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In Situ Bioremediation of Trichloroethylene

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Introduction

Volatile organic compounds (VOC) are present at over two-thirds of Superfund, Resource Conservation and Recovery Act of 1976 (RCRA), and U.S. Department of Defense sites (EPA 1996). Thus, remediation of VOCs is a top concern to the Environmental Protection Agency. Trichloroethylene (TCE) is a persistent VOC contaminant that infiltrates soil and ground water from improper disposal of dry cleaning agents, degreasing solvents, and paint strippers. TCE is a chlorinated aliphatic hydrocarbon, suspect of being carcinogenic and mutagenic (EPA 1997). TCE is labeled as a dense non-aqueous phase liquid (DNAPL) meaning it sinks into the soil subsurface by displacing water from soil pores and eventually sinking into the groundwater while leaving behind residual pockets that can contribute to long term contamination (Anderson and Anderson, 1996). Consequently, TCE must be remediated in both the groundwater and in the subsurface of soils.

Remediation methods for TCE are air stripping, carbon adsorption, soil venting, surface bioreactors, and in situ bioremediation. Air stripping, carbon adsorption, and surface bioreactors are used for contaminated water. Soil venting is used for contamination in the vadose zone, while in situ bioremediation can be used in the vadose zone and in the water table.

Water remediation methods are ex situ and consist of a system in which pumping contaminated water out from the water table is necessary. Air stripping is a process by which a constant air stream is sent through the contaminated ground water to force TCE from the water into its gas phase. The gas phase is then emitted into the atmosphere. This process changes TCE from one medium to another. This method is often combined with carbon adsorption. When air stripping alone is used, TCE levels are often above local drinking water standards. The treated water from air stripping is passed through a tank that is lined with a sorbant. This sorbant will bind TCE and remove it from the water. Contamination breakthrough occurs when the sorbant sites are full and the TCE will stay in the water. Changing the sorbant in the holding tank will solve this problem. The surface bioreactor pumps contaminated ground water into a tank system where methane and air are added and the TCE contaminant is then treated by methanotrophic microorganisms. These microorganisms will degrade TCE by an oxidation process (Russell *et al.*1992).

Soil venting is an in situ air stripping method. Air is pumped into the soil subsurface and vented or vacuum extracted. The gases that are given off or extracted are treated with a catalytic oxidizer before being released into the air. The catalyst material must be halogen tolerant. Halogens can be adsorbed to some catalysts and then the catalytic material is ineffective in the oxidation process (Lombard *et al.* 1993). The gases will be collected and sent though the catalytic material creating a reaction and oxidizing the organic vapors.

This paper describes an in situ bioremediation method to degrade TCE by increasing the indigenous methanotrophic population found in the soil. The methanotrophs degrade TCE

instead of transforming it into another medium, unlike air stripping. This method can also be used in the water table, treating the ground water, and in the subsurface, treating the soil.

Methanotrophic Microorganisms

Methanotrophs are aerobic microorganisms that can degrade TCE by co-metabolism. Co-metabolism is a process by which a microorganism breaks down a compound with out gaining an energy source. The non-energy-supplying compound may compete with an energy-supplying compound for a specific active site on an enzyme or may be used when the energy source is gone. For example, methanotrophs use methane as an energy source but TCE can compete with methane for a specific site on an enzyme. If TCE joins with the enzyme, the methanotroph will break it down but will not receive any form of energy from it. Therefore, although methanotrophs can metabolize TCE, they can not use it for energy to grow and multiply and will require an alternate energy source.

As methanotrophs metabolize methane, an increased amount of methane monooxygenase enzyme (MMO) is produced within their cells. MMO inserts molecular oxygen into TCE, removing the carbon-carbon double bond, creating an epoxide molecule (Figure 1). Due to the elimination of the carbon- carbon double bond, the epoxide will be unstable in the aqueous environment outside the cell and break down to formate, chlorinated acids, glyoxylate, and carbon monoxide. Methanotrophs and/or heterotrophs will then metabolize these products into final products of carbon dioxide and cell mass (Henry and Grbic-Galic 1994). There are two general categories of methanotrophs based on the type of MMO they contain. Type I methanotrophs express membrane-bound particulate MMO (pMMO) and Type II express soluble MMO (sMMO) (Bowman *et al.* 1993, Travis and Rosenberg 1997). The sMMO has a greater affinity to degrade a variety of compounds and can degrade TCE at a faster rate than pMMO (Oldenhuis *et al.* 1991).

