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Anaerobic Degradation of Chlorinated Hydrocarbons in Groundwater Aquifers or

*"Chlorinated Hydrocarbon Degradation"*

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TECHNICAL COMPLETION REPORT

Project Number UCAL-WRC-W-849

August, 1997

WATER RESOURCES  
CENTER ARCHIVES

UCI -- 1997

UNIVERSITY OF CALIFORNIA  
BERKELEY

University of California Water Resources Center

The research leading to this report was supported by the University of California Water Resources Center, part of Water Resources Center Project UCAL-WRC-W-849.

## ABSTRACT

Groundwater contamination by chlorinated hydrocarbons, such as tetrachloroethene (PCE) or trichloroethene (TCE), is a major concern throughout the United States. A developing strategy for the remediation of PCE and TCE contaminated aquifers is anaerobic biodegradation. From a TCE contaminated groundwater site, microorganisms were enriched with the ability to anaerobically convert PCE and TCE completely to ethene. Kinetic studies performed with this culture showed that degradation of PCE, TCE, and vinyl chloride (VC) was first order with respect to substrate concentration up to their solubility. It was also shown that, although VC inhibited TCE degradation, a finite degradation rate could be achieved in the presence of high VC concentrations.

PCE and TCE form dense-nonaqueous-phase liquids (DNAPLs) which can provide a persistent groundwater contamination source. Under saturating PCE and TCE conditions, these chlorinated ethenes were rapidly converted to ethene with little or no accumulation of VC. These results suggest that anaerobic remediation can potentially be used in the remediation of PCE and TCE DNAPLs.

## KEYWORDS

Groundwater quality, groundwater remediation, bacteria, chlorination, wastewater treatment, water quality, biological control and treatment

TABLE OF CONTENTS

Abstract .....	ii
Keywords .....	ii
Table of Contents .....	iii
List of Tables .....	iv
List of Figure.....	v
Problem and Research Objectives .....	1
Methodology .....	3
Principle Findings and Significance .....	5
Summary .....	25
Sources Consulted.....	25
Acknowledgement .....	25
Bibliography .....	26

LIST OF TABLES

Table 1: Degradation of trichloroethene with various carbon sources. ....6  
Table 2: Summary of measured kinetic parameters.....14  
Table 3: Production rate comparison under saturating and subsaturating conditions .....18

LIST OF FIGURES

Figure 1: Complete degradation of TCE.....7

Figure 2: Methane, glucose, and acetate profiles during TCE degradation.....10

Figure 3: PCE degradation rates .....11

Figure 4: TCE degradation rates .....12

Figure 5: VC degradation rates .....13

Figure 6: TCE degradation rates in presence of VC .....16

Figure 7: VC production under saturating and subsaturating conditions .....17

Figure 8: Ethene production under saturating and subsaturating conditions .....20

Figure 9: Methane production under saturating and subsaturating conditions .....21

Figure 10: VC and ethene production rates .....22

Figure 11: Methane production and TCE degradation rates .....24

## PROBLEM AND RESEARCH OBJECTIVES

Groundwater contamination by the chlorinated hydrocarbons tetrachloroethene (PCE) and trichloroethene (TCE) is a major problem throughout the United States. Due to their widespread use as degreasing agents, in the production of silicon wafers, in the dry cleaning industry, in household cleaning products, etc., and because of poor handling and disposal practices, these compounds are frequently found as contaminants in groundwater (Dyksen and Hess 1982; Love and Eilers 1982; Vogel, Criddle et al. 1987). A 1980 study of drinking water systems throughout the United States reported that 31 municipal wells in California were closed due to TCE contamination and that wells in 15 Pennsylvania communities were closed for the same reason (Lee, Thomas et al. 1988). Furthermore, vinyl chloride (VC), an intermediate in the PCE and TCE degradation pathways, is a known carcinogen. Since PCE and TCE are suspected carcinogens, and because of the toxicity of VC, these compounds are regulated by the United States Environmental Protection Agency and the Safe Drinking Water Act of 1986.

A common problem associated with the contamination of groundwater systems by PCE and TCE stems from the formation of dense-nonaqueous-phase-liquids (DNAPLs) (Mackay and Cherry 1989; Oolman, Godard et al. 1995). PCE and TCE form DNAPLs that sink through permeable groundwater aquifers until a non-permeable zone is reached at which point they pool. These DNAPL pools are sources of aquifer contamination that can persist for decades.

Anaerobic biodegradation strategies have the potential for the successful remediation of PCE and TCE contaminated aquifers. Several researchers have enriched cultures from sewage sludge that can dechlorinate PCE and TCE completely to ethene or ethane (Freedman and Gossett 1989; DiStefano, Gossett et al. 1991; DiStefano, Gossett et al. 1992; Holliger, Schraa et al. 1993). In our laboratory we have enriched a mixed culture of microorganisms from a TCE contaminated groundwater site that has the ability to anaerobically convert TCE to ethene (Bolesch, Nielsen et al. 1997). We have also shown that this culture has the ability to

dechlorinated PCE (Nielsen and Keasling 1997). To effectively utilize anaerobic degradation as a remediation strategy, several aspects of the process must be understood. Many of these aspects form the basis of our research objectives:

- 1) to enrich for anaerobic microorganisms which will reductively dechlorinate chlorinated alkenes completely in liquid cultures;
- 2) to determine the key nutrients necessary for complete dechlorination;
- 3) to determine what components of the liquid medium must be added to the groundwater to stimulate TCE degradation;
- 4) to determine if the same anaerobic biodegradation process can be used for chlorinated hydrocarbons other than TCE and PCE;
- 5) to determine what types of microorganisms are responsible for the degradation process;
- 6) to determine how intermediates in the degradation pathway affect degradation of the primary contaminant;
- 7) to determine the effect DNAPLs will have on degradation;
- 8) to determine if anaerobic biodegradation of TCE can be used at contaminated sites other than the one from which we have obtained samples;

## METHODOLOGY

### Culture Medium and Growth Conditions

Groundwater samples were collected from a TCE-contaminated site (Santa Clara County, California) by transferring 50 mL of groundwater to 160-mL serum bottles containing 50 mL of a minimal salts medium. To these bottles various carbon sources and TCE were added. From these samples, a mixed culture of microorganisms capable of degrading TCE completely to ethene under anaerobic conditions was obtained (Bolesch, Nielsen et al. 1997).

During cultivation, 100-mL aliquots of medium were kept in 160-mL serum bottles sealed with butyl-rubber septum under anaerobic conditions. To these bottles, 100  $\mu$ L of 1M glucose was added as the electron donor. Either 100  $\mu$ L of PCE-saturated water or 25  $\mu$ L of TCE-saturated water was added as the electron acceptor. Once the primary electron acceptor had been degraded, the headspace of the bottle was purged with nitrogen. The bottle was then inoculated with the desired electron acceptor and glucose. This process was repeated as necessary.

### Analytical Techniques

Gas chromatography was used to measure PCE, TCE, VC, ethene, and methane concentrations in the headspace of the serum bottles. PCE and TCE concentrations were measured using an HP 6890 GC fitted with a capillary column (HP-624; Hewlett Packard; San Fernando, CA) and an electron capture detector. For the kinetic studies, the VC, ethene, and methane concentrations were measured using an HP 6890 GC fitted with a capillary column (Poraplot-Q; Hewlett Packard; San Fernando, CA) and a flame ionization detector. For the saturation studies, the VC, ethene, and methane concentrations were measured using a Varian GC fitted with a packed column (Porapak-Q; Alltech; Deerfield, IL). The liquid concentration of



each compound was then calculated using Henry's Law and published values of the Henry's constant for each compound (Gossett 1987). The chromatographic peak areas obtained from the measurements were converted to concentrations by preparing standard curves for each compound of interest.

