11-15-2005

Long term monitoring of trichloroethylene degradation indicator parameters using sensors

Ravi Krishna Prasanth Gudavalli
Florida International University

FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

LONG TERM MONITORING OF TRICHLOROETHYLENE DEGRADATION INDICATOR PARAMETERS USING SENSORS

A thesis submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

in

ENVIRONMENTAL ENGINEERING

by

Ravi Krishna Prasanth Gudavalli

2005

To: Dean Vish Prasad
College of Engineering and Computing

This thesis, written by Ravi Krishna Prasanth Gudavalli, and entitled Long Term Monitoring of Trichloroethylene Degradation Indicator Parameters Using Sensors, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this thesis and recommend that it be approved.

Yelena Katsenovich

Shonali Laha

Berrin Tansel, Major Professor

Date of Defense: 15 November, 2005

The thesis of Ravi Krishna Prasanth Gudavalli is approved.

Dean Vish Prasad
College of Engineering and Computing

Dean Douglas Wartzok
University Graduate School

Florida International University, 2005

DEDICATION

I dedicate this thesis to my father late Mr. HanumanthaRao Gudavalli, to my mother Mrs. DhanaLakshmi Gudavalli and to the members of my family for their love and support to achieve this goal and throughout my file.

ACKNOWLEDGMENTS

I would like to express my deep appreciation to my academic supervisor, Dr. Berrin Tansel for her guidance, encouragement, support and criticism throughout the course of this work. I am also grateful to the members of the committee Dr. Shonali Laha, Dr. Yelena Katsenovich for their continuous guidance and helpful comments. I would like to thank all the faculty members and staff at the Environmental Engineering department for their help and cooperation.

I gratefully acknowledge Mr. Leonel Lagos at Applied Research Center for his support and giving me the opportunity to work with him in this project and facilitating me with all the requirements to complete my thesis. I would like to thank Mr. Celso Duran and Mr. Jose Varona for their help and support in all aspects to successfully completing my thesis. Special thanks are expressed to my friends and colleagues at Applied Research Center for their support, help, and companionship throughout the time we spend sharing the same working environment.

I am thankful to my friend Mr. Pradeep Appasani for his comments and for reviewing the write-up of my thesis. Last but not the least I am highly obliged to my mother Mrs. Dhanalakshmi Gudavalli for her constant support and affection, and her boundless dedication to my education. I am also grateful to by elder brother, Mr. Krishnakanth Gudavalli and my sister-in-law Mrs. Madhavi Gudavalli for their encouragement and support throughout. I am thankful to my roommates Mr. Harindra Vedala and Mr. Raghunandan Seelaboyina for their help and support.

ABSTRACT OF THE THESIS

LONG TERM MONITORING OF TRICHLOROETHYLENE DEGRADATION INDICATOR PARAMETERS USING SENSORS

by

Ravi Krishna Prasanth Gudavalli

Florida International University, 2005

Miami, Florida

Professor Berrin Tansel, Major Professor

Past operations at Savannah River Site (SRS) have resulted in significant amount of groundwater contamination with trichloroethylene. Natural attenuation of chlorinated solvents via reductive dechlorination is one of the most important processes occurring at SRS, which requires monitoring. Many traditional monitoring techniques require manual sampling and analysis at an onsite or offsite laboratory, which is costly and time consuming. Therefore the need for a system, which can accurately and cost-effectively conduct real-time analysis using automated sensors, is important. There are several characteristics of groundwater like pH, ORP, conductivity and chloride that may be monitored to assess the TCE degradation. To evaluate the effectiveness of the sensors to measure the required parameters, a series of tests were conducted by varying the parameters that can affect the performance of the sensors. Interference by the other ions is neither strong nor permanent but can cause interference during measurement. So a thorough testing of the ISE is necessary to obtain reliable data.

TABLE OF CONTENTS

LIST OF TABLES

TABLE PAGE

LIST OF FIGURES

FIGURE PAGE

1.0 INTRODUCTION

The U.S. Department of Energy (DOE) Savannah River Site (SRS) produced two atomic weapon fuels between 1953 and 1988. Plutonium was used to trigger the chain reaction and tritium to greatly amplify the force. The SRS occupies approximately 310 square miles of land adjacent to the Savannah River, principally in Aiken and Barnwell counties of western South Carolina. SRS is a secured U.S. Government facility with no permanent residents, which is located approximately 25 miles southeast of Augusta, Georgia and 20 miles south of Aiken, South Carolina. Five reactors were built on the site which produced 36 metric tons of plutonium. Also built were support facilities including two chemical separation plants, a heavy water extraction plant, a nuclear fuel and target fabrication facility and waste management facilities. SRS is located atop sediments of the Atlantic coastal plain composed of sand and clay. Surface water quality is easily monitored but groundwater is less accessible. Water flows readily through the sand layers but is retarded by the less permeable clay beds, creating a complex system aquifers. Figure 1 shows the various groundwater contaminated locations by the operations during the life of the SRS. The movement of water into the ground passing through the aquifer and into streams and lakes continues to carry contamination along with it resulting in spreading plumes.

The following describes the major contaminants detected at various locations in the SRS

- A-Area and M-Area: chlorinated volatile organics, radionuclides, metals
- Sanitary landfill and B-Area: chlorinated volatile organics, radionuclides, metals
- TNX area: chlorinated volatile organics, radionuclides, metals

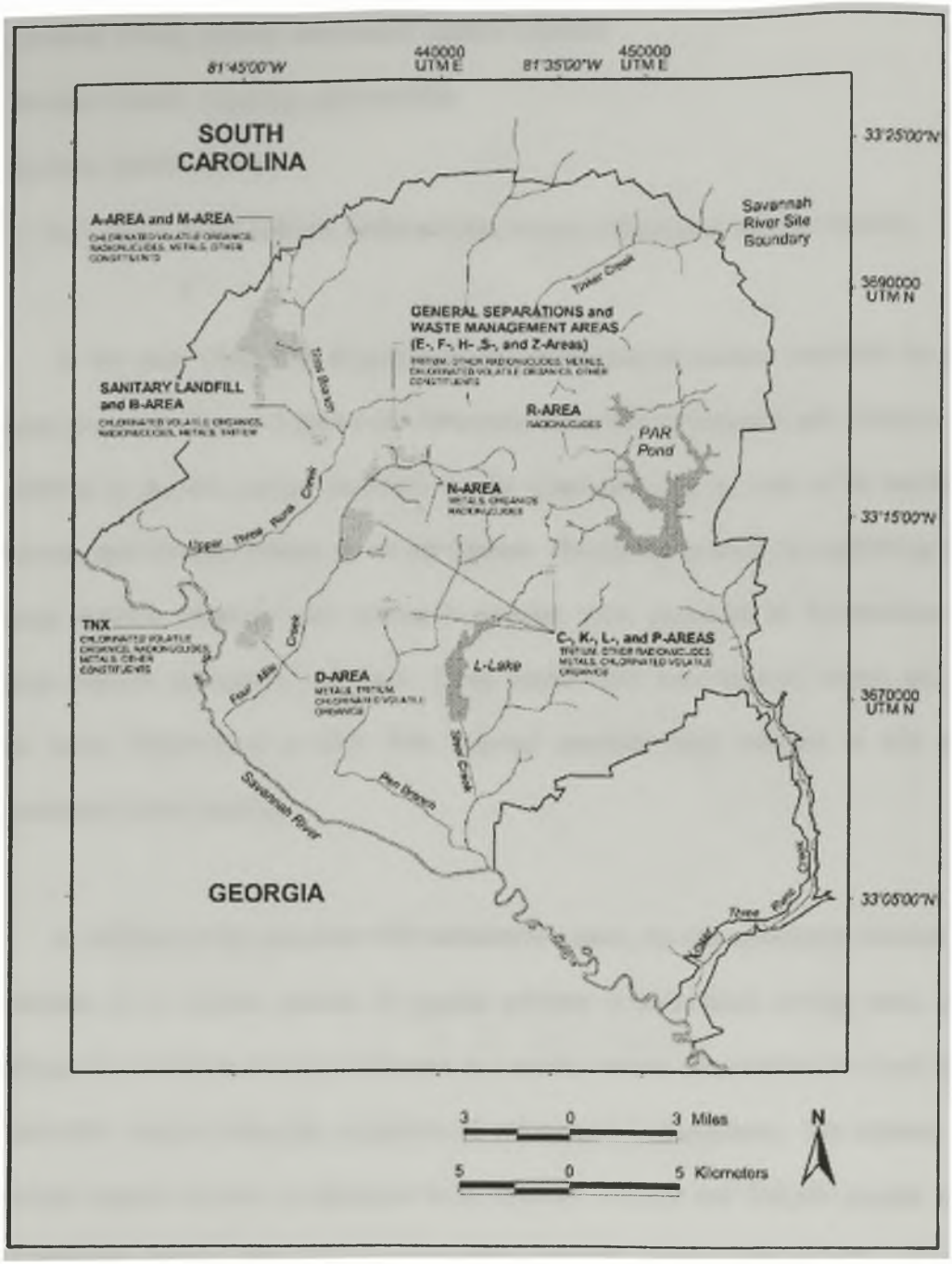

Figure 1: Various facilities monitored at the SRS.

- General separation and waste management areas (E, F, H, S, and Z areas): tritium, other radionuclides, chlorinated volatile organics

- D-Area: metals, tritium, chlorinated volatile organics
- N-Area: metals, organics, radionuclides
- R-Area: radionuclides
- C, K, L and P Areas: tritium, radionuclides, metals, chlorinated volatile organics

In the year 1988, SRS discontinued the production of nuclear materials for the defense programs. Figure 2 shows the Savannah River Site operational area. Purpose of the SRS is to provide nuclear materials for the space program, as well as for medical, industrial, and research efforts up to the present. During the process of supporting the national defense, chemical and radioactive wastes were generated as by-products of nuclear material production processes. These wastes have been treated, stored, and in some cases, disposed of at SRS. Past disposal practices have resulted in soil and groundwater contamination.

In addition to the concerns with radioactive wastes, the site operations resulted in the release of 2.1 million pounds of organic solvents to an unlined settling basin and discharge of 1.3 million pounds of solvents to a nearby stream. Contamination of soil and groundwater resulted from the migration of chlorinated hydrocarbons. The amount of dissolved organic solvents is estimated to be between 260,000 and 450,000 pounds and concentrations greater than 10 ppb have been detected. Among the volatile organic compounds (VOCs), trichloroethylene (TCE), tetrachloroethylene (PCE), and 1,1,1-trichloroethane (TCA) are of great concern.

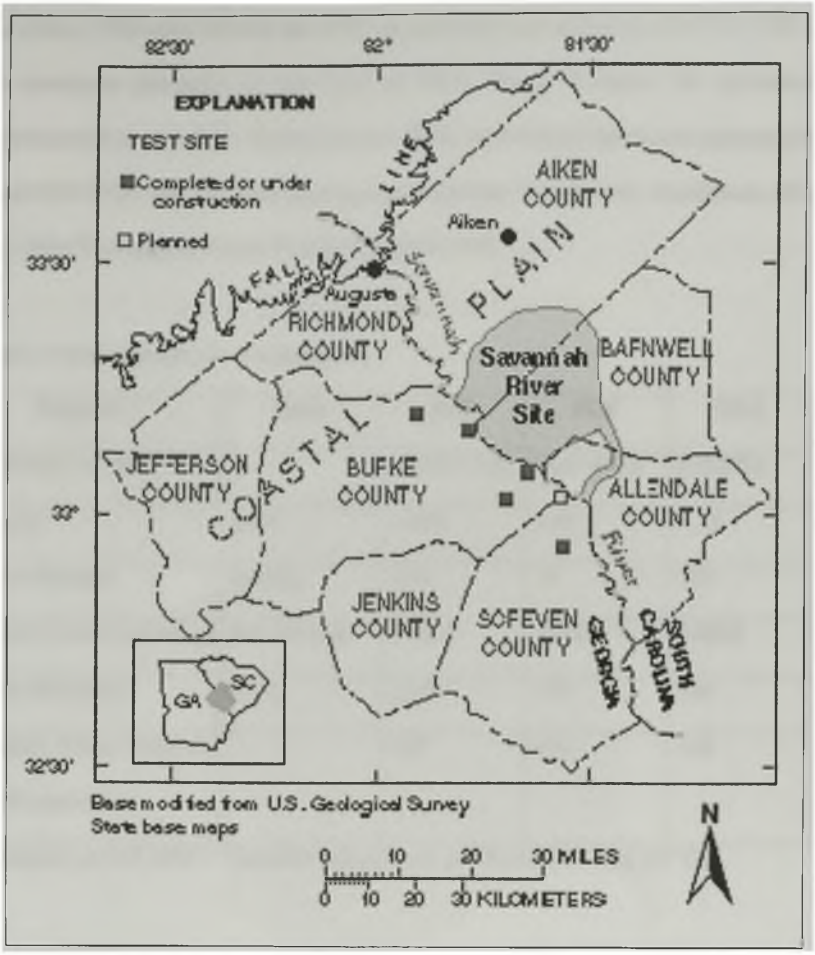

Figure 2: Savannah River Site operational Area.

Chemical properties of VOCs are listed in Table 1. VOC contamination appears throughout the vadose zone beneath the source areas with concentrations in fine-grained zones ranging up to 1,000 mg/g. Groundwater concentrations, ranging from saturated to less than 1 mg/L, are found throughout the area. Approximately a thickness of 150 feet

and about 1,200 acres of area has VOC-contaminated groundwater with 75% TCE and the remainder primarily in the form of PCE. Figure 3 shows the groundwater contamination area at SRS. During the year 1991, pure solvent dense non-aqueous phase liquid (DNAPL) was detected, causing complications to long-term remediation efforts. The DNAPL is approximately 95% PCE and 5% TCE.

Table 1: Chemical properties of Contaminants

Property *	Units	TCE	PCE	TCA
Empirical Formula	---	$ClCH=CCl_2$	$Cl_2C=CCl_2$	CH_3CCl_3
Density	g/cm^3	1.464	1.62	1.31
Vapor Pressure	mm Hg	57.9	19	124
Henry's Law Constant	atm m^3/mole	0.0091	0.0029	0.0016
Water Solubility	mg/L	1,100	150	300
Octanol- Water Partition Coefficient (K_{ow})	---	195	126	148

* Property at STP (STP = Standard Temperature and Pressure; 1 atm, 25° C)

Discovery of contamination adjacent to the settling basin in 1981 initiated a site assessment effort eventuality involving approximately 250 monitoring-wells over a broad area. A pilot groundwater remediation system began operation in February 1983. Full-scale groundwater treatment began in September 1985. Initial survey of the groundwater at SRS revealed the concentrations of VOC's greater than 50,000 ppb. During the year 2004, a maximum contamination of 37,300 ppb of TCE was detected. Initial cleanup

activities were initiated by the USDOE under a RCRA permit in 1985, following variety of measures including capping/solidification of various solid waste disposal sites and disposal basins, treatment of hazardous substances and groundwater pump and treat. Sites containing leaking underground storage tanks, chemical waste dumps and toxic chemicals require characterization and long term monitoring to protect environmental resources and to determine when remedial measures are needed.

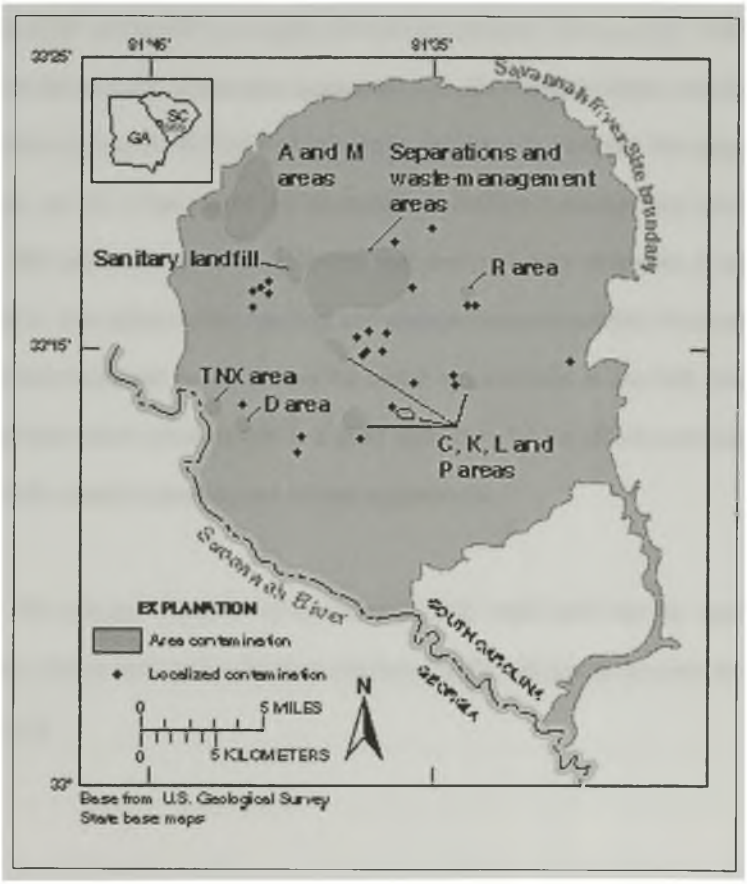

Figure 3: Groundwater contaminated area at SRS.

A typical long term monitoring plan consists of locating and installation of groundwater wells as well as developing a sampling and analysis scheme. The sampling frequency should be appropriate to detect the migration of the plume over time to protect receptors and to define trends in analyte concentrations and should account for groundwater flow and solute transport rates. Current sampling methods used at the SRS are costly and time-sensitive, and limitations in sampling and analytical techniques exist. DOE SRS requires manual collection of nearly 40,000 samples per year, which will cost between $100 and $1,000 per sample for off-site analysis. Wilson et al. (1995) reported that as much as 80% of the costs associated with site characterization and cleanup of a Superfund site can be attributed to laboratory analyses. In addition, the integrity of the analyses can be compromised during sample collection, transport, and storage as the VOCs that are intended to be collected and analyzed may evaporate if samples are exposed to atmosphere while handling and storage. Hence measured concentration at an off-site laboratory can be lower than the actual concentration at the field. Using the in-situ real time sensor network can be a good alternative for the off-site analysis, reducing the need for manual sampling and off-site analysis cost.

The primary objective of the research is to study and test the capabilities of sensors to detect and measure trichloroethylene (TCE) indicator parameters for long term monitoring.

2.0 LITERATURE REVIEW

Trichloroethylene (TCE) is a halogenated aliphatic organic compound, which due to its unique properties and solvent effects, has been widely used as an ingredient in industrial cleaning solutions and as a "universal" degreasing agent. Molecular structure of trichloroethylene (TCE) is shown in Figure 4. TCE, perchloroethylene (PCE), and trichloroethane (TCA) are the most frequently detected volatile organic chemicals (VOCs) in ground water in United States (Fischer et al., 1987), and are among 14 volatile organic compounds regulated under Safe Drinking Water Act Amendments of 1986. The maximum contamination limit for TCE is currently 5 ppb. Physical and chemical processes (e.g., air stripping and carbon adsorption) are used most frequently for remediation. However, interest has been growing in biological process because they offer the prospect of converting the contaminants to harmless products, rather then transferring them from one part of the environment to another.

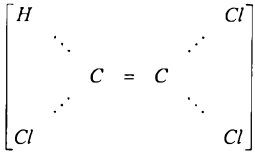

Figure 4: Molecular structure of trichloroethylene (TCE).

Contamination by chlorinated solvents exists in both vadose zone and saturated zone. Due to their high density and low solubility, chlorinated solvents move down to the bottom until an impermeable barrier is reached. In vadose zone, TCE associated with soil pore water enters gas phase or transformed into dense non-aqueous phase liquid

(DNAPL) because of its volatility. As TCE can transform into any of the three phases, the areal extent of spill may be more than the original spill.