Bacterial Cell Aqueous Environment

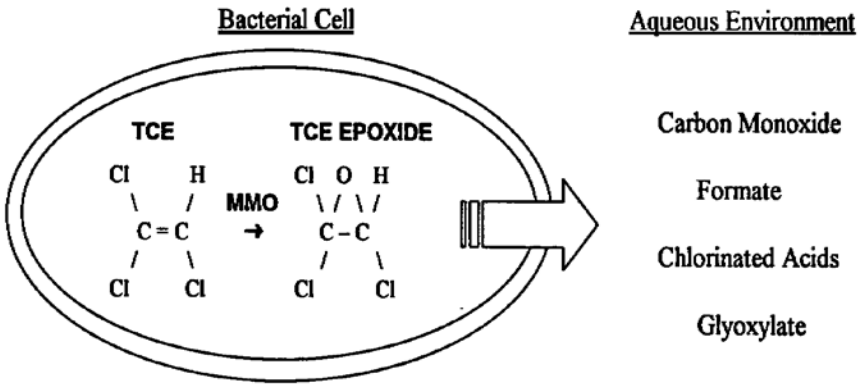

Figure 1. Proposed mechanism of TCE degradation by methanotrophs. Methane monooxygenase (MMO), an enzyme produced by methanotrophs, inserts an oxygen molecule into the TCE compound creating a TCE epoxide. The TCE epoxide is extruded from the cell into the aqueous environment. Due to the instability of the TCE epoxide in this environment, it breaks down to carbon monoxide, formate, glyoxylate, and chlorinated acids. These compounds are further metabolized by methanotrophs and/or heterotrophs to final products of carbon dioxide and cell mass. *Adapted from Henry and Grbic-Galic 1994.*

Degrading TCE by increasing the indigenous methanotrophic population with nutrient addition was first tested by Wilson and Wilson (1985) in the laboratory. They found that by adding natural gas to a sand column (containing indigenous microbes), methanotrophic populations increased and TCE was degraded to carbon dioxide. Expanding this idea to in situ bioremediation requires additional considerations such as: are the correct microbes present, what is the optimum engineering set up (how are the nutrients going to be supplied), and what are the optimal combinations of nutrients. Due to all the required considerations and precautions, testing in situ bioremediation through increasing methanotrophic populations has been in process for over fifteen years.

Site Study: Moffet Naval Air Station

The Moffet Naval Air Station, Mountain View, California was the location of a confined aquifer that was contaminated with TCE and 1,1,1-trichloroethane (TCA) concentrations up to 100 mg/l (Roberts *et al.* 1990). The site contained a shallow sand and gravel aquifer that had a clay layer above it, 4 meters thick, and a clay layer below it. Nine horizontal wells were placed through the plume. Two injection wells (one at each end of plume), three sampling wells placed 1.0, 2.2, and 4.0 meters from each injection well, and an extraction well in the middle (6 meters from both

injection wells). A detailed description of the contaminated site can be found in Roberts *et al*. (1990).

Methane- and oxygen-containing groundwater was alternately pulsed into the system through the injection wells to avoid clogging and to distribute the nutrients evenly (Roberts *et al.* 1990). Semprini *et al.* (1990) found, by analyzing water samples through out the experiment, that the indigenous population of methanotrophs did increase and TCE degradation increased to 20-30%. Simpler chlorinated aliphatic compounds had a higher rate of degradation: vinyl chloride 90-95%, *trans*-DCE 80-90%, *cis*-DCE 45-55%.