### Kinetic Studies

For each kinetic study, 6-mL aliquots of medium containing bacteria were added to 10-mL serum bottles that had been purged with nitrogen to create an anaerobic environment. Glucose was added to each bottle to a final concentration of 16.7 mM. Finally, PCE (Sigma; St. Louis, MO), TCE (Aldrich; Milwaukee, WI), or VC (VC: Alltech; Deerfield, IL) was added in varying amounts. Headspace samples were taken and analyzed at frequent intervals. The initial degradation rates were calculated from the slope of the time-concentration curves. When the effect of VC on TCE degradation was measured, the same amounts of TCE were added to each bottle.

### Degradation Studies

To compare rates of PCE and TCE degradation under saturating and subsaturating conditions, 100-mL aliquots of media containing bacteria were added to 160-mL serum bottles which had been purged with nitrogen to create an anaerobic environment. To each bottle 100  $\mu$ L of 1M glucose was added. For the experiments under subsaturating conditions, 25  $\mu$ L of TCE-saturated water or 175  $\mu$ L of PCE-saturated water was added to the bottles. These aliquots contain an equal number of moles of TCE and PCE. For the experiments under saturating conditions, 50  $\mu$ L of PCE or 250  $\mu$ L of TCE was added to the bottles. These amounts correspond to twice that necessary to saturate the aqueous phase. A visible organic phase

(bubble) at the bottom of the bottle confirmed saturation. The bottles were incubated at 30°C. Headspace samples were analyzed for VC, ethene, methane, PCE, and TCE.

## PRINCIPLE FINDINGS AND SIGNIFICANCE

### Enrichment of Anaerobic Microorganisms

Serum bottles were inoculated with 50 mL of the minimal salts medium (containing yeast extract) and 50 mL of groundwater from the contaminated site. Glucose, acetate, formate, and methanol were added as carbon sources. The cultures grown in glucose showed complete conversion to ethene (table 1). The cultures grown in acetate, formate, and methanol incompletely degraded TCE resulting in the products of DCE, VC, and ethene (table 1).

The cultures grown in glucose were used to inoculate serum bottles containing medium, yeast extract, and glucose. The culture showed TCE degradation activity after 10 days of incubation degraded 0.5 µg/ml TCE to below detectable limits in 20 days. In the three-month monitoring period, the intermediate product, *cis*-CDE, was degraded to VC and then to ethene. This culture was later enriched on TCE with increasing degradation rates (figure 1). This enriched culture degraded 33 µg of TCE in 12 days with an apparent TCE degradation rate of 20 µg/L/day. This rate compares to the TCE degradation rate of the unenriched culture. However, the time required for complete conversion of TCE to ethene is significantly less (weeks compared to months for the unenriched culture). In separate experiments, we have shown that anaerobic TCE degradation will not occur in medium not inoculated with ground water samples (data not shown).

Table 1

Degradation of Trichloroethene with Various Carbon Sources

Carbon Source	Chlorinated Ethene			
	TCE	DCE	VC	Ethene
Glucose	-	-	-	++++
Acetate	-	+	+	+
Formate	-	+	++	+
Methanol	-	+	++	+

Table symbols: none detected (-), small amount detected (+), moderate amount detected (++), and large amount detected (++++).

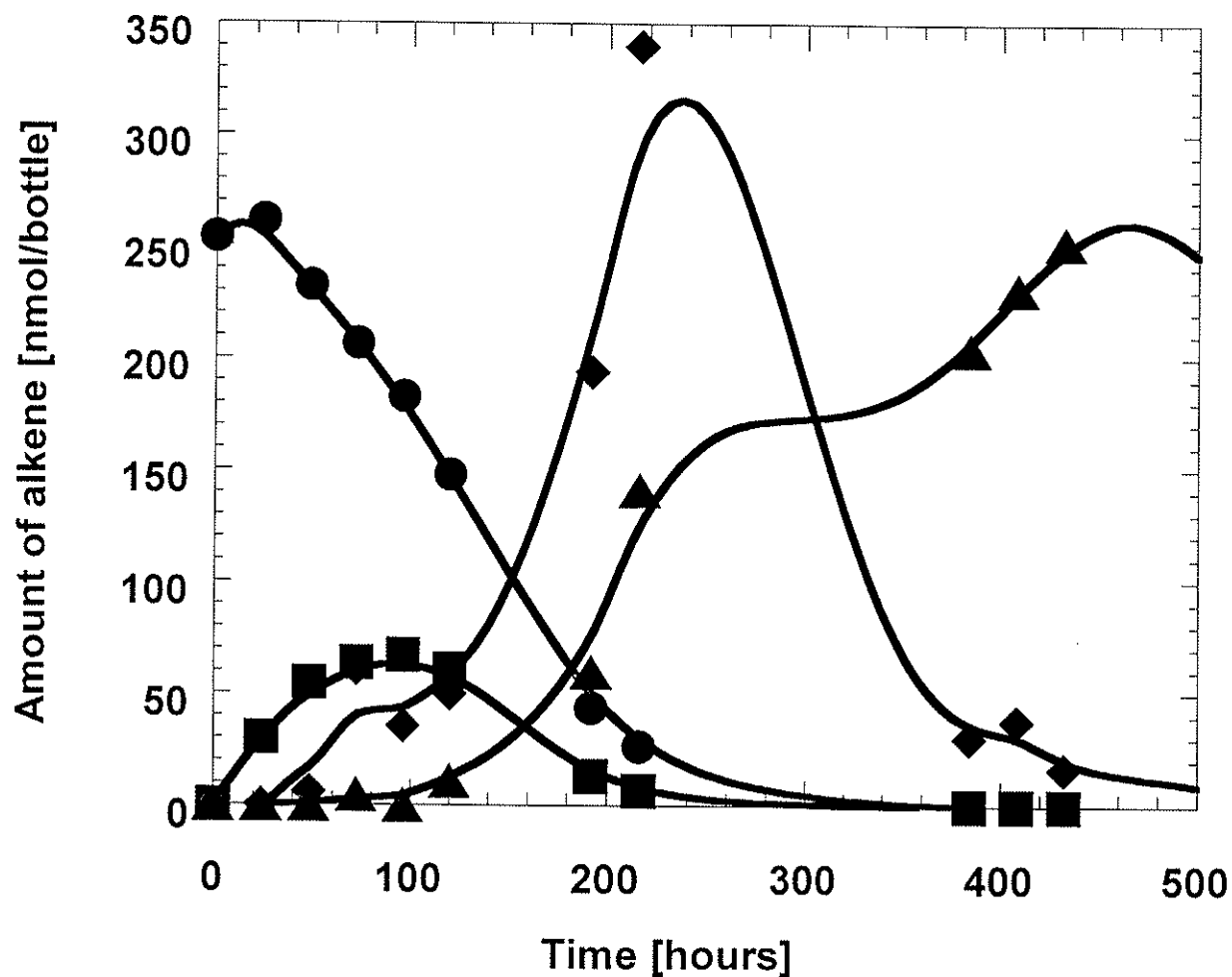


Figure 1. Complete degradation of TCE to ethene by an enriched groundwater culture using glucose as the carbon source. Filled circles: TCE. Filled squares: cis-DCE. Filled diamonds: VC. Filled triangles: ethene

The results presented above demonstrate complete reductive dechlorination of TCE to ethene in a laboratory groundwater microcosm inoculated from a contaminated groundwater aquifer. To the extent that the *in-situ* aquifer environment is similar to the microcosm studies, these results indicate the possibility of using reductive dechlorination as a method of remediating TCE contaminated groundwater sites once a suitable electron donor is supplied.

#### Key Nutrients for Dechlorination

A minimal salts medium was used in the culture studies to provide the nutrients needed for TCE dechlorination. The medium was amended with yeast extract to stimulate degradation. Glucose, acetate, formate, and methanol were added as electron donors (table 1). Of these electron sources, glucose proved the best at stimulating degradation.

