To the Graduate Council:

I am submitting herewith a dissertation written by Melissa E. Lenczewski entitled "Biodegradation of TCE in Fractured Shale and Saprolite." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Geology.

Larry D. McKay, Major Professor

We have read this dissertation
And recommend its acceptance:

Accepted for the Council:

Interim Vice Provost and
Dean of The Graduate School

BIODEGRADATION OF TCE IN FRACTURED SHALE AND SAPROLITE

A Dissertation
Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Melissa E. Lenczewski
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I would like to thank my committee: Larry McKay, Steve Driese, Phil Jardine, Gary Sayler, and Alice Layton.

I am most grateful to my family and friends that saw me through all these years at the University of Tennessee. Without there love and assistances I would never had made it.

Abstract

A series of investigations were conducted to examine biodegradation of trichloroethylene (TCE) contamination in fractured shale and in saprolite (formed from weathered sedimentary rocks). A plume of groundwater contaminated with trichloroethylene (TCE) was detected at the Oak Ridge Reservation (ORR) in eastern Tennessee adjacent to shallow waste trenches in fractured shale. Monitoring wells at the site indicated a downgradient decline in concentration of TCE and the appearance of its daughter products (*cis*-dichloroethylene (cDCE) and vinyl chloride (VC)), which suggests the likelihood that anaerobic biodegradation of TCE was occurring. This hypothesis is further supported by the existence of redox conditions, including iron reduction, sulfate reduction, and possibly methanogenesis, which are favorable for anaerobic biodegradation. Microbial community analysis using conventional enrichment methods and molecular methods also support this hypothesis by showing the presence of bacteria previously implicated in the anaerobic biodegradation of chlorinated solvents. This is believed to be the first study to show strong evidence of biodegradation of TCE in shale bedrock.

Additional investigations were performed using large undisturbed columns of fractured saprolite from an uncontaminated site about 1 km from the waste trenches. The experiment involved continuous pumping of groundwater containing dissolved phase TCE through one column containing the natural microbial communities (the biotic column), and through a second column in which the microorganisms had been inhibited. In effluent from the biotic column evidence of anaerobic biodegradation TCE appeared

within a few months. This included decreasing concentration of TCE in the effluent, appearance of daughter products (cDCE and VC), development of iron and sulfate reducing conditions, and appearance of iron and sulfate reducing bacteria. In the inhibited column there were no indicators of TCE degradation. It appears that TCE biodegradation processes in the shale and saprolite are very similar, and that they can occur spontaneously and rapidly without amendments to enhance biodegradation. Current Air Force Center for Environmental Excellence (AFCEE) protocols for determination of natural attenuation, which are based on monitoring of geochemical parameters, are appropriate for assessing the potential for TCE attenuation in the shale and shale saprolite found at the Oak Ridge Reservation.

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Chapter 1

Introduction

1.1 TCE in Fractured Porous Materials

Dense nonaqueous phase liquids (DNAPLs), especially chlorinated solvents such as trichloroethylene or trichloroethene (TCE), have been widely used as industrial solvents since the 1960s (Pankow and Cherry, 1996). Many of these solvents are frequently found in groundwater at industrial sites or landfills (Westrick et al., 1984). To date, most research on DNAPL behavior in the subsurface has been carried out in granular aquifer materials or fractured, low porosity rock. Very little research has been performed on DNAPLs in fractured shales or saprolite (formed from weathered sedimentary rock, which retains features from the parent bedrocks).

TCE was selected as the DNAPL for this research project for several reasons. First, it is a "typical" DNAPL, with respect to its physical and chemical properties, and can act as a model compound for behavior of other DNAPLs. Second, TCE is frequently found in high concentrations in groundwater at industrial sites and landfills (Westrick et al., 1984; Pankow and Cherry, 1996). Third, TCE is detrimental to human health and the environment at very low concentrations (5 ppb is the drinking water standard) (Environmental Protection Agency, 1985). Fourth, TCE is the most extensively studied DNAPL, with an extensive literature base on TCE biodegradation. Finally, a plume of groundwater contaminated with TCE was detected in fractured shale at Waste Area

Grouping 5 (WAG5), on the Oak Ridge Reservation (ORR), which was the inspiration for part of the research described in this dissertation (Chapter 2).

Most previous studies of transport and biodegradation of chlorinated solvents in groundwater have been carried out in relatively simple hydrogeologic settings, typically sand and gravel aquifers. In fractured and relatively porous materials, such as shale or saprolite, transport of immiscible and dissolved phase chlorinated solvents is strongly influenced by fracture and matrix pore structure. The immiscible DNAPL tends to follow the largest aperture fracture or pores (Kueper and McWorther, 1991; Pankow and Cherry, 1996; Cropper, 1998). The immiscible phase then rapidly dissolved (Figure 1-1) and diffuses into the fine-grained matrix (Parker et al., 1994; Pitner, 2000). The TCE can also diffuse back out of the matrix into the fractures, which slows the rate at which the contaminant is flushed out of the soil and rock, and greatly reduces the prospects for successful remediation using conventional source removal or "pump and treat" methods. Since pore structure and "matrix diffusion" play a major role in controlling distribution and concentration of TCE in fractured materials, it is quite possible that they may also influence biodegradation of TCE in these materials.

1.2 Biodegradation of TCE in Groundwater

In groundwater, the dissolved phase of many chlorinated solvents tends to slowly biodegrade under either aerobic or anaerobic conditions. The rate of biodegradation of chlorinated solvents is variable and depends on the properties of the contaminant, its distribution and the microbiological and geochemical environments in the subsurface.

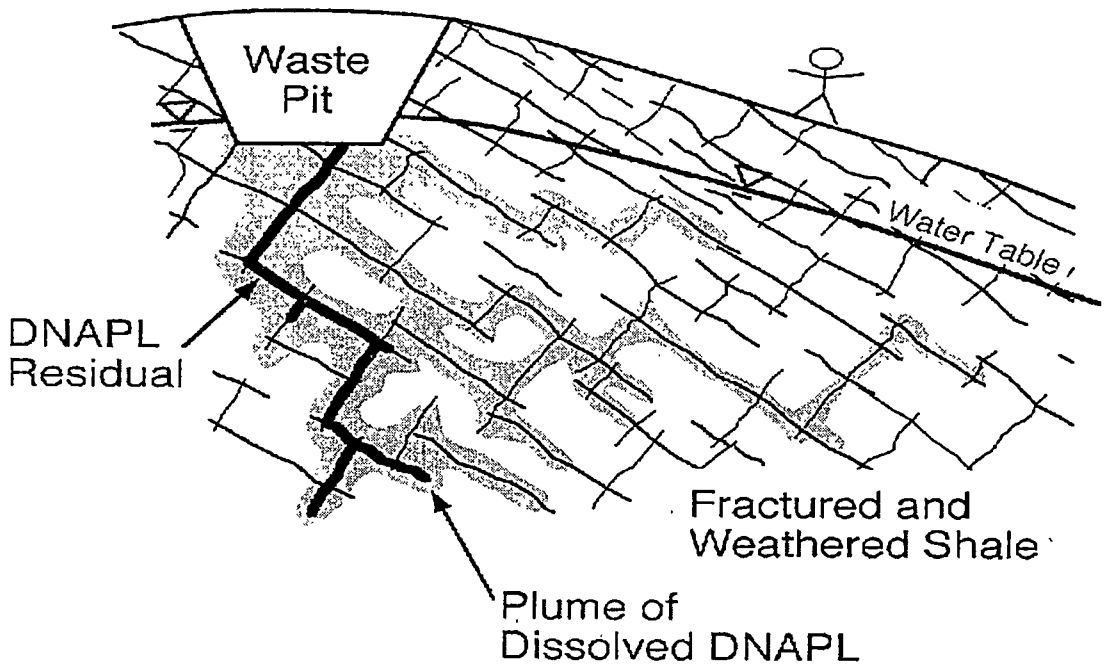


Figure 1-1. Conceptual model for DNAPL migration in the groundwater in fractured material.

Differences in TCE biodegradation are expected between fractured shale or saprolite and granular materials because of their differences in physical and geochemical properties.

Microbial dechlorination can be categorized into aerobic and anaerobic biodegradation pathways (Lee et al., 1998). In aerobic environments some bacteria, like methanotrophs, can cometabolically transform TCE to CO_2 and H_20. Cometabolism is the transformation of a compound by organisms that do not obtain energy or carbon for cell growth from the transformation of the compound, and hence require an alternative source of carbon and energy. Methanotrophic bacteria use methane as the sole carbon source. Aerobic biodegradation generally prefers less chlorinated compounds like dichloroethylene (DCE) and vinyl chloride (VC) (Vogel, 1994).

Anaerobic biodegradation transforms TCE to lesser-chlorinated compounds by reductive dechlorination, which involves the transfer of electrons to chlorinated ethylenes. The general transformation pathway is:

$$TCE \Rightarrow cDCE \Rightarrow VC \Rightarrow ethylene$$

As the number of chlorines decreases, the rate of dechlorination decreases and lesser-chlorinated compounds such as vinyl chloride accumulate (Fathepure and Tiedje, 1994). There are three different classes of anaerobic metabolism involved in reductive dechlorination of TCE. First, anaerobic iron reducing bacteria, such as *Geobacter* sp., utilize a wide range of hydrocarbon compounds to support microbial growth (Lovely and

Anderson, 2000; Lee et al., 1998; Krumholz et al., 1996). Some but not all *Geobacter* sp. also dehalogenate chlorinated solvents. Evidence for this metabolism includes the accumulation of reduced iron (Fe(II)) and the presence of these microorganisms in systems where biodegradation appears to be occurring. Second, certain dehalorespiring strains such as *Desulfitobacterium chlororespirans*, can use TCE as an electron acceptor for biologically useful energy generation (Sanford et al., 1996; Lee et al., 1998). These bacteria may produce cDCE as a final end product or may carry out complete dechlorination to ethylene. Lastly, TCE can be reductively dechlorinated by methanogens, sulfate reducing bacteria, and some iron reducting bacteria (Bagley and Gossett, 1990; Maymo-Gatell et al., 1997). These reactions are not thought to be energy yielding, but rather cometabolic, because only a small fraction of the total reducing equivalents derived from the oxidation of electron donors is used to reduce the solvent. Multiple dechlorination pathways are likely to operate at a site in heterogeneous materials like fractured shale and saprolite at the same time, thus in the natural environment is likely that some of these mechanisms will act together to naturally attenuate TCE.

1.3 Natural Attenuation of TCE

Natural attenuation is a remediation strategy that relies on existing physical, chemical, and microbial processes to reduce the concentration of a contaminant without human intervention (Brady et al., 1999). Loss of TCE mass through natural attenuation at contaminated sites is a commonly proposed strategy for remediation, but because of the importance of site-specific variations in the hydrogeological, geochemical, and microbial environments, predictions of biodegradation often have a high degree of uncertainty.

This is especially important in fractured material because of the erratic contaminant distribution and large seasonal variations in dilution and redox conditions that make it difficult to determine whether the plume is growing, stable or shrinking.

One critical step in natural attenuation is demonstrating whether contaminant mass and/or concentration is decreasing over time. This decrease may be due to contaminant sorption, dilution, volatilization, and biodegradation. During biodegradation the contaminant is destroyed or transformed into daughter products, instead of just changing states. Methods for determining whether or not anaerobic biodegradation of TCE is occurring in groundwater at a site include sampling for the presence of the daughter products cDCE and VC (discussed above), the presence of appropriate reducing conditions required for biodegradation, and identification of microbial communities that have the potential to carry out biodegradation (Lee et al., 1998).

Understanding the presence and distribution of redox conditions is fundamental to predicting the fate and transport of TCE in groundwater systems. Microbially mediated redox reactions affect the rate and extent of biodegradation processes, which in turn affect the mobility of organic contaminants. In anaerobic respiration, a series of alternative electron acceptors in the environment are used from the most oxidized to the most reducing (Pepper et al., 1996). In the case of TCE biodegradation, Fe(III), sulfate, and carbonate are used as electron acceptors forming Fe(II), sulfide, and methane, respectively (Bouwer, 1994). Chapelle and Bradley (1998) used redox conditions such as the occurrence of sulfate reducing and iron reducing environments as indicators of the

potential for natural attenuation of TCE and to determine the extent of anaerobic biodegradation at a site.

The presence of microorganisms that have the potential to biodegrade TCE can be determined by isolating the organisms directly from environmental samples or microcosms (Fries et al., 1997b; Wild et al., 1996; Sharma and McCarty, 1996; Maymo-Gatell et al., 1997) or through identification by molecular techniques (Fries et al., 1997a; Stapleton et al., 2000). Methanogenic, iron reducing and sulfate reducing bacteria have all been implicated in TCE reductive dechlorination and consortia of bacteria are often involved in complete biodegradation of TCE (Wilson et al., 1986; Freedman and Gossett, 1989; Smatlak et al., 1996; Bagley and Gossett, 1990; Lovely and Anderson, 2000; De Wever et al., 2000). For example, in a contaminated sandy aquifer Dojka et al. (1998) were able to link the presence of a methanogenic zone, through chemical analysis and molecular techniques, to the microorganism potentially responsible for the biodegradation. The combination of chemical and microbiological techniques can be used effectively to determine whether natural attenuation of chlorinated solvents is occurring at a site even though neither of these techniques may, by itself, provide definitive proof.

1.4 Goals, Hypotheses, and Objectives

The primary goal of this investigation was to determine whether TCE could biodegrade to cDCE and VC in fractured shale and saprolite. The research program was inspired by preliminary indications of *in situ* TCE biodegradation in groundwater in fractured shale bedrock near a waste pit at the WAG5 site on the ORR, Oak Ridge, TN (Jardine, personal

communication). The overall hypothesis was that microbial and geochemical conditions in the fractured shale and the overlaying mantle of saprolite are favorable for anaerobic biodegradation of TCE, so that once TCE is introduced, it will biodegrade "naturally" without addition of specially-selected bacteria, cometabolites or other chemicals.

The major chapters of this dissertation address this hypothesis from different perspectives, and are briefly outlined below.

Chapter 2- Natural Attenuation of Trichloroethylene in Fractured Shale Bedrock: The primary objective of this field-based investigation was to determine whether biodegradation of TCE was occurring under natural conditions in a plume of organic contaminants in fractured shale bedrock at the WAG5 site at ORR. A multiple analytical approach consisting of measurement of VOC concentration/distribution, redox conditions, and microbial techniques (enrichment and molecular techniques) was used. A second objective was to determine whether current monitored natural attenuation guidelines are effective in this type of complex geological setting.

Chapter 3- Biodegradation of TCE in Undisturbed Columns of Fractured Saprolite: This research is based on the hypothesis that microbially mediated anaerobic degradation of TCE in fractured shale saprolite can occur without any amendments to the system, other than the introduction of TCE. Redox conditions and microbial community composition in the saprolite and groundwater will naturally shift after the introduction of TCE to become favorable for biodegradation of TCE. The specific objectives of the

research are: 1) to determine if relative TCE mass loss occurs under biotic or inhibited conditions; 2) to determine if daughter products of anaerobic TCE degradation (cDCE and VC) appear in the effluent from the biotic and inhibited columns; 3) to determine if reducing conditions such as iron reduction or sulfate reduction, which are indicative of anaerobic biodegradation of TCE, develop with time after introduction of TCE in the biotic column; 4) to characterize the microbial community in the groundwater prior to and after introduction of TCE in order to determine if shifts in the community which favor biodegradation of TCE, occur; and 5) to determine whether current natural attenuation protocols are effective in this type of complex setting.

Chapter 4- Preliminary Investigations of Biodegradation of TCE in Fractured Saprolite: This chapter describes a preliminary biodegradation study that was carried out in a column of saprolite from ORR. The objectives were similar to those outlined in Chapter 3, but several problems were encountered that prevented the researcher from meeting all of these objectives. However, findings of this study, including methods development, were still valuable because they aided in the successful design of the subsequent experiment (Chapter 3). As well, more in-depth investigations of microbial community structure were carried out for the preliminary column experiment, which are expected to be relevant to future investigations. For these reasons, the preliminary experiment is presented as a separate chapter in the dissertation, rather than being incorporated in Chapter 3, or relegated to an appendix.

Chapter 2

Natural Attenuation of Trichloroethylene in Fractured Shale Bedrock

2.1 Introduction

Dense nonaqueous phase liquids (DNAPLs), especially chlorinated solvents such as trichloroethylene (TCE), have been widely used as industrial solvents since the 1960s. Many of these solvents are commonly found in groundwater at industrial sites or landfills (Pankow and Cherry, 1996). Natural attenuation of chlorinated solvents has been shown to occur in granular aquifer materials (Dojka et al., 1998; Chapelle et al., 1996; Kleopfer et al., 1985; Clement et al., 2000), but only a few studies have examined natural attenuation in fractured rock (Yager et al., 1997; Mobarry et al., 1999). To date, there have been no *in situ* studies of biodegradation of TCE in fractured shale bedrock, which is one of most commonly occurring rock types. As a result, the potential for biodegradation in this material must be largely inferred from studies in other materials. Although these comparisons are very useful, TCE biodegradation in fractured shale could be substantially different because of the different physical and geochemical properties of this material. Particularly, unconfined fractured bedrock aquifers are often subject to larger seasonal and short term variations in water table elevation, flow rate and redox conditions, which could influence biodegradation.

Anaerobic biodegradation transforms TCE to lesser-chlorinated compounds by reductive dechlorination. The general pathway is: TCE $\Rightarrow$ cDCE$\Rightarrow$ VC$\Rightarrow$ ethylene. The presence

of cDCE as a product of TCE breakdown is indicative of biodegradation while the presence of mixed cDCE and tDCE would indicate inhibited chemical breakdown. The rate of dechlorination decreases as the number of chlorine atoms decreases, thus lesser-chlorinated compounds such as vinyl chloride may accumulate in groundwater (Fathepure et al., 1994). Vinyl chloride (VC) is more carcinogenic than TCE, so biodegradation of TCE under anaerobic conditions would not be sufficient to eliminate health risks. Diverse microorganisms have been shown to anaerobically biodegrade TCE through different pathways, and consortia of bacteria are often involved in biodegradation of TCE. These include methanogenic, Fe (III)-reducing and sulfate reducing bacteria that have been implicated in TCE dechlorination (Wilson et al., 1986; Freedman and Gossett, 1989; Smatlak et al., 1996; Bagley and Gossett, 1990; Lovely and Anderson, 2000).

TCE is generally resistant to biodegradation in aerobic subsurface environments, contributing to its persistence in shallow polluted groundwater (Pankow and Cherry, 1996). Some aerobic bacteria, like methanotrophs, which utilize methane as a sole carbon source, can cometabolically transform TCE to CO_2. Cometabolism is the partial oxidation of the substrate, but the energy derived from oxidation is not used to support microbial growth, and thus an alternative source of carbon and energy is required. Aerobic biodegradation is generally more effective for less chlorinated compounds like DCE and VC (Vogel, 1994). Other aerobic bacteria cometabolize TCE using aromatic compounds such as phenol and toluene (McCarty et al., 1998; Harker and Kim, 1990; Fries et al., 1997b).

Monitored natural attenuation (MNA) is an alternative remediation strategy that relies on existing physical, chemical and microbiological processes to reduce the concentration of a contaminant without human intervention (Brady et al., 1999). To determine if MNA is a viable alternative for contaminated groundwater at a site, different site screening and technical guidelines have been established (Brady et al., 1999; Wiedemeier et al., 1998). The utilization of these screening tools leads to the development of a conceptual model for natural attenuation and can aid regulators in determining if natural attenuation is a viable alternative for remediation of a site. One of these screening tools is the Air Force Center for Environmental Excellence (AFCEE) protocol (Wiedemeier et al., 1998) for assessing biodegradation. The AFCEE protocol uses input parameters describing site redox conditions and daughter product concentrations to determine a numerical "score" for conditions at a site. This score serves only to indicate the likelihood of natural attenuation and provides site managers with a clear identification of likely data needed for MNA implementation.

Successful implementation of MNA strategy often requires an assortment of different types of monitoring and characterization data. One critical step is evidence of historical groundwater and/or soil chemistry data demonstrating decreasing contaminant mass and/or concentration over time at relevant sampling points. This decrease may be due to contaminant sorption, dilution, volatilization, and nonbiological or biological breakdown. Obtaining such data may take many years, especially in complex flow systems where long-term concentration trends may vary substantially in different parts of the system, or where they may be masked by seasonal variations. As a result, methods that assess the

microbial and geochemical conditions needed for degradation can also play an important role.

Methods for demonstrating biological activity at a site include sampling for intermediates of biological metabolism, demonstration of the presence of electron acceptors/donors, and description of the microbial community at the site, especially identifying organisms believed to be responsible for the metabolism of contaminants (Dojka et al., 1998). Chapelle and Bradley (1998) used redox conditions such as sulfate reducing and Fe (III) reducing environments, as indicators of the potential for natural attenuation of TCE and PCE in a sandy aquifer. Microorganisms that biodegrade TCE can be isolated directly from environmental samples or microcosms (Fries et al., 1997b; Wild et al., 1996; Sharma and McCarty, 1996; Maymo-Gatell et al., 1997) and their presence in groundwater can be used as indicators of the potential for biodegradation. The ability to monitor changes in microbial communities reflecting contaminant exposure has advanced with development of molecular based techniques. Only a few studies have been performed using molecular techniques to characterize microbial communities at field sites in which natural attenuation may be occurring (Fries et al., 1997a; Stapleton et al., 2000; Dojka et al., 1998). Using chemical analyses and molecular techniques Dojka et al. (1998) linked the presence of a methanogenic zone in a chlorinated solvent contaminated aquifer with the microorganism potentially responsible for the biodegradation. These studies show that a combination of chemical, microbiological and molecular techniques can be used effectively to assess natural attenuation of TCE in granular aquifers, but no

work has been carried out to determine if these procedures are equally effective in more complex hydrogeological environments, such as fractured shale aquifers.

The primary objective of this investigation is to determine whether TCE can biodegrade under existing conditions in contaminated fractured shale bedrock aquifer at the Oak Ridge Reservation in East Tennessee. A multiple analytical approach involving determination of VOC concentration/distribution, redox conditions, and microbial community structure (using enrichment and molecular techniques) was used to determine if biodegradation of TCE is occurring. A second objective is to determine whether current MNA guidelines are effective for assessing the potential for TCE biodegradation in a complex hydrogeological setting.

