An Interdisciplinary Approach to Trichloroethylene Pollution in Groundwater Systems Phase II: Computer Modeling

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The purpose of this research study is to investigate the reliability of aerobic bioremediation as a de-pollution method in groundwater systems containing viable levels of trichloroethylene and other chlorinated, toxic compounds. This portion of the study will use computer modeling to represent a full-scale bioremediation plan for a simulated groundwater system containing both TCE and Toluene through the use of the microorganism Pseudomonas putida F1. The project will conclude with a compilation of the combined years' study into a fully adaptable applications manual, including possible Superfund case-study scenarios.

Groundwater & Aquifer Systems

The basis for the existence of human life is groundwater, of which the American populace uses over 70 billion gallons a day. Groundwater, or water that is found in sand, gravel, or porous rock, is given its name since it is usually found in the geological strata, or aquifers, beneath the ground.

Approximately 4 percent of the water in the hydrologic (water) cycle may be found, at any given time, in the form of groundwater. Aquifers in the United States are estimated to contain some 100 quadrillion gallons of groundwater (Patrick et. al 1987).

Groundwater has many properties that vary according to its location. Total dissolved solids (TDS), hardness, salinity, pH, and naturally-occurring toxin levels are some of these properties. TDS is a measure of the amount of solid particles dissolved in the groundwater. Hardness looks at the magnesium and calcium levels. Salinity deals with salt, while pH is a measure of disassociation.

The amount of naturally-occurring toxins in a particular groundwater system is entirely dependent on the composition of the soil above the aquifer and the subsurface organisms existing in the area (Patrick et. al 1987).

The usability of groundwater as drinking water is a highly regulated area of industry in countries around the world. Groundwater systems are particularly easy methods of spreading diseases, pathogens, and problems among a populace. Some groundwater is highly pure, usable as drinking water without any treatment. Other groundwater requires several pathways of treatment and filtration before it can be distributed in a city (Patrick et. al 1987).

The EPA regulates the level of certain components of groundwater that are hazardous to public health through the National Primary and Secondary Drinking Water Standards. Groundwater is not only contaminated by naturally-occurring sources. Most groundwater contamination is a direct or indirect result of human interference in the environment. Sources of contamination can be divided into three categories: natural processes, human disposal practices, and human industrial runoff.

Today the most common form of contamination comes from industrial runoff, which occurs when factories or farms are negligent in containing the chemicals they use. These chemicals leak out of the contained area, by means of air or water, and find their way into the groundwater system (Patrick et. al 1987).

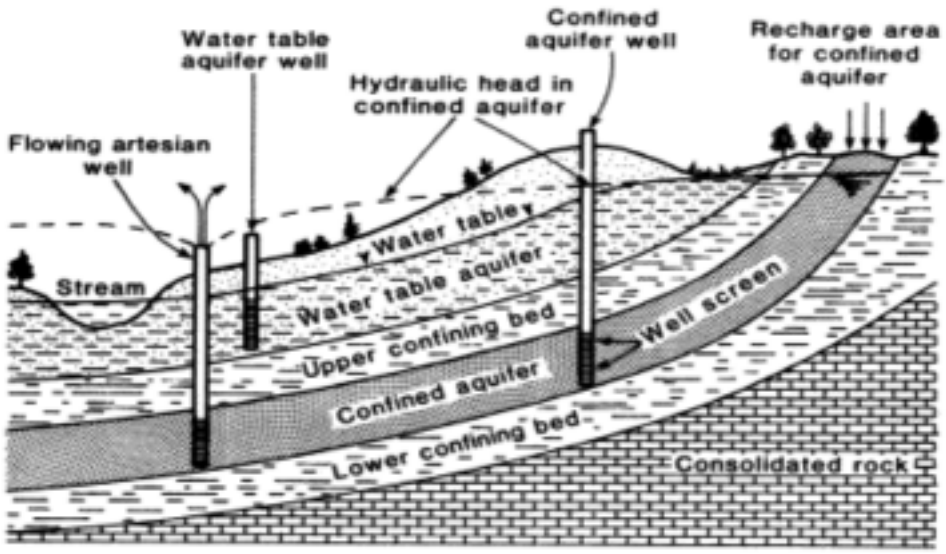

Figure 1: Diagram of a groundwater system and all the geological strata associated with its functions. Courtesy of Johnson Division (1975) in Groundwater and Wells.