2.1 Regulations

EPA regulates TCE under the Clean Air Act (CAA) as a hazardous air pollutant, the Safe Drinking Water Act (SDWA), the Superfund Amendment and Reauthorization Act (SARA), the Comprehensive Environmental Response, Compensation, and the Liability Act (CERCLA), and the Clean Water Act (CWA). Under SWDA, the maximum contamination level for TCE in drinking water is 5 ppb. TCE is regulated under Resources Conservation and Recovery Act as a halogenated organic compound (HOC) and under land disposal restrictions. Under the latter, hazardous waste that contains total concentrations of HCOs of at least 1,000 mg/L (liquids) or 1,000 mg/kg (non-liquids) are prohibited from land disposal. Priority data needs under SARA include exposure levels from humans living near hazardous waste sites and for other populations and epidemiological studies on health effects, including carcinogenity. TCE is on the CERCLA List of Hazardous Substances, with a reportable quantity of 100 lb (45.5 kg). TCE is regulated under the CWA as a priority pollutant in final discharge resulting from steam electric power generation. It is designated a hazardous substance if discharged to navigable waters. FDA regulations govern the presence of TCE in color additives, bottled water, and food, as extraction solvent residues, and as an indirect additive, and as byproduct from adhesives or other materials used in food packaging. The National Institute for Occupational Safety and Health (NIOSH) considers TCE to be a potential occupational carcinogen. NIOSH's recommended exposure limits are 2 ppm during use

of TCE as an anesthetic and 25ppm as a 10-hour time-weighted average (TWA) during all other exposures. The Occupational Safety and Health Administration's (OSHA's) permissible exposure limit for TCE in workroom air is 100 ppm as an 8 hour time weighted average (TWA), with a ceiling value of 200 ppm. OSHA also regulates TCE under Hazard Communication Standard and as a chemical hazard in laboratories.

2.2 TCE properties

Assessment of remediation technologies feasible for reclamation of subsurface environmental media contained with TCE must involve consideration of the compound's physical and chemical properties (i.e., distribution coefficients, reactivity, solubility, etc.). Table 2 represents the list of physico-chemical properties of TCE.

Table 2: Physico-chemical Properties of Trichloroethylene

Density	1.464 g/ml
Water Solubility	1100 mg/l
Henry's Law Constant (atm-m^3/mol @ 20° C)	0.0091
Molecular Weight	131.4
Boiling Point	86.7° C
Kow	195

These properties are directly responsible for behavior, transport and fate of chemical in the subsurface environment. Knowledge of a compound's physico-chemical tendencies can be used to predict and alter behavior and fate of that compound in the environment.

Density: (1.464 g/ml) Density can be defined as the concentration of matter, and is measured in mass per unit volume. In relation to liquids these units are grams per millimeter. TCE is heavier than water; therefore, a spill of sufficient magnitude is likely to move downward through the subsurface until lower permeable features impends its progress. This often results in formation of a pool of dense non-aqueous phase liquid (DNAPL). A density difference of about 1% above or below that of water (1.0 gm/ml) can significantly influence the movement of contaminants in saturated and unsaturated zones (Josephson, 1983).

Water solubility: Water solubility can be defined as the maximum concentration of a solute, which can be carried in water under equilibrium conditions and is generally given as ppm (parts per million) or mg/l (milligram per liter). The water solubility limit of TCE is 1100 mg/l, the maximum concentration of TCE that can be in aqueous solution at 20^0C. Water solubility of a compound has a direct relation on distribution coefficients and biodegradability. A compound that is relatively insoluble in water will prefer to partition into another phase; i.e., volatilize into soil, gas or sorb to organic material. Compounds that are not relatively insoluble are also not as readily available for transport across the bacterial membrane, and thus less subject to biological action.

K_{oc} value: K_{oc} is defined as the amount of sorption on a unit carbon basis. The K_{oc} can be predicted from other chemical properties of compounds such as water solubility and octanol water coefficient. The low K_{oc} value of 2.42 for TCE translates into little retardation by soil or aquifer organic material.

Henry's Law Constant: Henry's law states that the amount of gas that is dissolved in a given quantity of liquid at constant temperature and pressure is directly proportional to partial pressure of the gas above the solution. Henry's coefficients, as a result, describe the relative tendency of a compound to volatilize from liquid to air. The Henry's Law Constant for TCE is 0.0091, which is high enough when combined with its low solubility in water and high vapor pressure, for efficient transfer of TCE to the atmosphere. The evaporation half-life of TCE in water is on the order of 20 minutes at room temperature in both static and stirred vessels (Dilling, 1975; Dilling et al., 1975).

Environmental Distribution of Trichloroethylene:

It has been estimated that about 60% of the total TCE produced in the United States is lost to the atmosphere, with negligible discharge into water bodies (Cohen and Ryan, 1985). Trichloroethylene contamination exists in both vadose and saturated zones of the subsurface environment. This contamination results from spills, leaking transfer lines, storage tanks and poor environmental awareness. Because of its density and low Koc, TCE will ultimately move downward in the vadose zone until an impermeable barrier is reached. Such a scenario occurs when a TCE spill is of sufficient magnitude or deep enough in the vadose zone for volatilization to be restricted. Once in the vadose zone, TCE can become associated with soil pore water, enter the gas phase because of its Henry's constant, or exist as non-aqueous phase liquid (NAPL). It is therefore conceivable that upward or downward movement of TCE can occur in each of these three phases, thereby increasing areal extent of the original spill in both the vadose and saturated zones. While movement of large concentrations of TCE through the vadose

zone may be rapid, surface tension exerted at the capillary fringe may retard further downward movement of smaller spills.

Non-aqueous phase concentrations of TCE, which are large enough to overcome capillary forces, will move downward into the aquifer. Once the water table is penetrated, lateral flow may be mediated by the regional ground-water flow. Due to its high density, the movement of free-phase TCE is still directed vertically until lower permeability features are encountered. Once an impermeable layer is encountered, horizontal movement will occur. Such movement may even be directed against the natural ground-water flow by the effects of gravity. Since permeability is a function of the liquid as well as the medium, the vertical movement of TCE through an aquifer is determined by geological properties of the aquifer material; i.e., granular size of sand or clay lenses. Trichloroethylene will tend to pool near these impermeable features. Water passing over and around these pools solubilizes TCE that can be spread throughout the aquifer.

2.3 Treatment Technologies:

This section describes the various treatment technologies available for treatment of trichloroethylene.

2.3.1 Surface treatment Technologies

2.3.1.1 Air Stripping

Air stripping is an applicable technology for removal of TCE from contaminated water. A constant stream of air is used to drive TCE from solution, taking advantage of the low Henry's Law constant and water solubility. This process, however, only shifts the compound to another medium. New restrictions on venting of volatile organic

compounds to the atmosphere may preclude the use of such technologies or require treatment of the air-stripped off-gases by carbon adsorption. The air stripper allows percolation of large volumes of air through contaminated water. This has the effect of changing conditions to favor volatilization of TCE. Application limitations usually occur as the concentration of TCE falls below a threshold level where volumes of air larger than logistically possible are required to continue the stripping process.

In 1982, 10-12 United States utilities were using some form of aeration technique to strip volatile organic chemicals such as TCE from water supplies. The air strippers used were of three principal types: redwood slat aerators, packed towers and spray towers, all of which are designed to allow maximum contact of water, air and TCE (Robeck and Love, 1983). Since 1982, many sites and municipal supply systems have utilized air strippers (generally packed towers) as a method for removing VOCs from contaminated ground water.

2.3.1.2 Combined Air Stripping and Carbon Adsorption

A method for remediating water contaminated with TCE is to combine the technologies of air stripping and granular activated carbon (GAC) adsorption. This treatment train is attractive because it ameliorates shortcomings of both technologies. In the case of air stripping, the residual concentration of TCE in treated effluent may be above local or regional drinking water standards, thereby necessitating a "polishing" step prior to use or discharge. Limitations associated with GAC adsorption are:

- A given sorbent has a finite capacity for sorbtion of a given contaminant; once this limit is reached, contaminant breakthrough occurs. This breakthrough results from competition between contaminants and normal solutes for unbound sites; less tightly bound solutes are displaced by those solutes having a greater affinity. When loss of binding efficiency becomes great enough, breakthrough of contaminants occurs. Once breakthrough occurs, the sorbent must be changed or cleaned. Adsorption of TCE to GAC, based on equilibrium concentrations of 1 ppm at neutral pH and 20°C, is 28 mg/g (Amy et al., 1987).
- High dissolved organic carbon (DOC) and other contaminants can compete with TCE for binding sites available on the sorbent, thus increasing the likelihood of breakthrough. A concentration of 10-ppm natural organic matter in river water has been shown to reduce TCE adsorption by up to 70% (Amy et al., 1987).

Recognizing the limitations of sorption (the first may only be economically limiting), use of this technology, as a polishing step following air stripping can be a viable link in a surface treatment process for TCE. Air stripping can be used to remove the majority of TCE, followed by adsorption, which is used to polish the stripper effluent. This approach will lower construction costs of the air stripper and increase life expectancy of the adsorbent.

2.3.2 Subsurface Remediation Technology

2.3.2.1 Soil Venting

Soil venting is an in-situ air stripping technique used to remove volatile contaminants from the vadose zone. Air is forced into the soil subsurface through a series

of air inlets, then vented or extracted under vacuum through extraction pipes. Air laden with organic vapors moves along an induced flow path toward withdrawal wells where it is removed from the unsaturated zone and treated and/or released to the atmosphere (Baeher et al., 1988). Success of the method depends on rate of contaminant mass transfer from immiscible and water phases to the air phase, and on ability to establish an airflow field that intersects the distributed contaminant. In many cases, treatment of off-gases may be required due to air quality standards.

Trichloroethylene, due to its high potential for interphase transfer to the gaseous phase, should be an excellent candidate for soil venting technologies. Cary et al. (1989) suggested that by forcing air into the water table below the contamination and maintaining sufficient air entry pressure throughout a significant volume of soil, TCE should be trapped at the soil water interface and released to the gas phase. This release to the gaseous phase would favor transfer to the soil surface. Field results reported by Mehran et al. (1987) link the TCE concentration in soil gas with current levels in ground water; therefore, the theory suggested by Cary et al. would appear to be correct for contamination at or near the soil-water interface. This also may point to the possibility that movement of gaseous TCE from the saturated zone into the soil gas phase may act to further spread contamination. Soil venting technology for removal of TCE has not been fully tested in the field or, at least, has not been reported in refereed literature; however, results from two pilot-scale tests demonstrate the potential applicability of the technology for removal of TCE from the vadose zone (Coia et al., 1985 and Danko et al., 1989).

2.3.2.2 In-Well Aeration

Another remediation method based on interphase transfer potential of TCE is in-well aeration. In-well aeration can be accomplished using either an air-lift or electric submersible pump and sparger. The air-lift pump may or may not be used with a sparger. Technologies based strictly on volatilization of TCE, however effective at subsurface remediation, do not satisfy the intent of Section 121 of CERCLA. This Section states that "remedial actions in which treatment permanently and significantly reduces the volume, toxicity or mobility of the hazardous substances, pollutants, and contaminants, in a principal element, are to be preferred over remedial actions not involving such treatment." Air stripping simply transfers TCE from one medium (soil or water) to another (atmosphere) without any significant reduction in volume or toxicity.

2.3.2.3 Bioremediation

Biological remediation is one methodology, which has the potential to satisfy the intent of Section 121 of CERCLA. Although bioremediation of environments contaminated with TCE can best be described as in its infancy, this technology remains attractive since the possibility exists for complete mineralization of TCE to CO2, water, and chlorine instead of simply a transfer from one medium to another. In the case of biological degradation, bacteria may produce necessary enzymes and cofactors which act as catalysts for some of the chemical processes already described.

Aerobic Degradation: It has long been thought that TCE is resistant to degradation under aerobic conditions due to its already oxidized state. Recently, a number of

monooxygenases produced under aerobic conditions have been shown to degrade TCE (Nelson et al., 1987; Harker and Young, 1990). Under these conditions, there is no buildup of vinyl chloride, and complete mineralization is possible. An aromatic compound such as toluene or phenol is required, however, for induction of the enzymes responsible. As a result any application of this system would require the presence of a suitable aromatic compound or other inducers. The inducer requirement might be alleviated by manipulation of the proper genetic sequence for monooxygenase production, but other carbon and\or energy sources would probably be required for growth.

Methylotrophic degradation: The enzyme methane monooxygenase (MMO) produced by methylotrophic bacteria growing in the presence of oxygen at the expense of methane has a wide range of growth substrates and pseudo substrates; one of which is TCE, this enzyme epoxidates TCE. The resulting chemical complex is unstable and quickly hydrolyzes to various products dependent on the pH of the menstruum. TCE epoxide in phosphate buffer at pH 7.7 has a half-life of 12 seconds (Miller and Guengerich, 1982). If TCE Epoxidation by MMO follows enzyme kinetics similar to that of a true growth substrate and inhibitor rather than some enzyme modification due to its reaction with MMO, the question arises as to whether or not methane necessary for production of MMO will competitively inhibit TCE epoxidation (or more correctly overcome the TCE inhibition). If such is the case, only a certain percentage of TCE may be epoxidated before it is transported away from the bacteria in a flow situation. Since this process is co-metabolic, methane is a strict requirement. Removal of the true substrate will result in

rapid loss of production of MMO, thereby reducing the ability to epoxidate TCE. Methods to enhance this ability are genetic in nature. Genetic recombination resulting in constitutive expression of the MMO genes is required in order that methane will no longer be required for induction. Even if constitutive expression is achieved, small amounts of methane still may be needed as a carbon and energy source.

Anaerobic degradation: Because of the oxidized state of TCE, ecological condition under which degradation is most likely to occur is a reducing environment. Biological degradation (and possibly mineralization) of TCE under anaerobic conditions has been studied for a number of years (Bouwer and McCarty, 1983; Bouwer et al., 1981). The first reported biological attenuation of TCE was that of the obligate anaerobic methanogenic bacteria through a process known as reductive dehalogenation. Under anaerobic conditions, oxidized TCE can function as an electron sink and is readily reduced by electrons (or reducing equivalents) formed as a result of metabolism (oxidation) of organic electron donors by members of methanogenic consortia. Volatile fatty acids and toluene may serve as oxidizable substrates (electron donors), which can be coupled to reduction of halo-organic molecules such as TCE with the resultant removal of a chlorine atom (Sewell et al., 1990). Freedman and Gossett (1989) reported conversion of PCE and TCE to ethylene without significant conversion to carbon dioxide. The conversion occurred in an anaerobic system stimulated with electron donors such as hydrogen, methanol, formate and acetate, with added yeast extract. Presumably, the electron donors provided the electrons or reducing equivalents necessary for complete chlorine removal by reductive dehalogenation. PCE mineralization has been reported by

other authors (Vogel and McCarty, 1985), although it seems that in the absence of sufficient oxidizable organic compounds a buildup of DCE(s) or vinyl chloride also will occur (Bouwer and McCarty, 1983; Bouwer et al., 1981). Theoretically, under anaerobic conditions, with sufficient quantities of other readily oxidizable substrates and the necessary auxiliary nutrients, methanogenic consortia may be capable of converting TCE to harmless end products. More research is needed, however, to determine how effective this remediation may be, and what actual requirements are needed to drive the process. Advantages of anaerobic processes are: there appears to be no apparent lower concentration limit to activity nor is there a need to perfuse the subsurface with copious amounts of oxygen.

Mixed Consortia Degradation: Recently, it has been shown that mixed consortia of bacteria can effectively mineralize TCE (Henson et al., 1988; Wilson and Wilson, 1985). This involves co-metabolism of TCE (epoxidation) by bacteria that oxidize gaseous hydrocarbons such as methane, propane and butane, followed by hydrolysis of the TCE epoxide. The hydrolysis products are then utilized by other naturally occurring bacteria. Wackett et al. (1989) surveyed a number of propane oxidizing bacteria for their ability to degrade TCE. While TCE oxidation was not common among the bacteria surveyed, unique members could oxidize TCE. High concentrations (>15% v/v) of the gaseous hydrocarbons were found to inhibit co-metabolism of TCE. Oxygen concentrations also could be limiting in aqueous treatment systems since the gaseous, alkane-utilizing and heterotrophic population requires oxygen.

2.4 Background on Anaerobic Degradation Process

Under anaerobic conditions, chlorinated VOCs can be biodegraded by reductive dechlorination pathways, which entail the replacement of chlorine atoms by hydrogen to produce more reduced, less chlorinated products (Bouwer, 1992; Chapelle, 1993). Biodegradation of highly chlorinated VOCs such as TCE is known to occur under a range of anaerobic conditions (nitrate-reducing, iron-reducing, sulfate-reducing, and methanogenoic) but is believed to be faster and more likely to result in complete dechlorinaton to nontoxic end products of ethylene and ethane under methanogenic conditions compared to less reducing conditions (McCarty and Semprini, 1994).

Highly chlorinated VOCs mostly undergo reductive dehalogenation through co-metabolism, giving the microorganism neither energy nor growth (McCarty and semprini, 1994). Under anaerobic conditions the chlorinated VOCs can act as electron acceptors, and the presence of electron donors is needed to drive the reaction. Reductive dechlorination of tetrachloroethylene and TCE is not sustainable unless an electron donor, such as methanol, acetate, or gaseous hydrogen, is provided (Fathepure and Boyd, 1988; Freedman and Gossett, 1989).

TCE biodegrades under anaerobic conditions through hydrogenolysis, a reductive dechlorination process that sequentially produces isomers of 1,2- dichloroehtylene (12DCE), vinyl chloride (VC), and ethylene as shown in Figure 5. Ethane also has been reported as a degradation product (Belay and Daniels, 1987; de Burin et al., 1992). Of the three possible DCE isomers that can be produced from hydrogenolysis of TCE, several

studies have indicated that the cis-isomer of 1,2-dichloroethylene (cis-12DCE) predominates over trans-12DCE and that 1,1-dichloroethylene is the least significant intermediate (Bouwer, 1994).

Subsurface microorganisms can degrade fuel hydrocarbons through a primary substrate and chlorinated aliphatic hydrocarbons through electron donor, electron acceptor and co-metabolism, where chlorinated organic is fortuitous and there is no benefit to microorganism. At many sites, use chlorinated hydrocarbon as electron acceptors appears to be more predominant under natural conditions. Where as in case of biodegradation of chlorinated aliphatic hydrocarbons will be an electron-donor-limited process. Native organic carbon is used as electron donor and dissolved oxygen (DO) as electron acceptor. If anthropogenic carbon (e.g., fuel hydrocarbon) is present, then it will be used as an electron donor. When all the dissolved oxygen is consumed, anaerobic microorganism typically use additional electron acceptor in the following order of preference if present: nitrate, ferric iron oxyhydroxide, sulfate and finally carbon dioxide.

In general all types of bacteria are present at all sites. However all bacteria involved in all of the potential biodegradation pathways for chlorinated solvents are not necessarily present at every site. All of the bacteria needed for the reductive dechloronation of PCE or TCE to DCE are present at approximately 90% of all sites, where as all of the bacteria needed for the reductive dechlorination of PCE or TCE to ethene are present at approximately 75% of all sites.