2.2 Site Descriptions and Hydrology

This study was conducted in the southeastern portion of Waste Area Group 5 (WAG5) on the Oak Ridge Reservation (ORR) in Oak Ridge, Tennessee (Figure 2-1). The reservation is located in the Valley and Ridge geologic province and the study site consists of 0.5 to 2.5 m of saprolite (highly weathered bedrock) on top of interbedded shale and limestone of the Upper Cambrian age Dismal Gap Formation. Bedding dips towards the southeast at 30-35°. Approximately 30 wells were installed along a 35 m long transect between a cluster of waste-filled trenches and a seep along a stream (Figure 2-2). Three of the drilled wells were equipped with multilevel piezometers and an additional 24 drive-point wells were installed with a truck-mounted pneumatic hammer. The wells were installed as part of a previous study of groundwater flow and transport

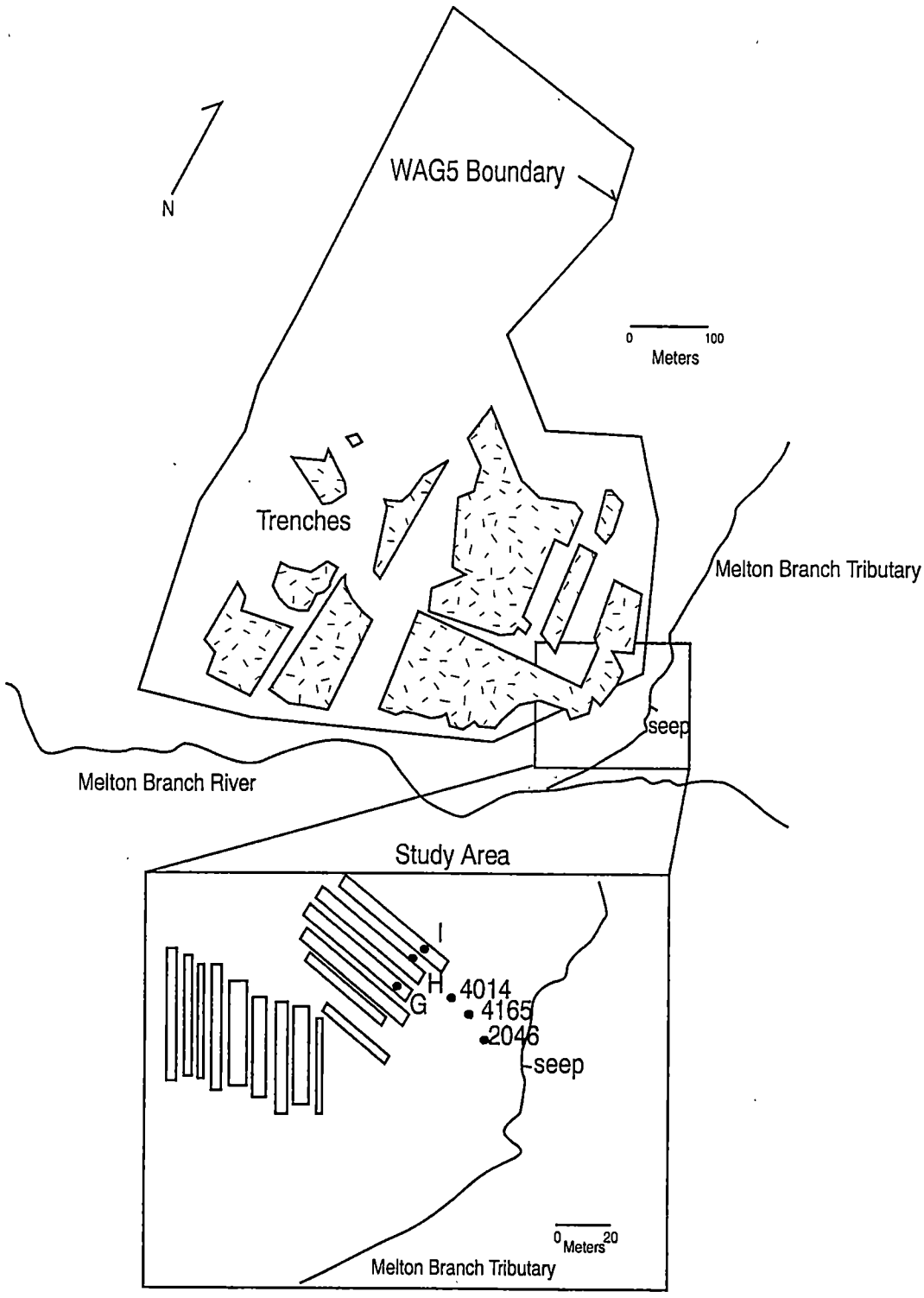


Figure 2-1. Location of waste trenches and well field at waste area grouping 5 (WAG5) at the Oak Ridge Reservation, Oak Ridge, TN (Jardine et al., 1999).

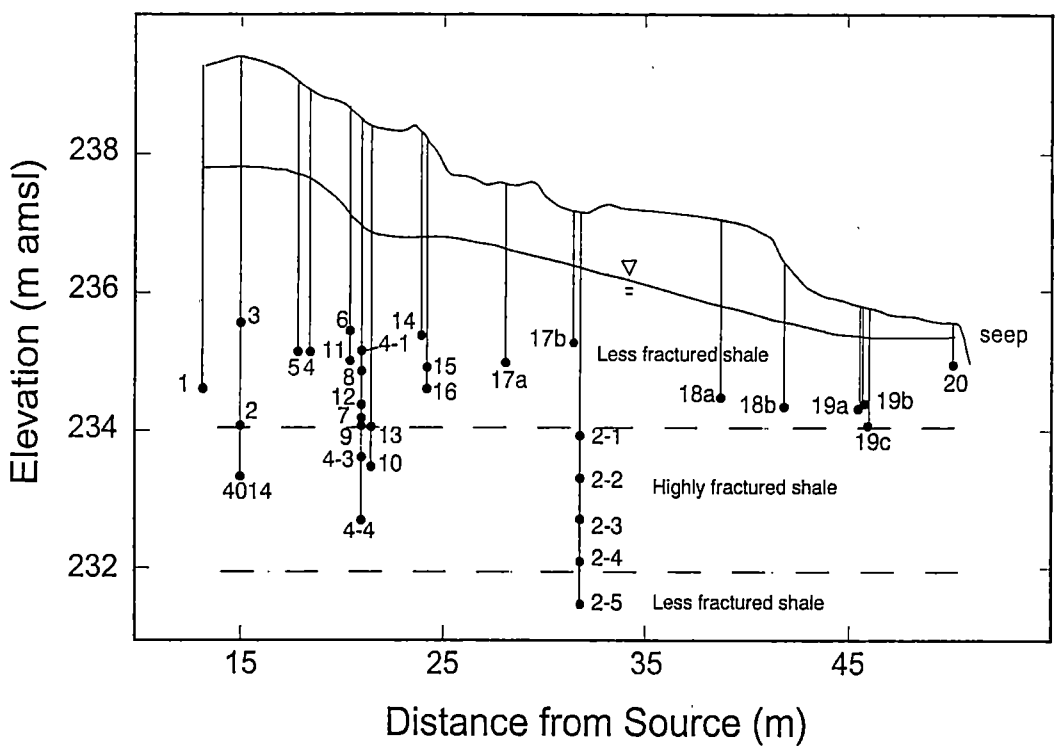


Figure 2-2. Cross-section of experimental field facility at WAG5 showing location and sampling depth of all groundwater monitoring wells (Jardine et al., 1999).

(Jardine et al., 1999). The studies by Jardine et al. (1999) revealed two different flow regimes within the bedrock at the site (Table 2-1 and Figure 2-2): a 2 m thick highly fractured zone, characterized by rapid flow, with specific discharge rates as high as 300 to 500m/yr, and a less fractured "matrix" zone above and below the fractured zone with specific discharge rates that were 3 to 10 times less than in the fracture zone. Hydraulic head values were continuously measured in the piezometers for periods of 180-550 days, indicating an average hydraulic gradient of 0.06 to 0.1 along the transect. Most of the fractures are less than a meter in length, but they are numerous and highly interconnected, resulting in fracture densities of up to 5/m in the unweathered bedrock (Dreier et al., 1987; Sledz and Huff, 1981). Hydrogeological investigations at WAG5 (Jardine et al., 1999) indicate that the groundwater flow direction is generally west to east, roughly along geologic strike of local bedding, and towards a crosscutting perennial stream. Previous investigations at ORR indicate that conductive fractures tend to be oriented along strike of bedding and that preferential flow tends to develop along strike (Lee et al., 1992).

2.3 Sample Collection and Analysis

2.3.1 Volatile Organic Carbon (VOC) and Dissolved Gases

Groundwater samples were collected from wells positioned in the trenches and downgradient from the well field (Figure 2-2). Groundwater samples for volatile organic carbon (VOC) analysis were collected in 40 mL vials with Teflon coated septa. The samples were taken so that the vials contained zero headspace and they were stored upside-down and submerged in water for no longer than 24 hours to prevent loss of any

Table 2-1: Physical properties of fractured and weathered shale at WAG5 ([1]Jardine et al., 1999;[2]Jardine et al., 1989).

Hydraulic conductivity[1]:	1.1×10^{-7} to 1.5×10^{-5} m/s
Hydraulic gradient[1]:	0.06 to 0.1
Infiltration rate (m/y)[1]:	1.33
Porosity[1]:	10 to25%
Fracture aperture[1]:	30 to 102μm
Fracture density[1]:	5 to 200/m
Fraction organic carbon[2]:	0.006

VOC by diffusion or volatilization. The VOC samples were analyzed using a Tekmar 3000 Purge and Trap system (Vernon, BC, Canada) with a VOCARB 3000 trap and a Hewlett Packard 5890-series II gas chromatograph with DB-624 column (Palo Alto, CA). The inlet temperature on the gas chromatograph was 180°C and on the detector was 300°C, with the column temperature held initially at 35°C for 5 minutes, then increased 5°C/minute to a final temperature of 90°C, where it was held constant for 2 minutes.

Groundwater was also sampled for measurements of dissolved concentrations of methane, ethane, and ethylene gas from the same wells where the VOC samples were collected. The samples were collected in 40 mL vials with Teflon-coated septa, with each vial containing a measurable amount of headspace. Sample and headspace volumes were determined by weight. The samples were vigorously agitated and stored upside-down and submerged in water, and were generally analyzed within four hours of collection. Sample analysis involved the direct injection of 25-500 μL of headspace gas, depending on concentration, from each vial into a Hewlett Packard 5890-series II gas chromatograph with a chrompak coated Poraplot Q column (Palo Alto, CA).

2.3.2 Inorganic Chemical Analysis

Groundwater from each well was analyzed for concentration of inorganic solutes at the same time as the VOC and dissolved gases. Average chemical properties of the groundwater are presented in Table 2-2. Dissolved oxygen (DO) was determined in the field using colorimetric indicators. For concentrations between 0 and 2 ppm, DO was analyzed with a Hach DR2000 field spectrophotometer (Loveland, CO). For DO

Table 2-2: Range chemical properties of the groundwater at WAG5 over a period of a year

pH	6.4-7.3
Temperature	12°C
Dissolved Oxygen	0.02-3 ppm
Sulfate	0.5 to 5 ppm
Sulfide	0.01-0.2 ppm
Total Iron	1 to 13 ppm
Iron (II)	0.5-13 ppm
Chloride	12-17 ppm
Phosphate	0 ppm
Alkalinity	500 ppm $CaCO_3$
Nitrate	<0.1 ppm
Nitrite	<0.05 ppm
Hydrogen	est. <1 nM
TOC	1-2 ppm
TCE	0-34 ppb
cDCE	0-50 ppb
VC	0-18 ppb
Methane	0-14 ppm

concentrations greater than 2 ppm, a colorimetric method using Chemetrics ampoules (Calverton, VA) was used. Nitrate, nitrite, total iron, Fe (II), and sulfide were also determined colorimetrically in the field using Hach (Loveland, CO) kits. Chloride, nitrate, sulfate, and phosphate were determined using an ion chromatograph (Dionex DX300, AS4A column, Sunnyvale, CA) using a spectral array detector at 190 nm. Major cations were determined using Inductively Coupled Plasma (ICP) and total organic carbon (TOC) was determined with a Shimadzu 5000 TOC analyzer. Alkalinity was determined in the field using the Gran titration method.

2.3.3. Microbiological Analysis and Molecular Analysis

Groundwater samples were collected in sterile 40 mL vials with Teflon coated septa for microbial enrichment studies. Samples from well numbers 1, 2, 10, 11, 14, 2-4, 2-5, 19c, and I (waste trench well) were tested. The samples were taken so that the vials contained zero headspace and they were stored upside-down for no longer than 72 hours at 5°C prior to testing. BART-IRB and BART-SRB media were obtained from Hach (Loveland, CO) for determination of iron- and sulfate-reducing bacteria. The methanogens were incubated in methanogen media (Atlas, 1995) and methanotrophs were incubated in media described by Pfiffner et al. (1997).

Molecular analysis for identification of microbial communities was determined at the same time that the enrichments were done from wells 10 and 11, and from positive enrichments for iron-reducing and sulfate-reducing bacteria. In this approach, the 16S rDNA was amplified from DNA extracted from groundwater samples and cloned to

create 16S rDNA libraries. Water was collected in sterile 40 mL vials with Teflon coated septa and filtered through a 0.2μm filter (Durapore, Millipore, Bedford, MY) then stored at –80°C until further processing. DNA was extracted by bead beating filters with a lysing matrix (Bio101, Carlsbad, CA) and STE buffer (10mM Tris (pH 8), 1 mM ETDA, and 100 mM NaCl) for 20 s and 4 m/s. Samples were then centrifuged for 2 minutes at 10,000 rpm with the supernatant liquid placed into a new sterile microcentrifuge tube and stored at –80°C until further processing.

To generate clone libraries, community rDNA was amplified by PCR with a eubacteria reverse oligonucleotide primer 1492R (5'-GGT TAC CTT GTT ACG ACT T-3') and a eubacteria forward oligonucleotide primer 27F (5'-AGA GTT TGA TCM TGG CTC AG -3') or the universal primer 530F (5'-GTG CCA GCM GCC GCG G-3') and the universal oligonucleotide reserve primer of 1392R (5'-ACG GGC GGT GTG TRC-3') (Lane et al., 1985). Extracted DNA was amplified in a reaction mixture with PCR beads (Amersham Pharmacia Biotech Inc. Piscataway, NJ) and a 20 nM concentration of each forward and reverse primer. Reaction mixtures were incubated in a Perkin Elmer Gene Amp PCR system 2400 thermal cycler (Wellesley, MA) at 94°C for 5 minutes followed by 15 cycles at 94°C for 30 seconds, 60°C for 45 seconds, and 72°C for 2 minutes and followed by a final extension period of 7 minutes at 72°C. Ten μL of DNA was visualized with a 2% agarose gel with ethidium bromide to determine if the reaction was successful. PCR products were cloned with the TOPO TA Cloning kit (Invitrogen Corp., Carlsbad, CA). Plasmids containing the correct DNA inserts were prepared for sequencing using RPM AFS Midi plasmid preparation kit (Bio 101, Carlsbad, CA).

Sequences were determined using 530F primer by the Molecular Biology Sequencing Facility located on the University of Tennessee campus, which is equipped with an Applied Biosystems 373A Automated Sequencer (Foster City, CA). Analysis of sequences was done using the GenBank (http://www.ncbi.nlm.nih.gov/) basic Blast search. Sequence alignments were performed using ClustalX (v. 1.64b) and the trees were constructed using TreeView (http://taxonomy.zoology.gla.ac.uk/rod/treeview.html).

2.4 Results and Discussion

2.4.1 VOC Concentration and Distribution

Observation wells indicate the presence of VOCs in the trenches and a trend towards lower concentrations of TCE and other VOCs with increasing distance from the contaminated trenches (Figure 2-3, Table 2-3). The origin and the types of waste disposed at WAG5 are unknown, so to determine which trench was most likely to be the source of the VOC, sampling wells were situated along the downgradient side of the waste trenches (Figure 2-1). Three of these wells (G, H and I) were the only wells with any detectable concentrations of TCE (0.003-0.3 μM), which suggests that the waste trenches located directly up gradient of the transect of monitoring wells (Figure 2-2) are the likeliest source of the TCE plume. The presence of the anaerobic daughter products of TCE (cDCE, VC, and ethylene) in the plume is a probable indicator that natural attenuation is occurring at the site (Table 2-3). Chemical DCE can exist as either a *cis* or *trans* mixture, however, in the wells only cDCE was observed, which is indicative of biological reductive chlorination of TCE. The highest concentration of TCE in the monitoring wells was 0.1 μM (well 11) with concentrations in most of the plume between

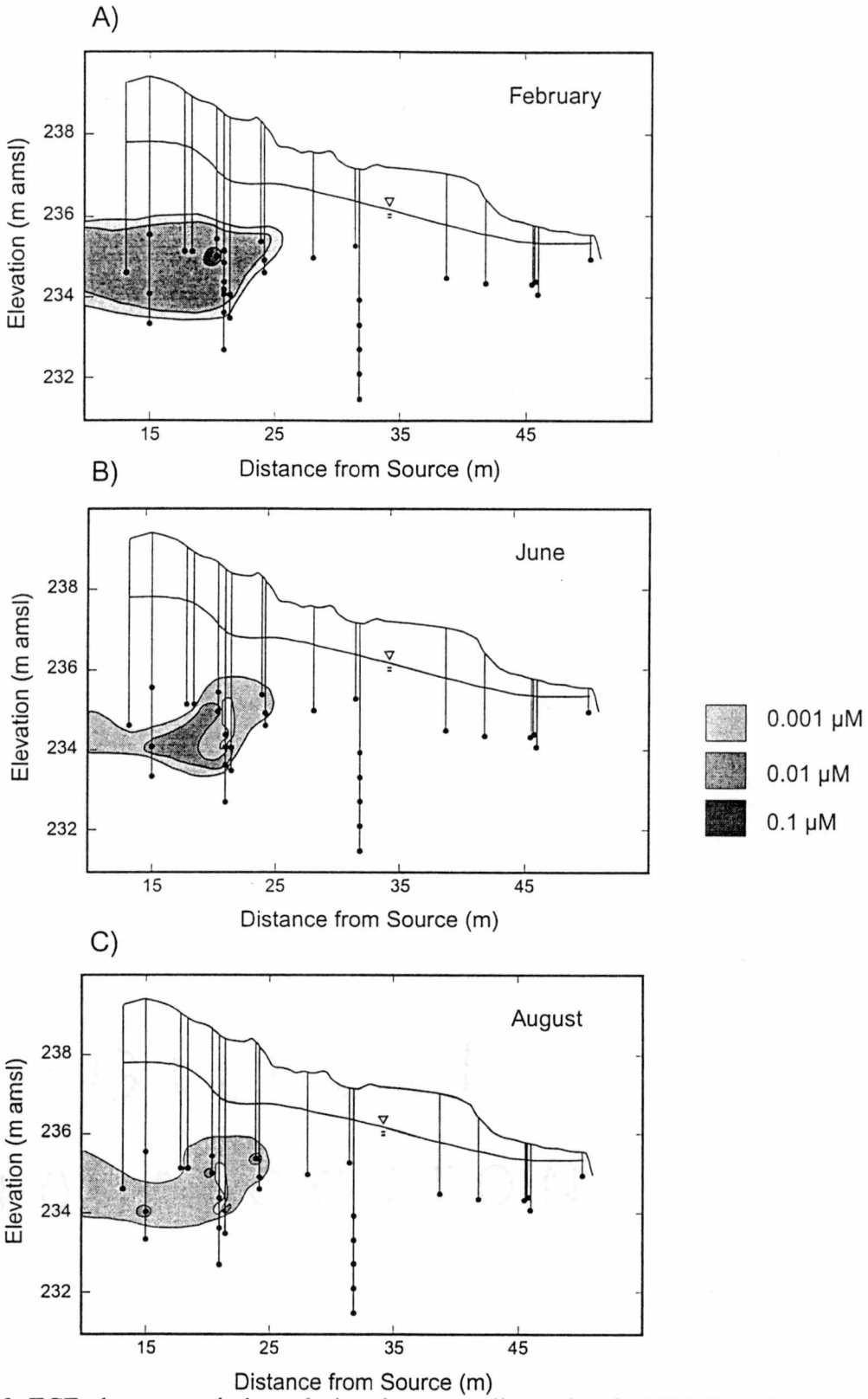


Figure 2-3. TCE plume morphology during three sampling points in 1999 (A. February, B. June, and C. August) at WAG5.

Table 2-3: VOC, chemical results, and AFCEE score from 5 wells at the WAG5 site.

Well	1		10		11		17a		19a	
Date	February	August	February	August	February	August	February	August	February	August
O_2 (ppm)	0.74	2.00	0.39	1.21	0.02	1.96	0.01	1.46	0.28	3.00
Fe(II) (ppm)	2.00	2.06	6.08	2.60	1.82	1.59	4.26	3.20	8.56	5.94
SO_4 (ppm)	3.15	1.99	3.75	2.79	4.49	3.54	5.61	4.36	6.26	6.14
S^{2-} (ppm)	0.01	0.01	0.00	0.00	0.05	0.14	0.05	0.05	0.01	0.01
CH_4 (ppm)	6.27	1.95	2.78	2.00	1.61	1.03	0.20	0.25	0.92	0.99
Cl- (C/Co)*	28.63	41.11	24.69	43.00	18.95	44.15	25.26	39.13	27.91	43.97
TCE (ppb)	3.50	0.46	0.00	0.00	13.20	34.20	0.00	0.00	0.00	0.00
cDCE (ppb)	12.50	5.02	5.80	3.22	49.14	28.80	23.94	14.05	0.00	0.80
VC (ppb)	13.76	4.10	10.14	2.90	20.71	5.55	17.40	3.32	12.14	1.70
AFCEE Score	19	14	20	12	22	14	19	11	17	11

* Co = 0.4 ppm

0.004-0.05 μM. The DCE and VC plumes are both longer than the TCE plumes, and DCE and VC concentrations are higher than the TCE concentrations throughout the site (Figure 2-4). These observations are both consistent with a conceptual model of anaerobic biodegradation of TCE, and the absence of TCE in the downgradient portion of the plume suggests that the process has either been occurring for many years or is very efficient. The temporal variations seen for TCE are also mimicked by the other VOCs (not shown), and are likely due to seasonal variations in recharge.