National Priority Listing and the Superfund Sites

Established by the federal government to clean up uncontrolled hazardous waste sites, Superfund, a public and privately sponsored program, strategically searches for, isolates, and cleans up contaminated lands. The contamination level of these lands is decided by committees using regulations set forth by the parent agency of the Superfund program, the EPA.

The National Priority Listings (NPLs) are a system managed by the Superfund program in which Superfund sites needing the most attention are published. All contaminated dumps listed in the NPLs are eligible for federal grant money and areas of research,

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Step One:
$HC_2Cl_3 + H+ \rightarrow H_2C_2Cl_2 + Cl^-$
Trichloroethylene → cis- & tans- 1,2-dichloroethene
Step Two:
$H_2C_2Cl_2 + H^+ \rightarrow H_3C_2Cl + Cl^-$
1,2-dichloroethene → Vinyl chloride
Step Three:
$H_3C_2Cl + H^+ \rightarrow H_4C_2 + Cl^-$
Vinyl chloride → Ethylene

Figure 2: The anaerobic biodegradation of trichloroethylene.

First Pathway:
$C_2HCl_3 + H_2O + e^- \rightarrow C_2HO^-_3 + 3H^+ + 3Cl^-$
Trichloroethylene + Water + Electron → Glyoxylate + 3 [H] ions + 3 [Cl] ions
Second Pathway:
$C_2HCl_3 + 4H_2O \rightarrow CHO^-_2 + 7H^+ + 3Cl^- + 2e^-$
Trichloroethylene + 4 Water → Formate + 7 [H] ions + 3 [Cl] ions + 2 electrons

Figure 3: The aerobic biodegradation of trichloroethylene.

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planning, and development. There are thousands of sites listed in the NPLs and the Superfund directory (Superfund FAQs, 2003).

As of October 4, 2004, there were 305 Superfund groundwater sites in the United States listed as being contaminated with viable amounts of trichloroethylene. Of these 305 sites, the majority are near landfills and either chemical companies or military bases (Active Superfund Sites, 2004).

Many methods have been employed over the years to attempt to rehabilitate contaminated groundwater systems. Most sources agree, however, that these methods are expensive, time-consuming, and tedious. The best way to keep groundwater clean is to prevent contamination.

New techniques, such as in-situ bioremediation (being investigated in this study) and in-situ chemical neutralization, are still in research phases and are not widely applied. The most common technique is the withdrawal of groundwater from the aquifer and its detoxification in a surface plant. This is a costly process that can take years to complete.

For volatile organic chemicals such as trichloroethylene, the most common withdraw and treat methods are air stripping, steam stripping, and phytoremediation. Air and steam stripping are disadvantageous, however, because they bring the trichloroethylene pollutant from the water into the air, where it decomposes into phosophogene, a lung irritant. Phytoremediation involves using plants and exploiting their symbiotic relationships with bacteria to attain detoxification. This again is a costly process that requires large treatment plants (Patrick et. al 1987; Macalady, 1998).

Trichloroethylene

Listed as a volatile organic chemical (VOC), trichloroethylene, which has a density of 1.46 g/mL, pressure of 58 mm, and a viscosity of .57 cP, is a colorless, room-temperature liquid of distinct odor and taste (Macalady, 1998). Using the trade names Triclene and Vitran, trichloroethylene (also spelled trichloroethene) is a synthetic chemical used as a degreaser and solvent. It can be found in typewriter correction fluid, paint removers, adhesives, and spot removers. The chemical formula for trichloroethylene is C_2HCl_3 (ATSDR- Trichloroethylene, 2000).

Trichloroethylene can be both spontaneously and catalytically metabolized. As stated previously, in the air it spontaneously degrades into the lung irritant phosphogene, while in water and soil, it can be degraded by means of both aerobic and anaerobic bacteria (ATSDR- Trichloroethylene, 2000).