Competition for the active site on MMO between methane and TCE was shown on mixed cultures from the Moffet Naval Air Station site (Henry and Grbic-Galic 1994). This competition for the active site led to the testing of alternate primary sources for microbes that would not compete with TCE. Semprini *et al.* (1991) tested formate and methanol as alternate sources, which resulted in an eventual decrease in TCE degradation. Formate was found to be an intermediate energy source not a growth substrate, which caused a decrease in methanotrophic populations and decreased the level of MMO, resulting in less TCE degradation. Their data suggested that methanol did not eliminate inhibition competition as did formate. When toluene and phenol were primary substrates, degradation of TCE, 1,1-DCE, and *cis*-DCE increased, while *trans*-DCE decreased (Hopkins *et al.* 1993, Hopkins and McCarty 1995). Although toluene and phenol showed promising results as substrates, extreme caution should be used when adding them to the groundwater because they are both toxic and regulated chemicals (Hopkins and McCarty 1995).

Study Site: Savannah River Site

The Savannah River Site (SRS) is a study site located in a 320 square mile nuclear production facility near Aiken, South Carolina, that started production in the 1950s. One area of the facility was used to degrease target and fuel elements that were used in the reactors. The degreasing solvents were dumped into a process sewer line that moved the contaminants to an unlined basin within the facility. The process sewer line leaked, contaminating the soil subsurface surrounding the basin. The solvents from the subsurface and basin then continued to leach into the groundwater creating a plume over one square mile (Hazen 1996).

It was estimated that 13 million pounds of chlorinated degreasing solvents were used from 1952–1982. It is assumed that 50-95% of the solvents evaporated during the degreasing processes, while the remaining went into the process sewer line. Dissolved solvents were detected in the groundwater in 1981. By 1985, the basin was no longer in use and process wastes from this facility were sent to a treatment facility (Marine and Bledsoe 1984). Remediation by air stripping started in 1985.

The Savannah River Site (SRS) used a similar bioremediation method as the Moffet Naval Air Base starting in 1992. The structure was changed to horizontal wells above and below the aquifer and a vacuum on the upper well to distribute the nutrients evenly (Figure 2) (Hazen *et al*. 1994, Palumbo *et al.* 1995). Soil and water samples were taken every three months to monitor the systems effectiveness and to make any needed changes had it been ineffective (Hazen 1996).

Methane and air pulsing was used, as in Semprini *et al.* (1990). Similar results were found at SRS as at the Moffet site; methane triggered an increase in methanotrophic populations (Palumbo *et al.* 1995, Pfiffner *et al.* 1997). Only type II methanotrophs were isolated from SRS, but the testing was done after methane injection, which may have produced an environment conducive to this microbial type (Bowman *et al.* 1993).