The amount of dechlorinated daughter products relative to TCE increased dramatically with increased distance from the trenches, again suggesting anaerobic biodegradation is occurring (Table 2-3). Reductive dechlorination of TCE in groundwater is pronounced with nearly 10 times more cDCE present relative to TCE (Figure 2-5a). Conversion of cDCE to VC appears to be the rate-limiting step in the dechlorination process as evidenced by the higher molar ratio of cDCE to VC (Figure 2-5b). The accumulation of cDCE in groundwater due to rate limiting VC production is a common occurrence during anaerobic biodegradation (Fathepure and Tiedje, 1994). Either VC persists in groundwater with essentially no ethylene production, or it is effectively dechlorinated with the production of ethylene (Figure 2-5c). The presence of significant amounts of groundwater cDCE and the additional presence of VC and ethylene strongly suggests that anaerobic biodegradation is a key process responsible for dechlorination of TCE at the site. This anaerobic biodegradation most likely follows the well-described pathway of TCE⇒ cDCE⇒ VC.

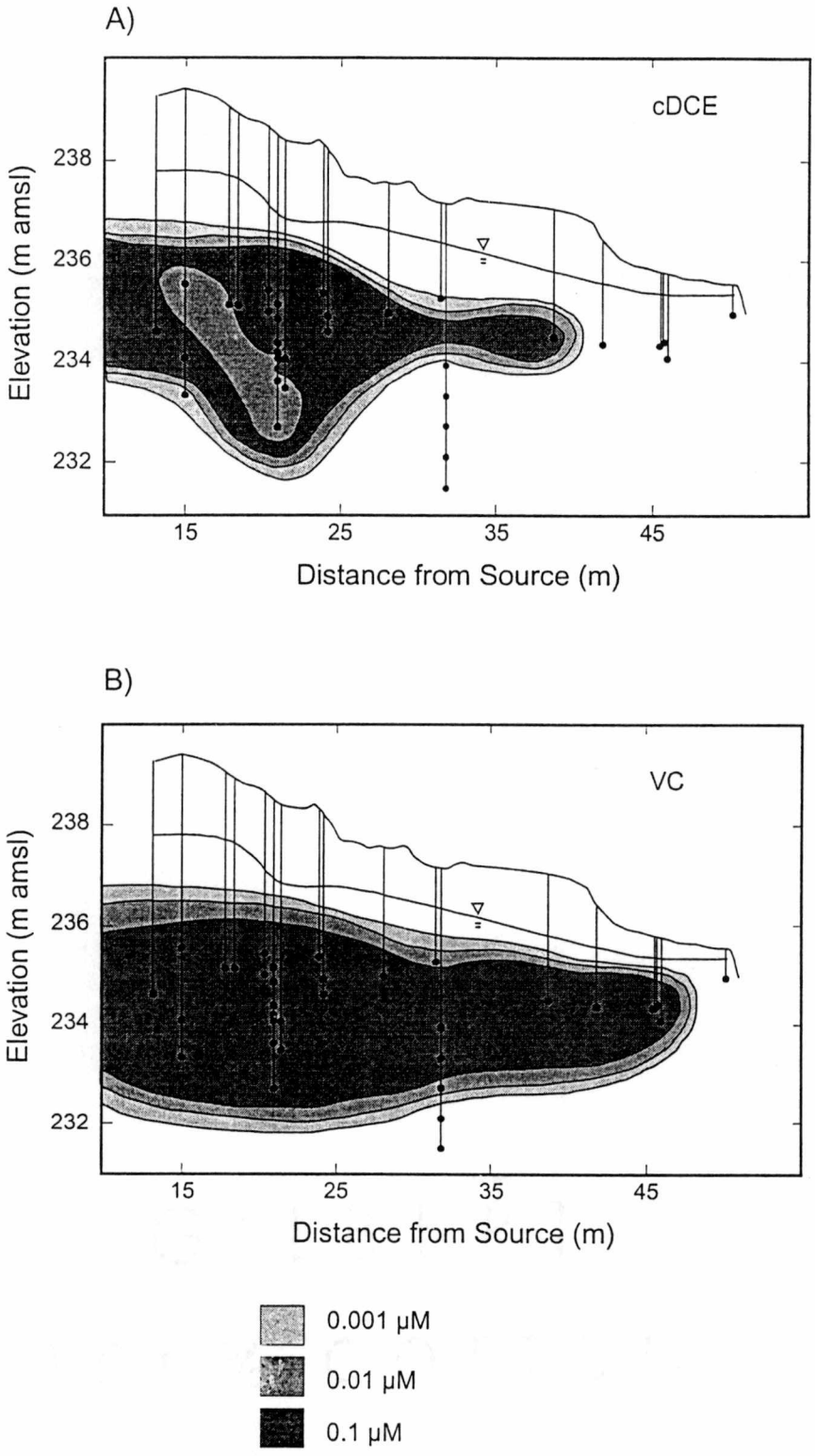


Figure 2-4. cDCE and VC plumes in February at WAG5.

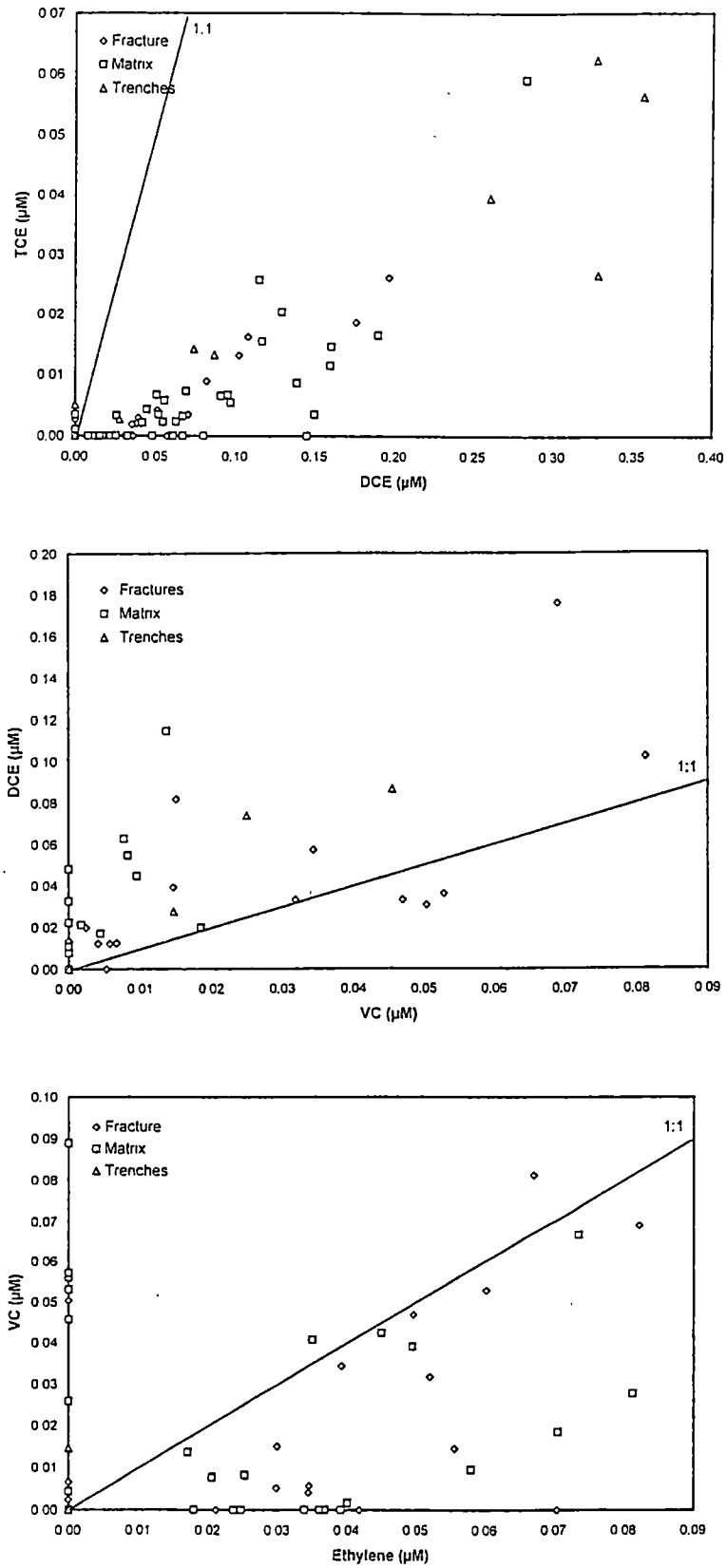


Figure 2-5. Concentrations of VOC relative to each other from sampling wells at WAG5. A) TCE vs. cDCE; B) cDCE vs. VC; and C) VC and ethylene.

Samples were taken over the course of a year to determine temporal variation in the plumes. A shrinking or stable plume is an indication of a natural attenuation of TCE. Figure 2-3 shows the shape of the TCE plume taken during February, June and August of 1999. The plume is changing seasonally, but there is not a clearly defined trend over this time period, thus concentration changes cannot alone be used as indicators of biodegradation. The trends observed for the VOCs are similar to those observed for tritium, in which concentrations were at a minimum in early April and highest in December (Jardine et al., 1999). Jardine et al. (1999) speculated that the decrease of tritium is due to seasonal increases in rainfall during the early spring, which cause fluctuations in the amount of dilution in the plume and could explain the rise and fall in the TCE concentrations. A "hot spot" of TCE was located down gradient from the suspected source zone at well 11 (Table 2-3). This region is located in the matrix flow zone as described by Jardine et al. (1999) and may not be as susceptible to seasonal variations in flow and dilution.

The methane concentrations across the field site are 1000 times higher than the concentrations of the other VOCs. The high concentration of methane in the waste trenches is suspected to be the result of biological waste buried at the site (Clapp, 1992). Some of the methane might be due to dechlorination of the chlorinated solvents, but this could be masked by the high concentrations of methane from other sources in trenches.

2.4.2 Redox Conditions

Redox conditions can be used as an indicator of the potential for natural attenuation of TCE (Chapelle and Brady, 1998). Reductive dechlorination of TCE occurs under anaerobic conditions in which Fe (III), SO_4, or CO_2 acts as the alternative electron acceptor (Wilson et al., 1986; Freedman and Gossett, 1989; Smatlak et al., 1996; Bagley and Gossett, 1990). Observations of anaerobic conditions (dissolved oxygen < 1 mg/L), decreasing concentrations of the alternative electron acceptors, and appearance of the reduced form of the alternative electron acceptors (Fe(II), S^{2-}, and CH_4) can indicate a high probability that reductive dechlorination is likely to occur at a site. If the site is aerobic and a cometabolite, such as methane, is present then less chlorinated compounds can be completely mineralized to CO_2. The WAG5 site is generally anaerobic throughout the year (Tables 2-2 and 2-3), which supports reductive dechlorination of the more chlorinated compounds with accumulation of less chlorinated compounds. At different times of the year some wells switch between anaerobic and aerobic conditions (Table 2-3), thereby allowing for transformation of the chlorinated solvents to CO_2 in some areas.

Different types of redox indicators have been implicated in anaerobic biodegradation of TCE such as iron reduction, sulfate reduction, and methanogenesis. The concentrations of reduced iron (Fe (II)) and sulfide were determined both spatially and temporally (Tables 2-2 and 2-3). Reduced iron concentrations range from 1-7 ppm throughout the site and overlap the sulfide plume with concentrations ranging from 10-50 ppb. The exception is at well 2-4 where the concentration of sulfide exceeds 200 ppb. The sulfide and Fe (II) plume (not shown) are roughly the same size and shape as the VC and

ethylene plumes (not shown). The iron reducing bacteria are the least sensitive to changing redox conditions (which can switch from anaerobic to aerobic) at the site and could account for much of the reductive dechlorination of the highly chlorinated solvents (Lovely and Anderson, 2000). This is supported by the higher concentration of reduced iron compared to the sulfide concentrations. Sulfate reducing bacteria may also be active in the same wells as the iron reducing bacteria, but would be more affected by the switching from aerobic to anaerobic conditions. As described earlier, evidence of methanogenesis cannot be obtained because of high background concentrations of methane at the site.

Another possible indicator of natural attenuation is an increase in the concentration of chloride, which is a product of reductive dechlorination of the chlorinated solvents. Chloride concentrations at the site range from 12-17 ppm and are above the typical background concentrations of 0.4-1.0 ppm measured in nearby uncontaminated areas (Table 2-2 and 2-3). Chloride concentrations in the waste trenches are much lower than in the plume suggesting that the chloride in the groundwater was not due to disposal of salt-rich wastes.

2.4.3 Monitored Natural Attenuation Evaluation

The potential for utilization of monitored natural attenuation (MNA) at the site was evaluated using the guidelines described by Brady et al. (1999) and Wiedemeier et al. (1998). The first step is to determine the potential for anaerobic biodegradation. The AFCEE protocol (Wiedemeier et al., 1998) uses different weighed analytical parameters

to determine a potential for biodegradation. Table 2-3 lists the values for different geochemical parameters from 5 selected wells during 2 different time periods with the AFCEE score. A score of –1 to 5 points shows inadequate evidence for anaerobic biodegradation; a score of 6 to 14 point shows limited evidence for anaerobic biodegradation; a score of 15 to 20 points is adequate evidence for anaerobic biodegradation, whereas a score greater than 20 point is strong evidence for anaerobic biodegradation. None of the samples showed inadequate evidence for anaerobic biodegradation. In February all of the wells showed strong to adequate evidence for anaerobic biodegradation whereas in August all the wells showed only limited evidence for anaerobic biodegradation. The main factor that influenced the shift was the change in dissolved oxygen concentration, which went from very reducing to aerobic. Depending on the time of year that an assessment is done a different potential for biodegradation will be obtained. This is especially likely to occur in fractured bedrock aquifers, where seasonal water table levels and flow rates are largely controlled by the fracture porosity, which is often much lower than the total porosity. This indicates that site evaluations for MNA in fractured rock aquifers should be carried out several times, to include both seasonally wet and seasonally dry periods.

2.4.4 Microbial Community Structure

Assessment of the microbial community structure also supports the hypothesis that anaerobic biodegradation is occurring at the WAG5 field site. Indirect evidence for the presence of iron-reducing bacteria, sulfate-reducing bacteria, methanogens, and methanotrophs was provided using geochemical redox indicators, as previously

described. Assessment of the microbial community at the site can also be done by direct enrichment of bacteria or identification of bacteria via detection of nucleic acid. Most probable numbers (MPN) is a form of enrichment. The MPN data for methanogens, iron-reducing bacteria, sulfate-reducing bacteria, and methanotrophs are presented in Table 2-4. The tested wells comprised 8 wells in total, with one well located in the trench. In most of the wells, the assays indicate that microorganisms capable of iron-reduction, sulfate-reduction, methanogenesis and aerobic cometabolism of TCE are present. At well 2-4 there was both a high concentration of sulfide and a high number of sulfate reducing bacteria. 16S rDNA from iron reducing and sulfate reducing enrichments were cloned to determine the identity of the microorganisms. The organisms were identified as either *Pseudomonas* or *Alcaligenes.* These organisms are not known to be iron- or sulfate-reducing bacteria, which suggests that enrichments may not accurately reflect the identity of the microorganisms present at a site.

Another method to identify bacteria that are found in a water sample is with clone libraries. The clone libraries constructed in this study were prepared using the small subunit of rDNA (16S rDNA) and then compared to databases to determine the identity of microorganisms that might not be culturable with enrichments. Two rDNA clone libraries were prepared from bulk DNA extracted from aquifer samples and analyzed to determine the degree of microbial diversity associated with the WAG5 site. The libraries were prepared from wells 10 and 11 (Table 2-5), representing fracture and matrix zones, respectively, in the aquifer. Well 11 was in the zone in which TCE was present at the highest concentration (0.1 μM), while well 10 was 2 meters below well 11 and was in a

Table 2-4: Most probable number (MPN) of bacteria in wells at WAG5 (CFU/mL).

Well	Methanogens	Iron Reducers	Sulfate Reducers	Methanotrophs
1	2.1	0.073	0.03	210
2-4	>24	1.5	4.6	39
2-5	>24	0.91	24	NA
2	2	0.036	2.4	NA
10	>24	0.15	0.15	>2400
11	4.3	0.036	4.6	NA
14	11	0.091	0	NA
19c	2.4	0.091	2.3	9.3

NA=not tested

Table 2-5: Clone libraries from well 10 and 11.

Clone Numbers[a]	Putative Division	Database Match[b]
11u27	*Euryarchaeota*	97% uncultured Archaeon WCHA1-38 (AF050612)
11u20	*Euryarchaeota*	
10u12	*Euryarchaeota*	
11u2	*Euryarchaeota*	
11u31	*Euryarchaeota*	
10u34	*Euryarchaeota*	
10u1, 11u5	*Euryarchaeota*	
11u30,37	Unidentified bacterium	
10u3	Unidentified bacterium	94% to clone group A17o from groundwater (X91440)
10u5	Clone Group A51P	
10u7,9,14,20,25,36	Clone Group A51P	
10e12,15,11e7,11u36	Clone Group A51P	
10e2,10u26	Clone Group A51P	96% Clone group S23 from groundwater (Z69327)
10u21	*Cytophagales*	
11e8	*Cytophagales*	94% *Flavobacterium* (M62798)
10e1, 11u18	*Cytophagales*	96% Clone BSV73 from anoxic soil (AJ229217)
11u32	*Cytophagales*	
10u4, 8, 15, 33	*Cytophagales*	
10e4	*Cytophagales*	
10u22	*Cytophagales*	98% Uncultured bacterium 81 (AF104275) Anaerobic benzene degradation in a petroleum contaminated aquifer
11u25	*Cytophagales*	
10u27	*Cytophagales*	
10e5, 6	*Cytophagales*	
10e16, 11e13	*Firmicutes*	98% tetrachloroethylene degrading *Clostiridium bifermentans* (Y18787)
10u16	OP11	
10u35	OP11	
10e3	OP11	
10u13	OP11	
11u4	OP11	
11u10	OP11	
11u3	OP11	
11e14	OP11	
10u18	OP11	
10u19	OP11	
10u24,25	OP12	94% Clone OPB54 from a Yellowstone hot spring (AF027087)
11e12	*Planctomycetales*	

10u23,11u29	*Proteobacteria (β)*	99% *Ralstonia picketti* (X67042)
10u29	*Proteobacteria (β)*	94% *Dechlorimonas* (AF170357)
10u30, 10e13	*Proteobacteria (β)*	
11u33	*Proteobacteria (γ)*	
11u7	*Proteobacteria (δ)*	
11u28	*Proteobacteria (δ)*	
10e17	*Proteobacteria (δ)*	
11e6,16	*Proteobacteria (δ)*	
11u38	*Proteobacteria (δ)*	98% WCHB1-12 uncultured bacterium form a hydrocarbon -and chlorinated-solvent contaminated aquifer (AF050534)
10e7,8,10,11,14	*Proteobacteria (δ)*	95% *Syntrophus* sp. Lyp (AF126282) anaerobic propionate-degrader
10u10	*Proteobacteria (δ)*	96% *Geobacter arculus* dissimilatory Fe(III)-reducing bacteria (U96917)
10u32	Undescribed	
11u25	Undescribed	
11u8	Undescribed	
10u6	Eukaryota	96% *Clavulina cristata* (AF026640)

[a]Clone number designations- All clones were from either well 10 or well 11, e= libraries constructed with PCR products using eubacterial primers 27f and 1492r, u= libraries constructed with PCR products using universal primers 530f and 1390r.

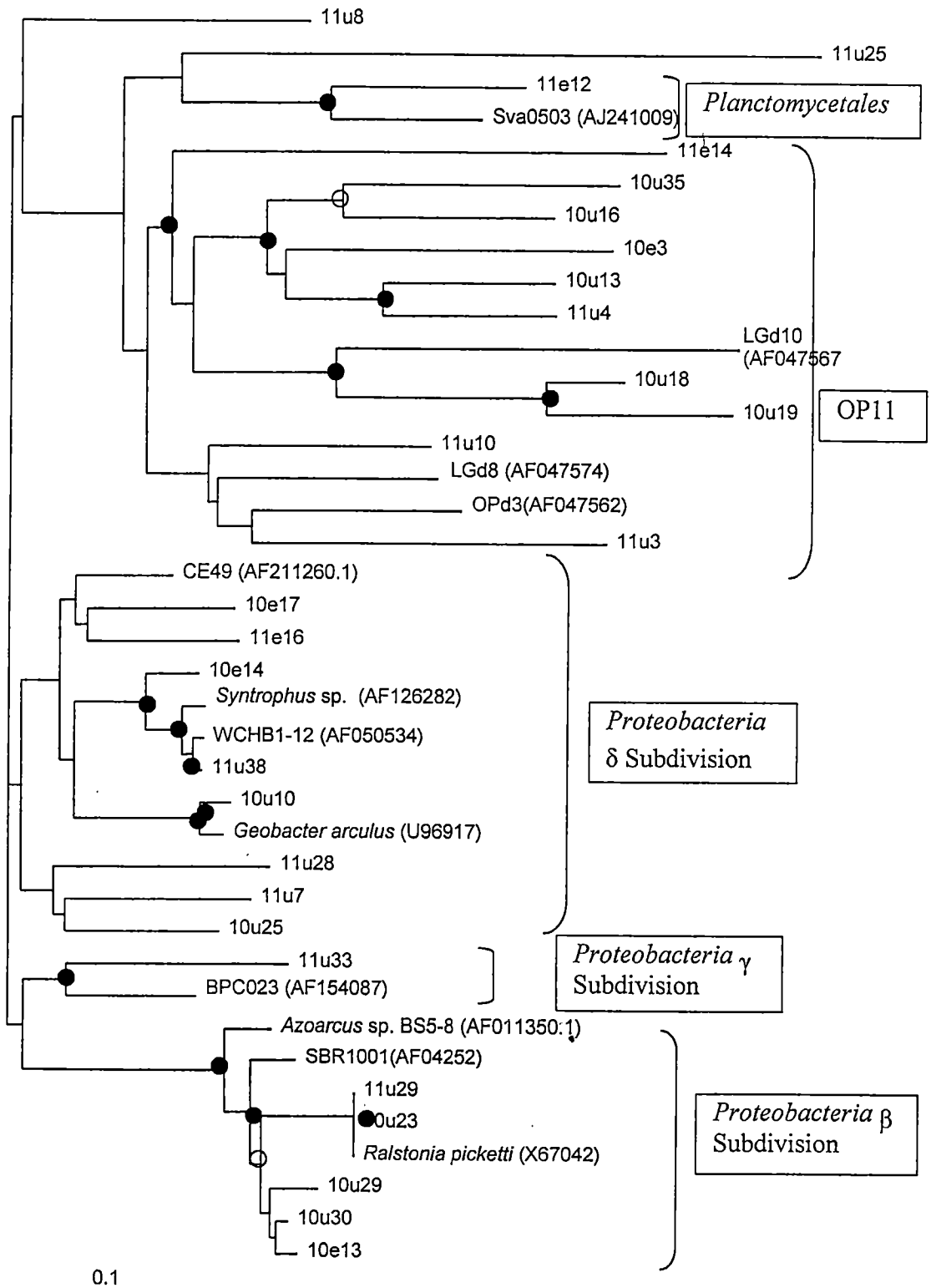
[b]Database matches greater than or equal to 94%.

zone with high amounts of reduced iron. Table 2-5 summarizes the distribution of sequence types by divisions and percent abundance in each library. Comparative analyses of the WAG5 aquifer sequences to known 16S rDNA sequences revealed a broad spectrum of bacterial and archaeal diversity. Of the 76 clones, 7 were archaeal, 68 were bacterial and 1 was a eucaryote. Of the clones that were sequenced, 23 were ≥ 94% identical to an rDNA sequence available in GenBank (http://www.ncbi.nlm.nih.gov/) as of March 2000. The profile of 16S rDNA sequences is consistent with an anaerobic environment. Many of the closest matches in Table 2-5 are with anaerobic bacteria. In addition, common bacteria in aerobic enrichments such as α, β, and γ subgroups of proteobacter are absent or low in abundance.