The anaerobic biodegradation, termed reductive dechlorination, can be achieved by any of the dehalonating species of bacteria (Dehalobacter, Dehaloccocides, etc.). This biodegradation is summarized in Figure 2.

Notice that this degradation requires three steps. The ultimate byproduct of the degeneration is ethylene, a double tetrahedron hydrocarbon. Some of the intermediate substances produced along the pathway, such as vinyl chloride, are, however, toxic and more fatal to humans than trichloroethylene itself. For this reason, dechlorination is not a recommended method for de-pollution of trichloroethylene-contaminated sites (Woodbury et al. 2000).

Trichloroethylene can also be biodegraded aerobically. This degeneration is best achieved using any member of the pseudomonas genus of microorganisms, which contain the enzyme toluene deoxygenase, which is responsible for the catalyzed transformation of trichloroethylene to the less volatile products glyoxylate and formate.

This reaction requires the addition of either one molecule of water and one electron or four molecules of water. Figure 3 summarizes these reactions (Nasirullah & Pant, 2003).

Trichloroethylene is quite toxic to humans and the environment. Its carcinogenicity, or ability to cause cancer, still remains a controversy. Currently, the ATSDR (Agency for Toxic Substances and Disease Registry) classifies trichloroethylene as "reasonably anticipated to be a human carcinogen."

Current studies show exposure to large amounts over elongated periods of time could potentially cause deficiencies or tumors in the kidney and liver (Wartenberg, 2002). Another study published in the journal *Environmental Health Perspectives* showed that trichloroethylene and other halogenated hydrocarbons could have impairing effects on fetal heart development in lab mice (Johnson, 2003).

Whatever the current position may be, the federal government does not recognize trichloroethylene as a carcinogen (ATSDR-Trichloroethylene, 2000); however, one of the products of its anaerobic decomposition, vinyl chloride, is a widely recognized carcinogen of extreme toxicity. For this reason, very few applications of anaerobic bioremediation of trichloroethylene exist (Kielhorn, 2000).

Toluene

A colorless liquid with a distinctive smell and taste, toluene is a naturally occurring solvent found in paint, paint thinner, gasoline, nail polish, lacquer, and rubber. It can be smelled in the air at 8 ppm or tasted in the water at 1 ppm. Toluene is a toxic substance that can cause severe brain damage. Insomnia, nausea, amnesia, poor judgment, and loss of appetite are among some of the common side effects of prolonged exposure. Extremely large amounts or a regular, unhealthy exposure can even cause unconsciousness or coma.

Toluene has a chemical formula of $C_6H_5CH_3$ and is commercially produced as methacide. Some of its chemical properties include a melting point of -95 K, a boiling point of 110 K, density of .8669 g/mL, and a solubility of 538.4 g per liter of room temperature water. It is said to have the same properties as another volatile organic: benzene (ATSDR- Toluene).

Pertaining to this experiment, only the catalyzed decomposition of toluene is signifi-

$C_6H_5CH_3 + 3NADH + O_2 \rightarrow 3NAD^+ + C_7H_{10}O_2$

Toluene + (reduced) Nicotineamide Adenine Dinucleotide + Oxygen → Toluene-cis 1,2-dihydrodiol + (oxidized) Nicotineamide Adenine Dinucleotide

Figure 4: The catalyzed decomposition of toluene.

cant. This decomposition is accelerated by the enzyme toluene deoxygenase, found in most strains of pseudomonas and microorganisms of the like. The reaction, which requires the presence of diatomic oxygen and electron acceptors, is shown in Figure 4.

The decomposition produces the product toluene-cis 1,2-dihydrodiol, a substance whose toxicity remains relatively unknown (Nasirullah & Pant, 2003).

As a volatile hydrocarbon, toluene has much in common with its counterpart, trichloroethylene. In fact, their main difference is that trichloroethylene can not be found naturally; it is purely a manmade substance.