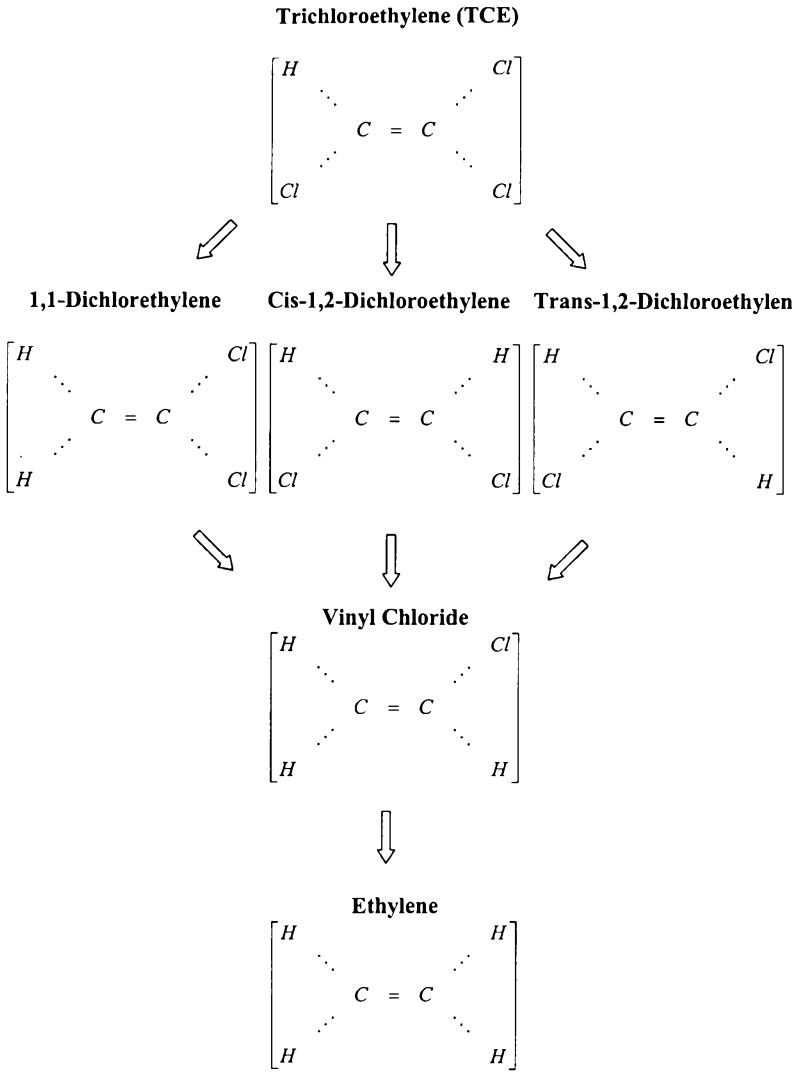

Figure 5: TCE biodegradation pathway under anaerobic degradation (Ref. Brower, 1994)

Biodegradation Mechanisms of Chlorinated Aliphatic Hydrocarbon

Reductive Dechlorination (Electron Acceptor Reaction): Reductive dechlorination is the most important process for the natural biodegradation of the more highly chlorinated solvents. Chlorinated hydrocarbon is used as an electron acceptor, not as a source for carbon, and chlorine atom is removed and replaced by hydrogen atom. In general, the process occurs by sequential dechlorination from PCA to TCE to DCE to VC to ethane. During reductive dechlorination, all three isomers of dichloroethene can theoretically be produced, however according to Bouwer (1994), under the influence of biodegradation, cis-1,2-di-chloroethene is a more common intermediate than trans-1,2-dichloroethene, and that 1,1-dichloroethene is least prevalent intermediate of the three dichloroethene isomers. The rate of reductive dechloronation decreases as the degree of chlorination decreases. Because chlorinated aliphatic hydrocarbons are used as electron acceptors, there must be an appropriate source of carbon for microbial growth.

Electron Donor Reactions: Microorganisms are generally believed to be incapable of growth using trichloroethene and perchloroethene as a primary substrate (Murray and Richardson, 1993). Under aerobic and some anaerobic conditions, less oxidized chlorinate aliphatic hydrocarbons can be used as the primary substrate in biologically mediated redox reactions (McCarty and Semprini, 1994). Microorganisms obtain energy and organic carbon from degraded chlorinated aliphatic hydrocarbons.

Co-metabolism: When a chlorinated aliphatic hydrocarbon is biodegraded via co-metabolism, the degradation is catalyzed by an enzyme or cofactor that is fortuitously

produced by the organisms for other purpose. Co-metabolic degradation of chlorinated aliphatic hydrocarbon may be harmful to the microorganism responsible for the production of enzyme or cofactor, since microorganism receives no known benefit from degradation (McCarty and Semprini, 1994).

Products of chlorinate solvents biodegradation

Carbon dioxide, water, and chloride are the products resulting from an aerobic mineralization of chlorinated solvents (Roberts 1989). Non-chlorinated products such as ethene, ethane and methane are produced by the reductive dechloronation of chlorinated compounds by the anaerobic degradation of chlorinated solvents. Complete dechloronation products such as ethene and chloride are not deemed to be a problem as the maximum contamination limit for chloride in groundwater is 1,000 mg/l; where as biodegradation of 100 mg/l of TCE would result in the release of approximately 80 mg/l of chloride over a long period of time.

2.5 Natural Attenuation

Natural Attenuation refers to naturally occurring process in soil and groundwater environments, which reduces the mass, toxicity, mobility, volume and concentration of a contamination without human intervention. The National Contingency Plan permits the use of natural attenuation as a remedy for superfund sites. Acknowledged by J. Willson, the natural attenuation will be affective as the sole remedy at approximately 20% of all chlorinated site. The processes that contribute to the natural attenuation of chlorinated solvents are:

Degradation: Degradation of most of the chlorinated solvents in groundwater occurs by the predominant oxidation-reduction reactions carried out by the bacteria. These reactions are referred to as biodegradation reactions. Biodegradation usually is the predominant process of natural attenuation at chlorinated solvents sites. The processes by which chlorinated solvents biodegrade are:

- Direct oxidation: chlorinated compound is used as electron source by breaking down into inorganic molecules such as carbon dioxide, water and chloride.
- Reductive dehalogenation: chlorinated compound is converted to another chemical by replacing the chlorine atoms with hydrogen atoms.
- Co-metabolism: chlorinated compound is converted into another chemical while microorganisms use other carbon compounds for their growth substrate.

Advection, Dispersion and Dilution: Transport of molecules dissolved in water is referred as advection. During advection, molecules will also spread along and away from the expected groundwater flow path. This is called dispersion and results from the mixing of groundwater and other molecules in individual pores and channels. The combination of these processes results in reduction of concentration of the molecules in the groundwater called dilution.

Diffusion: Diffusion is a dispersive process that results from the movement of molecules along the concentration gradient from higher concentrations to lower concentrations.

Sorption/Desorption: Molecules can adsorb onto and, in some cases, be absorbed by geologic materials. Over time, these molecules will desorb from the geologic materials in response to concentration gradients. Sorption affects the advective rate of molecules dissolved in groundwater.

Volatilization: The transfer of a molecule from a liquid phase or an aqueous solution to the vapor phase is known as volatilization. Chlorinated solvents are volatile organic compounds (VOC) that partition between liquid phase and gas phase, with less chlorinated compounds having a tendency towards higher volatility.

Stabilization: Process by which chemical molecules becomes chemically bound or transformed by a stabilization agent by reducing the mobility of the molecule in the groundwater, which is an irreversible reaction.

Evaluation of Natural Attenuation

Natural attenuation should be evaluated to some extent at every site, early in the process of investigation. Natural attenuation should be evaluated thoroughly when:

- Natural attenuation processes are observed or strongly expected to be occurring
- There are no human or ecological receptors that are likely to be impacted or potential receptors in the vicinity of the plumes are, or can be protected

The suggested lines of evidences are:

Documented decrease of contaminant mass at the site: Contamination loss is documented by reviewing the historical trends in contaminant concentration and distribution, in conjunction with site geology and hydrogeology to show that a reduction in the total mass of contaminant is occurring at the site.

Geochemical and Biochemical indicators: Examining the changes in the concentrations and distributions of geochemical and biochemical indicator parameters can show evidence to specific natural attenuation.

Direct microbial evidence: Microcosm studies are often used as the third line of evidence for natural attenuation.

Sequential order of PCE, TCE, DCE, VC and ethenes during the anaerobic degradation of PCE is shown in figure 6. BOD is supporting the growth of anaerobic bacteria as shown by the production of methane and acetate, and depletion of sulphate. Sulphate reducing and possibly iron reducing bacteria appear responsible for the initial dechlorination of PCE through to DCE. As the sulphate concentrations decreases, the activity of methanogenic bacteria increases. Under methanogenic/acetogenic conditions 1,2-DCE and VC are dechlorinated to ethene.

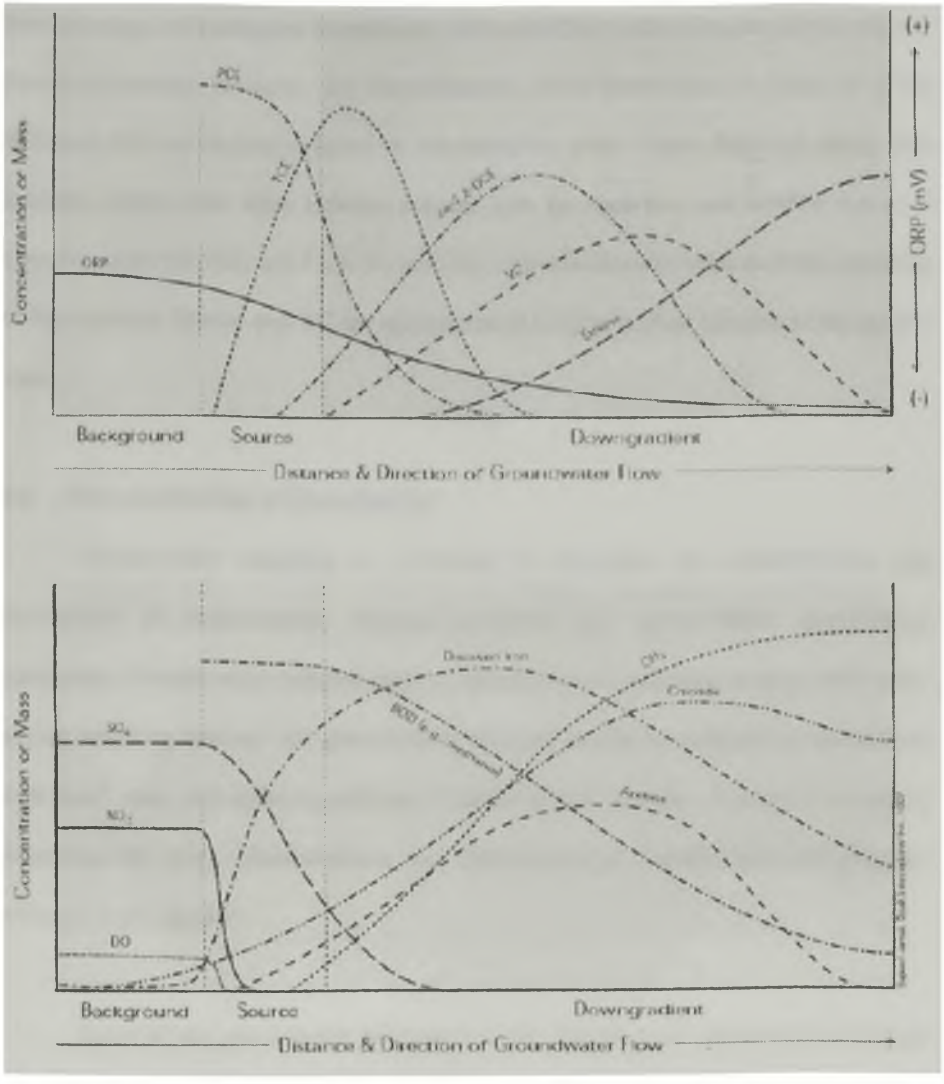

Figure 6: Common patterns of chlorinated solvents biodegradation in an anaerobic system
(Natural Attenuation of Chlorinated Solvents in Groundwater, May 1999)

Figure 7 shows the anaerobic zone developed in an aerobic groundwater system due to the metabolism of the BOD in the source area. In the anaerobic zone PCE is dechlorinated to TCE, DCE, VC and finally ethene. Methanogenic, sulphate reducing,

iron reducing, and acetogenic bacteria are active, and their interactions are responsible for the dechlorination. However, the dechlorination rate is insufficient to cause all of the TCE and DCE to be dechlorinated in the anaerobic zone. These chemicals along with methane, ethane, and vinyl chloride migrate into the transition and aerobic zones. In transition zone the TCE and DCE are partially cometabolized by methanotrophs growing on the methane. Ethene and VC are mineralized to CO_2 by aerobic bacteria in the aerobic zone.

2.6 Characterization of Groundwater

Ground-water sampling is conducted to determine the concentrations and distribution of contaminants, daughter products, and ground-water geochemical parameters. Ground-water samples may be obtained from monitoring wells or with point-source sampling devices. All ground-water samples should be collected in accordance with local, state, and federal guidelines. Volatile organic compound analysis is used to determine the types, concentrations, and distributions of contaminants and daughter products in the aquifer.

Some of the groundwater parameters to be characterized and monitored during biodegradation are:

Electron Acceptor: Dissolved oxygen (DO) is the most thermodynamically favored electron acceptor by microbes for the biodegradation of organic carbon. Reductive dechlorination will not occur if DO concentrations are above 0.5 mg/L.

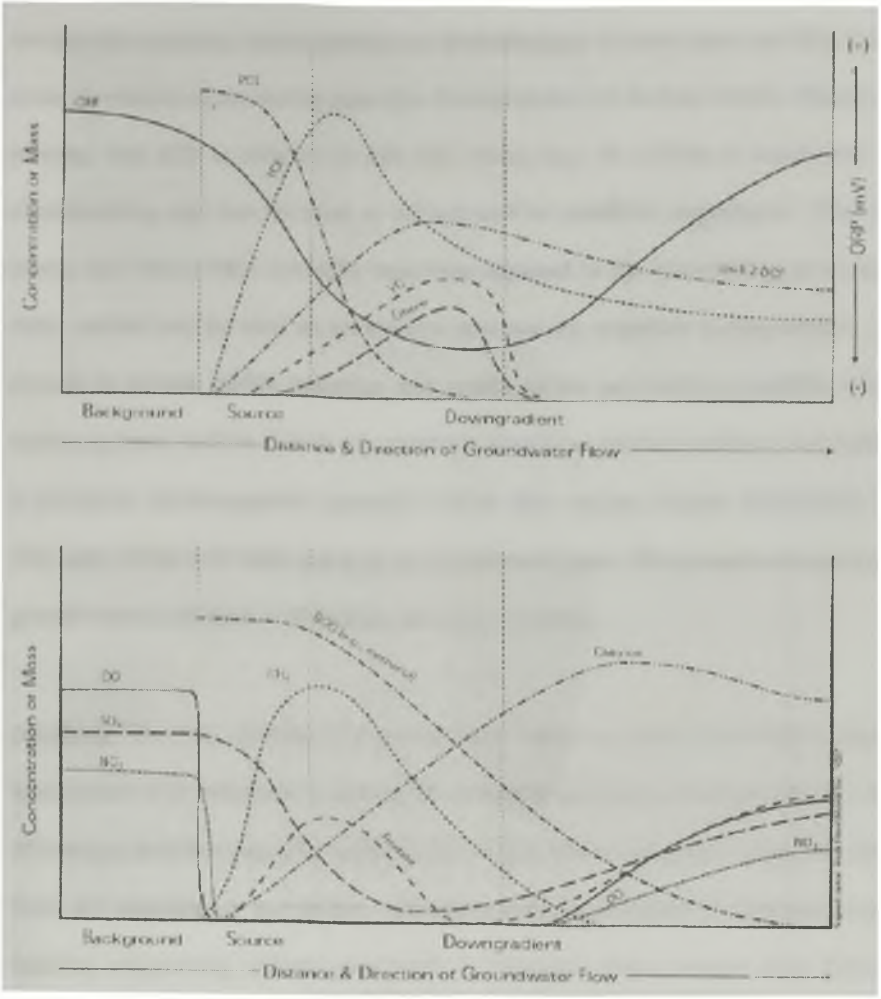

Figure 7: Patterns of chlorinated solvent biodegradation in sequential aerobic/anaerobic system
(Natural Attenuation of Chlorinated Solvents in Groundwater, May 1999)

During aerobic biodegradation of a substrate, DO concentrations decrease because of the microbial oxygen demand. After DO depletion, anaerobic microbes will use nitrate as an electron acceptor, followed by iron (III), then sulfate, and carbon dioxide. After DO has been depleted in the microbiological treatment zone, nitrate may be used as an electron

acceptor for anaerobic biodegradation via denitrification. In some cases iron (III) is used as an electron acceptor during anaerobic biodegradation of electron donors. During this process, iron (III) is reduced to iron (II), which may be soluble in water. Iron (II) concentrations can thus be used as an indicator of anaerobic degradation. After DO, nitrate, and bioavailable iron (III) have been depleted in the microbiological treatment zone, sulfate may be used as an electron acceptor for anaerobic biodegradation. This process is termed sulfate reduction and results in the production of sulfide. During methanogenesis, carbon dioxide (or acetate) is used as an electron acceptor, and methane is produced. Methanogenesis generally occurs after oxygen, nitrate, bioavailable iron (III), and sulfate have been depleted in the treatment zone. The presence of methane in ground water is indicative of strongly reducing conditions.

Alkalinity: The total alkalinity of a ground-water system is indicative of water's capacity to neutralize acid. Alkalinity is defined as "the net concentration of strong base in excess of strong acid with a pure CO_2-water system as the point of reference". Alkalinity results from the presence of hydroxides, carbonates, and bicarbonates of elements such as calcium, magnesium, sodium, potassium, or ammonia. These species result from the dissolution of rock (especially carbonate rocks), the transfer of CO_2 from the atmosphere, and the respiration of microorganisms. Alkalinity is important in the maintenance of groundwater pH because it buffers the groundwater system against acids generated during both aerobic and anaerobic biodegradation. Total alkalinity will be higher than the background alkalinity as increase in alkalinity resulting biodegradation.

Oxidation-Reduction (Redox) Potential: It is a measure of electron activity and an indicator of the relative tendency of a solution to accept or transfer electrons. Redox reactions in ground water containing organic compounds (natural or anthropogenic) are usually biologically mediated; therefore, the oxidation-reduction potential of a ground-water system depends on and influences rates of biodegradation. Knowledge of the oxidation-reduction potential of ground water also is important because some biological processes operate only within a prescribed range of redox conditions. The oxidation-reduction potential of ground water generally ranges from -400 to 800 millivolts (mV). Figure 8 shows the typical redox conditions for ground water when different electron acceptors are used. Oxidation-reduction potential can be used to provide real-time data on the location of the contaminant plume, especially in areas undergoing anaerobic biodegradation.

Temperature: Temperature of the groundwater directly affects the solubility of oxygen and other geochemical parameters. Oxygen is more soluble in cold water than in warm water. Temperature also affects the activity of bacteria. Rates of hydrocarbon biodegradation roughly double for every 10°C increase in temperature, over a temperature range of 5 °C to 25 °C. Groundwater temperature less than 5 °C tend to inhibit biodegradation.

pH: The pH of groundwater has an effect on the presence and activity of microbial population in the groundwater. Bacteria generally prefer a neutral or slightly alkaline pH

level, with an optimum pH range for most microorganisms between 6.0 and 8.0, however some microorganisms can tolerate a pH range from 5.0 to 9.0.