#### Required Liquid Medium Components

To achieve TCE degradation at a contaminated groundwater site, a carbon source must be supplied. Based on our studies, the preferable carbon source is glucose. Furthermore, yeast extract must also be supplied to stimulate degradation.

#### Anaerobic Degradation of other Chlorinated Hydrocarbons

Various chlorinated methanes and ethanes were added to culture bottles to act as electron acceptors. However, degradation of these compounds was not observed. Thus, this enriched culture appears to degrade solely chlorinated ethenes.

### Bacteria Involved in Degradation

To effectively utilize anaerobic dechlorination of TCE as a remediation strategy, it is helpful to know what types of bacteria are responsible for the overall degradation process. The by-products produced during degradation give insight into the types of microorganisms that might be involved. Two important by-products of TCE degradation in the presence of glucose are methane and acetate (figure 2). We propose that glucose is converted to acetate by an acetogen and that the methane production occurs due to the presence of a methanogen. These data also show that glucose is rapidly utilized and that dechlorination continues in the absence of glucose. This observation suggests that something other than glucose acts as the electron donor in all or part of the conversion of TCE to ethene.

### Effect of Degradation Intermediates

The kinetics of PCE and TCE degradation were examined by determining the initial “degradation” or “disappearance” rates as a function of the initial substrate concentrations. For concentrations of PCE, TCE, and VC up to their solubility limits, the initial reaction rate was first-order with respect to substrate concentration (figures 3, 4, and 5). If one assumes that the reductive dechlorination rate has a Monod dependence on the concentration of the chlorinated hydrocarbon, [S], then

$$\frac{d[S]}{dt} = -V_{\max} \frac{[S]}{K_S + [S]} [X]$$

where [X] is the biomass concentration. Since no conditions were found where the PCE, TCE, or VC degradation rates were zeroth-order with respect to the initial concentrations, one can only estimate  $V_{\max}/K_S$  (table 2). The results of the PCE and TCE kinetic studies indicate that no inhibition of either PCE or TCE degradation occurred at concentrations approaching saturation. The implication from these observations is that the possibility exists for the use of anaerobic

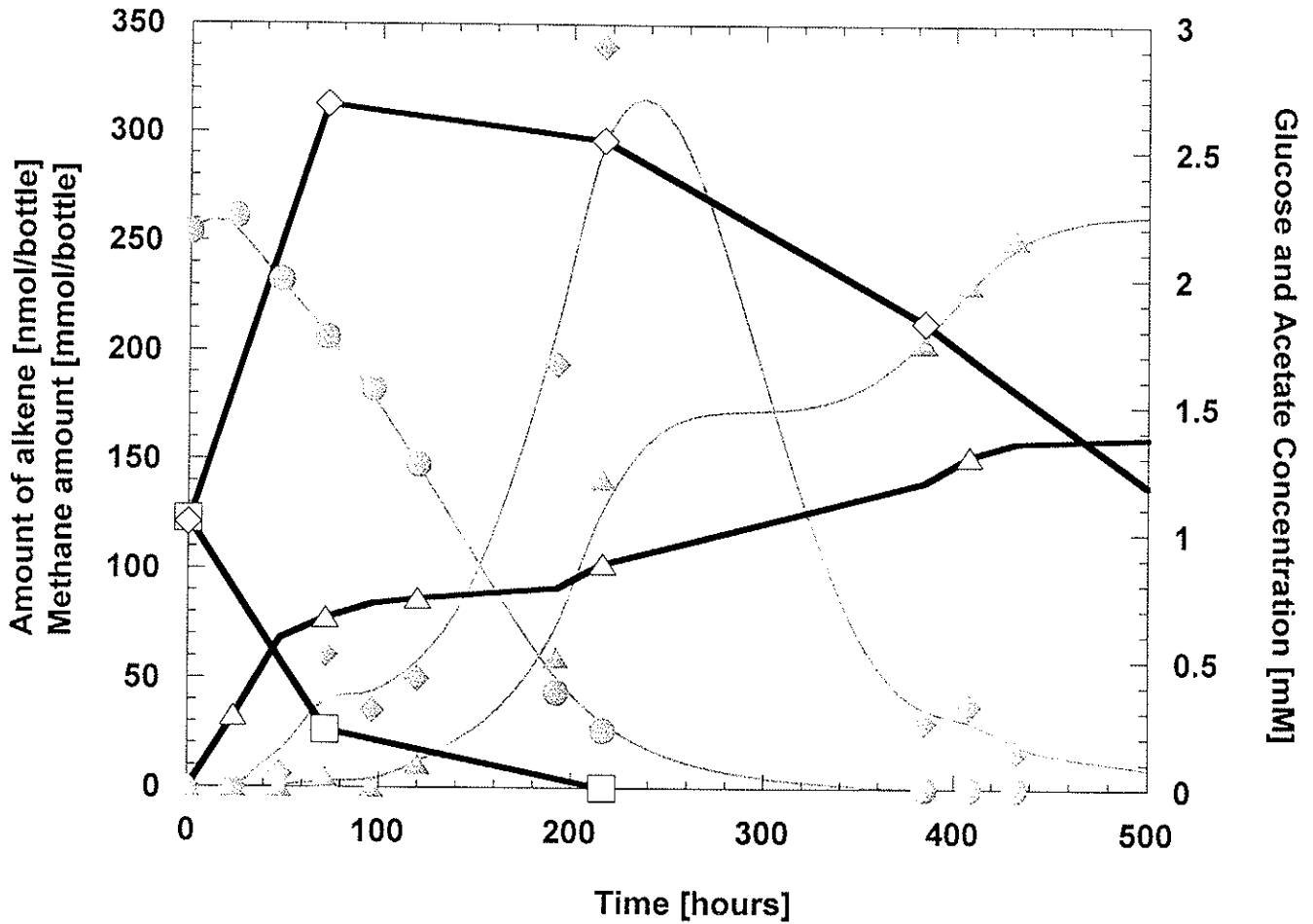


Figure 2. Methane production and glucose and acetate utilization during the complete degradation of TCE to ethene by an enriched groundwater culture using glucose as the carbon source. Filled circles: TCE. Filled diamonds: VC. Filled triangles: ethene. Open squares: glucose. Open diamonds: acetate. Open triangles: methane.