Over the past decade, much research has been indicated showing a chemiotactic interest between toluene and trichloroethylene by many archaeic bacteria. These include the experiments performed by Parales et. al 2000, Leahy et. al 1995, Mars et. al 1997, and Fries et. al 1997. Several other experiments have concentrated on the enzyme and microorganism generally used in this line of work: Pseudomonas putida.

Pseudomonas putida

Pseudomonas putida is a relatively versatile bacterium that can withstand extreme conditions and use a variety of carbon-based compounds for feeding. It belongs to the genus Pseudomonas, of the Domain Archea. Relative species include P. cepacia and P. arugenosa, of which P. arugenosa is the most dangerous. Its toxicity to humans, however, resides only in cystic fibrosis patients and even then can be easily treated with penicillin.

Due to their high survivability, endurance, and decomposing abilities, Pseudomonas strain bacteria, which are gram-negative, have been used over and over in laboratory work of the past half-century to demonstrate the positive use of controlled microorganisms in industrial and environmental cleanup (Macalady, 1998; Pareles et. al 2000). Ramos-Gonzales et. al 2003 best exemplified this continuing research in their several-years-long research study into biotransforming P. putida to meet the ever-increasing needs of human application.

This research study will seek to go one step further by transforming the Pseudomonas bacterium into a laboratory industrial plant producing hundreds of thousands of harmless, independent enzymes that can be placed directly into the groundwater system to systematically take care of pollution cleanup needs.

One of the main characteristics of P. putida necessary for the comprehension of this research study is the enzyme toluene deoxygenase. This enzyme, internally produced in Pseudomonas putida and various other similar strains of bacteria, is the machination responsible for the catalyzed degradation of trichloroethylene and toluene to simpler, less harmful compounds.

The enzyme was first published in research by Heald & Jenkins, 1994, from the De Montfort University, UK. Since then, it has become an increasing topic of research. The enzyme is the vital link in the reactions shown in Figures 3 and 4 (Heald & Jenkins, 1994; Nasirullah & Pant, 2003).

Hypothesis

Based upon the above literature, if, in a simulated and controlled groundwater system, Pseudomonas putida F1 is introduced, then assays taken at regular time intervals will show a logarithmic decrease of trichloroethylene over time, with Parameter Set 1 biodegrading the substrate the quickest. In addition, if, in the above described situation, the variables concerning the scale of the system (s), the velocity of the groundwater current (V), and the fraction organic carbon (F_{oc}) are independently manipulated, then an increase in groundwater velocity or scale will shorten the time needed to biodegrade the trichloroethylene, while a decrease in fraction organic carbon will achieve the same.

Methodology

In order to create the computer model simulating a groundwater system, the preexisting computer program, STANMOD, developed by the U.S. Salinity Laboratory in California, was used. STANMOD, which stands for Studio of Analytical Models, is a package of programs that allows for the modeling of solute transport and decomposition in aqueous or porous medium (Poeter 2003). The program uses information collected through a number of variables to solve the convection-dispersion solute transport equation.

Variable	Formula	Conventional Unit
C_i	*Constant*	ppm or mol
K_{oc}	*Index Value*	ml/g
F_{oc}	*Constant*	
K_d	$K_{oc} * F_{oc}$	
ρ_b	M_s / V_t	g/cm^3
Θ	V_w / V_t	
R	$1 + \frac{\rho_b * K_d}{\theta}$	
V	*Constant*	m/day
S	*Constant*	M
A	.10s	
D	$\alpha * V$	

Table 1: Parameter variables for creating a model.

These variables include V (velocity), D (dispersability), R (retardation rate), and μ (degradation factor). These four main parameters are, in turn, determined from a series of other variables. Table 1 summarizes the major variables that must be considered when using the STANMOD's CXFIT program to simulate a model.

The most important variable for this modeling study is μ, the degradation factor, which is the value extracted from the data collected in last year's research. Basically, it is the multiplicative constant in the equation of the graph of the results.