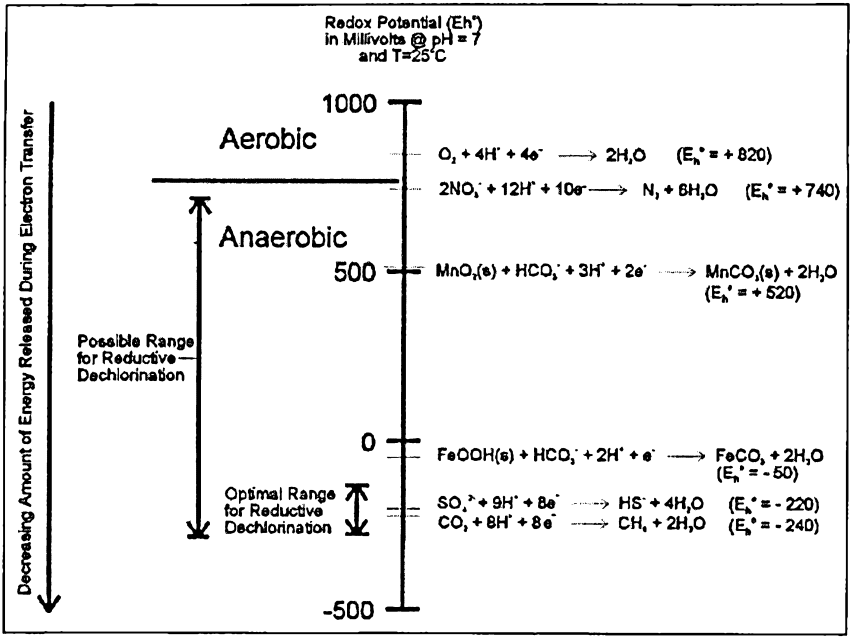

Figure 8: Redox potentials for various electron acceptors

Conductivity: Conductivity is a measure of the ability of a solution to conduct electricity. The conductivity of ground water is directly related to the concentration of ions in solution; conductivity increases as ion concentration increases. Conductivity measurements are used to ensure that groundwater samples collected at a site are representative of the water in the saturated zone containing the dissolved contamination. If the conductivities of samples taken from different sampling points are radically different, the waters may be from different hydrogeologic zones.

Chlorine: Elemental chlorine is the most abundant of the halogens. Although chlorine can occur in oxidation states ranging from Cl^- to Cl^{+7}, the chloride form (Cl^-) is the only form of major significance in natural waters (Hern, 1985). Chloride forms ion pairs or complex ions with some of the cations present in natural waters, but these complexes are not strong enough to be of significance in the chemistry of fresh water (Hern, 1985). The chemical behavior of chloride is neutral. Chloride ions generally do not enter into oxidation-reduction reactions, form no important solute complexes with other ions unless the chloride concentration is extremely high, do not form salts of low solubility, are not significantly adsorbed on mineral surfaces, and play few vital biochemical roles (Hern, 1985). Thus, physical processes control the migration of chloride ions in the subsurface. Reductive dechlorination results in the accumulation of inorganic chloride. In aquifers with a low background of inorganic chloride, the concentration of inorganic chloride should increase as the chlorinated solvents degrade. The sum of the inorganic chloride plus the contaminant being degraded should remain relatively consistent along the ground-water flow path.

3.0 SENSORS

A sensor is a device that receives and responds to an electrical signal or stimulus. Sensors are classified into two kinds Passive and Active. Passive sensor does not need any additional energy source and directly generates an electrical signal in response to an external stimulus, whereas active sensor requires external power for their operation, called an excitation signal.

Chemical sensors respond to stimulus produced by various chemicals and chemical reactions. Chemical sensors are intended for the identification and quantification of chemical species including both liquid and gas phases. Chemical sensors can be described using criteria and characterization general to all sensors such as stability, repeatability, linearity, response time, but two unique characteristics that separate them from other sensors are selectivity and sensitivity. Selectivity is the degree to which a sensor responds to only the desired species, with little or no interaction from other species. Sensitivity is the minimal concentrations and concentration changes that can be successfully and repeatedly sensed by the sensor.

One of the greatest difficulties in developing chemical sensors is that chemical reactions change the sensor, often in a way that is irreversible. Electrochemical cells employing liquid electrolytes lose a small amount of electrolytes with each measurement, requiring refilling of the electrolyte. Some of the simplest chemical sensor designs require that the sensing element chemically react with the analyte to effect a measurable

change in the indicator or signal, which often influences the device, causing stability problems.

3.1 Classification of Sensors

All chemical sensors can be classified into two major groups, direct (simple) sensors and indirect (complex) sensors.

Direct Sensors: Direct chemical sensors utilize any of a variety of chemical reaction phenomena that directly affect a measurable electrical characteristic such as resistance, potential, current and capacitance.

- Metal-Oxide chemical sensor: These are simple rugged devices that perform reasonably well with relatively simple electronic support. Bulk metal oxides have electrical properties that change in the presence of reducible gases such as methyl mercaption and ethyl alcohol. Metal oxide sensors change resistivity as a function of the presence of reducible gases.
- ChemFET: A chemFET is a chemical field-effect transistor that includes a gas-selective coating or series of coatings between its transistor gate and the analyte. This chemical element gives the device a control input that modifies the source drain conduction in relationship with selected chemical species.
- Electrochemical sensor: The electrochemical sensors, depending on the operating mode, they are divided into sensors which measure voltage (potentiometric), those which measure electric current (amperometric), and those which rely on the

measurement of conductivity or resistivity (conductimetric). A fundamental rule of an electrochemical sensor is that it always requires a closed circuit that is an electric current must be able to flow in order to make measurement.

- Potentiometric Sensor: This sensor uses the effect of concentration on the equilibrium of the redox reactions occurring at the electrode-electrolyte interface in an electrochemical cell. An electrical potential may develop at this interface due to the redox reaction which takes place at the electrode surface.
- Conductometric Sensors: An electrochemical conductivity sensor measures the change in the conductivity of the electrolyte in an electro chemical cell. An electrochemical sensor may be involved a capacitive impedance resulting from the polarization of the electrodes and faradic or charge-transfer process.
- Amperometric Sensors: The operating principle of the electrode is based on the use of an electrolyte solution contained within the electrode assembly to transport oxygen from an oxygen permeable membrane to the metal cathode. The cathode current arises from a two step oxygen reduction process.

- Enhanced Catalytic Gas Sensor: Enhanced catalytic gas sensors are experimental devices that employ active measurement techniques coupled with fairly simple electrochemical cell. The electrochemical cells are fabricated of ceramic metallic films and provide the reaction environment for potentiometric and amperometric measurements.

Complex Sensors: Complex (indirect) sensors employ chemistry influenced phenomena that do not directly affect an electrical characteristic and will require some form of

transducing to obtain electrical signal. These sensors involve chemical phenomena that change the state of an indicator as a function of some chemical reaction. These indicators require another transducer to convert the changing indicator to an electrical output.

- Thermal sensors: A temperature probe is coated with a chemically selective layer, upon introducing the sample; the probe measures the release of heat during the reaction between the sample and coating.
- Pellister Catalytic Sensors: These sensors operate on the principle similar to thermal enzymatic sensors. Heat is liberated as a result of a catalytic reaction taking place at the surface of the sensors and the related temperature change inside the device is measured.
- Optical Chemical Sensors: Optical sensors are based on the interaction of electromagnetic radiation with matter, which results in altering (modulating) some properties of the radiation. The presence of different chemicals in the analyte affects which wave lengths of light are modulated.
- Mass Detector: Chemical sensor that utilizes the very small mass change from absorbed chemical molecules to alter mechanical properties of a system is referred to as mass, gravimetric, or microbalance sensors.

3.2 Indicator Parameters

Possible indicator parameters that can be used for monitoring the degradation of trichloroethylene (TCE) include:

- Temperature
- Dissolved Oxygen (DO)

- Conductivity
- Redox or Oxidation Reduction Potential(ORP)
- pH
- Turbidity
- Chloride
- Nitrate/Nitrite
- SO_4
- Sulfide
- CO_2
- Total Organic Carbon (TOC)

Among these, the following seven indicator parameters were chosen for long term monitoring of the degradation of trichloroethylene (TCE):

- Temperature: Solubility of oxygen is dependent on temperature and also the activity of bacteria is also temperature dependent.
- pH: Presence and activity of bacteria is dependent on pH as bacteria are likely to grow better in neutral or slightly alkaline pH. Also release of hydrochloric acid during the breakdown of TCE and its daughter products increases the pH.
- Redox (ORP): Oxidation-reduction potential of a ground-water system depends on and influences rates of biodegradation.
- Dissolved Oxygen: Dissolved oxygen concentrations greater than 0.5 mg/l inhibits the reductive dechlorination, instead molecular oxygen acts as electron acceptor

- Conductivity: Provides the information about groundwater's ability to conduct electricity and is directly related to concentration of ions in the solution.
- Chloride: Reductive dechlorination increases the concentrations of elemental chlorine in groundwater.
- Nitrate: After DO is depleted, nitrate is used as electron acceptor.

3.3 Sensor Selection

After identifying the indicator parameters for long term monitoring of reductive dechlorination of TCE, a study was conducted to find the appropriate sensors and/or set of sensors coupled into a single instrument, During this study, considerations were made to narrow down the selection of sensors, depending on the requirements for the SRS, which including:

- Capital cost
- Durability
- Maintenance (including spare parts)
- Ease of use
- Reliability and accuracy

Taking all the parameters that guide in selecting the suitable sensors into consideration, Hydrolab® DataSonde 4a was determined to be the appropriate instruments for the project. DataSonde 4a is equipped with 6 sensors and one open port to add any other sensor if required in future. Figure 9 and Figure 10 shows the top view and

side view of the sensors and ports available in the DataSonde 4a, and specification are listed in Table 3.

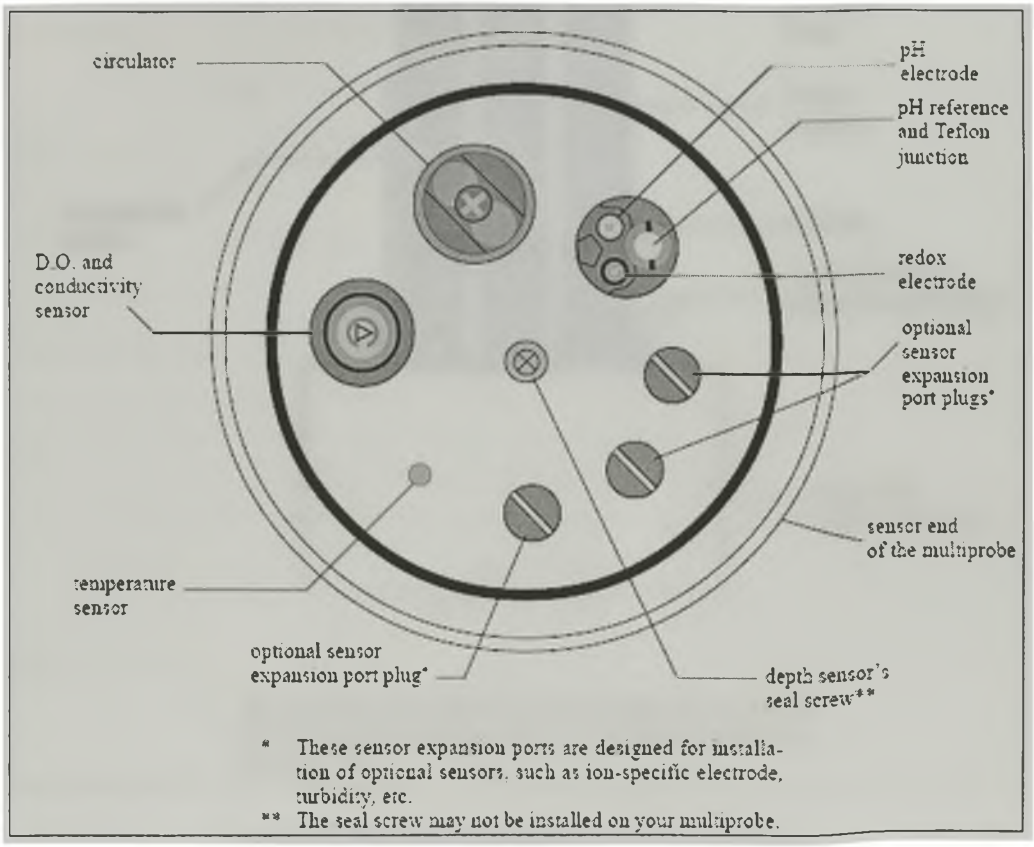

Figure 9: Hydrolab® DataSonde 4a sensors top
(Source: Hydrolab DataSonde 4a manual)

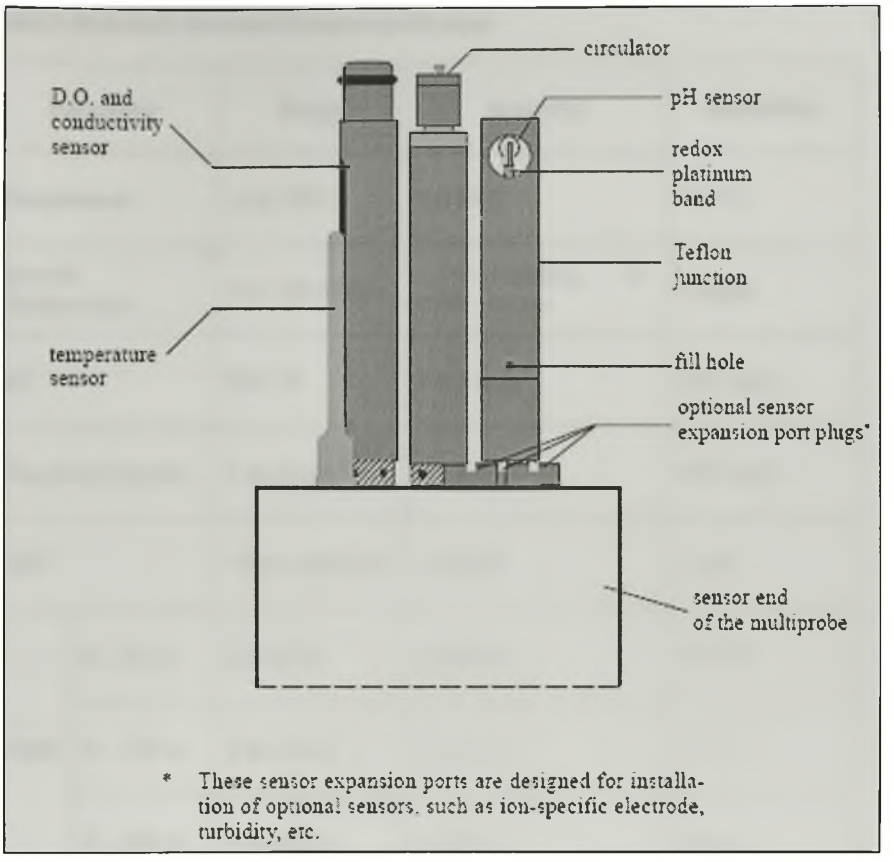

Figure 10: Hydrolab® DataSonde 4a sensors side view
(Source: Hydrolab DataSonde 4a manual)

Table 3: Hydrolab® DataSonde 4a sensor specifications

Parameter		Range	Accuracy	Resolution
Temperature		-5 to 50°C	± 0.10°C	0.01°C
Specific Conductance		0 to 100 mS/cm	± 1% of reading ± 0.001 mS/cm	4 digits
pH		0 to 14	± 0.2 units	0.01 units
Dissolved Oxygen		0 to 20 mg/l	± 0.2 mg/l	0.01 mg/l
ORP		-999 to 999 mV	± 20 mV	1 mV
Depth	0 – 25 m	0 to 25 m	± 0.08 m	0.01 m
	0 – 100 m	0 to 100 m	± 0.3 m	0.1 m
	0 – 200 m	0 to 200 m	± 0.6 m	0.1 m
Salinity		0 to 70 ppt*	± 0.2 ppt*	0.01 ppt*
Nitrate		0 to 100 mg/l-N	Greater of ± 5% of reading or ±2% mg/l-N	0.01 mg/l-N
Chloride		0.5 to 18,000 mg/L	Greater of ± 5% of reading or ±2% mg/l	4 digits

* Salinity is measure in parts per thousand, source Hydrolab DataSonde 4a manual

4.0 EXPERIMENTAL SETUP

A bench scale test loop setup was designed to imitate the field setup. Experiments were conducted in two phases.

4.1 Phase #1

First phase of the test setup is shown in Figure 11 and involves the use of the following components:

- Hydrolab® DataSonde 4a
- Hydrolab® Surveyor 4a
- Continuously stirred Bioreactor
- Laptop Computer

DATASONDE 4a: DataSonde 4a is designed for use in flow-through applications and consists of seven built-in expansion ports for measuring 15 parameters including:

- Temperature
- pH
- ORP
- Dissolved Oxygen
- Conductivity
- Nitrate
- Chloride
- Depth
- Salinity

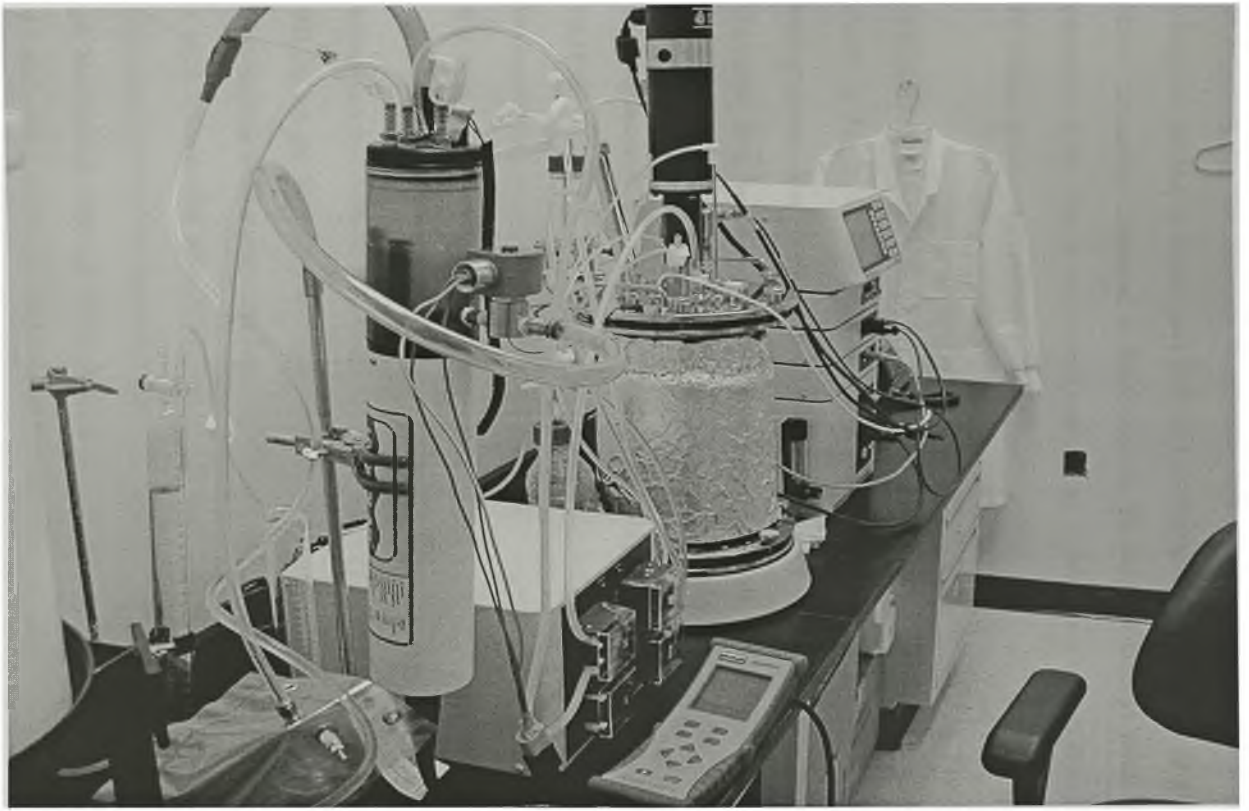

Figure 11: Experimental Lab setup

SURVEYOR 4a: The Surveyor 4a provides data logging and display in a rugged, waterproof (NEMA 6) display case that can be submerged for 30 minutes up to 6 feet below the surface. Connected to DataSonde 4a, the surveyor displays water quality parameters in real time or automatically stores data. It downloads the data directly to a PC without additional proprietary software.

