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Emerging Technology Summary

Pilot-Scale Demonstration of a Two-Stage Methanotrophic Bioreactor for Biodegradation of Trichloroethene in Groundwater

BioTrol, **Inc., developed a two-stage, methanotrophic, bioreactor system for remediation of water contaminated with trichloroethylene (TCE) and other chlorinated, volatile, aliphatic hydrocarbons. The first stage was a suspended-growth culture vessel with a bubbleless methane transfer device. The second stage was a plug-flow reactor fed with contaminated groundwater and effluent from the culture vessel. The system was tested at bench- and pilot-scale. When operating optimally, 89% of the influent TCE was degraded. Reactor kinetics were consistent with first-order biodegradation kinetics. Actual methane use in the pilot-scale reactor resulted in projected methane costs of $0.33 per 1000 gal of water treated. This cost could be reduced by modifications to the system. Calculated theoretical minimum methane costs were < $0.05 per 1000 gal. Variability in the degree of TCE degradation and difficulty in maintaining the activity of the microbial culture during continuous operation were noted. Sustained use of the technology will require modifications to culture conditions.**

This Summary was *developed by EPA's Risk Reduction Engineering Laboratory, Cincinnati, OH, to announce key findings of the SITE Emerging Technology program that is fully documented in a* separate *report (see Project Report ordering information* at *back).*

Introduction

Chlorinated, volatile, aliphatic hydrocarbons (Cl_x-VOCs) are the most commonly reported contaminants of groundwater. The reason for their widespread occurrence in the environment is their widespread use as solvents and degreasers. Since this problem came to light as recently as the early 1980s few approaches have been developed for remediating TCE-contaminated sites. Currently available remediation methods for subsurface environments include air sparging of the groundwater, vacuum extraction of contaminants from the vadose zone, and extraction of contaminated water for air-stripping. These techniques transfer contamination from the subsurface environment to either the air or to activated carbon, which must then be landfilled or incinerated. Landfilling the contaminated activated carbon transfers the contamination to another environment, and incineration is costly and requires considerable energy and capital equipment to completely oxidize volatile chemicals. Treatment systems based on oxidation of contaminants that use ultraviolet radiation

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in combination with a chemical oxidant (peroxide or ozone) are also available, but these methods are energy intensive and require addition of expensive chemicals.

A number of particularly promising new approaches rely on bacterial cooxidation of the Cl_x-VOCs during growth on another (primary) carbon source. One such bacterium is the obligate methanotroph Methylosinus trichosporium OB3b (hereafter, M.t. OB3b). This organism produces soluble methane monooxygenase (sMM0) when grown on single-carbon substrates (methane, methanol, or formate). SMMO is an enzyme of low substrate specificity capable of catalyzing a variety of oxidation reactions in addition to the oxidation of methane. Among those reactions are the oxidations of several Cl_x-VOCs (see Table 1 for list)-reactions often resulting in stoichiometric quantities of mineral end products (carbon dioxide, water, and chloride ion).

The full report addresses the use of *M.t.* OB3b for remediation of TCE-contaminated groundwater with the use of a two-stage bioreactor. In this system, cells produced at high concentration in a culture medium contacted contaminated groundwater in a plug-flow reactor. The objectives of the study were:

- to determine reactor design parameters at bench scale and to operate a pilot-scale reactor to achieve degradation of TCE and
- to determine operating values for parameters that influence the economic competitiveness of the system.

Since the economic viability of the system was dependent on the efficiency of methane utilization in the culture vessel, an innovative methane transfer method was used to increase methane transfer efficiency.

Procedure

In bench-scale experiments, the conceptual design was evaluated and starting values for the operational parameters of the bioreactor were determined. After the concept was confirmed at the bench, a pilot-scale reactor that used the design criteria established during the bench tests was constructed.

For the bench-scale system, cells were grown in a 2000 mL chemostat vessel in 1000 mL of culture medium and fed to fabricated glass columns where they contacted contaminated water. The total flow rate was adjusted by adding make-up water. Influent and effluent TCE concentrations were measured over the plug-flow reactor before and after initiation of cell culture flow to the column. Thus, conservation of TCE was established before introducing the cells.