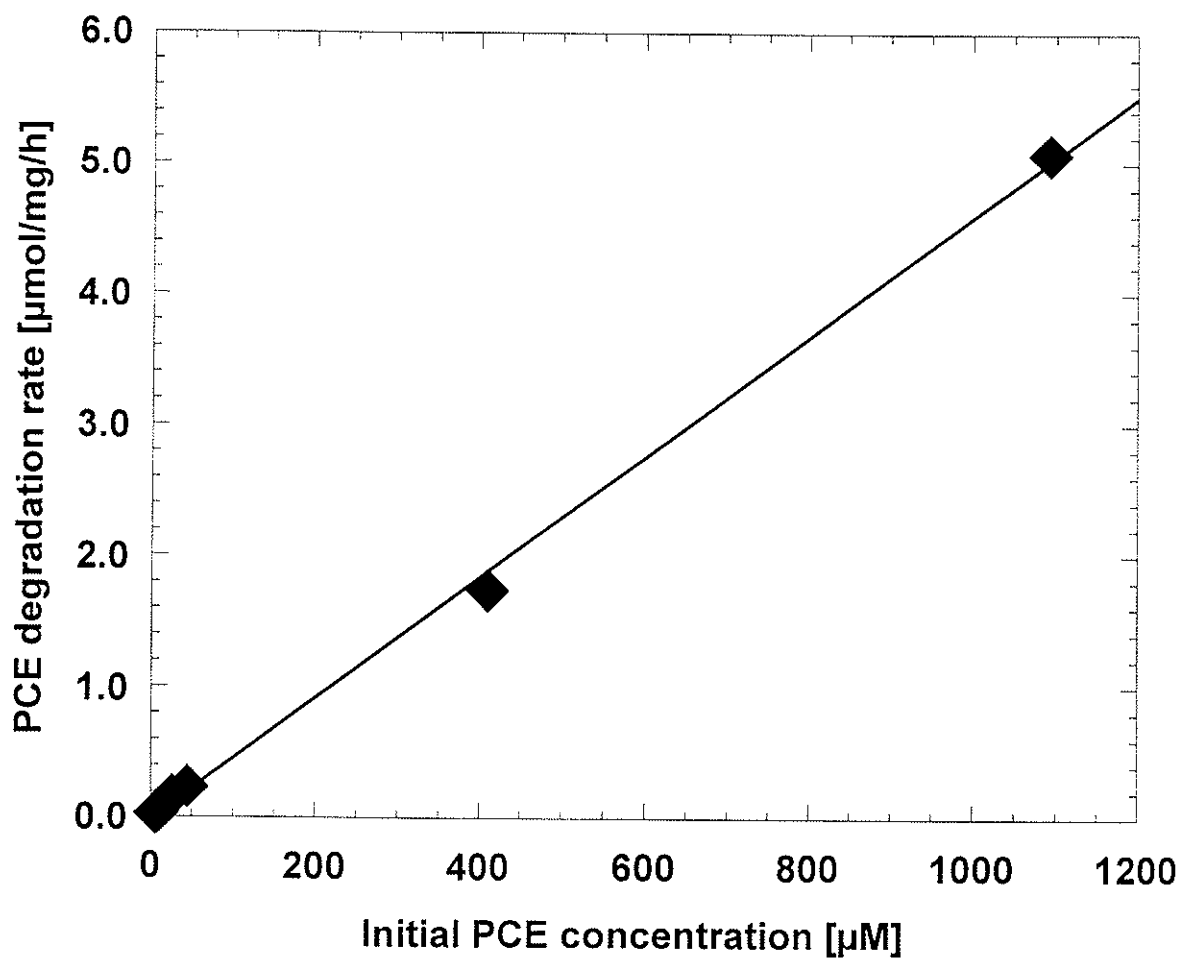


Figure 3. PCE degradation rate as a function of initial PCE concentration.



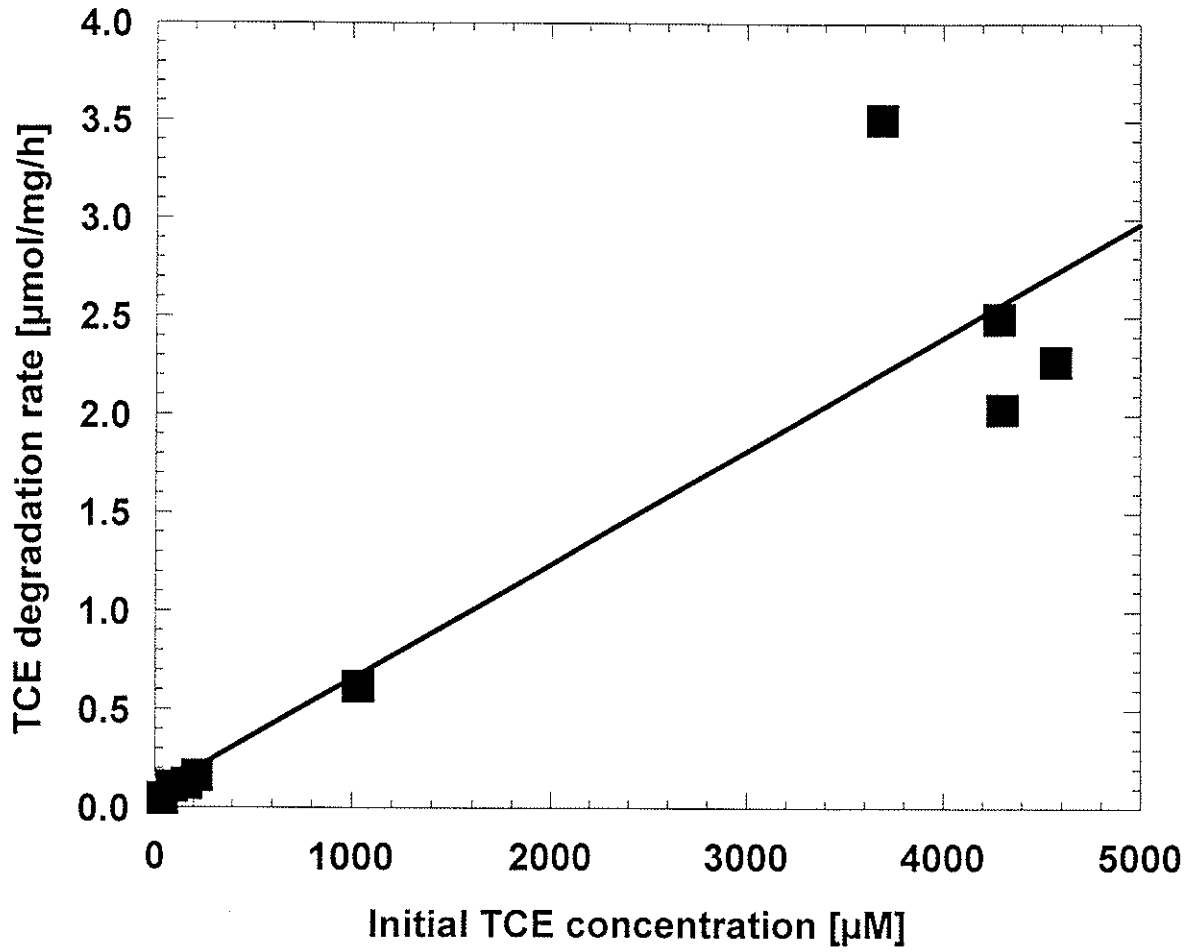


Figure 4. TCE degradation rate as a function of initial TCE concentration.