CONTINOUSLY STIRRED BIOREACTOR: A closed continuously stirred bioreactor as shown in Figure 12 has been used to cultivate the bacteria for anaerobic TCE biodegradation. The bioreactor was filled with growth medium and initially was inoculated with anaerobic bacteria from several sources: SRS TCE-contaminated sediment, sediment from industrial canal in Miami, and anaerobic digester. The reactor was maintained at 28°C temperature. The pH was controlled by automatic addition of Na_2HCO_3 to a value of 6.95 during the acclimatization of bacteria to anaerobic TCE biodegradation. During trend studies parallel test, pH was not controlled and reading of this value as well as DO concentration was compared with Hydrolab data. The basal medium for bioreactor (modified from Freedman and Gossett, 1989) consisted of the following compounds (in g/L of distilled deionized water): 1.5 g/L $NaHCO_3$, 0.2 g/L NH_4Cl, 0.1 g/L $K_2HPO_4 \cdot 3H_2O$, 0.055 g/L KH_2PO_4, 0.001 g/L resazurin, 0.039 g/L $Na_2S \cdot 9H_2O$ as a sulfur source and reductant, 0.02 g/L yeast extract, 0.1 g/L $MgCl_2 \cdot 6H_2O$, 1 mL/L vitamin stock solution (consists of 0.05 mg/L Cyanocobalamine), and 5 mL/L trace metal solution. The trace metal solution consisted of 0.005 g/L $FeCl_2 \cdot 4H_2O$, 0.005 g/L $MnCl_2 \cdot 4H_2O$, 0.001 g/L $COCl_2 \cdot 6H_2O$, 0.0006 g/L H_3BO_3, 0.0001 g/L $ZnCl_2$, 0.0001 g/L $NiCl_2 \cdot 6H_2O$, 0.0001 g/L $Na_2MoO_4 \cdot 2H_2O$, 0.002 g/L $CaCl_2 \cdot 2H_2O$.

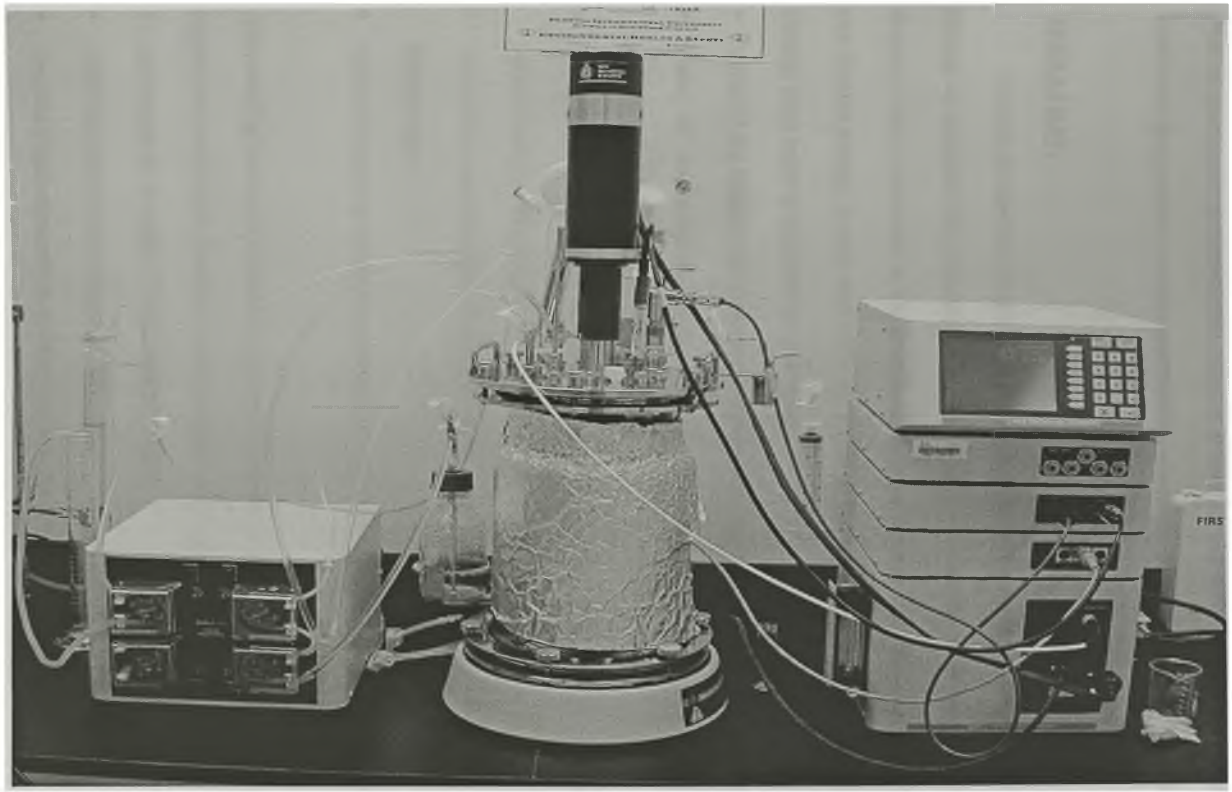

Figure 12: Continuously stirred Bioreactor

Prior to placing the solution in the reactor, it was purged with N_2 to create conditions suitable for the anaerobic bacteria and to create anaerobic condition inside the medium.

OPERATION:

Modifications were made to the DataSonde 4a flow through cell to add necessary fittings to complete the experimental setup. One 1-cm diameter hole and five 0.5-cm diameter holes were made in the DataSonde 4a flow through cell cap to connect fittings and flexible hosing. A hole was made on a side at the bottom of the flow-through cell. Fittings were made to facilitate the following:

- Valve #1 for flushing the sampling chamber after each use and during calibration.
- Valve #2 for connecting tedlar bag for collecting the gases formed during the reductive dechlorination of TCE, if any.
- Valve #3 for pumping the mixture of solution containing TCE solution and bacterial culture from continuously stirred bioreactor.
- Valve #4 is used for initial flushing of the oxygen present in the sampling chamber by nitrogen gas.
- Valve #5 is used to return the solution back into the continuously stirred bioreactor.

After making necessary adjustments to the DataSonde 4a flow-through cell, calibration of sensors was performed. Standards and materials used for calibration were:

- pH Standards: 4-buffer, 7-buffer and 10-buffer
- ORP Standard: Zobell's Standard Solution
- Specific Conductance/Salinity Standard: 1.413 mS/cm

- Chloride Standard: 46.2 mg/l and 319 mg/l
- Nitrate Standard: 4.613 mg/l and 46.13 mg/l
- Deionized Water
- Lint free cloth

Experiments were conducted by introducing into the Hydrolab's flow-through cell a feed solution from continuously stirred bioreactor consisting of basal medium with known concentration of TCE, yeast extract, and trace nutrients like nitrogen, phosphorus and vitamins. In addition, 1 mg/L of resazurin was added to indicate when the dissolved oxygen has been consumed and anaerobic conditions have been established. The resazurin in the solution turns colorless when ORP reaches approximately –150 mV. DataSonde 4a was connected to laptop by using HyperTerminal, with out any need for other software support.

The following problems were encountered during the experiment:

- Due the battery constraint on the Surveyor 4a, DataSonde 4a was connected to laptop PC for data collection while Surveyor 4a was only used for calibration.
- The circulator in the DataSonde 4a was drawing lots of power, taking this into account; an external DC supply was used to have the circulator running all the time.

DATA COLLECTION:

Data was collected for all the sensors available on the DataSonde 4a instrument during all the experiments. Data collection sheet was designed to record all the data collected, a sample data collection sheet is shown in Table 4.

DATA VALIDATION:

Samples were collected during the experiments to analyze them in the analytical laboratory for confirmation. 10-mL glass vials were used to collect samples for TCE, its degradation products, nitrate and chloride.

Table 4: Sample data collection sheet

Time (Hrs)	pH	DO (%)	Specific Conductivity (mS/cm)	ORP (mV)	Salinity (ppt)*	Nitrate (ppm)	Chloride (ppm)	TDS (gm/L)

* ppt: parts per thousand

4.2 Phase #2

A new solid-state combination Orion 96-17 ionplus chloride sensor that is known to have tolerance to sulfide ions, which can be present if TCE biodegradation occurs under anaerobic conditions was used.

Instruments used during the second phase of the experimental setup are as follows:

- Thermo Orion 96-17 ionplus chloride sensor
- WTW IonLab Multiparameter Benchtop Meter with conductivity probe
- Continuously Stirred Bioreactor (Same as used in Phase #1)

Thermo Orion Chloride Sensor: Thermo Orion® chloride sensor is a double junction ionplus sure-flow combination electrode, with a linear range of 0.4 mg/l to 79,900 mg/l. The sensor also has sufficient sensitivity (0.5 ppm), accuracy (± 2%), and robustness.

Conductivity Probe: A WTW IonLab Multiparameter Benchtop Meter with conductivity probe was used. The conductivity probe possesses the ability to detect 0.0 to 500,000µS/cm in ranges of 0.001/0.01/ 0.1/1µS/cm.

OPERATION:

Calibration of the chloride sensor was carried out using 5, 10, 100, and 500 ppm Cl^- solutions in deionized water at 25°C. The slope was calculated by using a linear regression method and found to be 60.8 mV/decade, which is in agreement with the theoretical value, 59.2 ± 2 mV/decade.

LABORATORY ANALYSIS

Chloride and Nitrate Analysis: For analyzing and confirming the concentrations of indicator parameters such as chloride and nitrate, samples from bioreactor were collected periodically into 10-mL glass vials. Two samples one at the beginning of experiment and one at the end of the experiment were collected from DataSonde 4a for measuring the concentrations of chloride and nitrate. HPLC Conductivity Detector was used to measure the concentrations of chloride and nitrate in the analytical laboratory. The HPLC equipment is a Waters 2690 HPLC system coupled to a 432 conductivity detector with an Alltech DS-PLUS™ auto suppressor. The software is Millennium version 3.2. The HPLC system is comprised of a pump, an injection valve and loop (200µl), an anion exchange column (ALLsep A-2 Anion 7u 100mm x 4.6mm, Alltech) and column oven. Instrumental settings are shown in table 5. Data was acquired by real- time chromatographic control and data acquisition system.

Table 5: Instrumental operating settings of HPLC-Conductivity System

HPLC Parameters		
Column	ALLsep A-2 Anion 7u 100mm x 4.6mm	
Mobile phase	2.8 mM $NaHCO_3$ and 2.2 mM Na_2CO_3	
Injected volume	100µl	
Flow rate	1.0 ml min^{-1}	
Detector settings	Sensitivity	0.0005 µ/cm
	Base Range	100
	Temperature	25^0C
Column Temperature: 25^0C		

The method used is modified EPA method 9056 and applies to quantitative determination of anions (F^-, Cl^-, NO_2^-, NO_3^-, SO_4^{2-}, HPO_4^{2-}, and Oxalate $C_2O_4^{2-}$) in

reagent water, surface water, ground water, and drinking water. To achieve comparable detection limits as ion chromatographic system must utilize suppressed conductivity detection. This technology of HPLC with conductivity detector can be used for both anion and cation analysis. The sulfate and oxalate is based on the principle of ion exchange chromatography. A water sample is injected into a stream of carbonate effluent by a Waters 2690 HPLC system and passed through an anion exchanger with quaternary amine functional groups, which functions as a resin in which a positive charge is able to bind negatively charged anions as they pass through the column. The affinity of binding is dependent on several variables including, but not limited to charge and size of the anion. The separated anions are first directed through an ion suppression system then to a conductivity detector. The column determines the type of charged species that will be separated. This technique uses conduction as the mode of the detection. The charged species will change the conductivity of the solution relative to the baseline of the buffer. This technique was highly improved by the ion suppression system. The ion suppression for anion analysis is a cartridge that actually reduces the conductivity of the carbonate buffer by neutralizing the base to water that has lower conductivity. Sample gets converted to acid form that has higher conductivity, improving sensitivity of the detection system.

TCE Analysis (GC/MS Method): Samples for TCE and its biodegradation products were collected into 40-mL glass vials from bioreactor and DataSonde 4a flow-through cell during trend study test. The concentrations of TCE and its degradation products, such as cis-DCE, trans-DCE, and vinyl chloride in water samples, were determined

simultaneously by the purge & trap GC/MS method SW-846, sample preparation method 5030B, and analytical method number 8260B. Analysis was performed on a Varian Star 3400cx GC/ Varian Saturn 2000 MS coupled with a Tekmar Dohrmann 3100 sample concentrator. The samples were introduced to the concentrator using an Archon Purge & Trap Auto Sampler with 5 mL injection volume from the 40-mL glass vials. A VOCARB 3000 trap is used with the program setting as: desorption time of 4 min, desorption temperature of 180°C, purge time of 11 min, bake time of 10min, and bake temperature of 225°C. Helium is used as carrier gas. Analytical separations were carried out on a WCOT Fused Silica Select 624CB capillary column (30m length x 0.25mm diameter, DF-1.4um). The temperature program was set as follows: start at 40°C for 1 min., then increase temperature at 8°C/min to 190°C and with 1 min final holding time.

Chemical Reagents: Reagent grade liquid TCE and cis-1,2 DCE (Aldrich Chem. Co., Milwaukee, WI) were used for preparing stock feed solutions and analytical standards. Vinyl Chloride (VC), (99+%, Scott Specialty Gases, Alltech Associates, Inc., Deerfield, IL) was used as an analytical standard. Methanol was used as electron donor and to develop analytical standards. Methanol (99.9% pure purge and trap grade) was obtained from Fisher Scientific. Yeast extract (Fisher Scientific) was used as a nutrient source. All chemicals for growth medium preparation were reagent grade and obtained from Fisher Scientific and Aldrich Chem. Co., Inc.

5.0 RESULTS AND DISCUSSION

5.1 Phase #1

During the execution of the first phase, two preliminary tests and two trend study tests were conducted. Ability to attain anaerobic conditions in the modified DataSonde 4a flow-through cell was the objective of the preliminary tests. The trend studies included individual and parallel tests using the modified DataSonde 4a flow-through cell and continuously stirred bioreactor served as actual TCE biodegradation system. Samples were collected from bioreactor and DataSonde 4a flow-through cell during the parallel experiment and were sent to analytical laboratory for analysis.

PRELIMINARY TESTS: The first test was started by transferring the TCE solution from Bioreactor to the DataSonde 4a and was conducted for a period of approximately 2 days (49 hours) until anaerobic conditions were achieved. During this test, temperature, pH, specific conductivity, and ORP parameters were measured using the modified DataSonde 4a flow-through cell. The purpose of this test was to prove that anaerobic conditions could be attained and maintained in the DataSonde 4a flow through cell. Data collected during the test is shown in Table 6.

Table 6: Data for preliminary test # 1

Time (hrs)	Temperature (°C)	pH	Specific Conductivity (mS/cm)	DO (%)	ORP (mV)
0	22.18	7.96	5.575	0.1	346
3	19.78	8.15	4.504	0.1	338
24	20.78	8.25	4.516	0.1	271
26	19.87	8.25	4.507	0.1	325
49	21.33	8.26	4.443	0.1	-187

DO and ORP data were chosen to determine the anaerobic behavior of this solution. By plotting ORP vs. time as presented in Figure 13 below, it was concluded that anaerobic conditions were achieved since the values of ORP started in positive range (346 mV) and ended in negative range (-187 mV), due to the depletion of oxygen and other electron acceptors in the reactor by the anaerobic bacteria presence. DO concentration measured remained at 0.1% saturation, indicating anoxic conditions. Anaerobic conditions were also confirmed by resazurin used is the system by changing the color of the solution to colorless from pink.

The second preliminary test was conducted over a period of approximately 24 hours. Experiment started by transferring the TCE solution from the bioreactor that served as actual TCE biodegradation system to the DataSonde 4a chamber. During the transfer of the TCE solution, concentration of oxygen of the solution increased up to 18%; however, within 3 hours anaerobic conditions were achieved, indicated by the negative ORP and very low DO readings provided in Table 7 below. The negative value of ORP and low value of DO%, as depicted in Figure 14 and Figure 15, indicate that anaerobic conditions have been achieved.

Table 7: Data for preliminary test # 2

Time (hrs)	Temperature (°C)	pH	Specific Conductivity (mS/Cm)	DO (%)	ORP (mV)
0	19.9	7.96	5.698	18.3	114
3	20.1	7.93	5.699	0.1	-171
5	20.17	7.91	5.698	0.1	-201
24	20.52	7.72	5.66	0.1	-272