For the pilot test, groundwater treated by air-stripping to remove TCE was obtained from a nearby army munitions facility and carried in a stainless-steel tank truck to BioTrol's pilot testing facility. The water was then piped from the truck to a 500 gal, polyethylene surge tank and metered into a stainless-steel plug-flow reactor at a controlled rate. A high-concentration TCE solution (prepared in degassed distilled water) was metered into the influent groundwater upstream from the influent sampling port. The TCE solution was held in a Teflon gas-sampling bag that collapsed as the TCE solution was pumped out. TCE and cells were added within the closed reactor system to avoid TCE losses by volatilization. Once again, TCE conservation was established before bacteria were introduced to the plug-flow reactor.

Culture medium containing a high density of cells (A_{600} = 1.8, or approximately 32 mg dry ceils/L) was pumped into the plug-flow reactor at a rate equivalent to 1/10 the rate of groundwater flow. The total flow to the plug-flow reactor was 1 L/min. (See Table 2 for operating parameters.) The TCE concentration was measured over the full length of the plug-flow reactor (at approximately 20-ft intervals). Reactor performance was determined on the basis of TCE concentrations throughout the reactor. Growth and activity of the microorganisms within the culture vessel were evaluated on the basis of culture density, color (visually), and sMM0 activity with the use of a colorimetric assay. All materials contacting the contaminated water were either stainless steel, Teflon, or glass.

Results and Discussion

A flow diagram of the reactor system is provided in Figure 1, and the operational parameters for the bench tests are shown in Table 2. The intention of the bench test was to determine parameters that would provide for stable, continuous treatment of TCE by *M.t.* OB3b. A culture-vessel dilution rate of 0.02/hr was established experimentally to maintain a steady-state *M.t.* OB3b concentration based on the growth rate of the bacteria in the chemostat. Growth of the organisms is, however, a function of methane availability, which is, in turn, a function of gas transfer efficiency. Gas transfer efficiency is variable based on the chemostat's characteristics (aerator and impeller dimensions, etc.) and, thus, will change during scale-up.

TCE biodegradation over several hours of treatment with the use of the optimized bench-scale reactor system is illustrated in Figure 2. Average influent and effluent TCE concentrations were 563 and 63 parts per billion (ppb), respectively, which correspond to an 89% TCE reduction.

Although these results signify an outstanding potential for TCE treatment through the reactor, instability of the pure culture of *M.t.* OB3b was noted. This suggests that ultimate modifications to the culture system would be needed to achieve stable, long-term treatment. The instability was noted as a sharp decrease in sMM0 activity by colorimetric assay followed by change in the color of the culture from yellow to dull green color. A mixed culture

Table 1 Summary of Compounds Degraded by Methylosinus trichosporium *OB3b*

Methanes	*Ethenes*
dichloro (methylene chloride)	*chloro (vinyl chloride)*
trichloro (chloroform)	*1,1-dichloro (vinylidene chloride)*
	t-1,2-dichloro (DCE)
	c-1,2-dichloro (DCE)
	trichloro (TCE)
Ethanes	***Other***
1,1-dichloro	*1,3-dichloropropene (-propylene)*
1,2-dichloro	*2,2,2-trichloroacetaldehyde (chloral hydrate)*
1,1,1-trichloro	

that included *M.t.* OB3b, various other morphologically diverse bacteria, and an abundance of ciliates 'was observed in the culture medium by phase-contrast microscopy. To achieve continuous treatment of TCE, this problem must be addressed; however, to determine the feasibility of the treatment concept before added effort was given to culture development, the pilot demonstration proceeded.

The primary objectives of the pilot-scale demonstration were (1) to determine whether TCE degradation activity similar to that observed during the bench test could be induced at pilot scale and (2) to evaluate the costs of operating the reactor system to treat TCE-contaminated water. Because the cost of methane gas was a primary concern, a bubbleless, gas transfer device was added to the reactor system to increase the methane transfer efficiency (Figure 1). Culture medium was circulated through the device for methane saturation and then returned to the culture vessel.

When operating optimally, 88% of the influent TCE was biodegraded by using the operating parameters shown in Table 2. TCE concentrations were measured at various distances down the plug-flow reactor to compare actual data to a first-order kinetic model. The results are shown in Figure 3. Clearly, with optimal performance, the first-order model adequately describes TCE removal from the contaminated water.