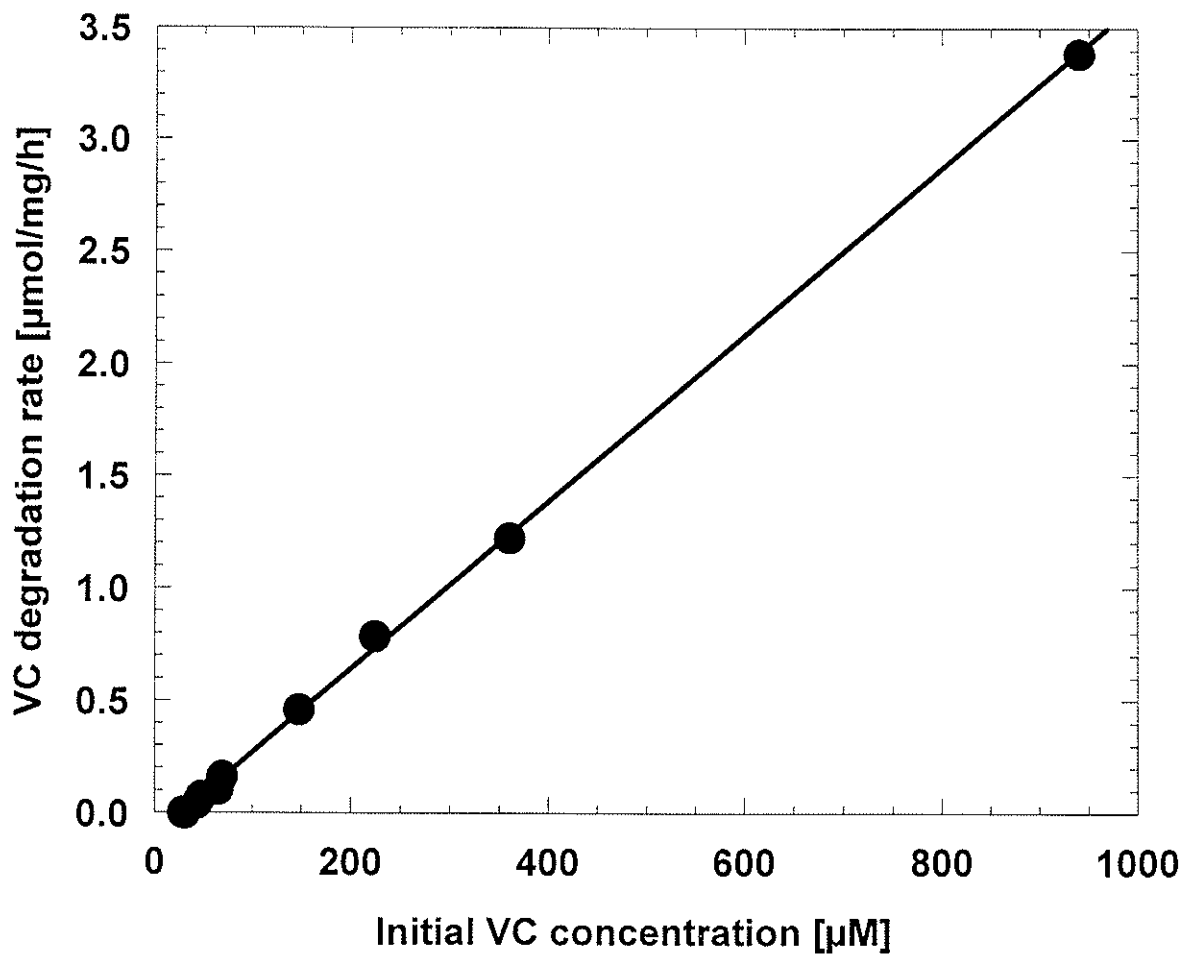


Figure 5. VC degradation rate as a function of initial VC concentration.

Table 2

Summary of Measured Kinetic Parameters

	$V_{\max}/K_S$ [nmol mg <sup>-1</sup> h <sup>-1</sup> μM <sup>-1</sup> ]
PCE	4.6
TCE	0.58
VC	3.7

PCE and TCE degradation in areas of high PCE and TCE concentration. Such areas would exist near PCE and TCE DNAPL pools

Since VC is a long-lived intermediate in the reductive dechlorination of TCE and PCE (Freedman and Gossett 1989; Bolesch, Nielsen et al. 1997), the effect of the initial VC concentration on the TCE degradation rate was investigated. The experimental results indicate that as the VC concentration increases, the TCE degradation rate decreases slightly (figure 6). Thus, some inhibition of TCE degradation by VC occurs. However, it is important to note that even at high VC concentrations, TCE degradation occurs. The fact that VC only slightly inhibits TCE degradation has a significant impact on the decision of whether to use anaerobic remediation as a strategy to remediate a TCE-contaminated aquifer. One concern is that as VC is produced, the TCE degradation rate would decrease and cease before the TCE is completely remediated from the contaminated site. However, the data obtained from this kinetic study indicate that TCE degradation will continue at a finite rate when large amounts of VC are present.

#### Degradation of DNAPLs

Since no inhibition of degradation occurred at high PCE and TCE concentrations, the kinetics of PCE and TCE degradation under saturating conditions were investigated. The production of VC and ethene was used as an indicator of degradation since it is assumed that both PCE and TCE are converted rapidly to VC and/or ethene and since the concentration of PCE and TCE should remain constant as long as a separate organic phase exists. Under subsaturating conditions, VC was produced and accumulated rapidly and was then converted to ethene (figure 7). However, under saturating conditions, VC accumulated to much smaller concentrations than under subsaturating conditions (figure 7) and the production rate of VC was approximately 80 times smaller than the VC production rate under subsaturating concentrations (table 3). Furthermore,

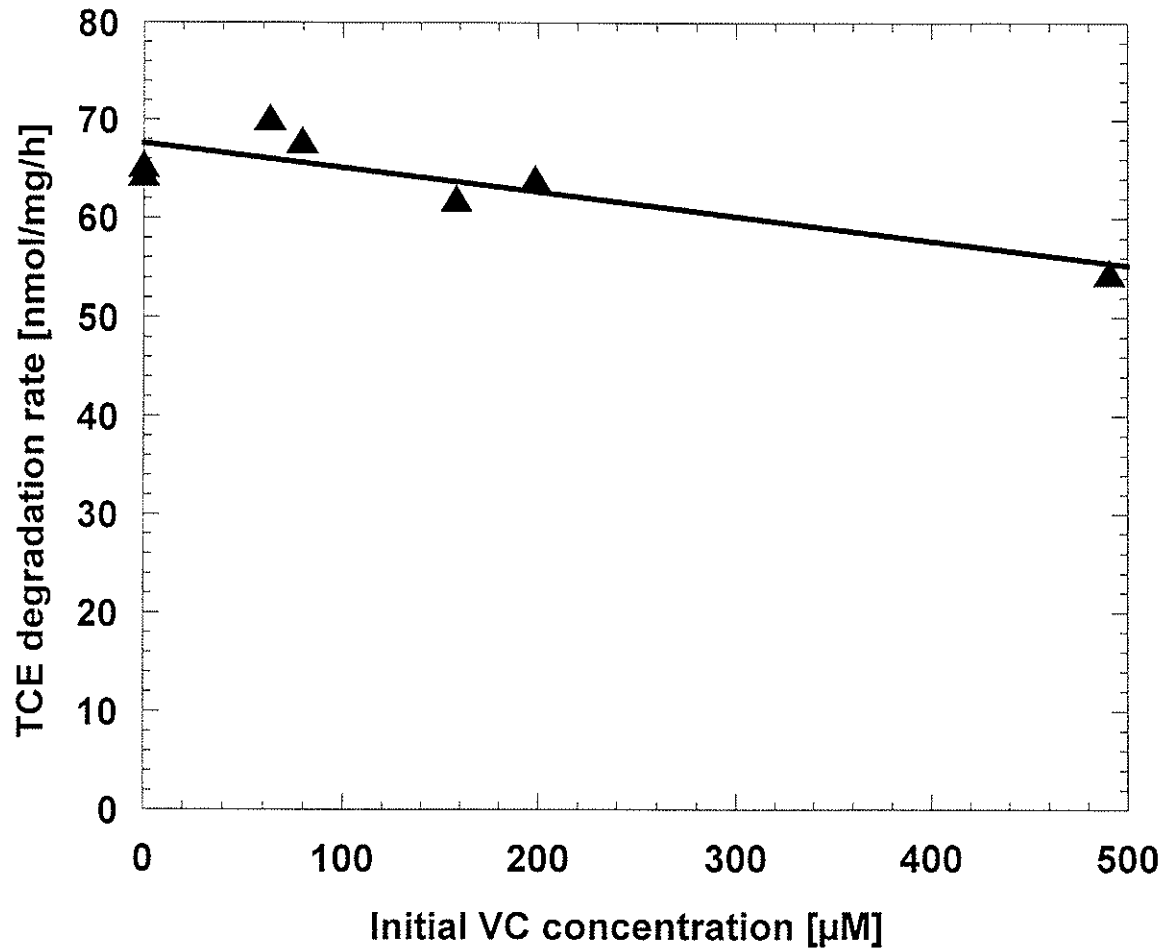


Figure 6. TCE degradation as a function of the initial VC concentration. The initial TCE concentration was 7.3 µM.