The equation for biodegradation is $c = e^{-kt}$, where c stands for the concentration and t stands for time elapsed. In order to determine k, however, a linear graph had to be created. This was achieved by taking the steps shown in Figure 5 to transform the data before regraphing it:

The data was regraphed with a forced intercept of 4 (the initial concentration had been 4.00 μl), resulting in the graph shown in Figure 6, created by Microsoft Excel. The linear trendlines were computer generated, and

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$\text{Log} (c = e^{-kt})$

$\text{Log} (c) = -kt$ OR

$y = mx + b$, where $b = c_i$

Figure 5: Transformation of biodegradation data.

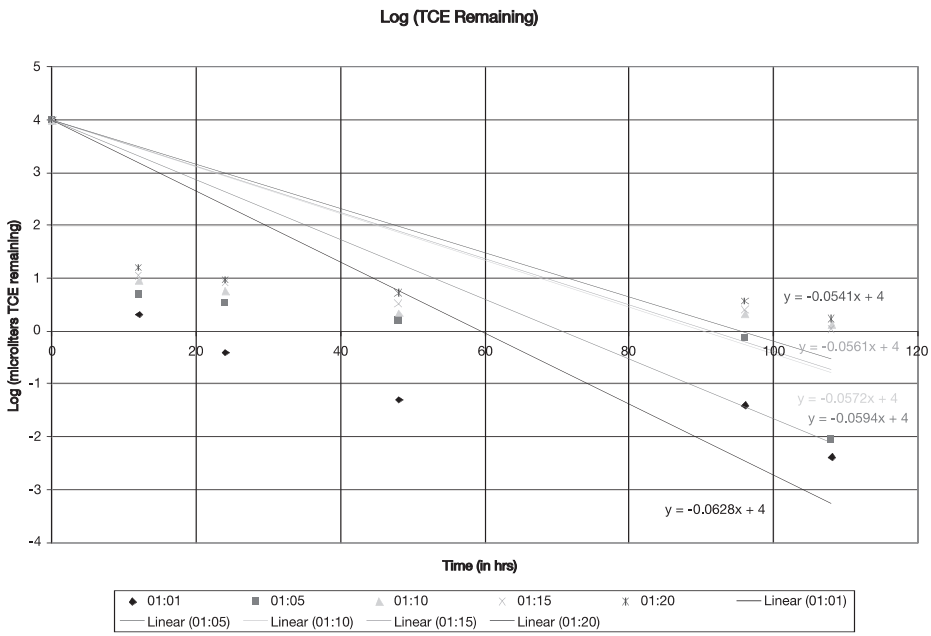

Figure 6: The graph for determining the possible values of constant k / μ.

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as a result, their equations are mathematically accurate and statistically correct in representing the field data.

With each μ-value now found, the next step in the process was to develop a system of experimental groups for applying and manipulating the variables. Aside from μ, every other variable was placed in a category called a scenario. By manipulating five of the variables—initial concentration (C_i), scale (s), velocity (V), θ, and F_{oc}—a total of 72 different scenario sets were created.

The five parameter sets (μ-values) were then applied to each scenario set using the STANMOD program. The resulting graph and the remaining trichloroethylene at t=200 was recorded in the data section. The graphs were generated by the program.

In order to measure independently, controls had to be chosen from the many values of the variables. The control for the study is Scenario/Trial No. 19. The standard scale size is 2,000 meters, the standard velocity is 1 m/day, the standard water content is 0.5 percent, and the average amount of organic carbon in a groundwater system is 0.1%. All these control conditions were met with Trial No. 19.

Parameter Set	Group Name *(tce:toluene) (ppm:ppm)*	μ-value
1	1:1	.0628
2	1:5	.0594
3	1:10	.0572
4	1:15	.0561
5	1:20	.0541

Table 2: μ-values.

Results

The complete results of the study can be found in a 72-page data book that includes a computer-generated graph and detailed information on each of the scenarios. In order to analyze the effect of several variables, some of the final (t=200) TCE concentrations of some scenarios were organized into tables. Tables 3 through 6 show the impact of the various variables on the final biodegradation. Notice that Trial No. 19 showed up in each of these tables. This was intentional, since Trial No. 19 is the ultimate control for the study.