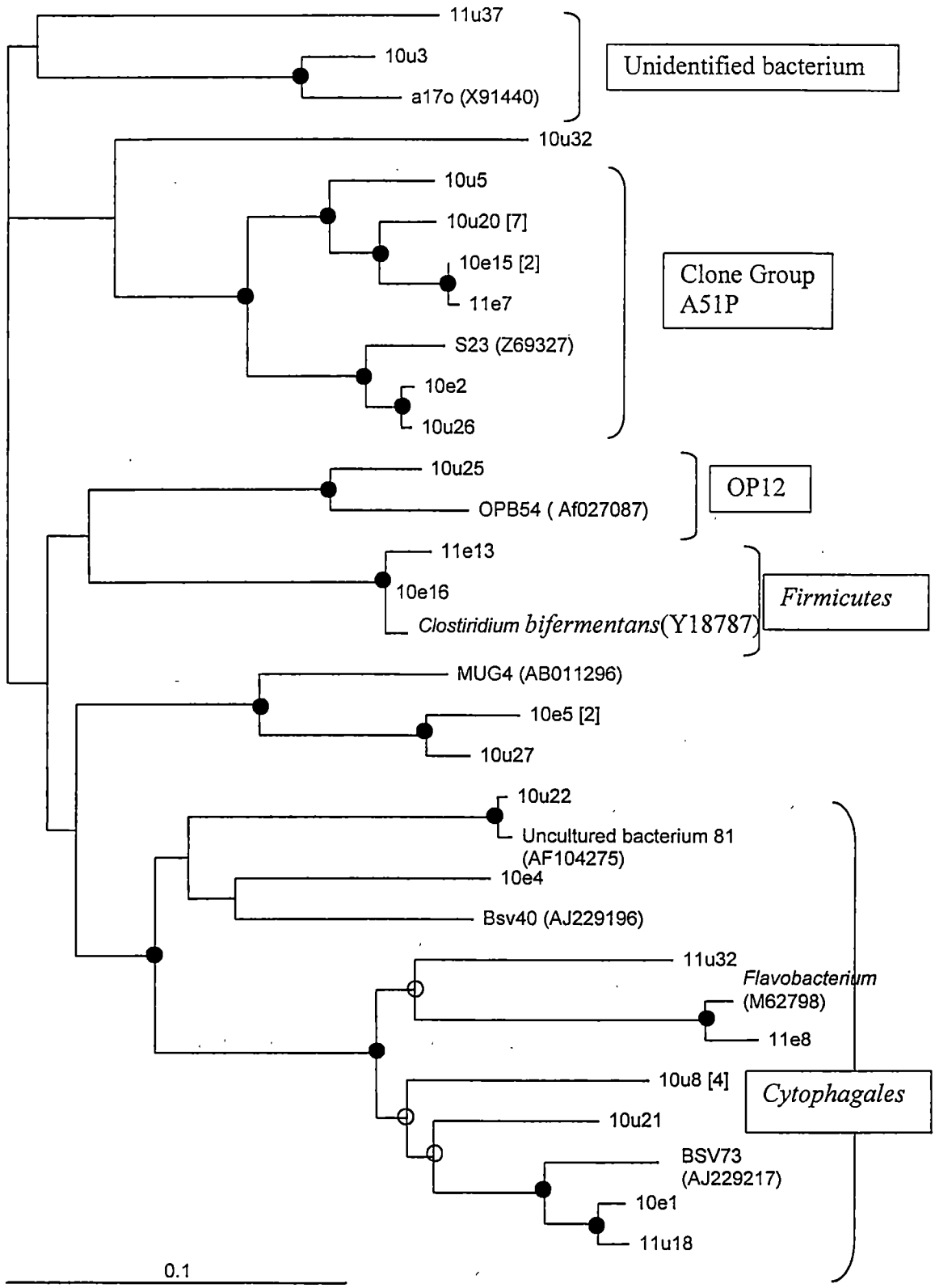
Figure 2-6 is an evolutionary distance tree of the bacterial domain showing the distribution of different divisions, which is a rough description of the bacterial biodiversity at this site. Phylogenetic groups represented include: Cytophaga (19.7%), Proteobacteria (β) (6.5%), Proteobacteria (γ) (1.3%), Proteobacteria (δ) (15.8%), Archeaon (9.2%), and Firmicutes (1.3%). Approximately 41% of the clone sequences from the WAG5 site could not be placed into known phylogenetic groups and 25 clones are affiliated with 2 candidate divisions that have no cultivated representatives (OP divisions) and A51P. A candidate division is a monophylogenetic group of rDNA sequences with no specific association with known divisions (Dojka et al., 1998). From this analysis is not possible to identify the microorganisms responsible for TCE biodegradation because microorganisms in different phylogenetic groups are capable of biodegrading TCE. However, some of the clones (10e16, 11e13, 10u29, and 11u38) are

Figure 2-6: Distance matrix trees showing phylogenetic relationships of 16S rDNA clones from wells 10 and 11. Putative divisions are listed outside the brackets for panels A, B, and C. Panel B was rooted with the Acidobacteria division, A. Proteobacteria and OP11, B. Cytophaga; Firmucutes, Clone group A51P, C. Archea. Numbers in brackets indicate number of nearly identical clones found in the same library. Branch points supported by bootstrap values (number trees with same branch order per 1000 trees generated) >75% are indicated by filled circles and those supported by >50% are indicated by open circles. Genbank accession numbers are in parenthesis and numbers of clones in the same library are in closed brackets.

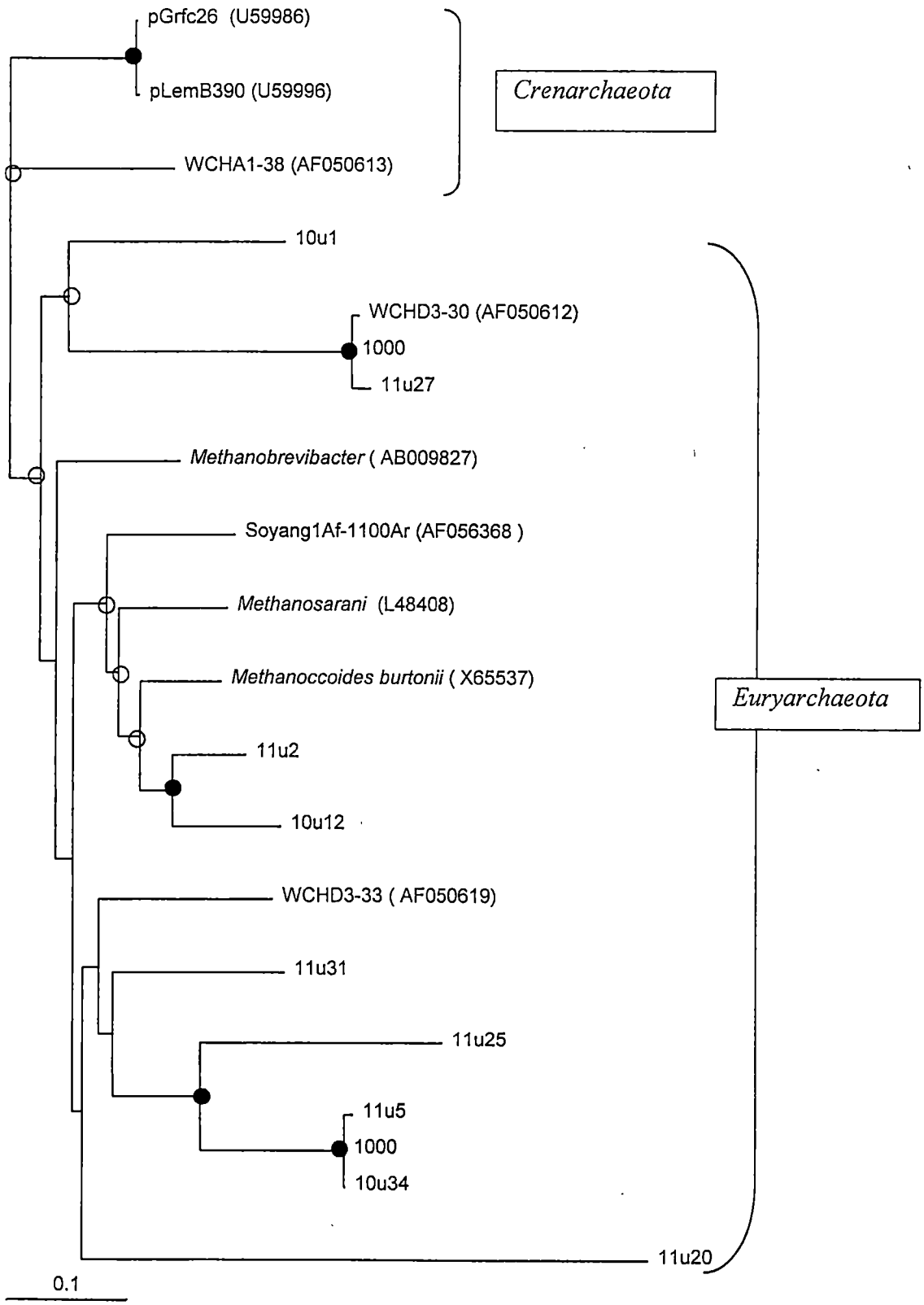
A.


11u8
11u25
11e12
Sva0503 (AJ241009)
Planctomycetales
11e14
10u35
10u16
10e3
10u13
11u4
LGd10
(AF047567
10u18
10u19
OP11
11u10
LGd8 (AF047574)
OPd3(AF047562)
11u3
CE49 (AF211260.1)
10e17
11e16
10e14
Syntrophus sp. (AF126282)
WCHB1-12 (AF050534)
11u38
10u10
Geobacter arculus (U96917)
11u28
11u7
10u25
Proteobacteria
δ Subdivision
11u33
BPC023 (AF154087)
Proteobacteria γ
Subdivision
Azoarcus sp. BS5-8 (AF011350.1)
SBR1001(AF04252)
11u29
0u23
Ralstonia picketti (X67042)
10u29
10u30
10e13
Proteobacteria β
Subdivision
0.1

B.


11u37
10u3
a17o (X91440)
Unidentified bacterium
10u32
10u5
10u20 [7]
10e15 [2]
11e7
Clone Group A51P
S23 (Z69327)
10e2
10u26
10u25
OPB54 (Af027087)
OP12
11e13
10e16
Clostiridium bifermentans(Y18787)
Firmicutes
MUG4 (AB011296)
10e5 [2]
10u27
10u22
Uncultured bacterium 81 (AF104275)
10e4
Bsv40 (AJ229196)
11u32
Flavobacterium (M62798)
11e8
10u8 [4]
10u21
BSV73 (AJ229217)
10e1
11u18
Cytophagales
0.1

C.


pGrfc26 (U59986)
pLemB390 (U59996)
WCHA1-38 (AF050613)
Crenarchaeota
10u1
WCHD3-30 (AF050612)
1000
11u27
Methanobrevibacter (AB009827)
Soyang1Af-1100Ar (AF056368)
Methanosarani (L48408)
Methanoccoides burtonii (X65537)
11u2
10u12
WCHD3-33 (AF050619)
11u31
11u25
11u5
1000
10u34
11u20
Euryarchaeota
0.1

similar to other clones or types of bacteria implicated in the anaerobic biodegradation of chlorinated solvents. A clone (11u38) was similar to a clone from a contaminated aquifer in which chlorinated-solvents were undergoing intrinsic bioremediation (Dojka et al., 1998). A *Clostridium bifermentans* has been identified as a PCE degrader (Change et al., 1999) and a *Dechlorimonas* has been identified as a chlorinated solvents degrader (Coates et al., 1999). Other identified organisms were sulfate-reducing bacteria and iron-reducing bacteria (>90% similarity) including *Thermodesulfovibrio, Geobacter sulferreducens* and *Geobacter arculus*. Different methanogen species were also identified. These cloned sequences provide a background database for future studies, including the enrichment and isolation of organisms involved in TCE transformation, and development of 16S rDNA oligonucleotide probes for monitoring specific populations.

2.5 Conclusions

The distribution of TCE, cDCE, and VC in monitoring wells at the WAG5 site on the Oak Ridge Reservation suggests that anaerobic biodegradation of TCE is occurring. Redox conditions are favorable for reductive dechlorination of TCE, cDCE and VC and the microbiological data reveal the presence of methanogens, iron-reducing and sulfate-reducing bacteria, all of which suggest that natural attenuation of TCE is occurring. Methanotrophs and dissolved oxygen are suggestive of potential aerobic biodegradation by cometabolism of TCE with methane. These data suggest two possible mechanisms for natural attenuation of TCE: first, anaerobic biodegradation of the highly chlorinated compounds and second, aerobic biodegradation in oxygenated zones, (e.g. seep, near the water table, and in fracture zones which receives periodic flushes of oxygenated storm

water). These processes result in nearly complete biodegradation of the TCE and its daughter products. This is believed to be the first field study to observe natural attenuation of TCE in fractured shale bedrock. It is unique, in that it combines VOC and redox potential data, with enrichment and molecular techniques, to examine natural attenuation at a hydrogeologically complex site. With only one of the techniques an incomplete view of natural attenuation at the site would be the result. With coupled geochemical-microbial analyses, it is possible to develop a more reliable conceptual model of natural attenuation. The large spatial and seasonal variations in geochemical conditions observed in this study are expected to occur in many shallow contaminant plumes in fractured aquifers and recognition of this is critical for successful assessment of the potential for remediation using monitored natural attenuation protocols.

Chapter 3

Biodegradation of TCE in Undisturbed Columns of Fractured Saprolite

3.1 Introduction

Most previous studies of natural attenuation and/or biodegradation of chlorinated solvents have been carried out in relatively simple aquifer materials, usually sand (Kleopfer et al., 1985; Chapelle et al., 1996; Dojka et al., 1998; Clement et al., 2000). The findings of these studies may not be appropriate for more complex physical/chemical systems. For example, groundwater systems in unconfined fractured rock aquifers are often subject to rapid changes in water table elevation, flow rate, and geochemistry, thus making it very difficult to use conventional indicators of biodegradation (such as mass or concentration decline and the presence of favorable redox conditions). Hence, there is a need for studies of biodegradation in complex materials like fractured shale.

Recent field studies of an existing plume of organic contaminants in fractured shale at the Oak Ridge Reservation (ORR) strongly suggest biodegradation of TCE can occur in this type of materials (Chapter 2). Sampling of wells at the field site indicated that TCE concentrations declined with distance from the waste pits and daughter products (cDCE and VC), which are suggestive of anaerobic biodegradation of TCE appeared further downgradient. Redox conditions at the field site indicated iron and sulfate reduction and possible methanogenesis, which were within the range typically considered favorable for anaerobic biodegradation of TCE (Bouwer, 1994; Chapelle and Bradley, 1998). Microorganisms found in groundwater at the field site include methanotrophs,

methanogens, iron and sulfate reducing bacteria, which have also previously been implicated in anaerobic biodegradation of chlorinated solvents (Wilson et al., 1986; Freedman and Gossett, 1989; Smatlak et al., 1996; Bagley and Gossett, 1990; Lovely and Anderson, 2000; De Wever et al., 2000).

Although the WAG5 study provides strong evidence for potential TCE biodegradation, there are still many unanswered questions, such as whether biodegradation could occur in both the shale bedrock (where the monitoring wells were located) and in the overlying 2 m of saprolite (highly weathered rock). As well, it is uncertain whether microbial communities and redox conditions in this environment can change quickly to support biodegradation or whether it take many years of exposure to contaminants. It is not currently feasible to carry out field experiments using TCE in uncontaminated bedrock/saprolite to answer these questions so methods are needed to represent these complex hydrogeological systems in the laboratory. Flow-through experiments in large undisturbed columns of saprolite from the Oak Ridge Reservation have proven successful for examination of solute transport (Reedy et al., 1996), colloid transport (Cumbie and McKay, 1998; Haun, 1998), DNAPL transport phenomena (Cropper, 1998; Pitner, 2000), and can be used for studying biodegradation of TCE. The advantages of undisturbed columns for determination of biodegradation of TCE are the following: the complex structure of saprolite is maintained in a controlled laboratory setting, flow-through systems mimic the natural environment, duration of experiments can be up to years, and a wide variety of geochemical and microbial characteristics can be tested.

This research is based on the hypothesis that microbially mediated anaerobic degradation of TCE in fractured shale saprolite can occur without any amendments to the system, other than the introduction of TCE. Redox conditions and microbial community composition in the saprolite and groundwater is expected to shift after the introduction of TCE to become favorable for biodegradation of TCE. The specific objectives of the research are: 1) to determine if relative TCE mass loss occurs under biotic or inhibited conditions; 2) to determine if daughter products of anaerobic TCE degradation (cDCE and VC) appear in the effluent from the biotic and inhibited columns; 3) to determine if reducing conditions such as iron reduction or sulfate reduction, which are indicative of anaerobic biodegradation of TCE, develop with time after introduction of TCE in the biotic column; 4) to characterize the microbial community in the groundwater prior to and after introduction of TCE, in order to determine if shifts in the community, which favor biodegradation of TCE occur; and 5) to determine whether current natural attenuation protocols are effective in this type of complex setting.

3.2 Material and Methods

3.2.1 Geological Setting

The experimental investigation was carried out using undisturbed samples of uncontaminated residual soil (saprolite) obtained from an existing research site in the proposed Solid Waste Storage Area #7 (SWSA7) at the ORR (Figure 3-1). The saprolite at SWSA7 has been extensively characterized from a physical and hydrogeological perspective (Solomon et al., 1992; Jardine et al., 1993; Cumbie and McKay, 1999; Driese

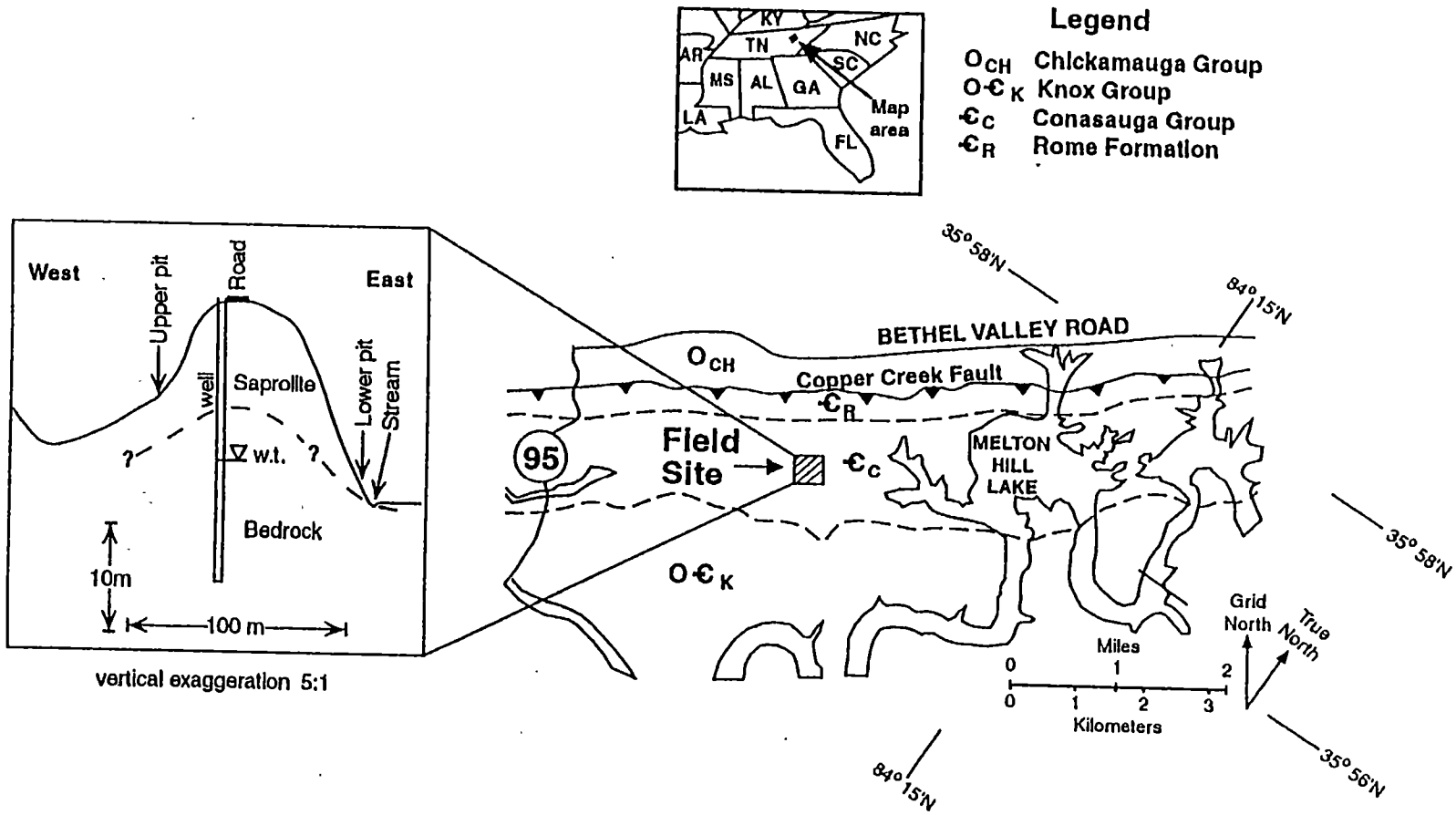


Figure 3-1. Site location within the Oak Ridge Reservation.

et al., in review). The saprolite is derived from *in situ* weathering of the underlying sedimentary bedrock, which is composed of interbedded shale, siltstone, and sandstones, which is part of the Dismal Gap Formation of the middle to upper Cambrian Conasauga Group (Hatcher et al., 1992). The carbonates have been leached, leaving a high porosity detrital matrix, which retains much of the structure of the parent bedrock. These structural features include bedding, which generally dips towards the southeast at 17 to 72°, and fractures caused by regional tectonic activity. Fractures occur both parallel and oblique to bedding with typical fracture spacing in the saprolite ranging from 0.005 to 0.9 m (Dreier et al., 1987; Solomon et al., 1992; Cumbie, 1997). Macropores formed by roots are also present in the saprolite to depths of >2 m in some places. The total porosity ranges from 15% to 58% (Dorsch and Katsube, 1999), with porosity due to fractures and biopores being approximately 1-2% (Cropper, 1998). In the upper portion of the saprolite many of these pores have been infilled with pedogenic clays or Fe/Mn oxides (Jardine et al., 1993; Driese et al., in review). Hydraulic conductivity values for columns previously collected at SWSA7 ranged from 2.7×10^{-4} to 4.5×10^{-9} m/s (Driese et al., in review).