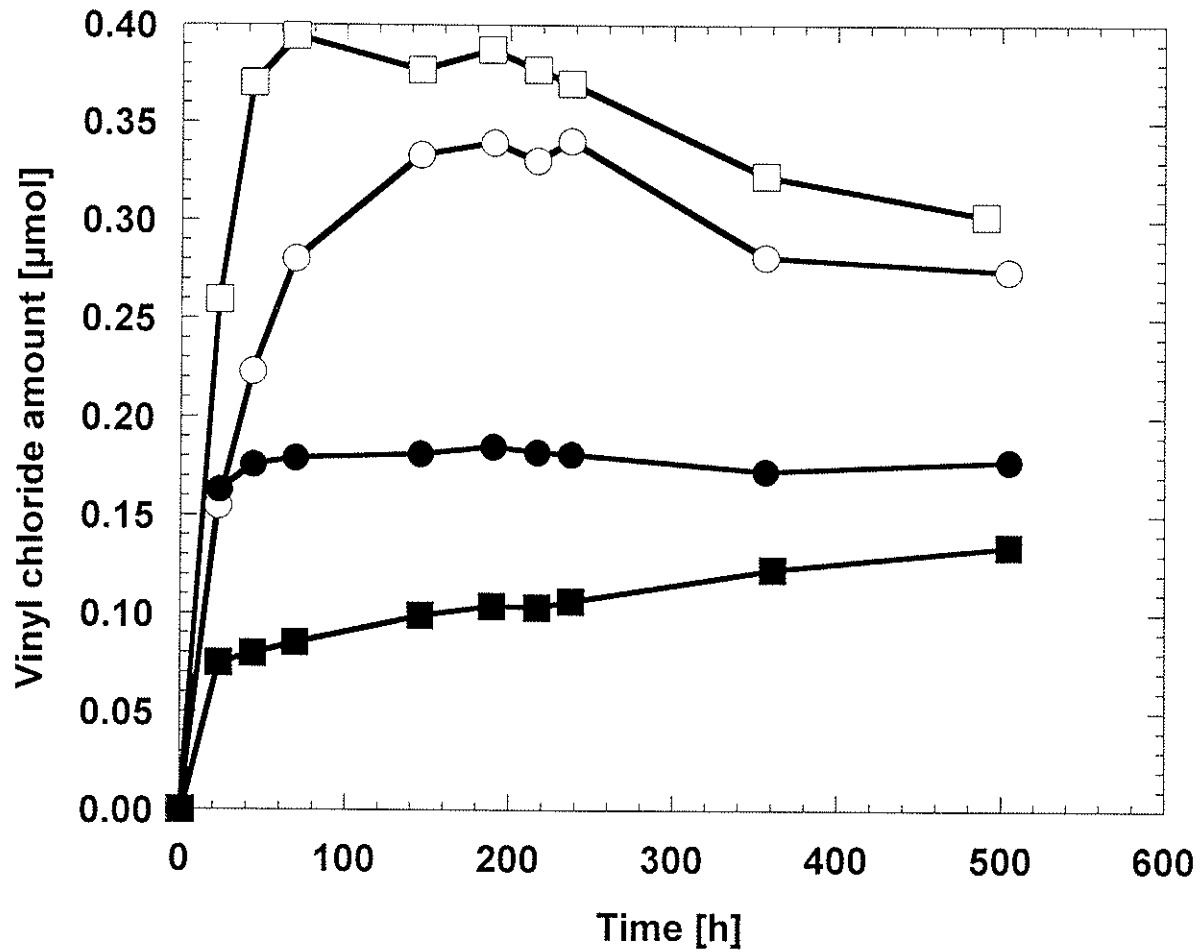


Figure 7. VC production during reductive dechlorination of PCE and TCE under saturating and subsaturating conditions. Filled circles: Saturating PCE conditions. Open circles: Subsaturating PCE conditions. Filled squares: Saturating TCE conditions. Open squares: Subsaturating TCE conditions.

Table 3

Production Rate Comparison under Saturating and Subsaturating Conditions

Production rates [nmol/h]	TCE		PCE	
	Subsaturating	Saturating	Subsaturating	Saturating
Vinyl chloride	8.59	0.12	5.19	0.05
Ethene	0.16	19.54	0.12	3.43
Methane	5300	~0	5000	~0

Production rates are based on the total amount of compound produced in each serum bottle as determined by headspace analysis

the production of ethene in the saturated system occurred quite rapidly and was approximately 120 times greater than the production of ethene in the subsaturated system (figure 8). Similar results were obtained in duplicate experiments (data not shown).

The results for PCE degradation under both saturating and subsaturating conditions were similar to those for TCE degradation (figures 7 and 8 and table 3). The production rate of VC under PCE subsaturating conditions was approximately 95 times that under saturating conditions (figure 7), whereas the ethene production rate under saturating conditions was approximately 30 times the production rate under subsaturating conditions (figure 8). Methane production rates obtained from this study indicate that methanogenesis is inhibited at high PCE and TCE concentrations (figure 9). At subsaturating PCE and TCE concentrations, the total methane production rate was 5.0  $\mu\text{mol/h}$ . However, under saturating conditions, the total methane production rate was approximately zero.

The subsaturating PCE and TCE concentrations used in the aforementioned studies were well below saturation. To determine whether the decrease in VC production rate and the increase in ethene production rate occurs at a single concentration or over a range of concentrations, the VC and ethene production rates were measured over a range of TCE concentrations. It can be seen from this data that the rate of conversion of TCE to ethene increases as the TCE concentration increases (figure 10).

The rapid conversion of PCE and TCE to ethene with little accumulation of VC under saturating conditions is an interesting and unexpected result. One possible explanation for this phenomenon is competition for electrons between the different organisms present in the mixed culture. Previous studies of reductive dechlorination by this culture have shown the rapid utilization of glucose and accumulation of acetate and methane (figure 2), suggesting the presence of acetogens and methanogens. Interspecies hydrogen transfer between acetogens and methanogens is well documented (Zinder 1993) It is possible that in the presence of



