expressed as percent VC removed, over an influent VC range of 0.70–0.97 mg/L (11–16  $\mu$ M). The easily distinguishable increase in VC transformation efficiencies with increase in methane concentration and the tight grouping of the results for each set of conditions illustrate the reliability and consistency of results with the microcosm procedures. The lack of significant differences between the VC transformation results from the 17-day and 9-day incubation periods, and the lack of residual methane in the microcosms after the initial exchange incubation indicates that both methane and VC transformation were complete within 9 days of feeding once an adequate population of methane-utilizing bacteria was established.

When influent VC concentrations were increased to 4 mg/L (64  $\mu$ M), the microcosms initially exhibited less VC transformation efficiency than at lower influent VC concentrations. However, after repeated exchanges at the higher concentration, transformation efficiencies in the OW18 microcosms matched or exceeded those at lower influent VC concentrations while OW29 microcosms remained much lower. As the influent VC concentrations

were again increased, it became clear that OW18 microcosms possessed greater VC transformation ability than the OW29 microcosms. The difference in VC transformation results may be due to the presence of organisms with different capabilities at the two locations.

For a given methane feed concentration, there appeared to be some maximum VC transformation capability by the microcosms, with OW18 microcosms exhibiting greater VC transformation at each methane concentration than OW29 microcosms. Since the maximum VC transformation was independent of influent VC concentration and directly related to methane utilization, methane utilization appears to drive the cometabolic activity.

In a previous study using a suspended mixed methanotrophic culture enriched from Moffett Field aquifer materials (18) to transform VC in the absence of methane, a  $T_y$  of 0.05 mg of VC/mg of  $\text{CH}_4$  (0.013 mol of VC/mol of  $\text{CH}_4$ ) was found (19). In another study, using a methanotrophic attached-film expanded-bed bioreactor, Nelson and Jewell (20) reported greater than 99% transformation of 3 mg/L (48  $\mu\text{M}$ ) influent VC, resulting in calculated transformation yields between 0.004 and 0.025 mg of VC/mg of  $\text{CH}_4$  (0.001 and 0.006 mol of VC/mol of  $\text{CH}_4$ ). These reactor culture enrichment values are much less than found in the soil microcosms. Here, in microcosms fed 1.5 or 4 mg/L (95 or 250  $\mu\text{M}$ ) methane, OW29 microcosms exhibited a  $T_y$  of about 1 mg of VC/mg of  $\text{CH}_4$  (0.26 mol of VC/mol of  $\text{CH}_4$ ) while OW18 microcosms achieved a  $T_y$  from 3 to 3.5 mg of VC/mg of  $\text{CH}_4$  (0.77–0.90 mol of VC/mol of  $\text{CH}_4$ ). Although the values may not be directly comparable due to the different systems from which they were obtained [different cultures and/or monooxygenases (18, 21), aqueous vs soil environment, the presence or absence of methane, etc.], the 20–70 times higher  $T_y$  values with the microcosms are significant. Significant also is the difference in  $T_y$  for cultures taken from different locations at the same site. Reasons for such widely different  $T_y$  values for VC transformation by methane utilizers deserves further study.

The soil microcosm results show that significant methane-stimulated biotransformation of VC can be obtained by the indigenous microorganisms at the St. Joseph site. While there appears to be spatial variability in the VC transformation abilities at the site, even the least responsive microcosms demonstrated the ability to transform milligram per liter levels of VC. If similar transformation yields can be obtained in the subsurface at other sites, the applicability of in-situ methane-stimulated VC transformation would appear most promising, giving incentive to solve the engineering problems related to methane-stimulated in-situ biotransformation of VC.

Overall, the microcosms performed well throughout the study. The small sample size did not adversely affect the ability for precise sample analysis. The results were consistently repeatable with duplicate microcosms exhibiting very similar behavior. Differences in VC transformation abilities due to different methane concentrations in the exchange solution and due to aquifer material obtained from different locations were easily discernible. The small size of the microcosms allowed more microcosms to be filled from a limited amount of available aseptically obtained aquifer material. Exchanges using the small microcosms required less time than those using larger soil columns (8), allowing more microcosms to be operated and resulting in more statistically reliable results. While the small microcosms worked well for the fine uniform sand from the St.

Joseph site, they have yet to be tested with larger or more graded materials. However, the simple basic design of the microcosms can be extended to larger test tubes if this is more suitable for a given aquifer material and sample volume required for analyses.