3.2.2 Flow-through Experiments in Undisturbed Saprolite Columns

Two undisturbed saprolite columns, 23 cm in diameter and approximately 25 cm in length, were collected for use in this study. The columns were excavated with hand tools and setup for flow-through experiments generally following the methods of previous researchers working in saprolite at ORR (Reedy et al., 1996; Howard, 1997; Cumbie, 1997; Haun, 1998; Cropper, 1998; Pitner, 2000). The columns were collected at a depth

of 1.5 to 2.5 m below ground surface, and approximately 1 m above the bedrock contact, which crop out in a nearby stream. After excavation of each column, a 25 cm diameter PVC casing was fitted over the column and the annulus was filled with a TCE-resistant epoxy (Ureol 6414; Ciba, Helsinki, Finland). The columns were then taken to the laboratory and disturbed material was trimmed from the ends in preparation for fitting with solvent-resistant end caps. The finished flow-through columns were then sealed to prevent exposure to the atmosphere, and then placed in an environmental chamber that was maintained at approximately the ambient soil temperature (12°C).

The columns were set up to carry out saturated flow-through experiments as shown in Figure 3-2. The injection system for each column consisted of a Tedlar sampling bag (SKC, Eighty Four, PA) connected to an HPLC pump using all stainless steel fittings. The flow rate was set at 0.2 mL/min, which corresponds to a specific discharge rate of 0.7 cm/day. This is within the range of specific discharge rates determined in previous field studies in saprolite and weathered shale at ORR (Lee et al., 1992; McKay et al., 1997). Water used for saturating the columns for influent during the subsequent flow through experiment was collected from bedrock well at the site. Ultra high pure grade helium was bubbled through the well water for 40 minutes before it was used. After saturation of the columns, sodium azide (0.65 g/L) and mercuric chloride (0.33 g/L) were added to the influent water of the inhibited control column to inhibit microorganisms, and it is referred to as the inhibited column. Sodium azide was only added to the influent water of the inhibited column for the rest of the life of the experiment. For the first 56 days, influent containing no TCE was injected into both columns to allow for monitoring

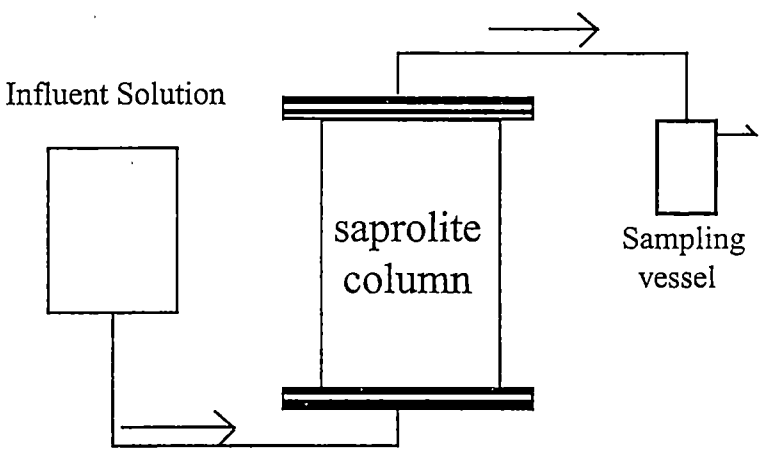


Figure 3-2. Column setup for biotic and inhibited columns. Saprolite column was placed in a 12°C chamber. Influent was injected into the column at 0.2 mL/min. Effluent was monitored for VOC, redox conditions, and microbial community.

of background conditions prior to addition of TCE. TCE was obtained from Fisher Scientific and a stock solution was prepared by mixing excess amounts of TCE with distilled water to create a saturated water solution of 1100 ppm. Starting on January 24, 2000, TCE from the stock solution was added to the influent of both columns for a final concentration of 1000 ppb. Influent samples were measured (see below) as 800-2500 ppb with an average value of 1780 ppb.

3.2.3 Volatile Organic Carbon (VOC) Analyses and Redox Measurements

The influent and the effluent from both columns were monitored for dissolved solvent concentrations (TCE, cDCE, tDCE, 1,1 DCE, and VC), redox conditions, and microorganisms. Influent samples were collected directly from the influent Tedlar bag. Effluent samples were taken from each column using a port at the base of a 150 mL stainless steel sampling vessel (Swagelok, Solon, OH). The system was designed so that samples could be collected with minimal exposure to the atmosphere. The samples were collected in 40 mL vials with Teflon coated septa. The samples contained zero headspace, and were stored upside-down for no longer than 2 weeks before analysis to prevent any loss of VOC by diffusion or volatilization. The VOC samples were analyzed using a Tekmar 3000 Purge and Trap system (Vernon, BC, Canada) with a VOCARB 3000 trap and a Hewlett Packard 5890-series II gas chromatograph with DB-624 column (Palo Alto, CA) and a PID detector. The inlet temperature in the gas chromatograph was 180°C and in the detector was 300°C. The column temperature was held initially at 35°C for 5 minutes, and then increased at a rate of 5°C/minute to a final temperature of 90°C, which was maintained for 2 minutes.

Redox conditions were determined by monitoring the effluent for dissolved oxygen, Fe(II), sulfate, sulfide, pH, and Eh. Dissolved oxygen content was determined with a YSI dissolved oxygen meter (Yellow Spring, OH). Redox analyses for Fe (II), sulfate and sulfide were determined using Hach (Loveland, CO) kits for the specific chemicals. Chloride concentration was determined by an ion specific probe (Corning, Corning, NY). pH and Eh were measured in the effluent water using an Orion portable pH-Eh meter, model 250A (Boston, MA).

3.2.4 Microbial Analyses

The influent and effluent water were tested for the presence of iron reducing and sulfate reducing bacteria using BART-IRB and BART-SRB media obtained from Hach (Loveland, CO). Molecular analysis for identification of individual microbes in the microbial communities was carried out on selected effluent water samples. This involved collection of 50 mL of water in a sterile 50 mL vial, which was filtered through a 0.2μm filter (Durapore, Millipore, Bedford, MY) and then stored at –80°C until processed. DNA was extracted by bead beating filters with lysing matrix (Bio101, Carlsbad, CA) using 1 mL of STE buffer (10mM Tris (pH 8), 1 mM EDTA, and 100 mM NaCl) for 30 s and 4 m/s. Samples were then centrifuged for 2 minutes at 10,000 rpm, and the supernatant was placed into a new sterile microcentrifuge tube stored at –80°C until further processing. Effluent water samples were screened using rapid profiling techniques and specific primers. Extracted DNA from effluent water was amplified in a reaction mixture with Ready-to-Go™ PCR beads (Amstersham Pharmacia, Piscataway, NJ) and a 10 nM concentration of each of the primers listed below. Reaction mixtures

were incubated in a Perkin Elmer (Wellesley, MA) Gene Amp PCR system 2400 thermal cycler.

Confirmation that the PCR reactions from the effluent samples of the inhibited column were not inhibited by the presence of sodium azide and the biotic and inhibited effluent not by TCE was done. Previously negative inhibited effluent sample (elapsed time 189 days), positive biotic effluent sample (elapsed time 106 days), plus influent with and without TCE were spiked with DNA at different concentrations (200, 20, 2 and 0.2 ng/μL). All spiked samples were positive. This indicated that the PCR reactions effluent from the inhibited was truly negative and were not inhibited by the presence of TCE or sodium azide, and thus did not contain DNA down to concentrations of 0.2ng/μL.

Specific primer sets for different types of microorganisms were also tested (Table 3-1). PCR primer sets for the 16S rDNA gene of six phylogenetic groups of sulfate reducing bacteria were used as described by Daly et al. (2000). Determination of methanogens was done using specific PCR primer set (Shinzato et al., 1999) ME855F (5'-TTA AAG GAA TTG GCG GGG GA-3') and ME1354R (5'-TGA CGG GCG GTG TGT GCA AG-3'). The PCR amplification conditions comprised of 40 cycles at 94°C for 30 sec, 60°C for 30 sec, and 72°C for 90 sec. Partial *Geobacteraceae* 16S rDNA sequences (Snoeyenbos-West et al., 2000) were amplified with bacterial forward primer 8F (5'-AGA GTT TGA TCC TGG CTC AG-3') and *Geobacteraceae*-specific reverse primer 825R (5'-TAC CCG CRA CAC CTA GT-3') in the first round of a semi-nested PCR protocol, followed by 530F and Geo 825R in the second round. The PCR condition was

Table 3-1. 16S rDNA-targeted PCR primer sequences specific for methanogens (Shinzato et al., 1999), iron reducing bacteria (Snoeyenbos-West et al., 2000) and sulfate reducing bacteria subgroups (Daly et al., 2000).

PRIMER	SEQUENCE 5'-3'[b]	GROUP	TYPE	EXPECTED SIZE PRODUCT
ME855F	TTA AAG GAA TTG GCG GGG GA	NA	Methanogens	500bp
ME1354R	TGA CGG GCG GTG TGT GCA AG			
8F	AGA GTT TGA TCC TGG CTC AG	NA	*Geobacteraceae*	815bp
825R	TAC CCG CRA CAC CTA GT			
Gx.182F	AGA CCT TCG GCT GGG ATG CT	NA	*Geothrix*	250bp
Gx.472R	AGG TAC CGT CAA GTA ACA SS			
DFM140	TAG MCY GGG ATA ACR SYK G	Group 1	*Desulfotomaculum*	700bp
DFM842	ATA CCC SCW WCW CCT AGC AC			
DBB121	CGC GTA GAT AAC CTG TCY TCA TG	Group 2	*Desulfobulbus*	1120bp
DBB1237	GTA GKA CGT GTG TAG CCC TGG TC			
DBM169	CTA ATR CCG GAT RAA GTC AG	Group 3	*Desulfobacterium*	840bp
DBM1006	ATT CTC ARG ATG TCA AGT CTG			
DSB127	GAT AAT CTG CCT TCA AGC CTG G	Group 4	*Desulfobacter*	1150bp
DSB1273	CYY YYY GCR RAG TCG STG CCC T			
DCC305	GAT CAG CCA CAC TGG RAC TGA CA	Group 5	*Desulfococcus-Desulfonema-Desulfosarcina*	860bp
DCC1165	GGG GCA GTA TCT TYA GAG TYC			
DSV230	GRG YCY GCG TYY CAT TAG C	Group 6	*Desulfovibrio-Desulfomicrobium*	610bp
DSV838	SYC CGR CAY CTA GYR TYC ATC			

[a]16S rDNA positions, *E.coli* numbering
[b]Ambiguities: R(G or A); Y(C or T); K(G or T); M(A or C); S(G or C); W(A or T)

touchdown primer annealing from 65 to 55°C (decreasing 0.5°C), followed by 10 cycles at 55°C. *Geothrix* species were also determined with a semi-nested primer. The primers for the first round of PCR were 8F and the *Geothrix*-specific reverse primer Gx.472R (5'-AGG TAC CGT CAA GTA ACA SS-3'). The *Geothrix*-specific forward primer Gx.182F (5'-AGA CCT TCG GCT GGG ATG CT-3') and Gx.472R were the primers for the second round. 20 μL of DNA from all reactions were then was visualized in a 2% agarose gel with ethidium bromide. Gels were visualized on an Alpha Innotech Corp. (San Leandro, CA) and software provided with the instrument was used to analyze the bands and determine the size of each band.

3.3 Results and Discussion

3.3.1 VOC Concentration Changes as Indicators of Biodegradation

The concentration of TCE in the effluent water reached the influent concentration (C/Co=1) after approximately 57 days for the biotic column and 75 days for the inhibited column (Figure 3-3). The differences in TCE arrival times likely reflect small variations in physical factors, such as the size or frequency of occurrence of fractures and root holes in the two columns. The relative concentration of TCE in effluent from the biotic column dropped below C/Co=1 after 60 days and continued to decrease until about 150 days, after which it remained relatively constant at a value of approximately 0.5 by 245 days. Concentration loss of TCE was not observed in effluent from the inhibited column, which maintained a relative concentration of approximately 1 throughout the remainder of the experiment. Because TCE concentration loss was only observed in the biotic column,

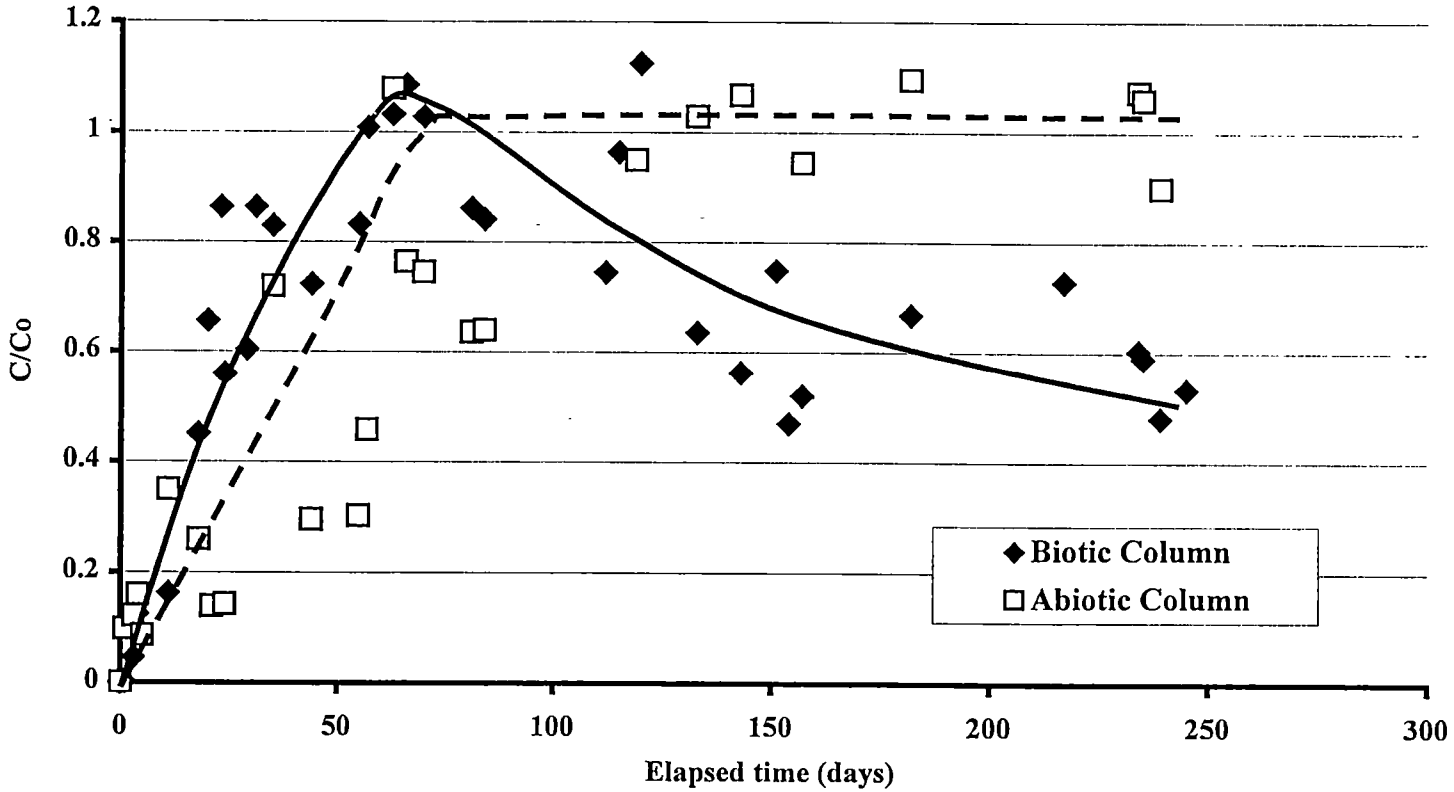


Figure 3-3. Relative concentration of TCE in effluent from biotic and abiotic columns with elapsed time since injection of TCE.

this indicates that the concentration loss most likely occurred due to microbial degradation of the TCE.

In the biotic column, cDCE was first detected in effluent after 31 days and concentrations stabilized at 30-100 ppb after 50 days (Figure 3-4). VC was detected in effluent after 234 days and the maximum VC concentration was 10 ppb (Figure 3-4). These compounds were not observed in the inhibited control column (Figure 3-5). Chloride concentrations were higher than influent concentrations (Table3-2), which would also indicate reductive dechlorination. The presence of the daughter products cDCE and VC in the biotic, but not the inhibited column, again indicates that anaerobic biodegradation of TCE was occurring. The rate of cDCE production, once cDCE was observed in the effluent was 0.6-μM cDCE/day. A mass balance for TCE over the life of the biotic column (250 days) indicated that 73% of the input TCE (12.5 mol) was observed in the effluent as TCE (9.1 mol), 3% (0.4 mol) as cDCE, and 0.1% (0.02 mol) as VC. In the inhibited control 76% of the input TCE (10.1 mol) has been accounted for in the effluent (7.6 mol). The unaccounted for TCE mass in both columns (24%) could be due to other natural attenuation processes such as sorption, diffusion, or volatilization.

3.3.2 Redox Indicators of Biodegradation

Measurements of redox conditions in effluent from the biotic and inhibited columns were utilized to assess the nature of TCE degradation in the saprolite. If anaerobic biodegradation is responsible for the observed VOC concentration changes, then the effluent should contain appropriate electron acceptors to facilitate this process. In the

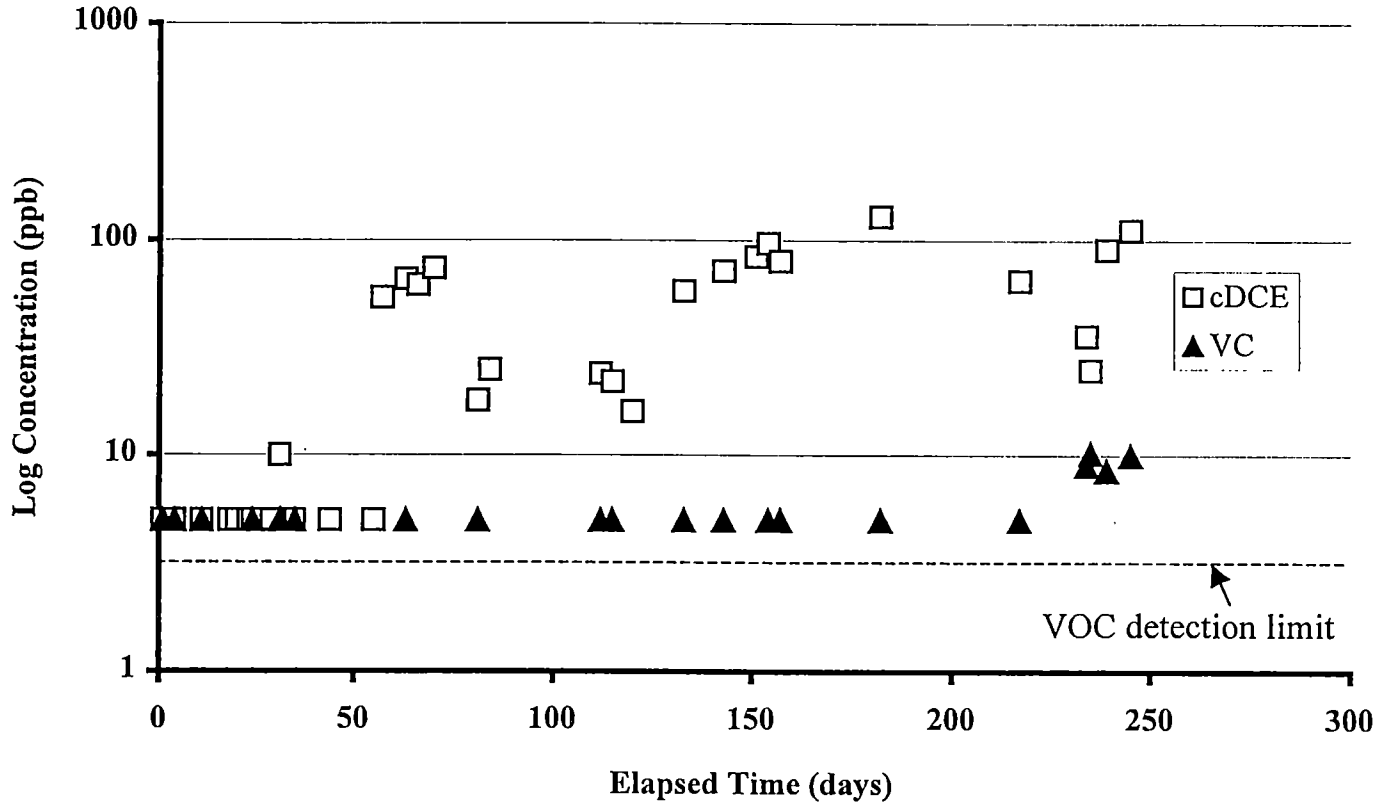


Figure 3-4. VOC concentrations in the biotic column.