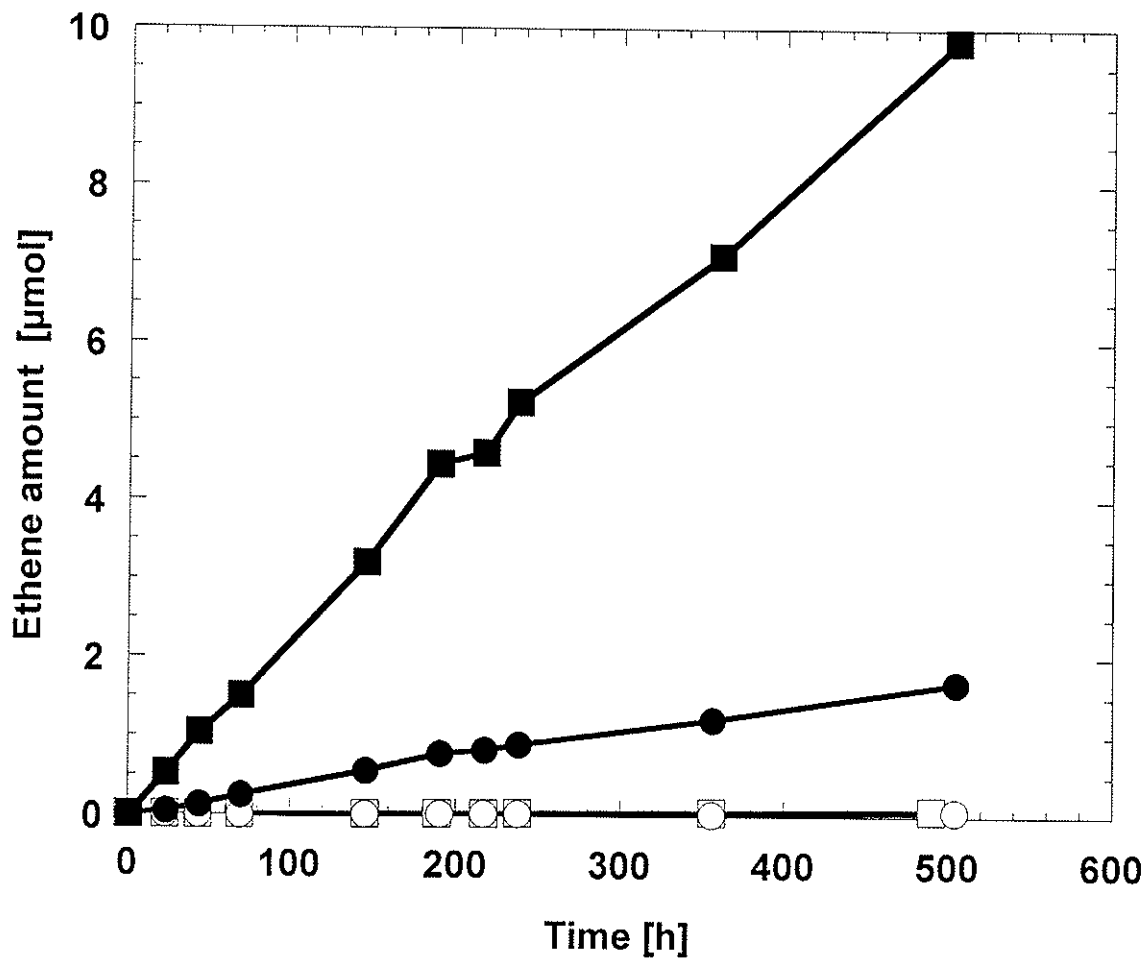


Figure 8. Ethene production during reductive dechlorination of PCE and TCE under saturating and subsaturating conditions. Filled circles: Saturating PCE conditions. Open circles: Subsaturating PCE conditions. Filled squares: Saturating TCE conditions. Open squares: Subsaturating TCE conditions.

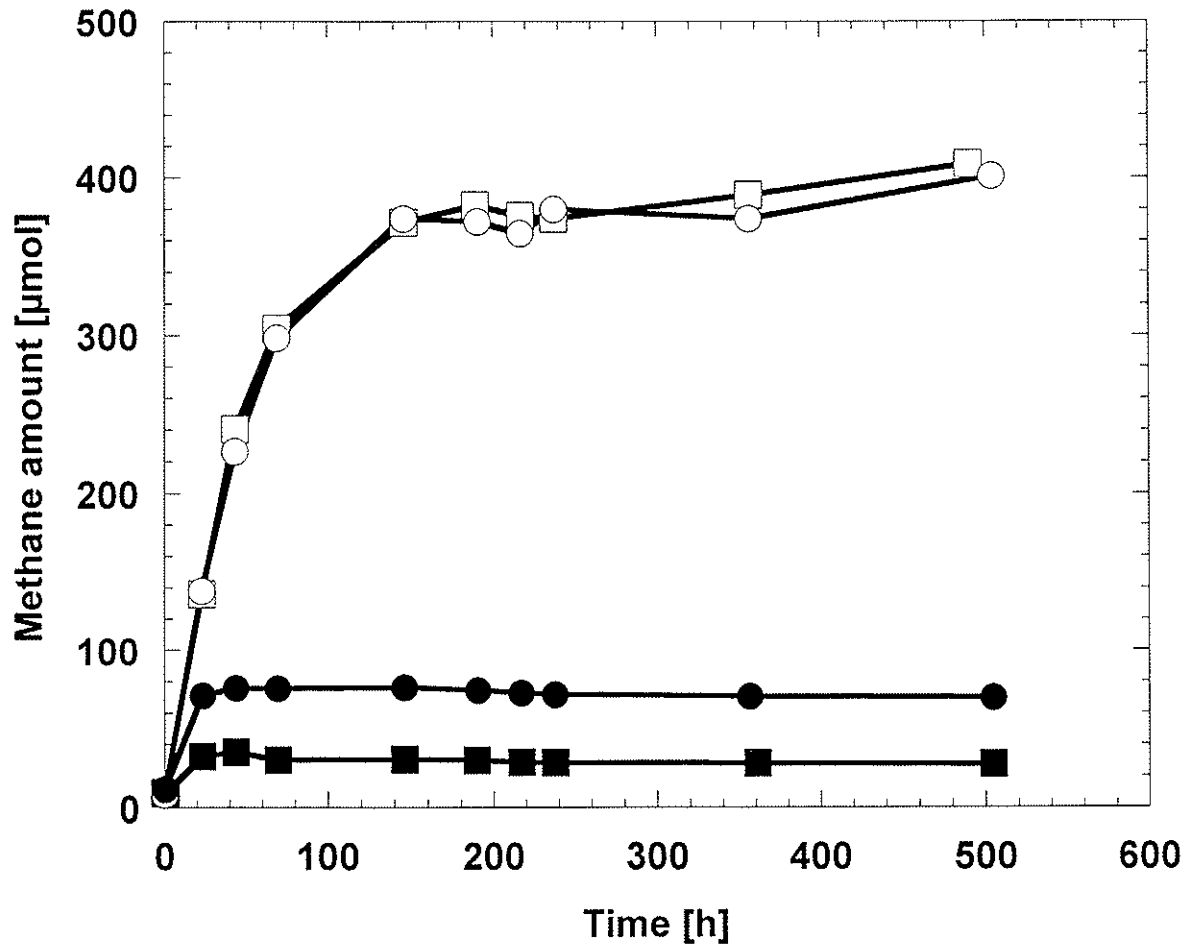


Figure 9. Methane production during reductive dechlorination of PCE and TCE under saturating and subsaturating conditions. Filled circles: Saturating PCE conditions. Open circles: Subsaturating PCE conditions. Filled squares: Saturating TCE conditions. Open squares: Subsaturating TCE conditions.