## Acknowledgments

This study was supported with financial assistance from Allied-Signal, Inc., Bendix Automotive Systems—North America; the Gas Research Institute; and the Robert S. Kerr Environmental Research Laboratory; U.S. Environmental Protection Agency, through the U.S. EPA-sponsored Western Region Hazardous Substance Research Center at Stanford University. The content of this paper does not necessarily represent the views of these organizations. Special appreciation is extended to John T. Wilson, Lowell Leatch, and other members of the staff at the Kerr Laboratory who provided advice on this study and the technical assistance required for collecting samples of aquifer material from the St. Joseph site.

## Literature Cited

- (1) Wilson, J. T.; Wilson, B. H. *Appl. Environ. Microbiol.* **1985**, *49*, 242–243.
- (2) Little, D. C.; Palumbo, A. V.; Herbes, S. E.; Lidstrom, M. E.; Tyndall, R. L.; Gilmer, P. J. *Appl. Environ. Microbiol.* **1988**, *54*, 951–956.
- (3) Tsien, H. C.; Brusseau, G. A.; Hanson, R. S.; Wackett, L. P. *Appl. Environ. Microbiol.* **1989**, *55*, 3155–3161.
- (4) Oldenhuis, R.; Oedzes, J. Y.; van der Waarde, J. J.; Janssen, D. B. *Appl. Environ. Microbiol.* **1991**, *57*, 7–14.
- (5) National Research Council. *In Situ Bioremediation When does it work?*; National Academy Press: Washington, DC, 1993; p 207.
- (6) Siegrist, H.; McCarty, P. L. *J. Contam. Hydrol.* **1987**, *2*, 31–50.
- (7) Roberts, P. V.; Semprini, L.; Hopkins, G. D.; Grbic-Galic, D.; McCarty, P. L.; Reinhard, M. EPA Report 600/2-89/033. U.S. EPA: Washington, DC, 1989; 214 pp.
- (8) Lanzarone, N. A.; McCarty, P. L. *Ground Water* **1990**, *28*, 910–919.
- (9) McCarty, P. L.; Semprini, L.; Dolan, M. E.; Harmon, T. C.; Tiedeman, C.; Gorelick, S. M. In *On-Site Bioreclamation*; Hinchee, R. E., Olfenbutter, R. F., Eds.; Butterworth-Heinemann: Stoneham, MA, 1991; p 539.
- (10) Gossett, J. M. *Environ. Sci. Technol.* **1987**, *21*, 202–208.
- (11) Dolan, M. E.; McCarty, P. L. Manuscript in progress.
- (12) Hartmans, S.; de Bont, J. A. M.; Tramper, J.; Luyben, K. C. A. M. *Biotechnol. Lett.* **1985**, *7*, 383–388.
- (13) Davis, J. W.; Carpenter, C. L. *Appl. Environ. Microbiol.* **1990**, *56*, 3878–3880.
- (14) Phelps, T. J.; Malachowski, K.; Schram, R. M.; White, D. C. *Appl. Environ. Microbiol.* **1991**, *57*, 1252–1254.
- (15) Castro, C. E.; Wade, R. S.; Riebeth, D. M.; Bartnicki, E. W.; Belser, N. O. *Environ. Toxicol. Chem.* **1992**, *11*, 757–764.
- (16) Hartmans, S.; de Bont, J. A. M. *Appl. Environ. Microbiol.* **1992**, *58*, 1220–1226.
- (17) Malachowsky, K. J.; Phelps, T. J.; Teboli, A. B.; Minnikin, D. E.; White, D. C. *Appl. Environ. Microbiol.* **1994**, *60*, 542–548.
- (18) Alvarez-Cohen, L.; McCarty, P. L.; Boulygina, E.; Hanson, R. S.; Brusseau, G. A.; Tsien, H. C. *Appl. Environ. Microbiol.* **1992**, *58*, 1886–1893.
- (19) Dolan, M. E.; McCarty, P. L. In *Bioremediation of Chlorinated and Polycyclic Aromatic Hydrocarbon Compounds*; Hinchee, R. E., Leeson, A., Semprini, L., Ong, S. K., Eds.; Lewis Publishers: Chelsea, MI, 1993; p 525.
- (20) Nelson, Y. M.; Jewell, W. J. *J. Environ. Eng.* **1993**, *119*, 890–907.
- (21) DiSpirito, A. A.; Gullledge, J.; Shiemke, A. K.; Murrell, J. C.; Lidstrom, M. E.; Krema, C. L. *Biodegradation* **1992**, *2*, 151–164.

Received for review August 8, 1994. Revised manuscript received March 24, 1995. Accepted March 28, 1995.\*

ES940502A

\* Abstract published in *Advance ACS Abstracts*, May 1, 1995.