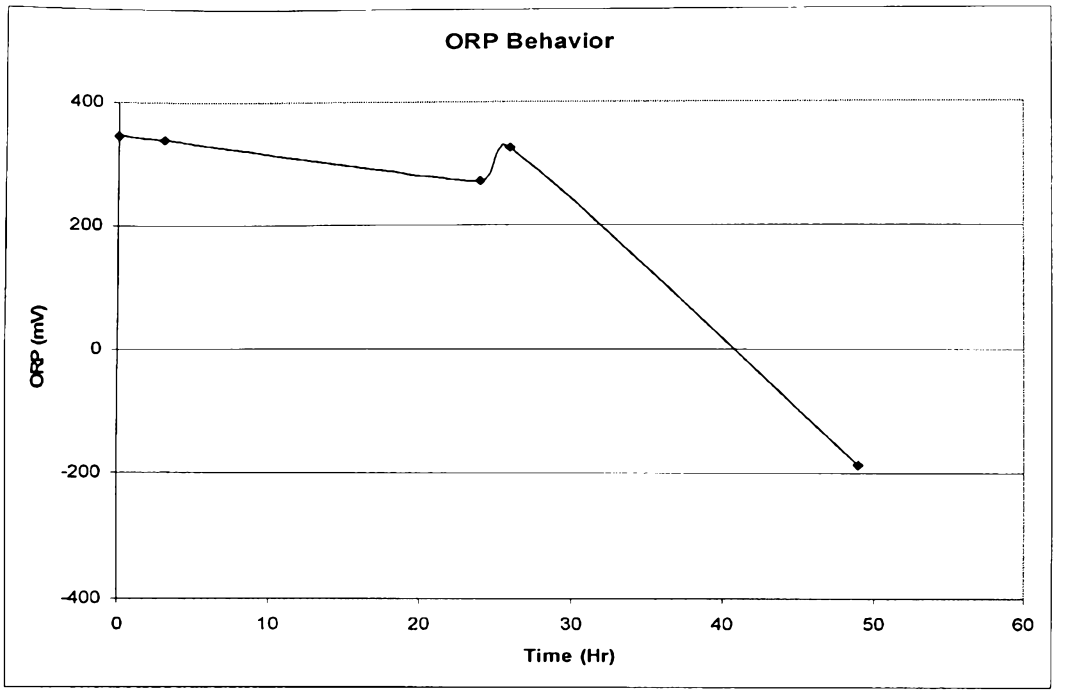

Figure 13: ORP behavior during preliminary test # 1

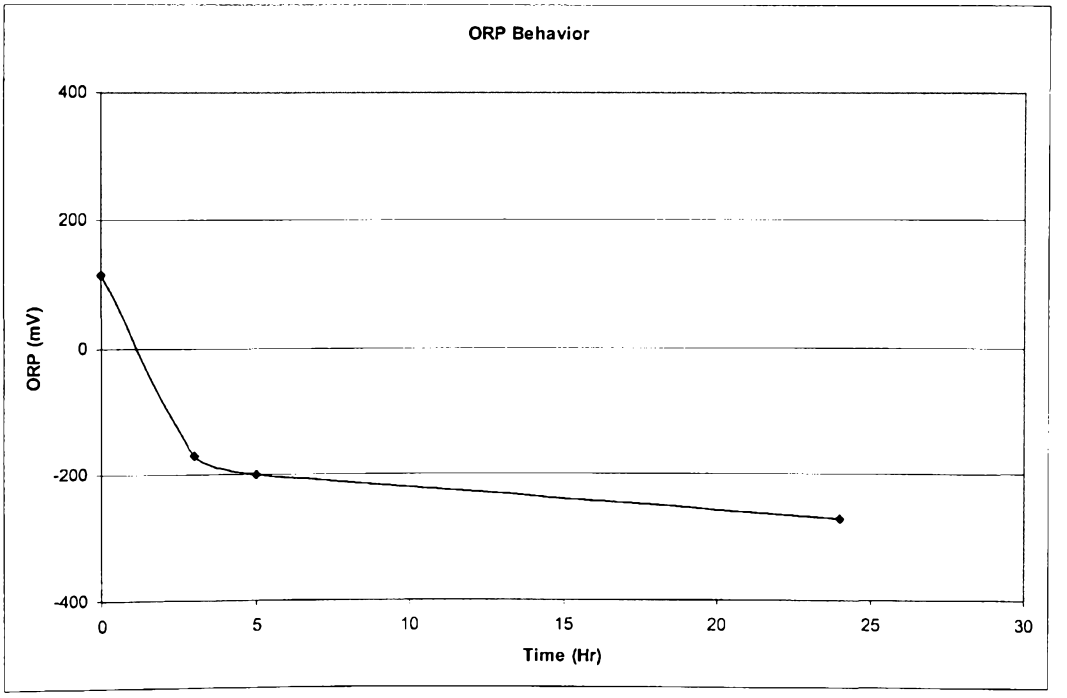

Figure 14: ORP behavior during preliminary test #2

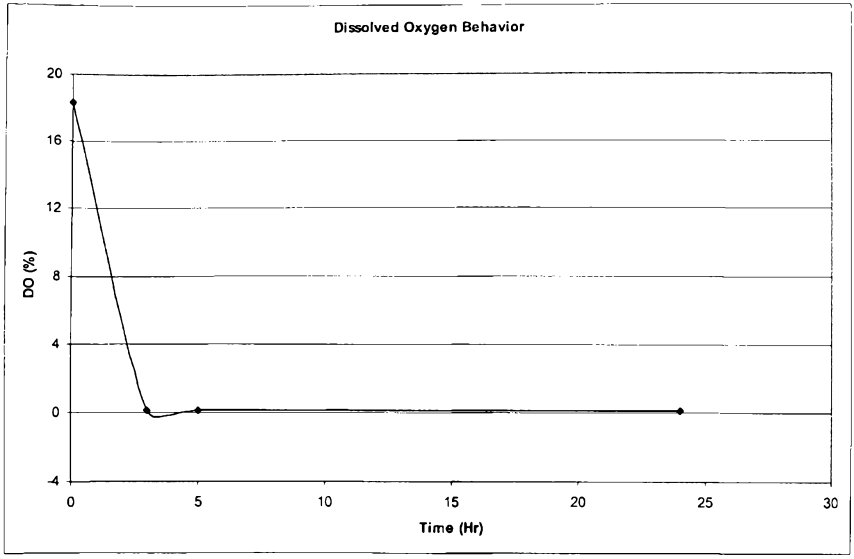

Figure 15: DO behavior during preliminary test#2

Based on the results from the second preliminary test it was concluded that the modified DataSonde 4a flow through-cell chamber was able to support anaerobic conditions during the dechlorination of TCE.

Trend Study Tests: In order to study the trend of TCE indicator parameters during anaerobic biodegradation, two experiments were conducted. The first experiment consisted of pumping a solution with acclimatized bacteria into the DataSonde 4a flow-through cell and collecting data on the computer. These results are presented in the following "individual experiment" section. This first test was conducted over a period of approximately 4 days using a TCE concentration of about 22 mg/l. The second set of experiments is presented in the section called "parallel experiment", and was conducted over a period of 2 weeks. For this test a TCE concentration of about 87 mg/L was used.

The DataSonde 4a flow through cell and bioreactor were maintained with the same concentration during this two week period and samples were collected on a regular basis and sent to the analytical laboratory for further confirmatory testing of selected indicator parameters. The laboratory data is presented in the section "analytical data results".

Individual experiment: In an individual experiment with duration of about 4 days (74 hours), 100 µl of TCE from a stock solution of 111.09 g/L was added to 540 ml of artificial ground water containing anaerobic bacteria to obtain a TCE concentration of approximately 22 mg/L. Data was collected at two hour intervals. The data obtained during the individual experiment is presented in Table 8 shown below.

Table 8: Individual Test Data

Time (Hrs)	pH	DO (%)	Specific Conductivity (mS/cm)	ORP (mV)	Salinity (ppt)*	Nitrate (ppm)	Chloride (ppm)	TDS (gm/l)
0	7.72	0.1	5.663	-272	3.12	22.13	1544	3.615
2	7.69	0.1	5.648	-256	3.11	21.05	1577	3.615
4	7.68	0.1	5.648	-256	3.11	21.34	1663	3.615
22	7.58	0.1	5.607	-255	3.09	19.23	1512	3.589
24	7.58	0.1	5.608	-261	3.09	18.39	1554	3.589
46	7.57	0.1	5.609	-248	3.09	18.42	1615	3.589
48	7.57	0.1	5.614	-268	3.09	18.94	1659	3.592
50	7.57	0.1	5.616	-270	3.09	17.89	1694	3.594
52	7.57	0.1	5.619	-272	3.1	17.44	1734	3.596
70	7.6	0.1	5.613	-245	3.09	27.61	1485	3.592
72	7.6	0.1	5.617	-249	3.1	28.99	1510	3.594
74	7.6	0.1	5.622	-259	3.1	30.34	1567	3.597

*ppt – Parts per thousand

Data indicated that pH values remained fairly constant over the 74-hour test period, varying between 7.58 and 7.72. The DO readings during the experiment were also constant at 0.1 % saturation indicating there is no oxygen present in the solution, which

indicates anaerobic conditions. ORP readings indicate the presence of anaerobic conditions. The negative values of ORP shown in Figure 16 assured that the anaerobic conditions were present during the experiment. Even though there are some disturbances in the readings, they are pretty much in the negative range, which confirms that anaerobic conditions were achieved during the experiment. During the experiment, DataSonde 4a recorded higher than expected nitrate reading values as shown in Figure 17. Values for nitrate were expected to be non-existence since only chloride salts were used for the preparation of the basal medium solution for bacteria culture acclimatization. Nitrate analyses performed in analytical laboratory determined value less than detection limit. DataSonde 4a manual does not provide any information about interferences that might change reading of nitrate selective electrode.

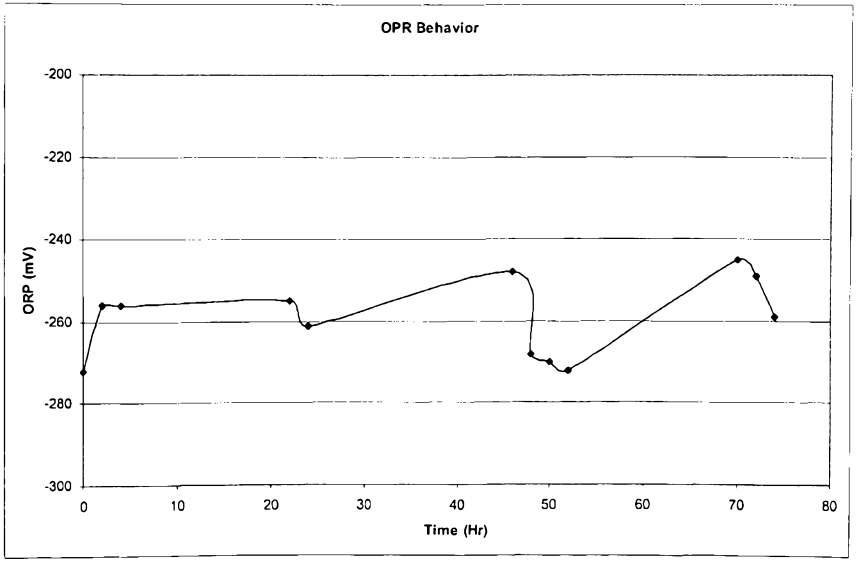

Figure 16: ORP behavior during Individual experiment

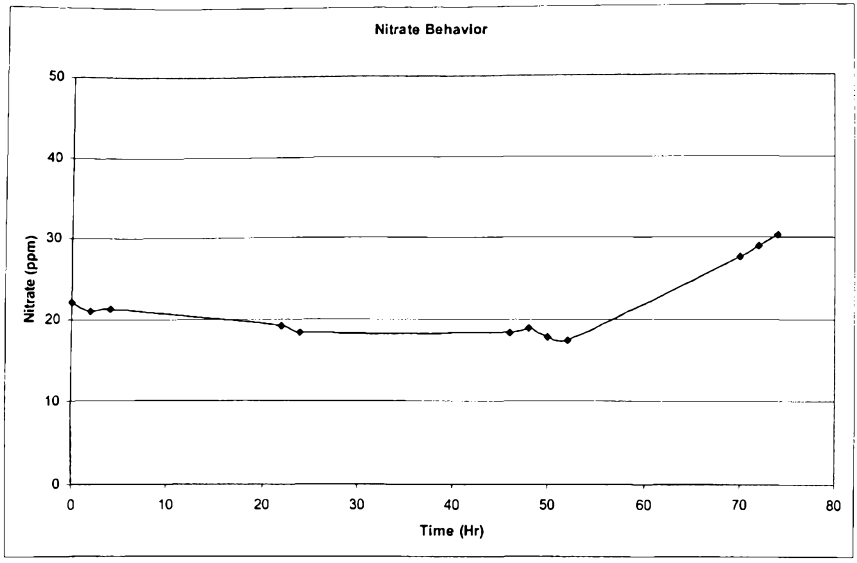

Figure 17: Nitrate Behavior during individual Experiment

The chloride readings observed from the DataSonde 4a instrument as shown in Figure 18 did not agree with expected values, as they are very high. Amount of 179 mg/l chloride concentration was determined in analytical laboratory when experiment was completed. DataSonde 4a manual does not provide any information about interferences that might change reading of chloride selective electrode. It was known from technical specification that the presence of bromide, sulfide and iodide ions will cause the Chloride electrode to malfunction by giving the interferences. Basal medium for Bioreactor contains less then 1 mg/l of sulfide. We do not know what concentration of sulfide ions might interfere with chloride reading and if it increase or decrease reading.

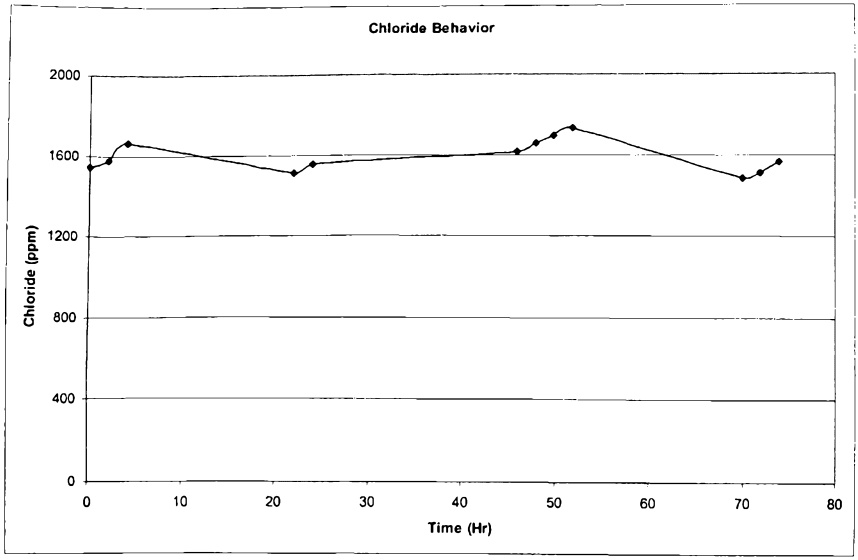

Figure 18: Chloride behavior during individual experiment

Parallel experiment

DataSonde 4a sensors were cleaned and recalibrated before the experiment. During the parallel experiment, about 87 mg/l of TCE was added to both Bioreactor and DataSonde 4a flow-through cell chamber. Experiment was conducted and readings were collected for a period of two weeks (290 hours). Samples were collected on a regular basis for further confirmatory testing of the selected indicator parameters. Samples to determine nitrate, chloride, TCE and its byproducts were collected from bioreactor and sent to analytical laboratory for confirmatory analysis. The analytical laboratory data is presented in "analytical data results" section. Readings were recorded on regular basis during the experiment and are tabulated in Table 9. During the experiment, slight decrease in pH was observed. This decrease may be due to formation of acid during the anaerobic

condition by replacement of the chloride in TCE with hydrogen. These readings as well as DO concentrations measured were consistent with bioreactor's main screen data and it is served as conformation for received data. The values of ORP are in the negative range, again confirming the anaerobic conditions. The negative values of ORP as well the values of DO of 0.1 % show that there is no oxygen present in the reactor.

Table 9: Parallel Experiment Data Collection Sheet

Time (Hrs)	pH	DO (%)	Specific Conductivity (mS/cm)	ORP (mV)	Salinity (ppt)*	Nitrate (ppm)	Chloride (ppm)	TDS (m/l)
0	7.74	0.1	4.95	-162	2.71	48.08	191.4	3.17
4	7.73	0.1	4.95	-193	2.72	45.48	196.6	3.17
20	7.71	0.1	4.93	-259	2.7	49.93	199.3	3.15
28	7.72	0.1	4.95	-261	2.71	47.91	204.7	3.17
44	7.70	0.1	4.97	-268	2.73	44.22	270.6	3.18
52	7.70	0.1	4.96	-277	2.72	46.85	356.5	3.17
68	7.70	0.1	4.97	-283	2.73	45.84	1047	3.18
76	7.70	0.1	4.96	-283	2.72	49.91	1320	3.17
92	7.69	0.1	4.98	-283	2.73	49.32	4402	3.18
100	7.68	0.1	4.98	-283	2.73	48.29	7377	3.19
164	7.57	0.1	4.98	-278	2.74	54.07	38983	3.19
172	7.55	0.1	4.97	-276	2.73	56.08	37508	3.18
188	7.52	0.1	4.99	-272	2.74	54.13	43410	3.19
196	7.49	0.1	4.98	-270	2.73	56.45	38004	3.19
213	7.46	0.1	4.97	-268	2.73	57.23	35225	3.18
220	7.46	0.1	4.99	-270	2.74	56.68	35039	3.19
236	7.42	0.1	4.98	-266	2.73	54.99	27153	3.19
244	7.41	0.1	4.99	-266	2.74	53.64	22749	3.19
260	7.38	0.1	5.00	-264	2.74	50.42	16398	3.20
268	7.35	0.1	5.00	-259	2.74	50.18	13639	3.20
284	7.29	0.1	5.01	-256	2.75	40.25	6815	3.20
290	7.29	0.1	5.02	-253	2.75	39.46	6526	3.21

*ppt: patrs per thousand

DataSonde 4a measures specific conductivity and calculates salinity and TDS values, the graphs of these three parameters shows almost the same pattern with corresponding values. As shown in Figure 19 through Figure 21, there is an increase in values for these three parameters, due to the release of chloride from TCE degradation.