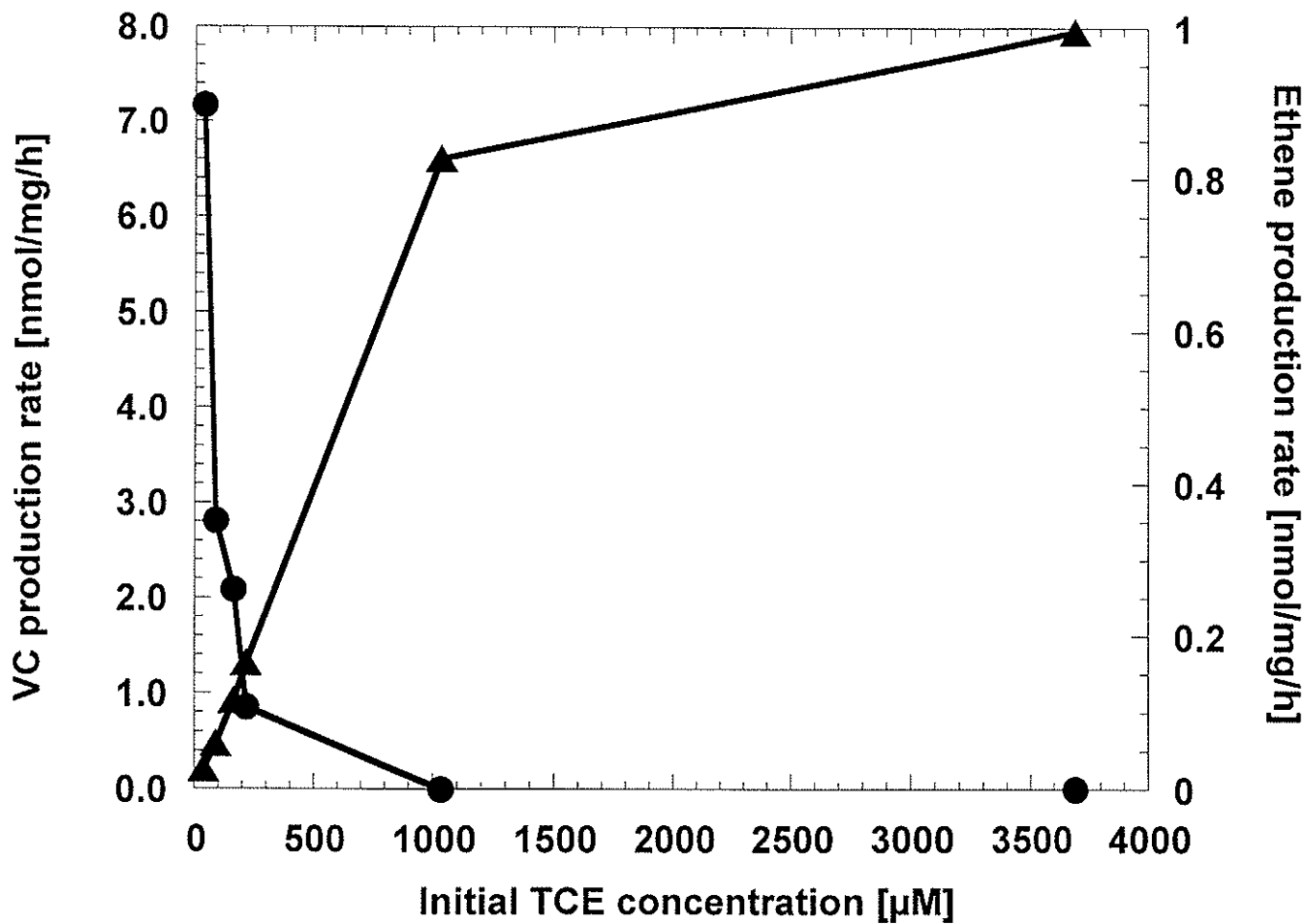


Figure 10. VC and ethene production rates as a function of initial TCE concentration. Filled circles: VC. Filled triangles: ethene.

subsaturating concentrations of TCE or PCE, the degrader must compete with the methanogen for hydrogen produced by acetogens. However, in the presence of saturating concentrations of TCE or PCE, methanogenesis would be inhibited (a phenomenon that has been documented by DiStefano and coworkers (DiStefano, Gossett et al. 1991)) and the dechlorinating organism would not need to compete for hydrogen. The results obtained from the kinetic studies lend support to this explanation: as the TCE concentration increases, methane production decreases (as methanogenesis becomes inhibited), VC accumulation decreases, and ethene production increases (figures 10 and 11).

#### Use of Anaerobic Degradation at Other Sites

Geomatrix Consultants, Inc. (San Francisco, CA) obtained permission from the California Environmental Protection Agency to use this treatment method at the contaminated site where the microorganisms were originally obtained. Corn syrup and yeast extract were pumped into the groundwater at the contaminated site, the bacteria present were enriched, and the redox potential was reduced to the levels necessary for TCE degradation. However, the rich carbon source also increased the viscosity of the groundwater making it difficult to measure the chlorinated hydrocarbon levels. Geomatrix will attempt to apply this method at a TCE contaminated site in Brazil. Furthermore, we anticipate its use at other TCE contaminated sites.

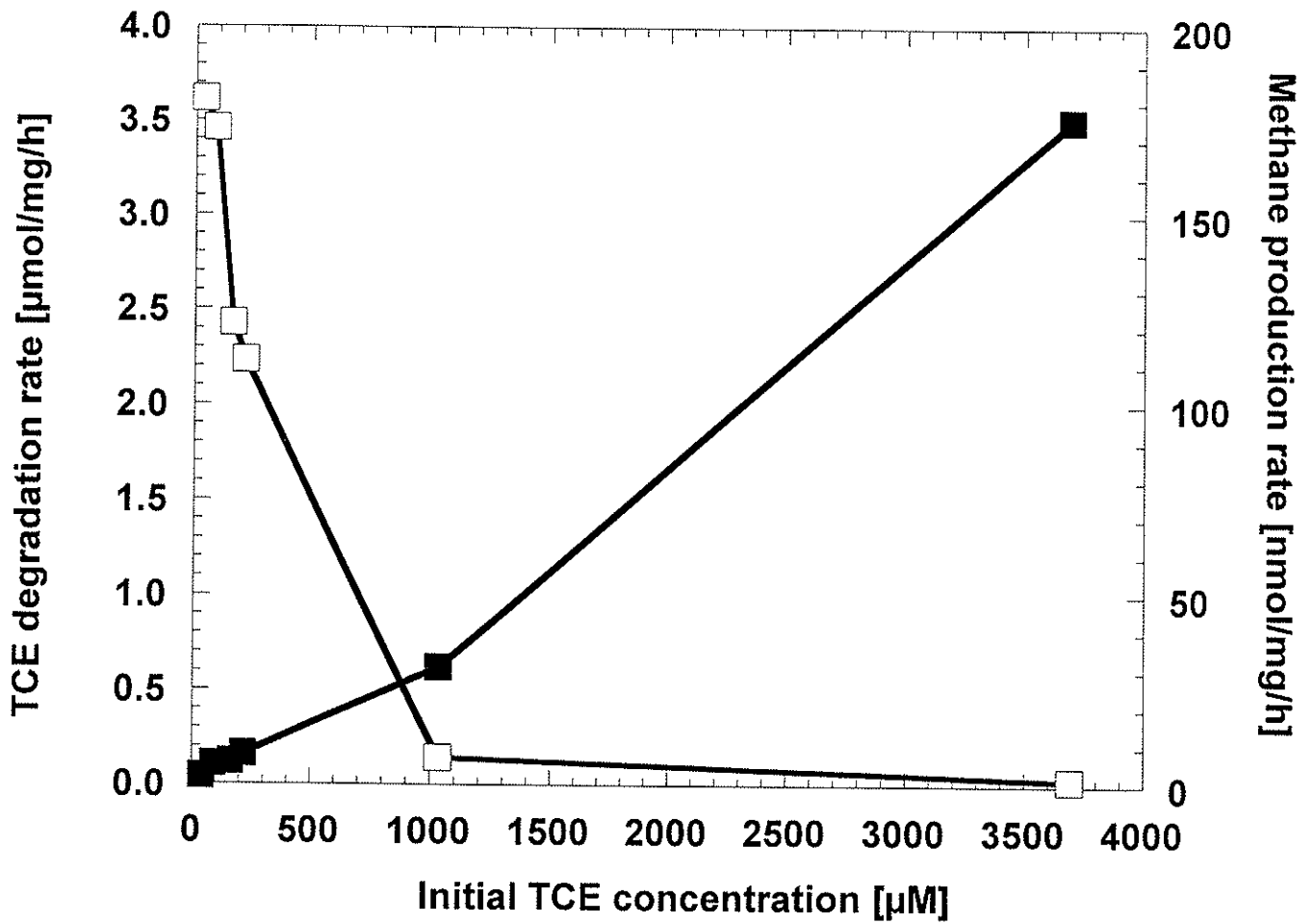


Figure 11. Methane production rates and TCE degradation rates as a function of initial TCE concentration. Filled squares: TCE. Open squares: methane.

## SUMMARY

Groundwater contamination by PCE and TCE is a major concern throughout the United States. One possible means of remediating such contaminated sites is anaerobic biodegradation. We have enriched a culture of microorganisms from a TCE contaminated groundwater site that has the capability to anaerobically degrade PCE and TCE, via the intermediate VC, completely to ethene using glucose as the electron donor. Kinetic studies performed with this mixed culture showed that PCE, TCE, and VC degradation is first-order with respect to substrate concentration up to their solubility. Furthermore, a finite TCE degradation rate in the presence of VC was demonstrated. This culture also showed the unexpected behavior of rapidly converting PCE and TCE to ethene with little or no accumulation of VC under saturating PCE and TCE conditions. These results suggest that using anaerobic degradation to remediate PCE and TCE DNAPLs has potential.

## SOURCES CONSULTED

We collaborated extensively with Geomatrix Consultants who provided the TCE dechlorinating organisms as well as financial support.

## ACKNOWLEDGEMENT

The research leading to this report was supported by the University of California Water Resources Center, as part of Water Resources Center Project UCAL-WRC-W-849

We also acknowledge Chevron and Geomatrix Consultants for their support of this work.

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