TCE disappearance was monitored through the reactor system during two separate operations. Operations 1 and 2 lasted 10 and 8 days, respectively. Some degree of TCE treatment (minimum 14%) was accomplished on each day of each operation. Typically, 5 or 6 days of near-optimal reactor performance (and approximately first-order reactor kinetics) were followed by a few days of decline before sMM0 activity was essentially eliminated. This was true for both bench- and pilot-scale systems. Day-to-day treatment efficiency, however, changed considerably, even though the growth rate (and thus, the cell concentration in the culture vessel) remained constant. This implicates the physiological conditions of the bacterial culture, which are probably affected by fluctuating concentrations of metabolic byproducts (such as methanol) or by competition from other organisms in the culture vessel.

During the pilot demonstration, methane was used at a rate of 240 ml/min at standard temperature and pressure. The apparent yield based on this flow rate and cell production rate was 3 x 10^{-3} g cells/g theoretical oxygen demand. Since methane is a highly usable substrate for these organisms and since typical yields on carbon substrates are $>10^{-1}$ g cells/g BOD, a high degree of methane stripping was suspected. Based on methane cost of $0.50/hundred ft^3, the calculated cost of methane during the pilot demonstration was approximately $0.33/1000 gal of water treated.

Although methane was added via the gas transfer device, air was still introduced through sparging devices within the culture vessel. The efficiency of methane use would probably be improved by adding both air and methane through the gas transfer device and, thus, sparging would be avoided. In addition, during this test, the vessel was stirred by an agitator that could be avoided or at least minimized. These improvements would increase the efficiency of methane use and, thus, reduce the cost associated with methane supply. (Theoretically, minimum rates of bacterial methane consumption would result in methane costs of < *$0.05/1000* gal of treated water.) Thus, it is expected that using this technology would result in lower treatment costs than would either advanced oxidation or carbon adsorption/disposal.

Table 2. *Bioreactor Operational Parameters for Bench and Pilot-Scale Systems*

Parameter	*Units*	*Bench Unit*	*Pilot Unit*
Culture Vessel:			
Volume	*L*	*1*	*300*
Cell dilution rate	hr^{-1}	*0.02*	*0.02*
Cell density	A_{600}	*6.1*	*1.8*
Methane flow rate	$L \cdot hr^{-1}$	*1.05*	*14.4*
Air flow rate	$L \cdot hr^{-1}$	*21*	*6000*
Plug Flow Contactor:			
Length	*m*	*0.61*	*30*
I.D.	*cm*	*2.54*	*5*
Volume	*L*	*0.31*	*60*
Culture medium flow (q_c)	$L \cdot hr^{-1}$	*0.0280*	*6*
Groundwater flow (q_w)	$L \cdot hr^{-1}$	*0.252*	*60*
Total bioreactor flow (Qb)	$L \cdot hr^{-1}$	*0.280*	*66*
HRT	*hr*	*1.1*	*0.91*

Conclusions and Recommendations

It was concluded that:

- methanotrophic TCE degradation in this two-stage bioreactor system is feasible, and
- the cost of methane necessary to support TCE biodegradation is not excessive in relation to the costs of other technologies available for destructive TCE removal from water.

The extent of degradation of TCE from day to day was highly variable. Conversely, the culture density (A_{600}) was relatively stable at approximately 1.8. Although the culture was not axenic, *M.t.* OB3b concentrations in the culture medium remained high through the end of the test runs. Thus, the fluctuations in TCE degradation activity more likely resulted from changes in the expression of sMM0 by the culture. Future studies should focus on stabilization of the culture for consistent, long-term treatment of chlorinated, volatile, aliphatic hydrocarbons.