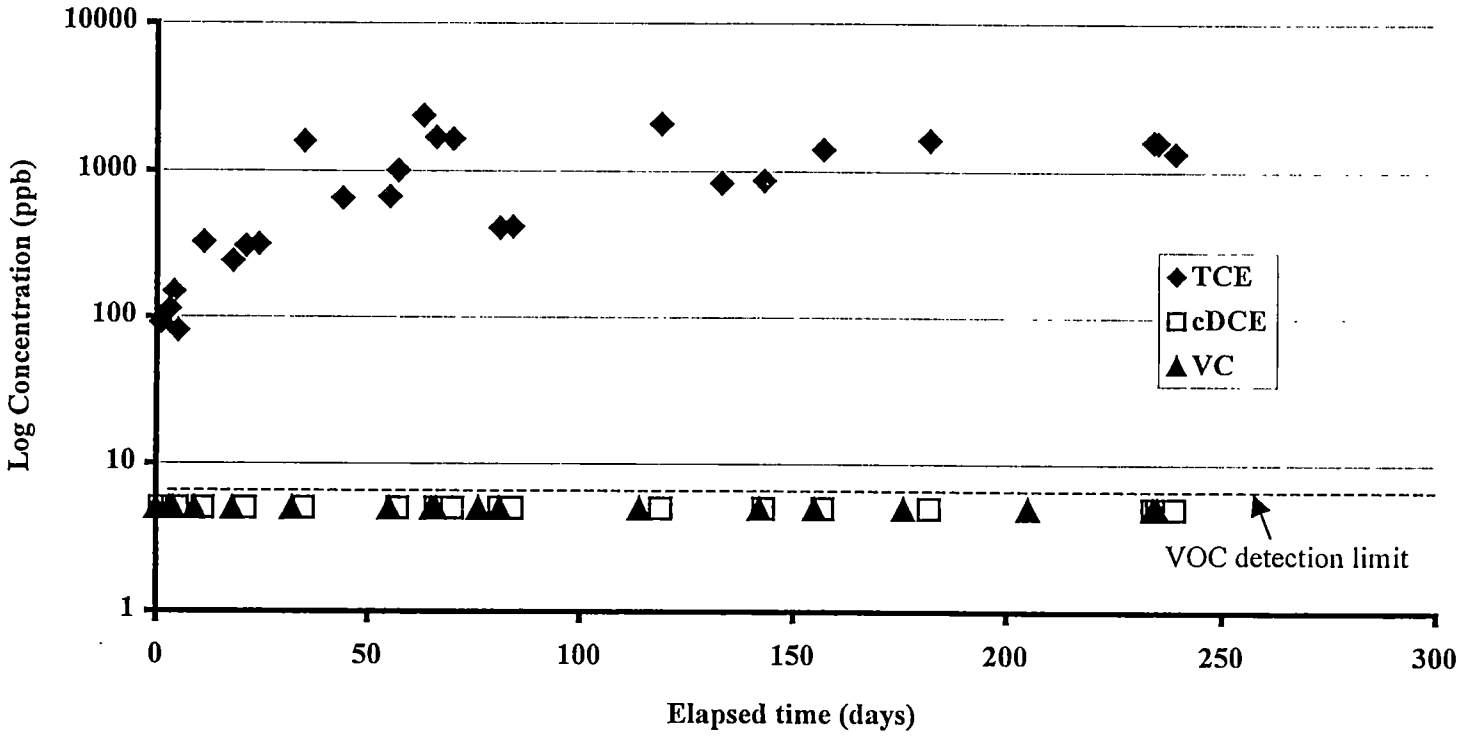


Figure 3-5. VOC concentrations in the inhibited column.

Table 3-2: VOC, chemical results, and AFCEE score for selected time points in effluent from the biotic column.

	Elapsed time (days) -40	9	87	136	248
O_2 (ppm)	3.41	1.54	2.3	1.5	1.42
Fe(II) (ppm)	0	0.4	1.2	1.2	1.6
SO_4 (ppm)	53.7	53.7	56.18	58.7	62
S^{2-} (ppm)	0	0	0.02	0.02	0.17
Cl- (C/Co)*	1	1	1	7.49	4.5
TCE (ppb)	0	263	1565	840	1703
cDCE (ppb)	0	0	25	58	112
VC (ppb)	0	0	0	0	10
AFCEE Score	0	2	6	8	15
AFCEE assessment of natural attenuation	inadequate	inadequate	limited	limited	adequate

*Co=0.667 ppm

case of TCE biodegradation, Fe(III), sulfate, and carbonate can all be used as electron acceptors (Bouwer, 1994). Two methods were used to determine which electron acceptors are being utilized in the columns. The first method is based on Eh-pH diagrams. As shown on Figure 3-6, the influent was above the $Fe(OH)^3/Fe^{2+}$ (iron reduction) stability line, indicating that the influent conditions were not iron reducing (Chapelle et al., 1996). Effluent water from the biotic column and the inhibited control column are shown in Figures 3-7 and 3-8, respectively. Eh-pH values in the effluent samples from the inhibited column were generally above the iron-reduction stability line, except for some early samples indicating that iron reduction was not occurring in the inhibited column. By comparison, the effluent from the biotic column showed a trend towards more reducing conditions, with longer exposure of TCE. This indicates that the environment within the biotic column is suitable for reductive dechlorination of TCE.

The second method used to determine which electron acceptors are involved in degradation is direct measurement of appropriate electron acceptors and their reduced form in the groundwater. To investigate this, influent and effluent water from both the biotic and inhibited columns were monitored for dissolved oxygen, reduced iron, sulfate and sulfide. For the inhibited column concentrations in the influent and the effluent water were approximately the same for dissolved oxygen (3.5-5.5 ppm), sulfate (49-68 ppm), reduced iron (below detect) and sulfide (below detect) indicating that these potential electron acceptors were not being utilized for degradation. This contrasts with effluent from the biotic column, which showed substantial differenced between influent and effluent for some of these indicators. The dissolved oxygen content of effluent from

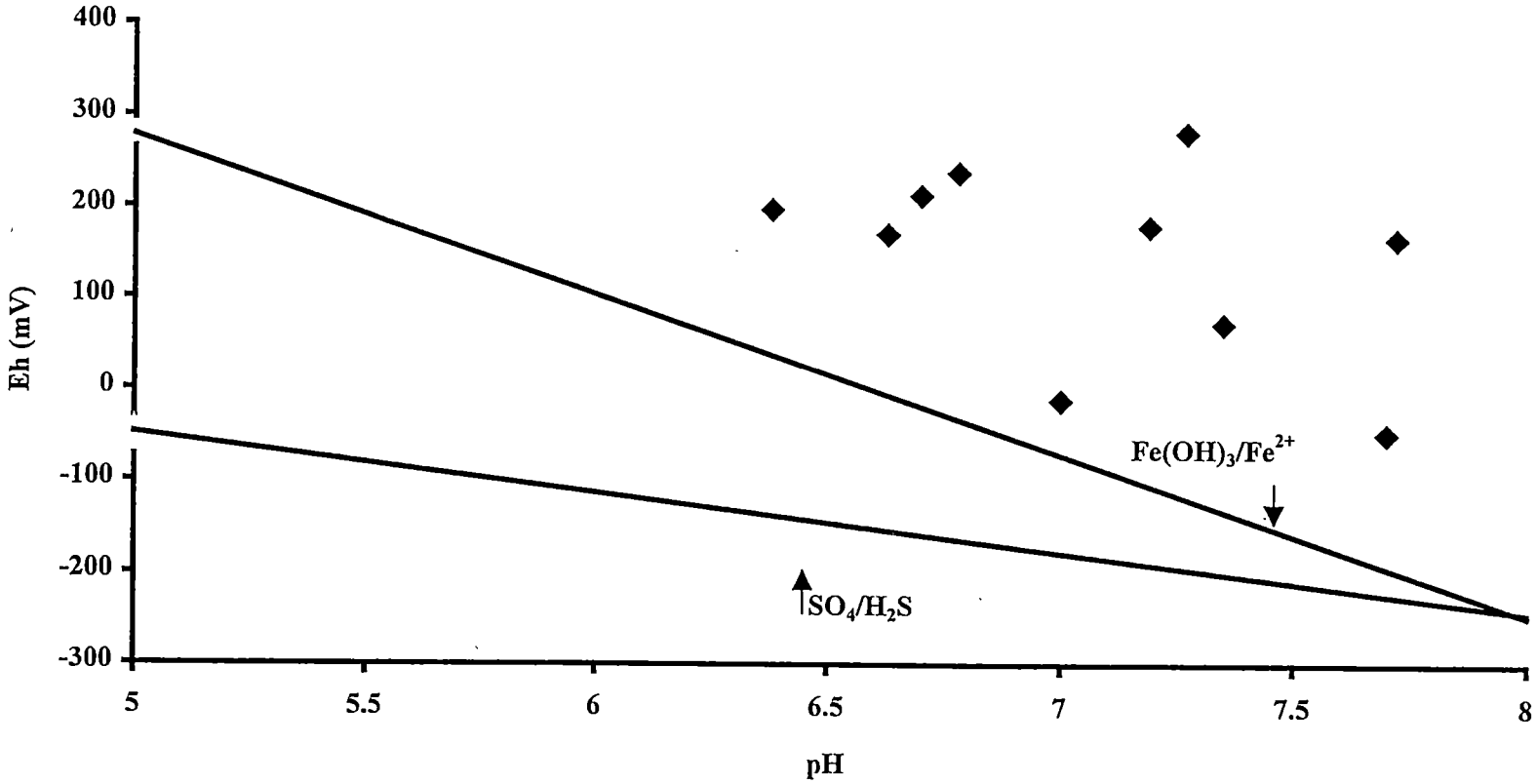


Figure 3-6. pH/Eh stability diagram for the influent water showing equilbria for the Fe-$(OH)_3/Fe^{2+}$, SO_4/HS- redox couples (Chapeile et al., 1996).

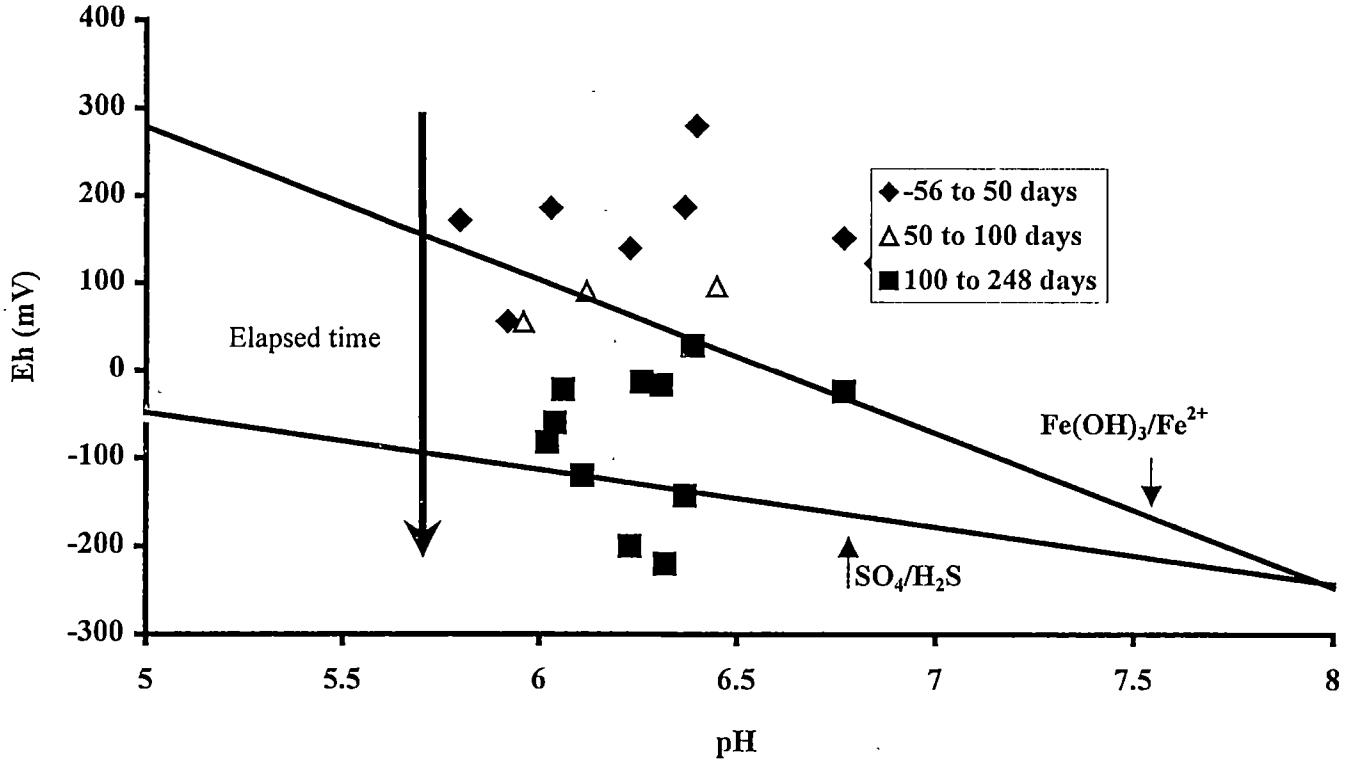


Figure 3-7. pH/Eh stability diagram for effluent water from the biotic column showing equilbria for the Fe-$(OH)_3/Fe^{2+}$, SO_4/HS- redox couples (Chapelle et al., 1996). Elapsed time refers to the time since the introduction of TCE to the influent water.

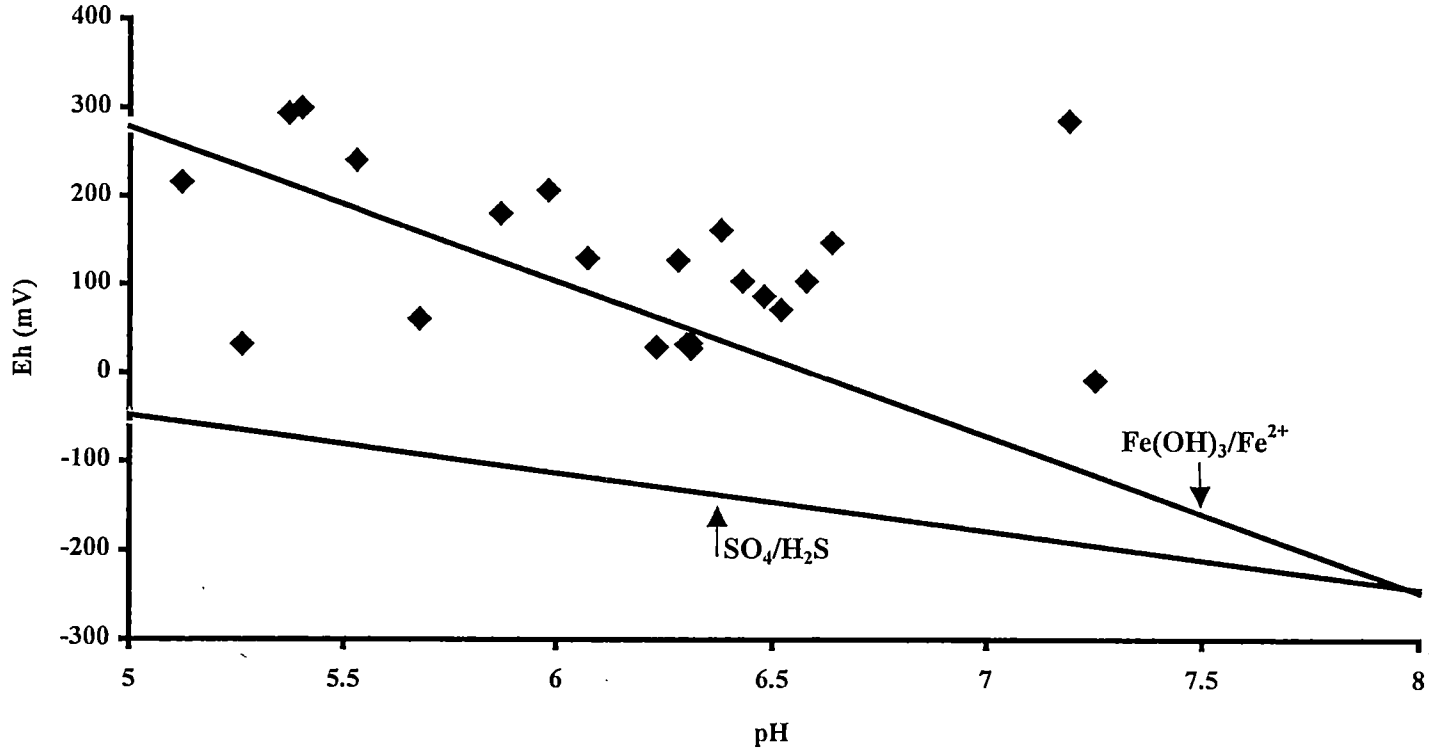


Figure 3-8. pH/Eh stability diagram for effluent water from the inhibited column showing equilbria for the Fe-$(OH)_3/Fe^{2+}$, SO_4/HS- redox couples (Chapelle et al., 1996).

the biotic column prior to TCE injection was around 3.5 ppm, and it dropped to below 1.5 ppm after 9 days after injection of TCE. The drop in dissolved oxygen leads to anaerobic conditions, but is not necessarily associated with TCE biodegradation. Figure 3-9 shows concentrations of reduced iron and sulfide in the biotic column. Prior to TCE injection, Fe(II) was not observed in the effluent water but after injection of TCE the reduced iron concentration steadily increased, reaching a value of 1.6 ppm within 250 days. This suggests that Fe(III) was being utilized as the electron acceptor during biodegradation of TCE. Sulfide concentrations remained below detection until day 65, and then briefly increased to a concentration of 0.01 ppm, which is just above the detection limit. The concentration then decreased below detection limit again until day 136, at which point it increased to approximately 0.1 ppm. This suggests that the sulfate was also being utilized in biodegradation of TCE, even though a decrease in sulfate concentration was not detected.

3.3.3 Microbial Community Analysis

The last factor needed to verify if anaerobic biodegradation of TCE is occurring in the biotic column is to determine if appropriate microorganisms necessary for biodegradation are present. Methanogenic, iron reducing and sulfate reducing bacteria have all been implicated in TCE dechlorination and consortia of bacteria are often involved in biodegradation of TCE (Wilson et al., 1986; Freedman and Gossett, 1989; Smatlak et al., 1996; Bagley and Gossett, 1990). Effluent water was analyzed to determine the types of bacteria that were present by enrichment and molecular techniques using specific primers. Enrichments for sulfate and iron reducing bacteria were performed on effluent

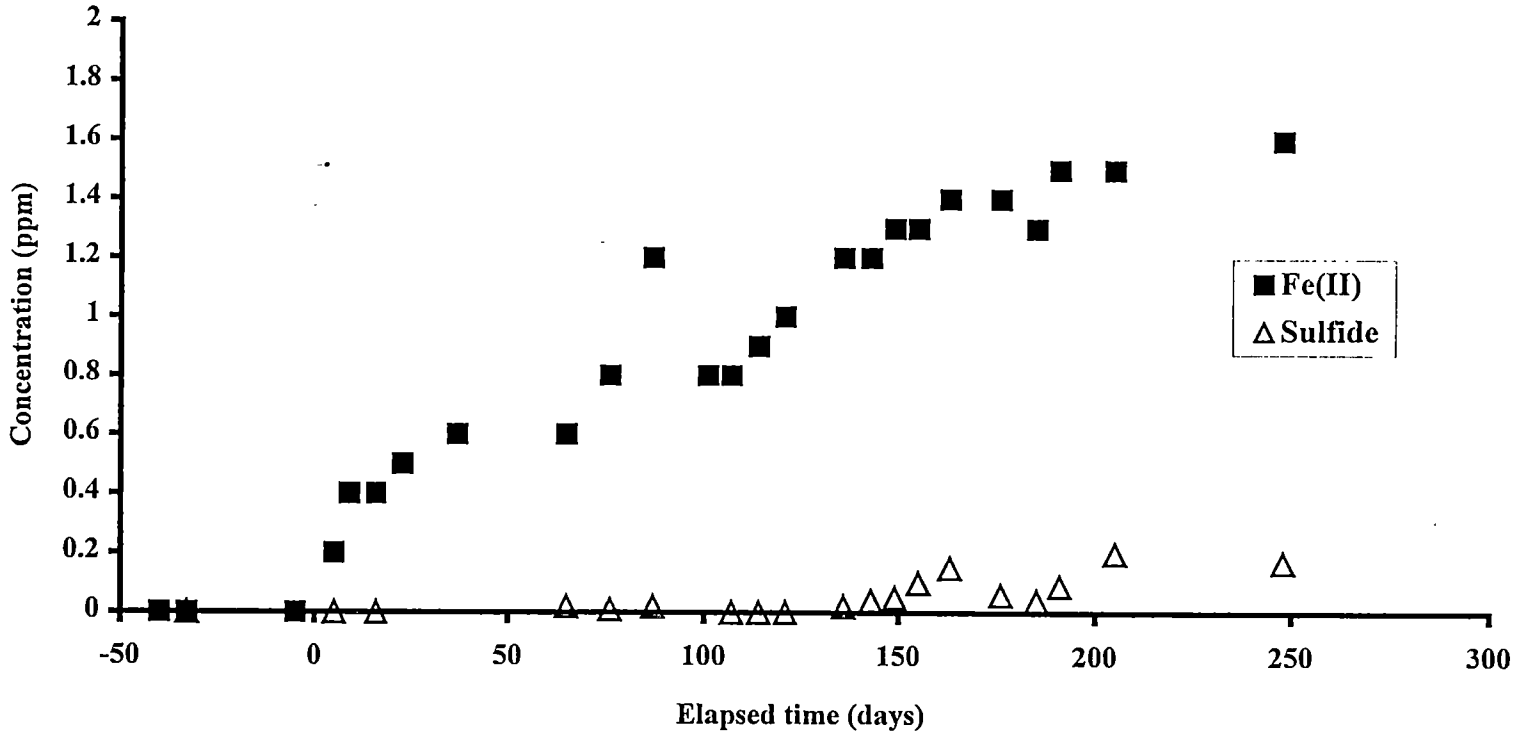


Figure 3-9. Fe (II) and sulfide concentrations in effluent from the biotic column.

samples from the biotic and inhibited column. As expected, all samples from the inhibited column were negative for the presence of iron reducing bacteria and sulfate reducing bacteria. In the biotic column, enrichments for iron reducing bacteria were positive in the effluent in every sample throughout the duration (205 days) of the experiment. Iron reducing bacteria were not present in the influent water. This indicates that iron reducing bacteria which are likely responsible for at least part of the TCE biodegradation, are naturally found in the saprolite and survived the injection of TCE. Sulfate reducing bacteria were detected only after 205 days. The presence of sulfide in the effluent water would indicate that sulfate reducing bacteria should be present after 65 days. The finding that iron reducing bacteria were present in the biotic column is similar to findings by Lovely and Anderson (2000). The observed that when anaerobic groundwater was exposed to organic contaminants, iron reducing bacteria came to dominant the community. However, the present study did not examine concentration or relative abundance of iron reducing bacteria, so we cannot determine whether they dominate the microbial community in the saprolite.