Analysis

The results show a number of trends between the different variables and constants in the simulated groundwater system. First, the original, laboratory-exemplified trend in the five different parameter sets (or groups) was preserved. The first parameter set (group 1:1) always showed the quickest decline in trichloroethylene concentrations. For this reason, it would no doubt be beneficial to use that trichloroethylene-to-toluene ratio when applying these cleanup techniques in the real world.

Second, the data showed and indicated some notions of the trends that were hypothesized to exist. The s variable appears to have no, or very little, effect on the outcome of the situation. On the other hand, the V variable does show a somewhat medium-sized decrease in the trichloroethylene concentrations when the velocity of the current of the groundwater is increased.

Theta's mathematical relationship was the most statistically correct. The data showed that when θ is higher, the trichloroethylene concentrations go lower. The fraction organic carbon (F_{oc}) played the largest influence on the biodegradation out of all the factors. A slight drop in the F_{oc} causes a massive decrease in trichloroethylene levels.

Conclusions

The first hypothesis, that Parameter Set 1 would biodegrade the trichloroethylene the quickest and that there would be a logarithmic decrease in trichloroethylene over time, was correct and is therefore accepted. The first hypothesis was similar to the hypothesis from last year, except that it specifically addressed the properties of the computer.

The second hypothesis was not so correct. It was made up of four parts, the first three of which suggested that any of the variables s, V, or θ, when increased, could easily quicken the pace of bioremediation. The latter part suggested that the F_{oc} variable worked the opposite.

The hypothesis was correct concerning variables V, F_{oc}, and θ; however, it incorrectly assumed that variable s had anything to do with the rate of bioremediation. An analysis of the data presented by variable s shows that despite the huge increases in its value, the final biodegradation concentrations remained almost the same, within a few hundredths or thousandths of each other. For this reason, the second hypothesis is rejected. It should have left out any reference to the variable s.

If the study were to be repeated, many things could be done differently. First, a larger variety and quantity of field data should be collected in order to assure the accuracy of the model and to allow the construction of a complex rather than simple model. Second, a 3-D mapping and modeling function should be used to give the model more dimension.

A possible extension for the program would be to create an inverse parameter that would allow an official to come up with cleanup solutions by simply entering data regarding the current status of the contaminated area and the extent of contamination. The inverse function could then determine every other variable that would be needed to quickly and efficiently clean up the mess.

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The Impact of Variables

Trial No.	Value of S	Cf at t=200 P1	P2	P3	P4	P5
1	500	0.000062245	0.00010513	0.00014757	0.00017483	0.00023797
19	2000	0.000062506	0.00010557	0.00014819	0.00017557	0.00023896
37	4000	0.000062506	0.00010557	0.00014819	0.00017557	0.00023896
55	8000	0.000062506	0.00010557	0.00014819	0.00017557	0.00023896

Table 3: The final degradation concentrations related to scale size.

The Impact of Variable V

Trial No.	Value of V	Cf at t=200 P1	P2	P3	P4	P5
19	1	0.000062506	0.00010557	0.00014819	0.00017557	0.00023896
25	5	0.000061252	0.00010345	0.00014521	0.00017205	0.00023417
31	10	0.000040568	0.000068516	0.000096177	0.00011395	0.00015509

Table 4: The final degradation concentrations related to velocity.

The Impact of Variable Theta

Trial No.	Value of Theta	Cf at t=200 P1	P2	P3	P4	P5
19	0.5	0.000062506	0.00010557	0.00014819	0.00017557	0.00023896
21	0.6	0.000042837	0.000073844	0.00010504	0.00012527	0.00017257
23	0.7	0.000032141	0.000056275	0.000080855	0.000096919	0.00013474

Table 5: The final degradation concentrations related to volumetric water content.

The Impact of Variable Foc

Trial No.	Value of F_{oc}	Cf at t=200 P1	P2	P3	P4	P5
19	0.001	0.000062506	0.00010557	0.00014819	0.00017557	0.00023896
20	0.0001	5.3212E-06	0.000010269	0.000015714	0.000019439	0.000028618

Table 6: The final degradation concentrations related to organic carbon.