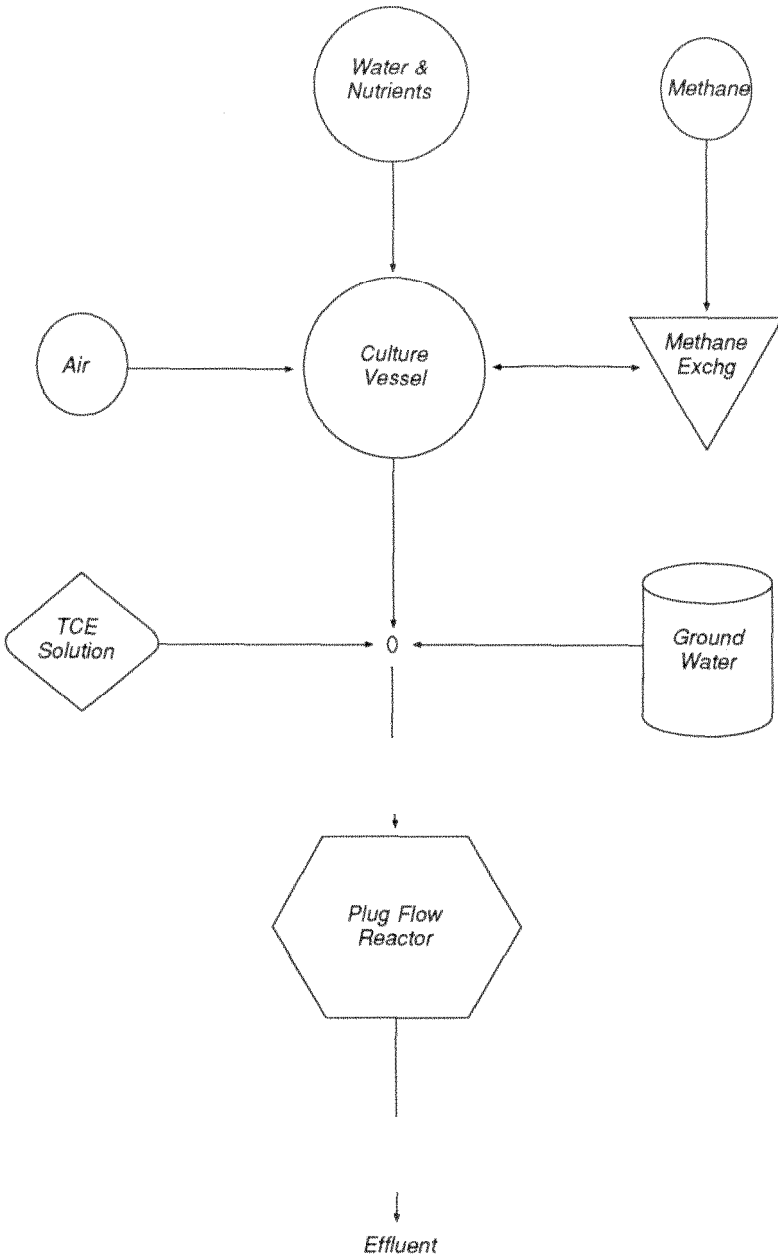

Figure 1. Schematic diagram of the bioreactor system. In the bench-scale system methane and air were introduced to the culture vessel through a glass air diffuser. The diagram includes a representation of the bubbleless gas-saturating device ("Methane Exchg") that was used in the pilot-scale system.