Using effluent samples from the columns, PCR products were obtained using specific primers for iron reducing bacteria (*Geobacter* and *Geothrix)* implicated in biodegradation of organic compounds, plus six different phylogenetic groups of sulfate reducing bacteria, and methanogens. The inhibited column effluent did not contain any of these organisms while effluent from the biotic column was positive for these organisms in every sample. This is consistent with the enrichment results for iron reducing bacteria, as described above. This indicates the presence of these organisms, but again, as was the

case for the enrichment investigations, does not provide measurements of concentration or relative abundance compared to the whole microbial community. Sulfate reducing bacteria PCR primers were used to determine the main type of sulfate reducing bacteria associated with exposure to TCE. Of the 6 primer sets tested, only group number 5 (Daly et al., 2000) produced a PCR product from effluent samples from the biotic column (Figure 3-10). Group number 5 consists of *Desulfococcus-Desulfonema-Desulfosarcina*, which are members of Proteobacteria δ subdivision and are likely responsible for sulfide observed in the effluent from the biotic column near the end of the experiment (Figure 3-9). The last group of microorganisms that have been implicated in TCE biodegradation is methanogens. These organisms were not detected in the effluent lines of either the biotic or inhibited column suggesting that reducing conditions in the columns were not acceptable for these organisms, or that they were not be present.

3.3.4 Natural Attenuation Evaluation

A method for assessing the potential for natural attenuation in groundwater was evaluated using the guidelines described by Brady et al. (1999) and Wiedemeier et al. (1998). The Air Force Center for Environmental Excellence (AFCEE) protocol (Wiedemeier et al., 1998) uses different weighed analytical parameters, such as redox conditions to determine potential for biodegradation of TCE. The score serves only to suggest the likelihood of natural attenuation in groundwater at a contaminated site and provides a clear identification of data needed for natural attenuation implementation. Table 3-2 lists the values for different geochemical parameters from five effluent samples taken from the biotic column at different times throughout the experiment along with the AFCEE score.

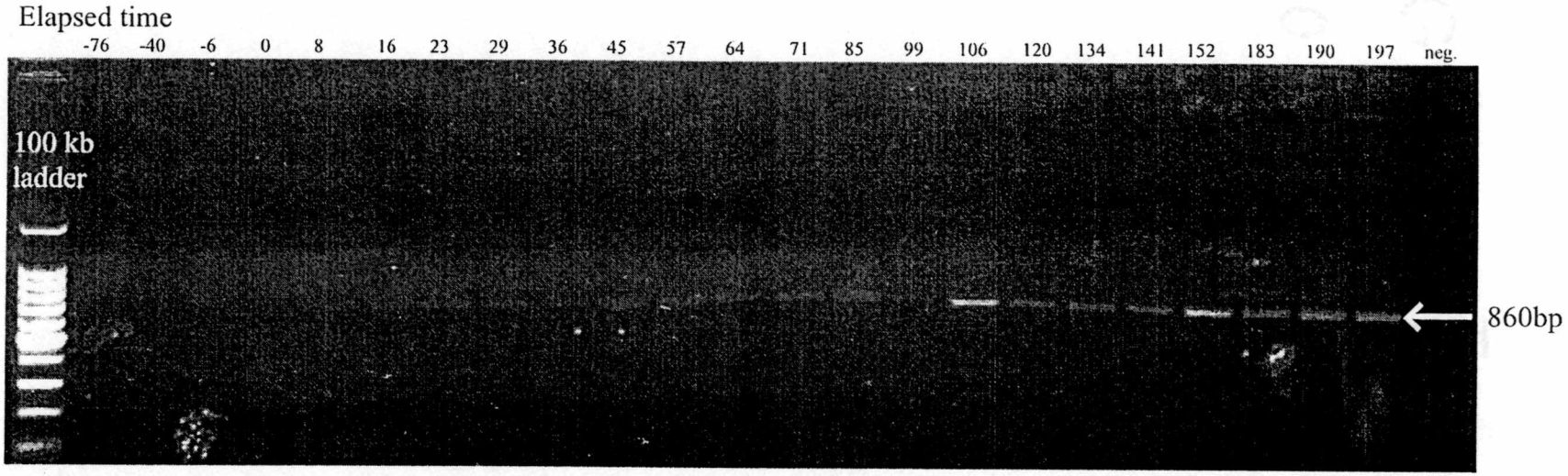


Figure 3-10. Sulfate Reducing bacteria group 5 *Desulfococcus-Desulfonema-Desulfosarcina* from the effluent of the biotic column.

A score of –1 to 5 shows inadequate evidence for anaerobic biodegradation; 6 to 14 points shows limited evidence for anaerobic biodegradation; a score of 15 to 20 points is adequate evidence for anaerobic biodegradation; and a score greater than 20 points is strong evidence for anaerobic biodegradation. The AFCEE score for the biotic column increased throughout the experiment, starting out as inadequate evidence for anaerobic biodegradation (-56 days to 84 days), limited evidence at 87 to 205 days and by the end of the experiment indicated that there was adequate evidence for anaerobic biodegradation. The AFCEE score for the inhibited column showed inadequate evidence for anaerobic biodegradation throughout the experiment. The AFCEE scores are consistent with the previously described evidence of TCE biodegradation in the biotic, but not the inhibited column. The AFCEE score only indicated that anaerobic biodegradation was occurring after 87 days while other indicators (presence of daughter products, reducing conditions, redox indicators) suggested that biodegradation started earlier.

3.3.5 Comparison to Field-Scale Evidence of Biodegradation at WAG5

This investigations of TCE biodegradation described in this chapter were inspired by a previous field study (Chapter 2), which found strong evidence that biodegradation of TCE and its daughter products was taking place in an existing plume of organic contaminants in fractured and slightly weathered shale at the WAG5 site on the ORR. There are many similarities between the findings of the laboratory and field studies, as described below. The geologic settings at the WAG5 site and the nearby SWSA7 site are nearly identical, except that there is a thicker mantle of saprolite overlying bedrock at the

SWSA7 site. The field study focused on the fate of contaminants in the upper 3-4 m of the bedrock, while the lab study used columns collected in the saprolite approximately 1 m above the saprolite-bedrock contact. The lowermost saprolite and the upper bedrock are, in many respects, geochemically similar, so a comparison of the findings of the two studies is warranted.

At the WAG5 site, there is a decline in concentration of TCE with downgradient distance from the waste trench, and increases in concentration of compounds that are indicative of TCE biodegradation including cDCE and VC. In the biotic column TCE concentration decreased with time and cDCE and VC, which were not initially present, both appeared after TCE was added to the column and their concentrations were still rising at the end of the experiment. The production of ethylene and methane might also have occurred in the biotic column, given a longer period of time. Redox conditions at the WAG5 site indicated that iron reduction, sulfate reduction and possibly methanogenesis were occurring, and these conditions are favorable for anaerobic biodegradation of TCE. In the biotic column, redox conditions shifted towards a more reducing environment after the injection of TCE, with iron reduction occurring first followed by sulfate reducing conditions. This suggests that similar shifts in redox conditions might have occurred at the WAG5 site in the first few months or years after TCE was disposed of in the waste trenches. Methanogenesis was not observed in the biotic column, which could either be due to insufficient time for highly reducing conditions to develop, or it may be that methane observed at the WAG5 was from the presence of other wastes in the trenches. Microbial community analysis at WAG5 revealed the presence of methanotrophs,

methanogens, iron reducing bacteria, and sulfate reducing bacteria, whereas in the biotic column only iron reducing and sulfate reducing bacteria were present. Methanotrophs were not examined for in the biotic column because these organisms are aerobic bacteria and the columns were intended to examine only anaerobic biodegradation. Methanogens were not detected in this experiment but were detected at the field site, suggesting that either reducing conditions in the columns were not acceptable for these organisms, that they might not be present, or that the methanogens are associated with other organic waste at the WAG5 site. In both the field study and the lab study there is strong evidence of anaerobic biodegradation of TCE, and it appears that this process can occur in both the shale bedrock and the overlying saprolite.

3.4 Conclusions and Implications

This study demonstrates that microbially mediated degradation of TCE under iron reducing and sulfate reducing conditions can spontaneously occur in fractured saprolite derived from sedimentary rock. The decrease in TCE mass and the appearance of daughter products of TCE (cDCE and VC) in the biotic column, but not in the inhibited column, are evidence of anaerobic biodegradation of TCE. The earliest evidence of biodegradation (appearance of cDCE) occurred within 30 days of the introduction of TCE into the previously uncontaminated biotic column, indicating that the necessary shifts in microbial communities and redox conditions can occur rapidly. Within the biotic column relative concentration of TCE decreased by up to 50% after traveling through only 25 cm of saprolite and it is quite possible that for longer transport distances and residence times biodegradation would proceed to an even a greater extent than was observed.

The shift in redox conditions (appearance of Fe(II) and sulfide) that occurred during biodegradation of TCE in the biotic column suggestions that there might be a correlation between these conditions and processes. As well, iron reducing and sulfate reducing bacteria were detected in the effluent water in the biotic column. However, these factors do not necessarily imply that iron reduction or sulfate reduction were directly involved in TCE biodegradation. Iron reducing bacteria, such as *Geobacter* sp., have been implicated in the oxidation of TCE in several recent investigations (Lovely and Anderson, 2000; Krumholz et al., 1996). Evidence from this experiment suggest that these types of microorganisms may be involved in the biodegradation, but further research needs to be done to determine the direct link between these organisms and biodegradation of TCE. TCE can also be reductively dechlorinated by sulfate reducing bacteria through a process that is thought not to be energy yielding but rather co-metabolic because only a small fraction of the total reducing equivalents derived from the oxidation of electron donors is used to reduce the solvent (Bagley and Gossett, 1989; Maymo-Gatell et al., 1995). The concomitant presence of sulfate reducing bacteria, sulfide, and TCE daughter products (cDCE and VC) suggest that these organisms might also be involved the biodegradation of TCE, but again future research is needed to determine if there is a direct link.

There is a tendency to ascribe a single dominant dechlorination pathway to biodegradation in an aquifer or soil based on bulk parameters such as presence of Fe(II) or specific type of microorganism (Lee et al., 1998). However, field sites in fractured shale and saprolite are often complex heterogeneous mixtures of aerobic and anaerobic

microenvironments that result from seasonal water table fluctuations and differences in permeability, lithology, channeling of water flow, and contaminant source zone characteristics (Jardine et al., 1999; Lee et al., 1998). Hence, it is possible that multiple dechlorination pathways such as iron reduction and sulfate reduction could simultaneously or sequentially operate in heterogeneous materials.

This experimental study and a companion field study (Chapter 2) both indicate that the current AFCEE protocols for natural attenuation, which are based on monitoring of geochemical parameters, are appropriate for assessing the potential for TCE attenuation in the shale and shale saprolite found at the Oak Ridge Reservation. The two studies, one in the shale and the other in the overlying saprolite, indicate that these materials are geochemically and microbially similar, and that TCE biodegradation likely occurs in much the same manner in both materials.

Chapter 4

Preliminary Investigations of Biodegradation of TCE in Fractured Saprolite

4.1 Introduction

This chapter describes a preliminary biodegradation study that was carried out in a column of saprolite from ORR. The principal hypothesis of this research is that microbial and geochemical conditions in the uncontaminated saprolite are favorable for anaerobic biodegradation of TCE, so that upon introduction of TCE into the saprolite, it will biodegrade "naturally" without addition of specially selected bacteria, cometabolites or other chemicals. The objective of this preliminary study was to determine the adequacy of the experimental design for determination of biodegradation of TCE. Determination of potential for biodegradation was assessed by a multiple analytical approach consisting of monitoring VOC concentration, redox conditions, and evaluation of microbial techniques (enrichment and molecular techniques). Several problems were encountered during the study that prevented the researcher from clearly assessing the nature of biodegradation occurring in the column. However, this research did form the foundation of latter experiments to evaluate biodegradation of TCE in fractured saprolite (Chapter 3).

4.2 Material and Methods

4.2.1 Geological Setting

The experimental investigation was carried out using an undisturbed sample of uncontaminated residual soil (saprolite) obtained from an existing research site in the proposed Solid Waste Storage Area #7 (SWSA7) at the ORR (Figure 4-1). The saprolite at SWSA7 has been extensively characterized from a physical and hydrogeological perspective (Solomon et al., 1992; Jardine et al., 1993; Cumbie and McKay, 1999; Driese et al., in review). The saprolite is derived from *in situ* weathering of the underlying sedimentary bedrock, which is composed of interbedded shale, siltstone, and sandstone, which is part of the Dismal Gap Formation of the Middle to Upper Cambrian Conasauga Group (Hatcher et al., 1992). The carbonates have been leached, leaving a high porosity detrital matrix, which retains much of the structure of the parent bedrock. These structural features include bedding which generally dips towards the southeast at 17 to 72°, and fractures caused by regional tectonic activity. Fractures occur both parallel and oblique to bedding, with typical fracture spacing in the saprolite ranging from 0.005 to 0.9 m (Dreier et al., 1987; Solomon et al., 1992; Cumbie, 1997). Macropores formed by roots are also present in the saprolite to depths of >2 m in some places. The total porosity ranges from 15% to 58% (Dorsch and Katsube, 1999) with porosity due to fractures and biopores being approximately 1-2% (Cropper, 1998). In the upper portion of the saprolite many of these pores have been infilled with pedogenic clays or Fe/Mn oxides (Jardine et al., 1993; Driese et al., in review). Hydraulic conductivity values for columns previously collected at SWSA7 ranged from 2.7×10^{-4} to 4.5×10^{-9} m/s (Driese et al., in review).

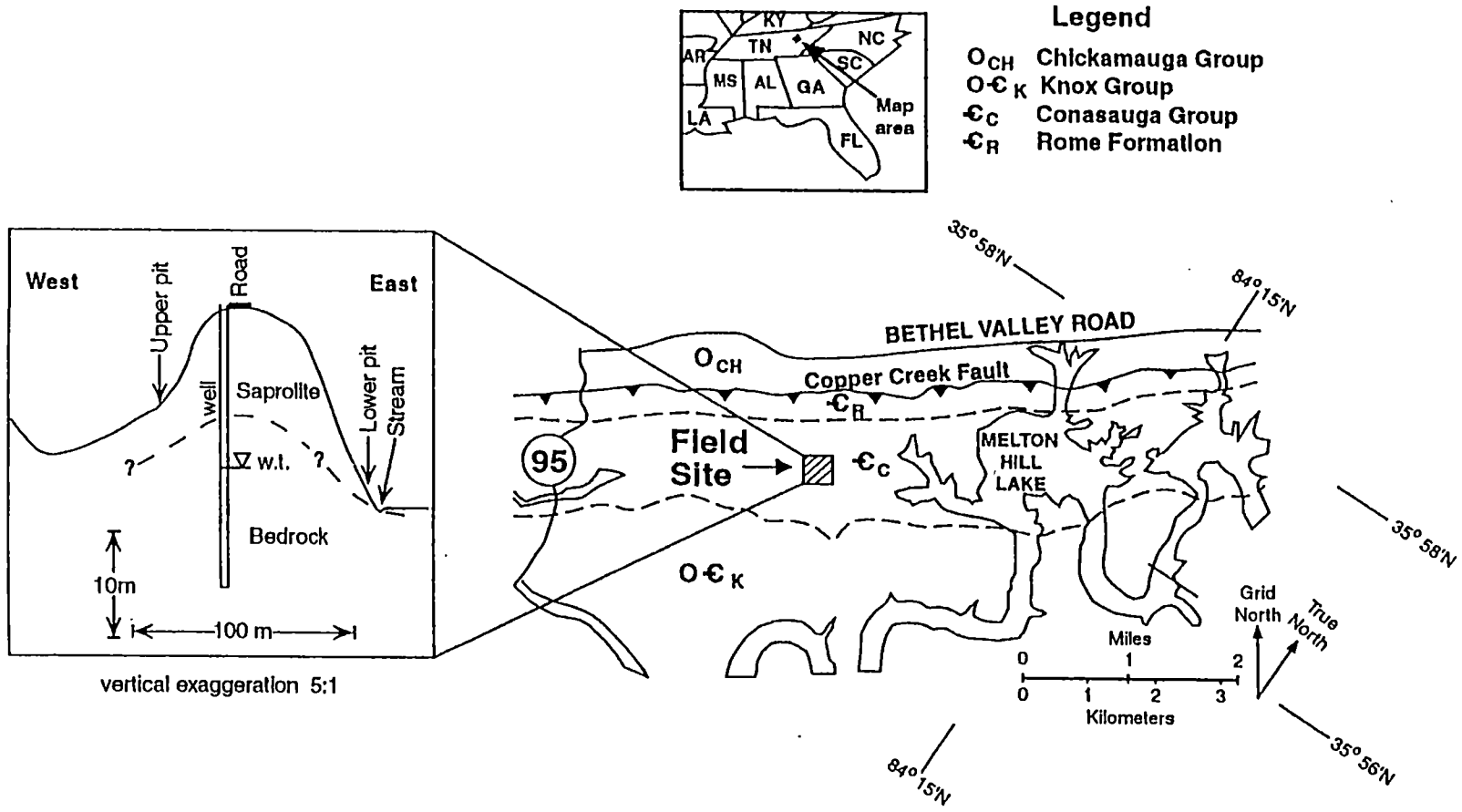


Figure 4-1. Site location within the Oak Ridge Reservation.

4.2.2 Flow-through Experiments in Undisturbed Soil Column

The 23 cm diameter, undisturbed saprolite column collected for use in this study was excavated with hand tools and setup following the methods of previous researchers working in saprolite at ORR (Reedy et al., 1996; Howard, 1997; Cumbie, 1997; Haun, 1998). The method was modified to make the casings and endcaps TCE solvent-resistant (Cropper, 1998; Pitner, 2000). The column was collected at a depth of 1.5 to 2.5 m below ground surface, and approximately 1 m above the bedrock contact, which crop out in a nearby stream. The sample was excavated in the shape of a cylinder with hand tools to keep disturbance to a minimum. A 25 cm diameter PVC casing was fitted over the saprolite column and the annulus was filled with Ureol 6414 solvent-resistant epoxy (Ciba, Helsinki, Finland). After collection, the column was immediately taken to the laboratory and disturbed material was trimmed from the ends in preparation for fitting with solvent-resistant end caps. The final lengths of the columns were 25 cm. The column was placed in a 12°C environmental chamber and sealed to prevent exposure to the atmosphere.

The column was setup to carry out saturated flow-through experiment as shown in Figure 4-2. The injection system consisted of a Tedlar sampling bag (SKC, Eighty Four, PA) connected to an HPLC pump using all stainless steel fittings. The flow rate was set at 0.2 mL/min, which corresponds to a specific discharge rate of 0.7 cm/day. This is within the range of specific discharge rates determined in previous field studies in deep saprolite and weathered shale at ORR (McKay et al., 1997; Lee et al., 1992). The sample was

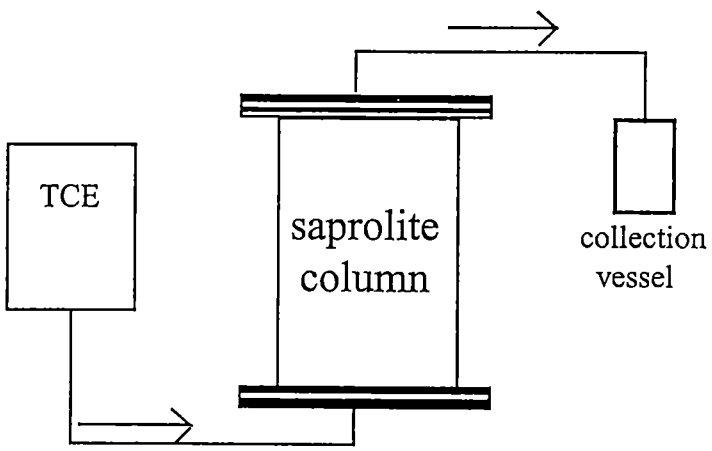


Figure 4-2. Column setup. Saprolite column was placed in a 12°C chamber. Various concentrations of TCE ranging from 0-13 ppm were injected into the column at 0.2 mL/min. Effluent was monitored for VOC, redox conditions, and microbial community.

saturated with groundwater from bedrock well at the SWSA7 site. Ultra high pure grade helium was bubbled through the well water for 40 minutes before it was used for the influent solution. After 48 days of injection of TCE-free influent water, the TCE injection started. All times in this experiment are elapsed times relative to the TCE injection. The TCE injection consist of 58 days at 0.1 ppm followed by around 21 days of 13 ppm (estimated) followed by 7 days of 0.1 ppm followed by 40 days of 1 ppm followed by 135 days of <0.01 ppm. Due to operational problems, exact documentation of injection concentration had to be estimated during the highest TCE concentration (Chris Knight, personal communication).

4.2.3 Monitoring of VOC and Redox Conditions

The effluent from the column was used for monitoring of dissolved solvent output, biodegradation products, redox conditions, and microorganisms. Samples were taken from a sampling port at the base of 150 mL stainless steal sampling vessel (Swagelok, Solon, OH) fitted at the effluent end of the column and sealed to an Erlenmeyer flask for overflow. Care was taken to collect VOC samples with minimal loss. The samples were collected in 40 mL vials with Teflon coated septa for VOC. The samples contained zero headspace, and were stored upside-down for no longer than 2 weeks to prevent any loss of VOC by diffusion or volatilization. The samples were analyzed using a Shimadzu GC-14A (Japan) gas chromatograph (GC). The inlet temperature for the gas chromatograph was 200°C and the detector was 200°C. The initial column temperature was 35°C for 5 minutes then increased 5°C/minutes to a final temperature of 100°C for 5 minutes. Dissolved oxygen was determined with YSI dissolved oxygen meter (Yellow

Spring, OH). Redox analysis for Fe (II), sulfate and sulfide were determine using Hach (Loveland, CO) kits for the specific chemical, following manufacture's directions. Chloride was determined by ion specific probe (Corning, Corning, NY). pH and Eh were measured in effluent water using an Orion portable pH-Eh meter model 250A (Boston, MA).