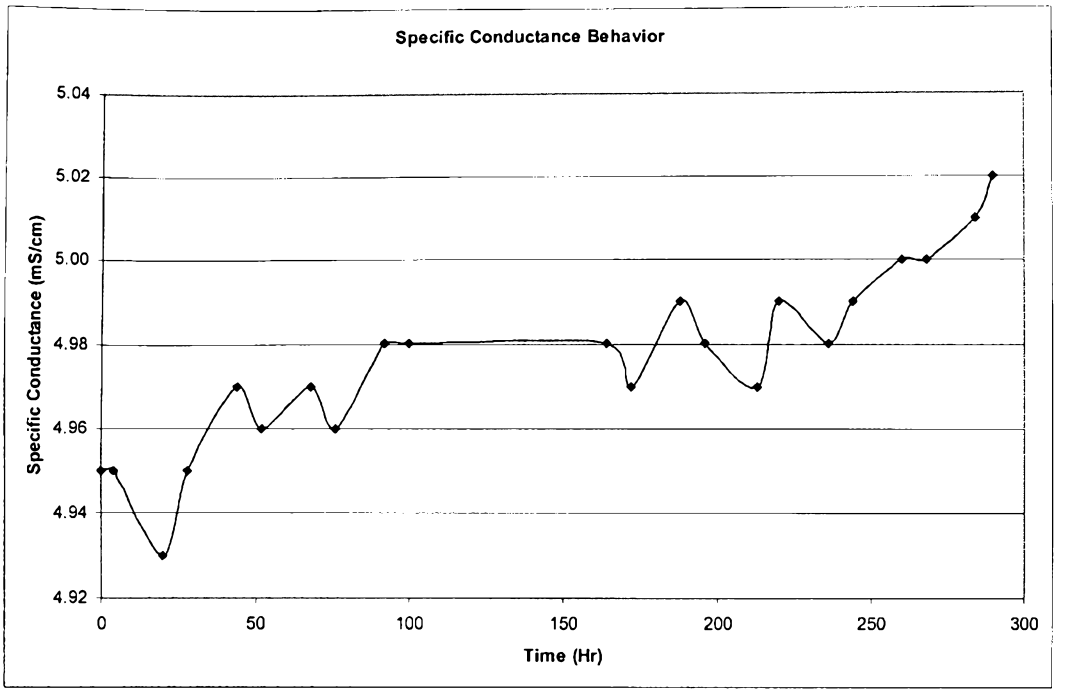

Figure 19: Specific conductivity behavior during parallel experiment

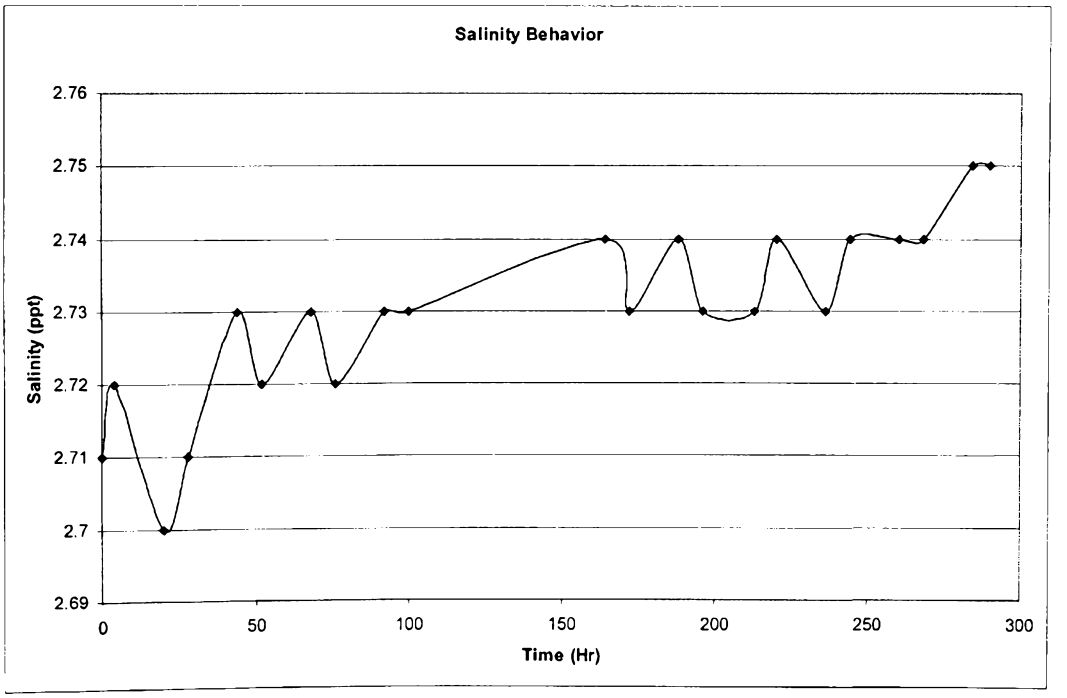

Figure 20: Salinity behavior during parallel experiment

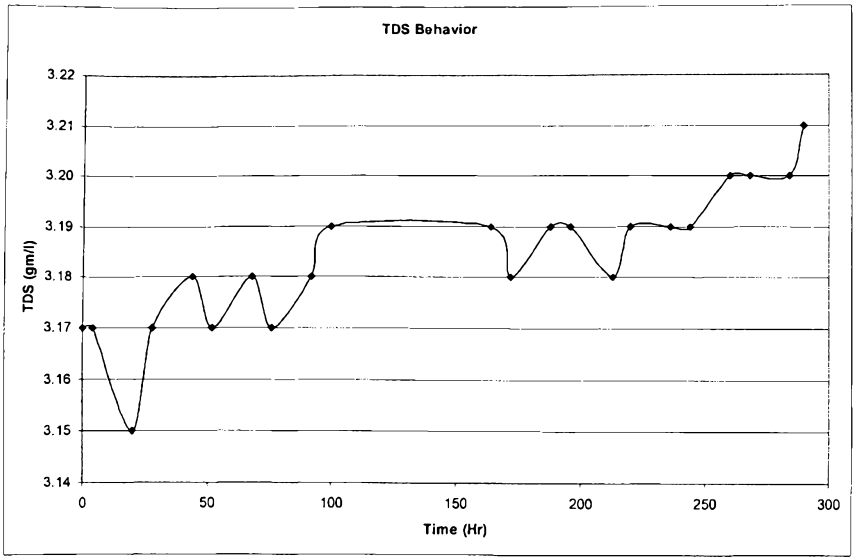

Figure 21: TDS behavior during parallel experiment

Behavior of behavior of nitrate during this experiment is shown Figure 22. The data obtained from the nitrate sensor is not considered valid since only chloride salts were used during the preparation of the solution and no nitrate was used. Therefore nitrate readings should be negligible. This is also confirmed by the laboratory analysis samples, which found nitrate less than detection limit in the solution. The chloride sensor behaved in an unusual manner. The concentration of chloride first increased, reached a maximum value of 43410 mg/l and then decreased again, which is not possible in the anaerobic degradation of TCE. This data is presented in Figure 23 below. At this point, it can be concluded that the DataSonde 4a chloride sensor did not performed as expected. Laboratory results for chloride are presented in "analytical results data" section and indicate a chloride concentration in the range of approximately 179 mg/l – 250 mg/l.

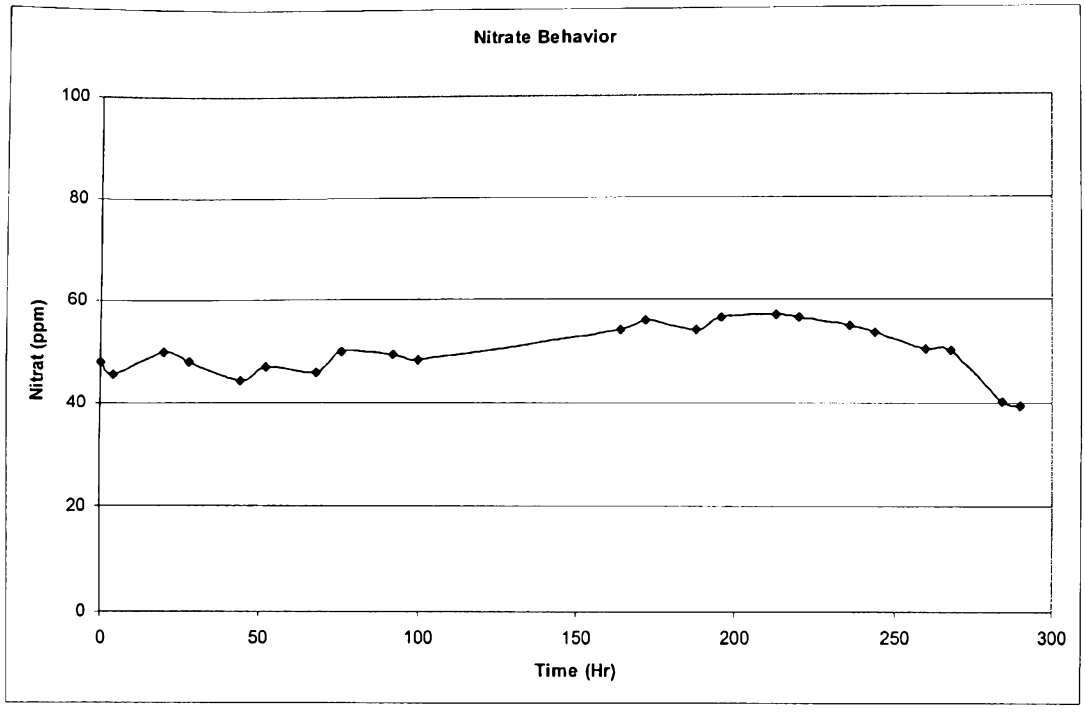

Figure 22: Nitrate during parallel experiment

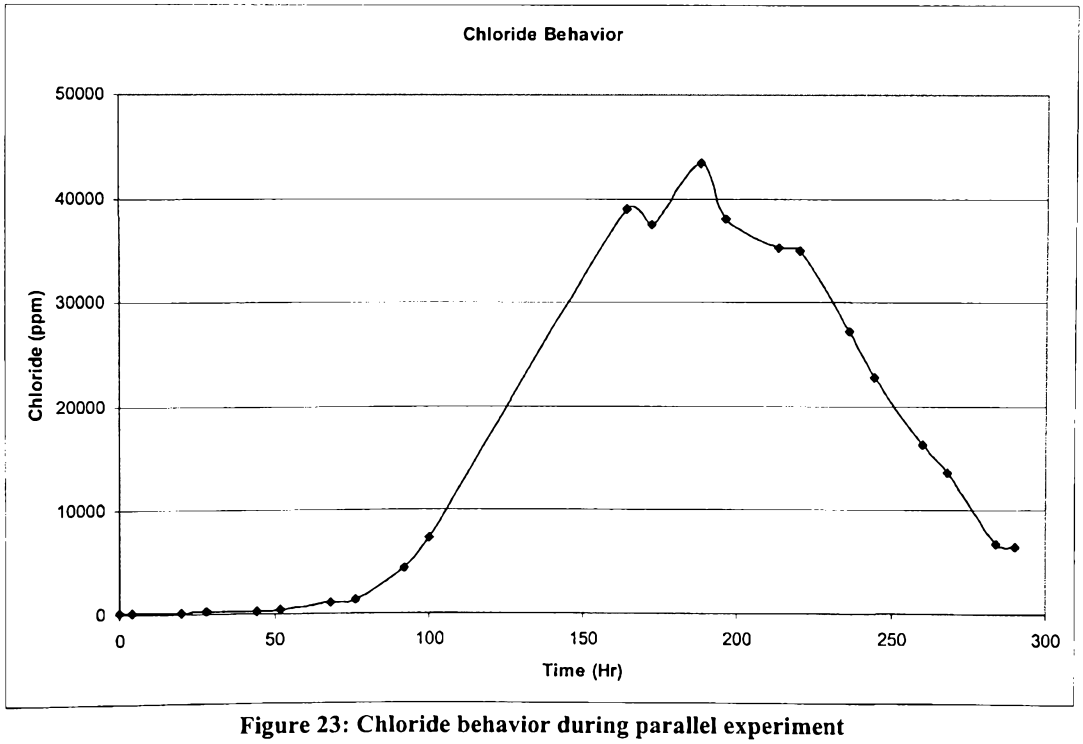

Figure 23: Chloride behavior during parallel experiment

Laboratory Analytical Data:

The samples collected during the parallel experiment from bioreactor and DataSonde 4a were analyzed at analytical laboratory. The laboratory analysis was conducted for nitrate, chloride, TCE and its byproducts. Nitrate values were found to be negligible (as expected) in bioreactor and the results for chloride are tabulated in Table 10 and graphically shown in Figure 24. The analytical laboratory tested for TCE and its breakdown products such as 1,1 DCE, trans DCE, and cis DCE, and vinyl chloride and results are presented in Table 11. Figure 24 shows an increase of chloride concentration during the life of the experiment. The concentration of chloride should increase, as the hydrogen will replace the chlorine in TCE during dehalogenation as the process of anaerobic degradation of TCE.

TCE samples from DataSonde 4a were collected at the beginning and at the end of the 2-week experiment. The results are presented in Table 11 below. Concentration of byproduct in bioreactor and DataSonde 4a represent different steps of biodegradation process due to different concentrations of biomass used in both systems. In both systems the concentrations of TCE decreased during the experiment, but concentration of other byproducts varied. In bioreactor the largest concentration was found for vinyl chloride, in DataSonde 4a for cis-DCE. Probably, for this type of bacteria consortia that is used for experiment, two weeks time is not sufficient to complete all biodegradation of the byproducts. Chloride results obtained from analytical lab for both systems showed similar concentrations.

Table 10: Analytical Lab Results for Chloride Samples from Bioreactor

Sample #	Analytical lab reading for chloride
1	179.04
2	201.52
3	208.03
4	198.14
5	192.31
6	199.75
7	177.88
8	203.82
9	207.52
10	245.76
11	220.27
12	214.43
13	244.35
14	220.07
15	221.31
16	213.3
17	219.77
18	250.61

Table 11: Concentration of TCE and its Breakdown Products

Compound	Concentration at the starting of the experiment (mg/l)	Bioreactor's concentration at the end of experiment (mg/L)	Hydrolab concentration at the end of the experiment (mg/l)
Vinyl Chloride	19.616	4.709	0.942
1,1,DCE	0.0268	<0.001	0.0065
Trans, DCE	0.2562	0.245	0.2185
Cis, DCE	0.178	0.186	13.53
TCE	87.203	0.004	6.176
Cl	0.179	250.61	251.21

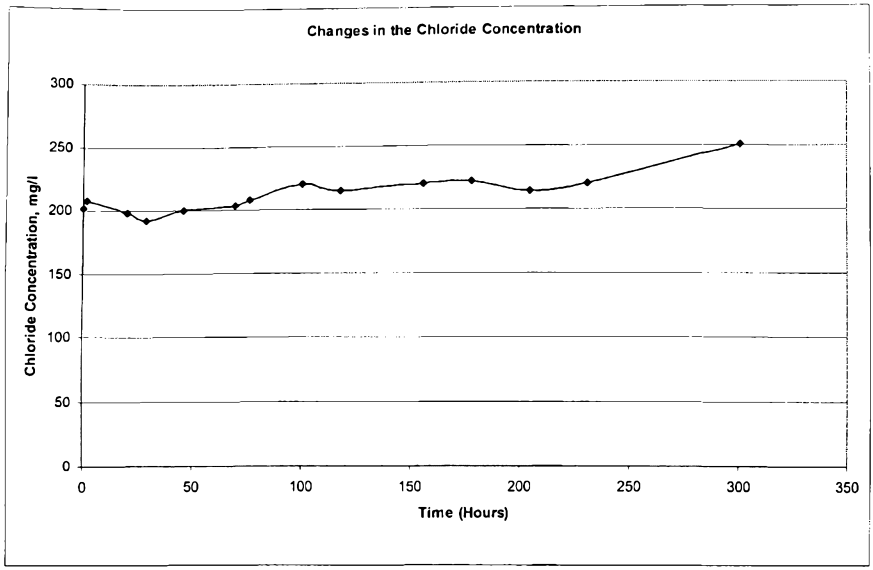

Figure 24: Chloride behavior from analytical lab results during parallel experiment

5.2 Phase #2

A new Thermo Orion 96-17 solid state combination electrode was used for measuring the chloride ion concentration. The pH/ORP meter was used to measure the chloride sensor reading in mV. WTW Inolab conductivity sensor was used for measuring the conductivity. Series of experiments were conducted with the sensor and tests were also conducted to determine the correlation between increase in the chloride concentration and conductivity and to study sulfide interferences with the chloride sensor.

Anaerobic bioreactor test:

The performance of chloride sensor was studied in an actual TCE biodegradation system under anaerobic conditions in a stirred bioreactor. The bioreactor was soil-free

and contained dehalogenating enriched culture grown in a methanogenic medium. Solid-state Orion 96-17 Ionplus combination chloride sensor was inserted into bioreactor through the available port on the head plate. 3 mL of the 142.53 g/L TCE stock was injected to the bioreactor resulting in 85.52 mg/L TCE in the solution. Background conductivity in the bioreactor was 2.18 mS/cm. The results of chloride ISE measurements are shown in Figure 25.

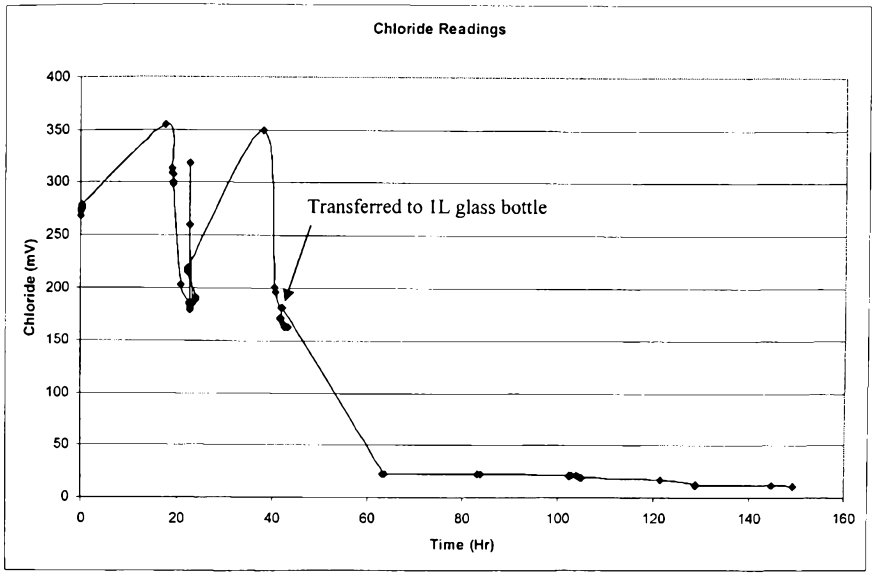

Figure 25: Chloride Readings during second phase

From the beginning of the experiment, fluctuations in the voltage corresponding to chloride concentration data were observed in the range of 170-360 mV. A large part of the experimental system included electrical devices, such as, stirrers and water-jacketed heaters, which were made of metal and were not properly grounded. It was found that

metallic parts of the bioreactor produced some electrical current that caused voltage instability and fluctuation. After 42 hours of operation 0.6 L of solution containing an active dehalogenating enrichment culture was transferred from bioreactor to a 1L glass reactor to avoid the interferences caused by the metallic parts. There were no metallic parts present in the glass reactor in contact with the electrolyte. After the solution transfer, data stabilized around 22 mV and slowly decreased during the next 86 hours to 11 mV as shown in Figure 25. Concentrations of the chloride at the beginning and the end of experiment determined in the analytical laboratory were 248 mg/L and 283.3 mg/L, respectively. Results of this experiment testified that metal parts present in the measurement cell could disrupt ion selective electrode performance and change readings abruptly.

Conductivity for the experimental period was increased from 2.09 mS/cm to 2.2 mS/cm as shown in Figure 26. Each milligram of chloride addition increased the specific conductivity in the range of 1.5-4.7 μS/cm (0.0015-0.0047mS/cm). Multi-parameter meter conductivity data expressed in mS/cm units with two decimals is not enough to show changes in μS/cm level by the addition of 1 mg of chloride or less during TCE degradation. In order to track more detailed correlation between conductivity and chloride in the solution with high concentration of ions, conductivity readings in mS/cm levels should be expressed with at least three decimals.

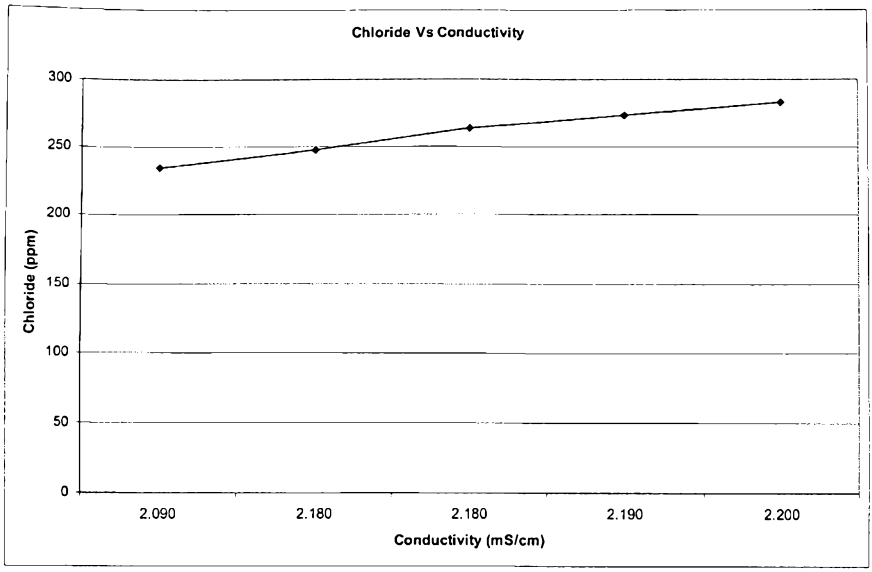

Figure 26: Correlation between chloride concentration and conductivity

The next experiment was conducted in the same 1.0 L glass reactor filled with fresh 0.6 L basal medium containing an active dehalogenating culture with the injection of 140μL of the 142,530ppm TCE stock to attain 33.26mg/L TCE. During the first three days, the voltage readings did not change due to some remaining oxygen that was still present in the reactor and relatively low temperature of the solution (20°C). When anaerobic conditions were established and the color of basal medium turned to colorless as a result of the presence of resazurin. As shown in Figure 27, chloride electrode voltage readings slowly decreased from 37 mV to 14 mV and then remained stable thereafter. When voltage reading is stabilized, the chloride concentration was determined as 283.29 mg/L.