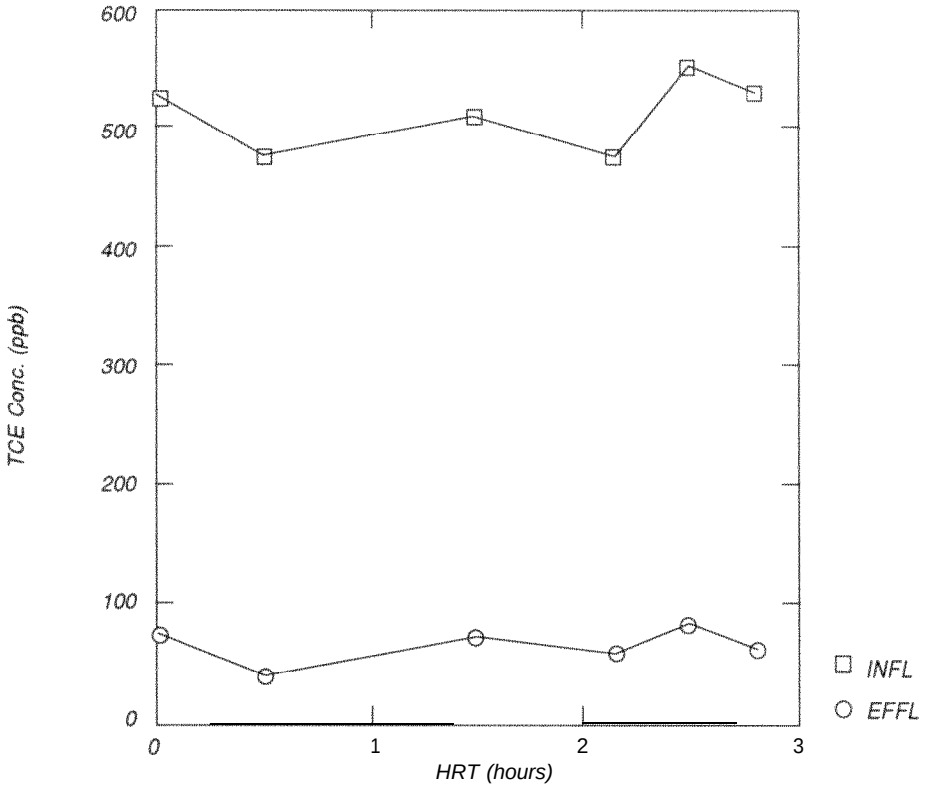

Figure 2. Bench-scale TCE concentrations in plug-flow reactor influent and effluent streams. Hour zero corresponds to the first TCE measurement after the flow of bacterial culture to the reactor began.

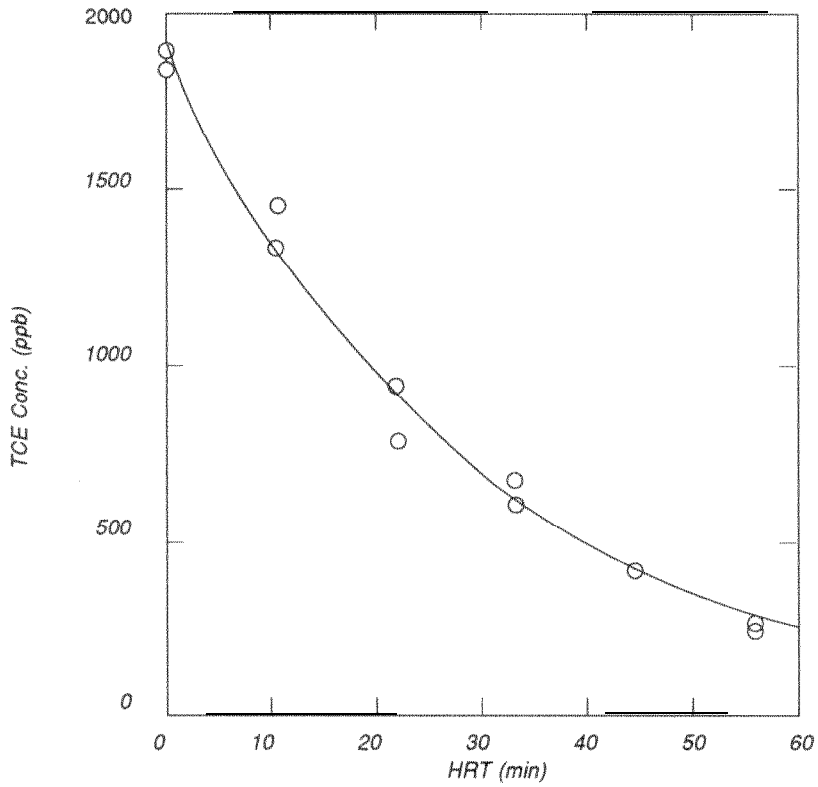

Figure 3. TCE concentrations at various distances down the length of the plug-flow reactor as a function of the ***hydraulic*** *residence time to thatport. Results are from the third day of operation of the pilot reactor, showing approximation of reactor performance to first-order kinetics. The parameters (estimated by nonlinear regression ± S. E. of the estimate) were* S_0 *= 1896 ± 40 ppb and K, = 3.43 ± 0.14 x* 10^{-2}*/min (2.06 ± 0.08/hr). The cell concentration in the plug flow reactor was 3.6 mg dry weight/L.*

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