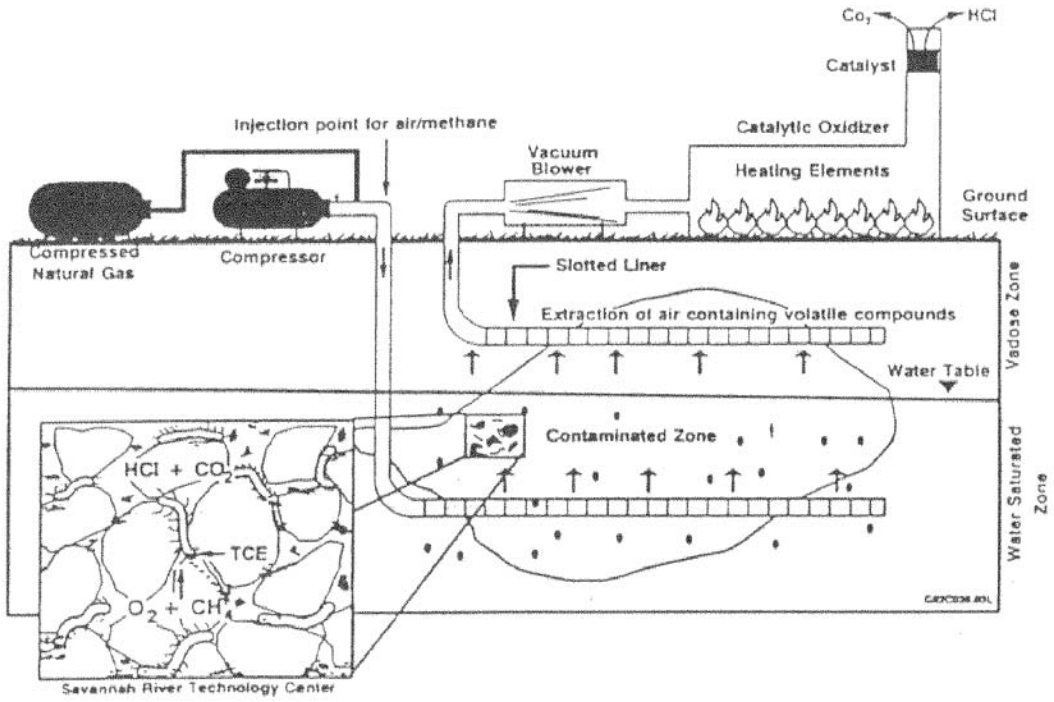

Figure 2. Horizontal wells for methane, air, and nutrient injection and extraction used in the Savannah River Site demonstration of in-situ bioremediation of TCE. Methane, air, and/or nutrients are injected into the lower horizontal well. The upper horizontal well extracts air and gases from the vadose zone under vacuum conditions. Any alternative gases collected are treated by the catalytic oxidizer and released into the atmosphere. *Adapted from Hazen et al. (1994). Used with permission from Battelle Press.*

Different nutrient dosing was tested at SRS to find the optimum conditions for high degradation of TCE. Before methane was added, less than 10% of TCE was mineralized and after adding methane greater than 50% was mineralized (Palumbo *et al.* 1995). Continuous methane (4%) injections had similar mineralization rates as pulsed methane injections (Pfiffner *et al.* 1997), confirming that methane can cause competitive inhibition in TCE degradation as suggested by Henry and Gblic-Galic (1994) and Semprini *et al.* (1990). Adding phosphate (as triethyl-phosphate) and nitrogen (as nitrous oxide) with the methane-pulsed injections resulted in greater than 90% TCE mineralized (Palumbo *et al.* 1995). Before adding nutrients, a site evaluation of natural nutrients should be completed because some soils have excess phosphorus that will be used by microbes.

Using empirical data from the SRS study, Travis and Rosenberg (1997) created a computer model for methanotrophic bioremediation of TCE. The model results showed bioremediation had a 25% increase of TCE degradation over air-stripping alone. This model considered predation of methanotrophs by protozoan and nutrient disbursement. Their model simulations showed an overestimation of degradation by 25% without predation considerations. The simulations also showed that when nitrogen was added, microbes would cluster around the injection wells and prevent methane from dispersing into the outer fields. Clay lenses also showed restricted flow of nutrients through the soil medium. Model simulation can assist researchers in predicting how methanotrophic bioremediation will perform under different soil textures and climate conditions.

Conclusion

In situ bioremediation of TCE is a plausible clean-up method but careful pre-treatment of site evaluation and analysis is required. Performing column tests to analyze indigenous microbes and soil texture problems are preliminary procedures that must be completed before remediation begins. Defining the contaminants in the subsurface and groundwater is important in determining a realistic bioremediation goal. Testing for heavy metals should be considered. Heavy metals may reduce the desired microbial population. For example, high levels of copper are conducive to type I methanotrophs, which are less efficient at TCE degradation than type II (Semprini 1997). A site evaluation problem that needs more research is defining the dense non-aqueous phase liquid (DNAPL) area. Estimating the geometry of DNAPL zones is crucial, so as not to contaminate previously uncontaminated ground water from connecting streamtubes (Jackson and Mariner 1995).

Sutfin and Ramey (1997) reported that a client owning a fabricating facility in northeast Missouri had been remediating TCE contaminants by air stripping but had little success in five years. They used the same set up as at SRS (Chlorinated Treatment by Methane Injection is its patented name) and, after three months, the ground water volatile organic carbons were reduced by 60-80%. This supports the use of in situ bioremediation of TCE.

In situ bioremediation of TCE, by increasing indigenous methanotrophic populations, does not create a potential health hazard from the transportation of contaminants and the contaminants are not volatilized into the atmosphere. Preliminary project costs are higher using this bioremediation method, but clean-up is more efficient and has a shorter timetable reducing the overall project cost. Research using different climates and soil textures must be conducted if more efficient procedures are to be found. In winter conditions, microbes are less active due to low temperatures, which reduces the effectiveness of this method. Overall, the in situ bioremediation of TCE is successful and can be very effective and cost efficient over air stripping alone, air stripping and carbon adsorption, soil venting and surface bioreactors.

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