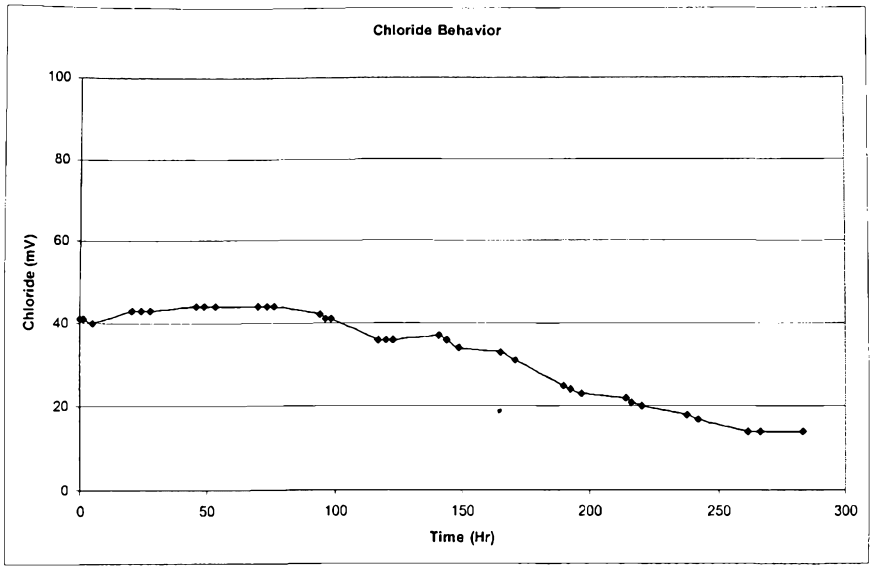

Figure 27: Chloride probe voltage reading

Sulfide in groundwater at concentrations greater than 1 mg/L is one of the geochemical parameters that indicate TCE biodegradation pathway is possible. However, in the case of the chloride measurement with the ISE, the presence of sulfide is a potential interference. To measure sulfide interference using the chloride probe, the next experiment was conducted with the same magnetic stirred 1 L-glass reactor, which included the active dehalogenating enrichment culture grown in a methanogenic medium. Before the experiment was started, 0.6 L of preliminarily nitrogen purged fresh basal medium solution was added to the reactor followed by the injection of 33.26ppm of TCE (140µl of the 142.53 g/L TCE stock). When anaerobic condition in the reactor was established and the color of the resazurin that was present in the basal medium turned to colorless, the solution was supplemented with 1.55 mg/L of sulfide (S^{2-}).

In the following 5 minutes, the voltage readings decreased from 38 mV to 28 mV and after 140 hours of operation reached –25 mV to –21 mV as shown in Figure 28. By the end of the experiment, the chloride concentration in the solution measured in the analytical laboratory increased from 153.58 mg/L to 160.25 mg/L. This concentration did not show a correlation with the negative values of voltage and indicated interference from sulfide added to the TCE biodegradation system at a concentration of 1.55 mg/L.

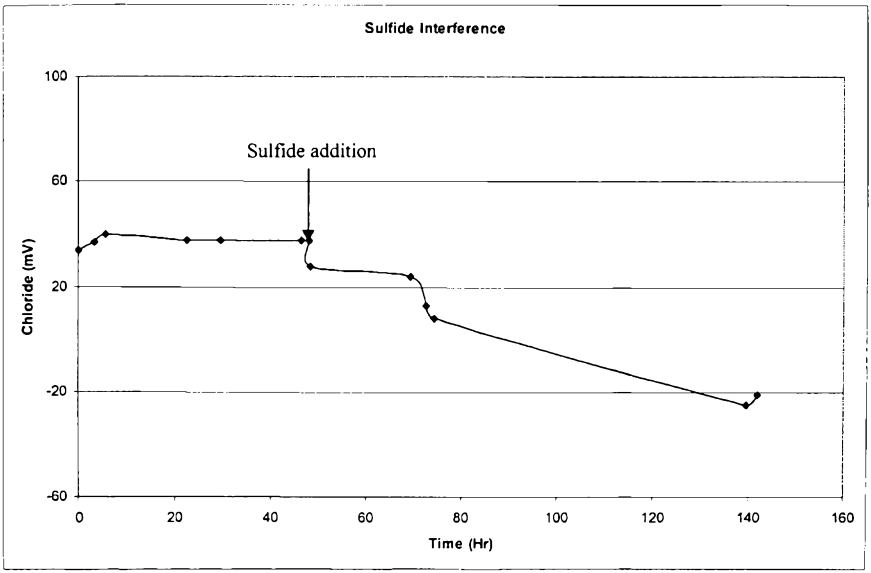

Figure 28: Chloride probe ORP reading in presence of 1.55mg/l sulfide

The experiment was repeated, lowering sulfide concentration to 0.6 mg/L. Before the start of the new experiment, the chloride probe was washed with deionized water and dried with Kim wipes to clean the biomass and thin dark-colored coating from membrane surface. After cleaning and maintaining the probe, the voltage reading turned positive again. The starting chloride concentration and the voltage were 153.05 mg/l and 47 mV,

respectively. When anaerobic conditions were achieved and the voltage reading slowly decreased to 46 mV, 0.6 mg/L of S^{-2} was added to the reactor. During the first 30 minutes, the chloride probe voltage readings were lowered from 46 mV to 2mV indicating the interference from sulfide. The probe was taken out, washed and cleaned with Kim wipes to remove black coating deposit from the membrane surface and inserted back into the reactor. For the next two hours, the voltage reading increased to reach its starting point and stabilized around 52 to 53 mV and was almost stable, as shown in Figure 29. Chloride concentrations before and after sulfide addition suggest that dehalogenation had not occurred and probably was inhibited by sulfide toxicity.

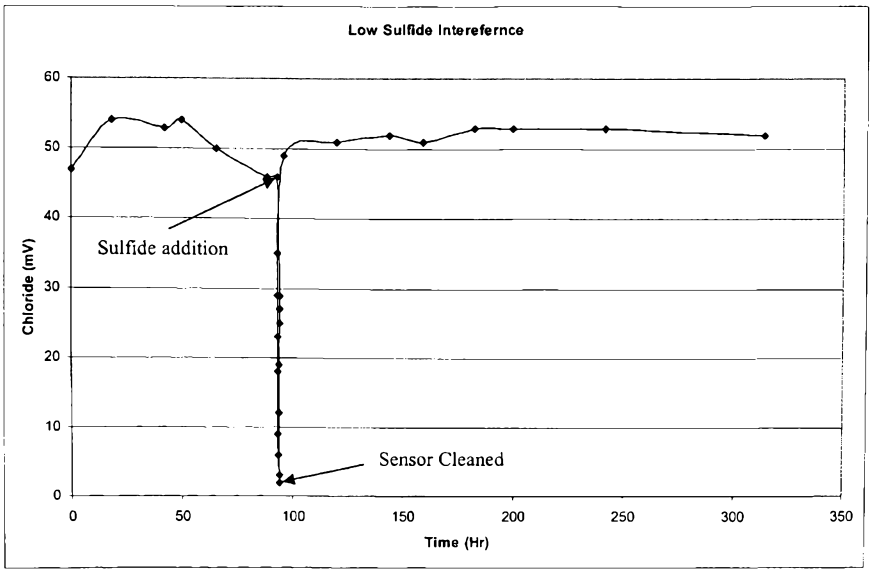

Figure 29: Changes in voltage reading in presence of sulfide

Sulfide inhibition has never been observed under field conditions even when large quantities of sulfate were reduced (Lee et al. 1998). A possible explanation for the apparent absence of sulfide inhibition in the field experiments is that sulfide could precipitate with iron contained in the soil. The SRS aquifer soil and sediment contain significant amounts of iron that can probably suppress sulfide inhibitory effect. Maybe sulfide interference with ISE can also be avoided in the presence of aquifer sediment and soil by binding hydrogen sulfide with iron. To further investigate the interference effect of sulfide, iron, and SRS aquifer sediment on the ISE probe performance, additional experiments need to be conducted.

6.0 CONCLUSIONS

During experimental work, DataSonde 4a sensor readings were compared with analytical results obtained from samples of the bioreactor.Some sensors show reliable data, but some did not. It was determined that DO concentration and pH values obtained from DataSonde 4a instrument were consistent with reading from main screen of bioreactor. Checking ORP value with another instrument proved reliability of ORP data. Readings from DataSonde 4a sensors for all these parameters showed expected and reliable values. Nitrate and chloride readings, comparison with previous parameters, have never been consistent with expected or analytical laboratory results. It could be due to interference with sulfide ions, which was present at small concentrations in the basal medium solution. If DataSonde 4a is used, nitrate and chloride reading have to be compared all the time with historical data or analytical results.

Sulfide interference for chloride selective electrode has to be considered in an area with active sulfate reduction process where groundwater may contain sulfide ions. Conductivity, TDS, and salinity results showed almost the same pattern with corresponding values. It is known that SRS groundwater has very specific quality with almost no alkalinity and small organic content. DataSonde 4a's conversion factor to calculate salinity and TDS from conductivity may not express correlation precisely for SRS conditions. In this case, conversion factors for specific area should be determined independently in an analytical lab by comparison with data obtained for TDS and salinity from the DataSonde 4a's specific conductivity sensor readings.

The chloride probe can be used to detect the chloride concentration instead of Hydrolab® DataSonde 4a. Metallic parts, such as, a head plate or stirrer in the flow-through cell with the ISE should be avoided. Metallic parts may produce some electrical current that can distort ion selective electrode reading.

Hydrogen sulfide added to the reactor caused interferences when measuring the chloride concentration using an Orion 96-17 Ionplus chloride sensor. Sulfide was also shown to inhibit dehalogenation. These effects can be suppressed in the presence of the SRS aquifer sediment or soil by binding hydrogen sulfide with iron. To further investigate the interference effects of sulfide, iron, and SRS aquifer sediment on the ISE probe performance, additional experiments need to be conducted.

Due to sulfide interference, the chloride probe may require some additional maintenance. Gently polishing with fine paper and cleaning the solid-state elements with deionized water and changing the reference solution can bring the electrode back to its regular performance. Conductivity measurements can be used to track chloride concentration changes, but calibration has to be performed in the solution with similar ion concentration. In order to develop a more detailed correlation between conductivity and chloride, conductivity readings in mS/cm levels should be expressed using at least three decimals places. In this case conductivity readings, due to low (approximately 0.25 mg/L - 1 mg/L or even less) chloride concentration released during TCE biodegradation, will be sensitive enough to show changes on μS/cm level.

REFERENCES

Amy, G.L., R.M. Narbaitz and W.J. Cooper. 1987. Removing VOC's from Groundwater Containing Humic Substances by Means of Coupled Air Stripping and Adsorption. Journ. AWWA 49:54.

Baeher, A.L., G.E. Hoag and M. C. Marley. 1988. Removing Volatile Contaminants from the Unsaturated Zone by Inducing Advective Air-phase Transport. J. Cont. Hydro. 4:1-26.

Barbara H. Wilson, John T. Wilson and Darryl Luce May 1997. Design and Interpretation of Microcosm Studies for Chlorinated Compounds. Proceedings of the Symposium on Natural Attenuation of Chlorinated Organics in Ground Water, United States Environmental Protection Agency, EPA/540/R-97/504, May 1997.

Bouwer, E.J. 1994. Bioremediation of chlorinated solvents using alternate electron acceptors. In: Norris, R.D., R.E. Hinchee, R. Brown, P.L. McCarty, L. Semprini, J.T. Wilson, D.H. Kampbell, M. Reinhard, E.J. Bouwer, R.C. Borden, T.M. Vogel, J.M. Thomas, and C.H. Ward, eds. Handbook of bioremediation. Boca Raton, FL: Lewis Publishers.

Bouwer, E.J., B.E. Rittman, and P.L. McCarty. 1981. Anaerobic degradation of halogenated 1- and 2-carbon organic compounds. Environ. Sci. Technol. 15 (5):596-599.

Cary, J.W., J.F. McBride and C.S. Simmons. 1989. Trichloroethylene Residuals in the Capillary Fringe as Affected by Air-Entry Pressures. J. Environ. Qual. 18:72-77.

Chapelle, F.H. Ground-water microbiology and geochemistry. New York, NY: John Wiley & Sons.

Chapelle, F.H. 1996. Identifying redox conditions that favor the natural attenuation of chlorinated ethenes in contaminated ground-water systems. In: Proceedings of the Symposium on Natural Attenuation of Chlorinated Organics in Ground Water, September 11-13, Dallas, TX.

Cohen, Y. and P.A. Ryan. 1985. Multimedia Modeling of Environmental Transport:Trichloroethylene Test Case. Environ. Sci. Tech. 19:412-417.

Coia, M.F., M.H. Corbin and G. Arastas. 1985. Soil Decontamination through In Situ Air Stripping of Volatile Organics - A Pilot Demonstration. Proceedings of the Petroleum Hydrocarbons and Organic Chemicals in Groundwater Prevention, Detection and Restoration. Houston, Texas. Nov. 13-15.

Dilling, W.L. 1975. Interphase Transfer Processes. II. Evaporation Rates of Chloromethanes, Ethanes, Ethylenes, Propanes and Propylenes from Dilute Aqueous Solutions, Comparisons with Theoretical Predictions. Environ. Sci. Tech. 11:405-409.

Dilling, W.L., N.B. Tefertiller and G.J. Kollos. 1975. Evaporation Rates and Reactivities of Methylene Chloride, Chloroform, 1,1,1-Trichloroethane, Trichloroethylene, Tetrachloroethylene and Other Chlorinated Compounds in Dilute Aqueous Solutions. Environ. Sci. Tech. 9:833-838.

Ellis, D. E., E. J. Lutz, G. M. Klecka, D. L. Pardieck, J. J. Salvo, M. A. Heitkamp, D. J. Gannon, C. C. Mikula, C. M. Vogel, G. D. Sayles, D. H. Kampbell, J.T. Wilson and D.T. Maiers. 1996. Remediation Technology Development Forum Intrinsic Remediation Project at Dover Air Force Base, Delaware. Symposium on Natural Attenuation of Chlorinated Organics in Groundwater. Dallas, September 11-13. EPA/540/R-96/509, pp 93-97.

Fischer, A.J., E.A. Rowan and R.F. Spalding. 1987. VOCs in Groundwater Influenced by Large Scale Withdrawals. Ground Water 25:407-413.

Freedman, D.L., and J.M. Gossett. 1989. Biological reductive dechlorination of tetrachloroethylene and trichloroethylene to ethylene under methanogenic conditions. Appl. Environ. Microbiol. 55:2144-2151.

Harker, A.R. and Y. Kim. 1990. Trichloroethylene Degradation by Two Independent Aromatic Degrading Pathyways in Alcaligenes eutrophus JMP 134. Appl. Environ. Micro. 56:1179-1181.

Henson, J.M., M.V. Yates, J.W. Cochran and D.L. Shackleford. 1988. Microbial Removal of Halogenated Methanes, Ethanes and Ethylenes in an Aerobic Soil Exposed to Methane. FEMS Micro. Ecol. 53:193-201.

Josephson, J. 1983. Subsurface Contaminants. Environ. Sci. Tech. 17:518A-521A.

Kitanidis, P.Kl, L. Semprini, D. H. Kampbell and J.T. Wilson. 1993. Natural anaerobic bioremediation of TCE at the St. Joseph, Michigan, Superfund site. In: U.S. EPA, Symposium on Bioremediation of hazardous wastes. IPA/600/R-92/126. pp. 47-50.

Lovley, D.R., F.H. Chapelle, and J.C. Woodward. 1994. Use of dissolved H2 concentrations to determine distribution of microbially catalyzed redox reactions in anoxic groundwater. Environ. Sci. Technol. 28:1255-1210.

Lovley, D.R., and S. Goodwin. 1988. Hydrogen concentrations as an indicator of the predominant terminal electron-accepting reaction in aquatic sediments. Geochim. Cosmochim. Acta 52:2993-3003.

McCarty, P.L., and L. Semprini. 1994. Ground-water treatment for chlorinated solvents. In: Norris, R.D., ed. Handbook of bioremediation. Boca Raton, FL: Lewis Publishers. pp. 87-116.

McCarty, P. L., and J. T. Wilson. 1992. Natural anaerobic treatment of a TCE plume at the St. Joseph, Michigan, NPL site. In: U.S. EPA, Bioremediation of hazardous wastes. EPA/600/R-93/054. pp. 57-60.

McCarty, P. L., L. Semprini, M.E. Dolan, T. C. Harmon, C. Tiedeman, S. M. Gorelick. 1991. In Situ Methanotrophic Bioremediation for Contaminated Groundwater at St. Joseph, Michigan. In: *In Situ* and On Site Bioreclamation, R. Hinchee and R. Olfenbuttel (Eds.). Buttersworth-Heineman, Stoneham, MA.

McCarty, P.L., P.V. Roberts, M. Reinhard, and G. Hopkins. 1992. Movement and transformations of halogenated aliphatic compounds in natural systems. In: Schnoor, J.L., ed. Fate of pesticides and chemicals in the environment. New York, NY: John Wiley & Sons.

Mehran, M., R.L. Olsen and B.M. Rector. 1987. Distribution Coefficient of Trichloroethylene in Soil Water Systems. Ground Water 25:275-282.

Miller, D.E. and R.W. Callaway. 1991. Biological Treatment of Trichloroethylene Vapor Streams. Submitted to Environmental Science & Technology.

Murray, W.D., and M. Richardson. 1993. Progress toward the biological treatment of C1 and C2 halogenated hydrocarbons. Crit. Rev. Environ. Sci. Technol. 23(3):195-217.

Nelson, M.J.K., S. Montgomery, W.R. Mahaffey and P.H. Pritchard. 1987. Biodegradation of Trichloroethylene and Involvement of an Aromatic Biodegradation Pathway. Appl. Environ. Micro. 53:949-954.

Perry L. McCarty Biotic and Abiotic Transformations of Chlorinated Solvents in Ground Water, Proceedings of the Symposium on Natural Attenuation of Chlorinated Organics in Ground Water, United States Environmental Protection Agency, EPA/540/R-97/504, May 1997.

Proceedings of the Symposium on Natural Attenuation of Chlorinated Organics in Ground Water, United States Environmental Protection Agency, EPA/540/R-97/504, May 1997.

Robeck, G.G. and O.T. Love. 1983. Removal of Volatile Organic Contaminants from Groundwater. Environ. Micro. 53:949-954.

Sewell, G.W., S.A. Gibson and H.H. Russell. 1990. Anaerobic In Situ Treatment of Chlorinated Ethenes. pp.67- 79. In: In-Situ Bioremediation of Ground Water and Contaminated Soils. Water Pollution Control Federation.

Todd H. Wiedemeier, Matthew A. Swanson, and David E. Moutoux Overview of the Technical Protocol for Natural Attenuation of Chlorinated Aliphatic Hydrocarbons in Ground Water Under Development for the U.S. Air Force Center for Environmental Excellence. Proceedings of the Symposium on Natural Attenuation of Chlorinated Organics in Ground Water, United States Environmental Protection Agency, EPA/540/R-97/504, May 1997.

Vogel, T.M. and P.L. McCarty. 1985. Biotransformation of Tetrachloroethylene to Trichloroethylene, Dichloroethylene, Vinyl Chloride, and Carbon Dioxide Under Methanogenic Conditions. Appl. Environ. Micro. 49:1080-1083.

Wackett, L.P., G.A. Brusseau, S.R. Householder and R.S. Hanson. 1989. Survey of Microbial Oxygenases: Trichlorethylene Degradation by Propane-Oxidizing Bacteria. Appl. Environ. Micro. 55:2960-2964.

Wilson, J.T., D. Kampbell, J. Weaver, B. Wilson, T. Imbrigiotta, and T. Ehlke. 1995. A review of intrinsic bioremediation of trichloroethylene in ground water at Picatinny Arsenal, New Jersey, and St. Joseph, Michigan. In: U.S. EPA. Symposium on Bioremediation of Hazardous Wastes: Research, Development, and Field Evaluations, Rye Brook, N.Y. EPA/600/R-95/076.

Wilson, J.T., J.W. Weaver, and D.H. Kampbell. 1994. Intrinsic bioremediation of TCE in groundwater at an NPL site in St. Joseph, Michigan. In: Symposium on Intrinsic Bioremediation of Groundwater, United States Environmental Protection Agency EPA/540/R-94/515, August 1994, Denver, CO.