4.2.4 Microbial Community Structure Analysis

Iron reducing and sulfate reducing bacteria were tested in the influent and effluent water. BART-IRB and BART-SRB media obtained from Hach (Loveland, CO) were used for determination of iron and sulfate reducing bacteria, respectively. Molecular analysis for identification of individual microbes in the microbial communities was determined from effluent water samples. 25-500 mL of effluent water was collected in sterile vials and then filtered through a 0.2μm filter (Durapore, Millipore, Bedford, MY). Samples were then stored at –80°C until further processing. DNA was extracted by bead beating filters with lysing matrix (Bio101, Carlsbad, CA) and 1 mL of STE buffer (10mM Tris (pH 8), 1 mM EDTA, and 100 mM NaCl) for 20 s and 4 m/s. Samples were centrifuged for 2 minutes at 10,000 rpm. The supernatant was placed into a new sterile microcentrifuge tube and stored at –80°C until further processing.

In this experiment, PCR products were obtained from effluent samples using specific primers for target organisms. Extracted DNA from effluent water was amplified in a reaction mixture with Ready-to-Go™ PCR beads (Amstersham Pharmacia, Piscataway, NJ) and a 10 nM concentration of each of the primers listed below. Reaction mixtures

were incubated in a Perkin Elmer (Wellesley, MA) Gene Amp PCR system 2400 thermal cycler. PCR primer sets for the 16S rDNA gene of six phylogenetic groups of sulfate reducing bacteria were used as described by Daly et al. (2000; Table 4-1). 10 μL of template DNA from each PCR reaction was then was visualized in a 2% agarose gel with ethidium bromide. Gels were visualized on an Alpha Innotech Corp. (San Leandro, CA) and software provided with the instrument was used to analyze the band patterns and determine the size of each band.

Clonal libraries were constructed from community rDNA PCR-amplified used Ready-to-Go™ PCR beads (Amstersham Pharamicia, Piscataway, NJ) and 10 nM of 16S forward primer 530F (5'-GTG CCA GCM GCC GCG GTA A-3') (Lane et al., 1985) and the 16S reverse primer of 1392R (5'-ACG GGC GGT GTG TRC-3') or with RISA primers (Cook et al., 1999). Reaction mixtures were incubated at 94°C for 5 minutes followed by 15 cycles 94°C for 30 seconds, 60°C for 45 seconds, and 72°C for 2 minutes and then by a final extension period of 7 minutes at 72°C. 10 μL of PCR product then was visualized in a 2% agarose gel with ethidium bromide. RISA fragment size for 5 of the RISA clones (40R-1, 40R-6, 40R-4, 40R-9, and 40R-10) were determined by amplifying plasmid preparations with RISA primers. PCR products were cloned with a TOPO TA Cloning kit (Invitrogen Corp., Carlsbad, CA) in accordance with manufacturer's directions. Plasmid DNAs containing inserts were prepared for sequencing using RPM AFS Midi plasmid preparation kit (Bio 101, Carlsbad, CA). Clones were screened prior to sequencing using random fragment length polymorphism (RFLP) to determine unique clones. The enzyme Hha and Rsa1 (0.2 μL) were added to 18 μL plasmid preparations

Table 4-1. 16S rDNA-targeted PCR primer sequences specific for SRB subgroups (Daly et al., 2000).

PRIMER	TARGET SITE[a]	SEQUENCE 5'-3'[b]	GROUP	TYPE	EXPECTED SIZE PRODUCT
DFM140	140-158	TAG MCY GGG ATA ACR SYK G	Group 1	*Desulfotomaculum*	700bp
DFM842	842-823	ATA CCC SCW WCW CCT AGC AC			
DBB121	121-142	CGC GTA GAT AAC CTG TCY TCA TG	Group 2	*Desulfobulbus*	1120bp
DBB1237	1237-1215	GTA GKA CGT GTG TAG CCC TGG TC			
DBM169	169-183	CTA ATR CCG GAT RAA GTC AG	Group 3	*Desulfobacterium*	840bp
DBM1006	1006-986	ATT CTC ARG ATG TCA AGT CTG			
DSB127	127-148	GAT AAT CTG CCT TCA AGC CTG G	Group 4	*Desulfobacter*	1150bp
DSB1273	1273-1252	CYY YYY GCR RAG TCG STG CCC T			
DCC305	305-327	GAT CAG CCA CAC TGG RAC TGA CA	Group 5	*Desulfococcus-Desulfonema-Desulfosarcina*	860bp
DCC1165	1165-1144	GGG GCA GTA TCT TYA GAG TYC			
DSV230	230-248	GRG YCY GCG TYY CAT TAG C	Group 6	*Desulfovibrio-Desulfomicrobium*	610bp
DSV838	838-818	SYC CGR CAY CTA GYR TYC ATC			

[a]16S rDNA positions, *E.coli* numbering
[b]Ambiguities: R(G or A); Y(C or T); K(G or T); M(A or C); S(G or C); W(A or T)

and incubated at 30°C overnight. Samples were then visualized on a 2% agarose gel and compared to other clones that had been sequenced and screened using RFLP. Unique fingerprints were then sequenced. Sequences were obtained by the molecular biology sequencing facility, located on the University of Tennessee campus, equipped with an Applied Biosystems (Foster City, CA) 373A Automated Sequencer. Analysis of sequences was done using the GenBank (http://www.ncbi.nlm.nih.gov/) basic Blast search. Sequence alignments were performed using ClustalX (v. 1.64b) and the trees were constructed using TreeView (http://taxonomy.zoology.gla.ac.uk/rod/treeview.html).

4.3 Results

4.3.1 VOC Concentration

The effluent water concentration was monitored for the presence of VOCs (Figure 4-3). Concentrations of TCE in the effluent increased to 0.1 ppm by 39 days after the injection of TCE began. After 58 days elapsed time, the influent concentration was inadvertently increased to a much higher concentration of around 13 ppm for 2 weeks, then was dropped to 0.1 ppm for 1 week, then raised to 1 ppm until day 135. The effluent concentration remained around 13 ppm during the period of fluctuating influent concentration. After 135 elapsed days, TCE-free influent water was injected into the column and the concentrations of TCE in the effluent decreased slowly with time. The slow decline in concentration is most likely due to diffusive exchange of TCE between the matrix and the fractures. This type of behavior is characteristic of solute transport in fractured saprolite (Reedy et al., 1996; McKay et al., 1997).

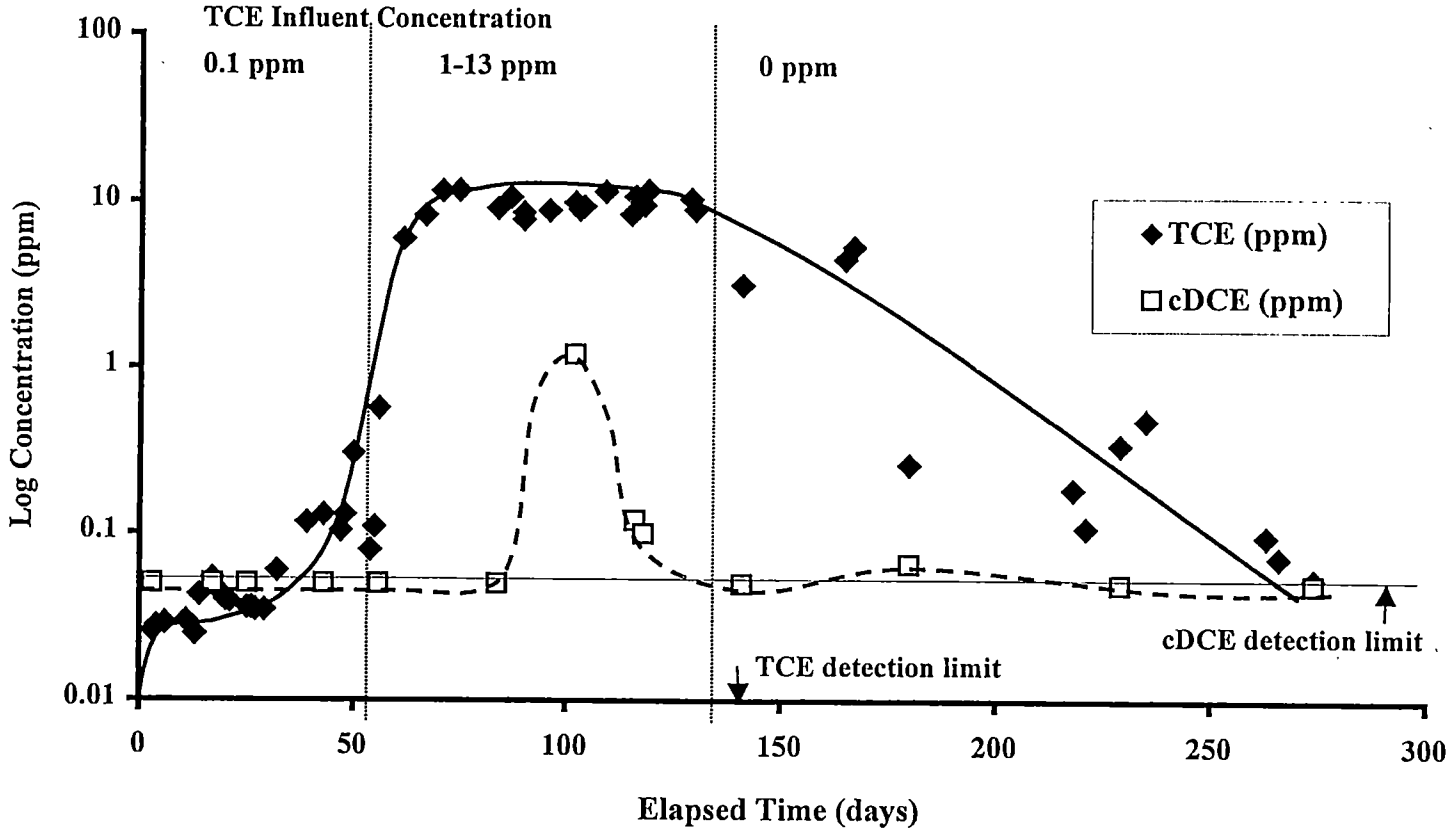


Figure 4-3. VOC concentration in effluent water samples. Elapsed time is days after TCE injection.

One indicator of biodegradation is whether there is a decrease in the concentration of TCE along a groundwater flow path. The decrease in concentration of TCE observed after day 135 cannot be attributed to biodegradation but is mostly due to the flushing with the lower concentration influent water. A mass balance was attempted to determine if there was an overall mass loss of TCE in the column that could be ascribed to biodegradation but the calculated mass of TCE recovered in the effluent (20 mol) was actually greater than the mass estimated from the influent. This result reflects a high degree of uncertainty in the TCE concentration in the influent.

A potential indicator of biodegradation was the appearance of one of the daughter products of TCE biodegradation, cDCE. cDCE was first detected after 102 days (Figure 4-3) and was detected in few effluent samples. At day 135, contaminant-free influent water was injected into the column, possibly diluting cDCE below the detection limit. The presence of cDCE, however, provides evidence that reductive dechlorination of TCE had occurred in the column and therefore biodegradation may occur in fractured saprolite.

4.3.2 Redox conditions

Redox conditions can be used to indicate the potential for natural attenuation of TCE (Chapelle and Brady, 1998). Reductive dechlorination of TCE occurs under anaerobic conditions in which Fe(III), SO_4, or CO_2 acts as the alternative electron acceptor, forming Fe(II), sulfide, and methane, respectively (Wilson et al., 1986; Freedman and Gossett, 1989; Smatlak et al., 1996; Bagley and Gossett, 1990). There are two methods to

determine which electron acceptors are being utilized. The Eh-pH diagram for the influent and the effluent water is shown on Figure 4-4. The influent is above the $Fe(OH)_3/Fe^{2+}$ stability line (Chapelle et al., 1996) indicating that it was not under iron reducing conditions , whereas all the effluent samples remain below the iron reduction stability line, thus indicating iron reducing conditions.

The determination of appropriate electron acceptors can also be directly measured in the effluent water. Influent concentrations of sulfate were 47.3 ± 17.2 ppm, whereas Fe(II) and sulfide were below detection. Figure 4-5 indicates the concentration of Fe(II) and sulfide for the effluent water in elapsed time after the injection of TCE. Fe(II) was not observed for the 48 days prior to injection of TCE, but only after the start of 0.1 ppm TCE injection into the column. The concentration of reduced iron increased during the first 30 days and then reached a steady state concentration of 1.2 ppm. After day 138 levels of Fe(II) increased until the end of the experiment at a concentration of 3.5 ppm. This indicates that iron reduction was occurring in the column. Sulfate concentration varied throughout the study (42.0 ± 10.8 ppm), corresponding to the natural fluctuations in the influent water. Sulfide was only detected in one sample from the effluent, which may or may not indicate sulfate reducing conditions.

4.3.3 Microbial community structure

Methanogenic, iron reducing and sulfate reducing bacteria have all been implicated in biodegradation of TCE and consortia of bacteria are often involved in biodegradation of TCE (Wilson et al., 1986; Freedman and Gossett, 1989; Smatlak et al., 1996; Bagley and

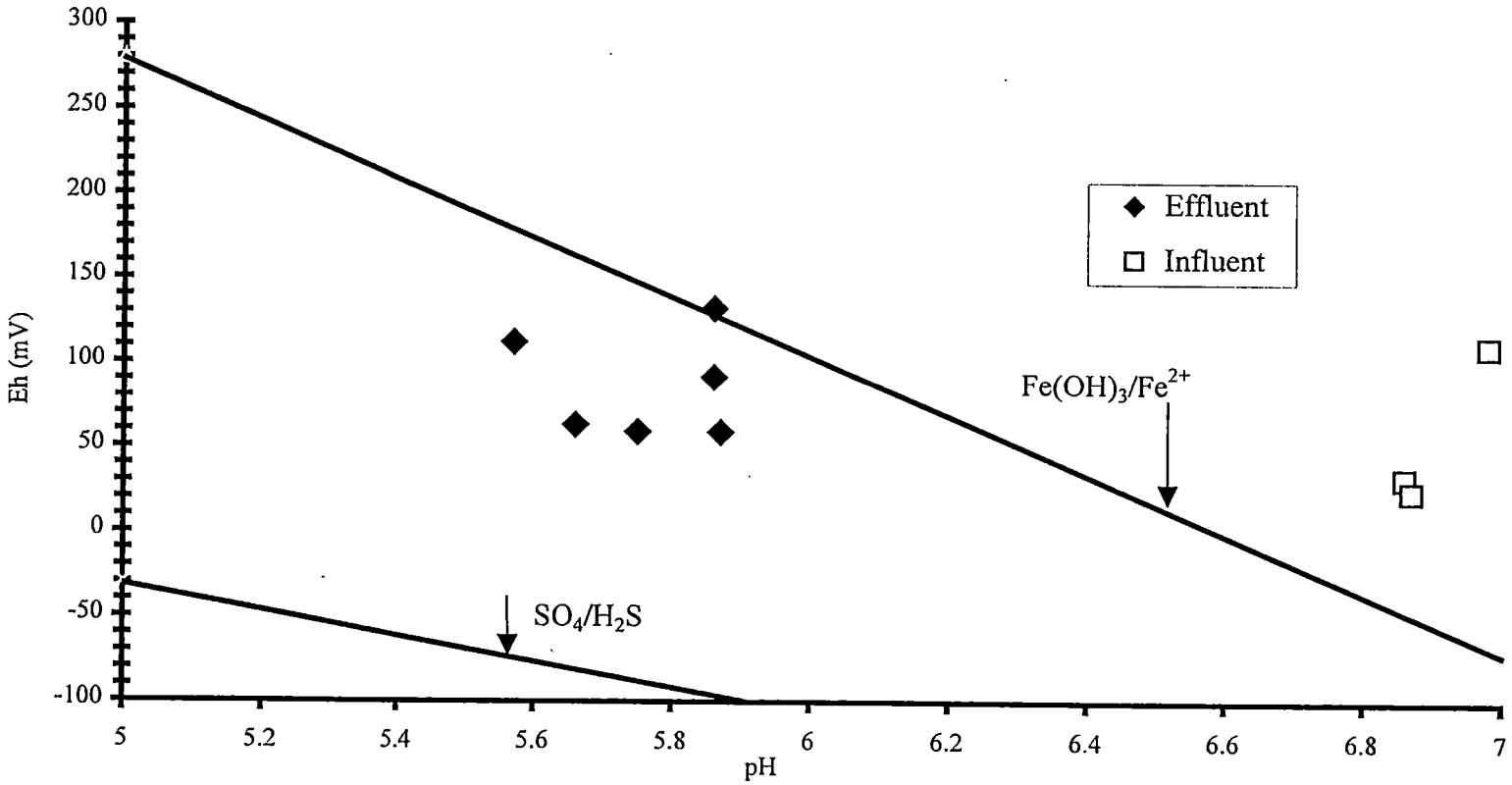


Figure 4-4. pH/Eh stability diagram showing equilbria for the Fe-(OH)3/Fe2+, SO42-/HS- redox couples (Chapelle et al., 1996).

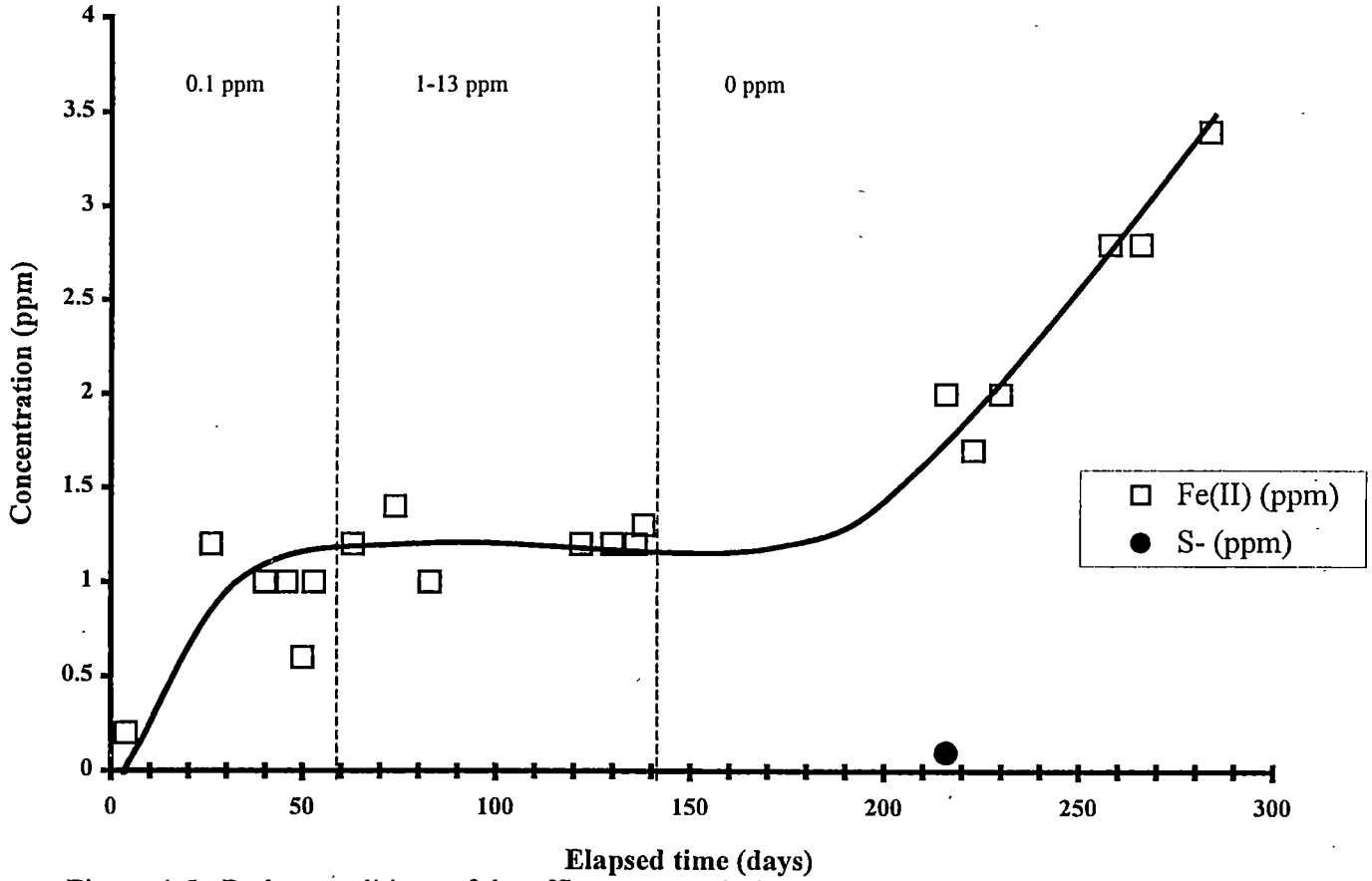


Figure 4-5. Redox conditions of the effluent water during the course of the experiment. Elapsed time is days after TCE injection. Prior to TCE injection Fe (II) was not observed in effluent.

Gossett, 1990; De Wever et al., 2000). Assessment of the microbial community was done by direct enrichment of bacteria and identification of bacteria via detection of nucleic acid.

Enrichments for iron reducing bacteria were positive in every sample throughout the duration of the experiment. Lovely and Anderson (2000) showed that after exposure of a microbial community to an organic contaminant such as hydrocarbons under anaerobic conditions, iron reducing bacteria were dominant. This result confirms the presence of iron reducing bacteria, but does not provide a concentration or abundance relative to the entire microbial community, therefore after a TCE spill it is not clear if these organisms dominate the system from this analysis. This evidence is also consistent with the Fe(II) data, which also suggest iron reducing conditions.

Sulfate reducing bacteria were detected by enrichment and by specific primers. The enrichments were positive during the course of the experiment, thus indicating the presence of these organisms. Specific primers were used to determine the main type of sulfate reducing bacteria found in this column with exposure to TCE (Daly et al., 2000). Of the 6 phylogenetic groups tested, only group number 5 produced a band from the effluent after exposure to TCE (Figure 4-6). Group number 5 consists of *Desulfococcus-Desulfonema-Desulfosarcina* which are members of Proteobacteria δ subdivision. This suggests that sulfate reducing bacteria are present, although reducing conditions in the column were not suitable for these organisms, as indicated by the lack of sulfide.

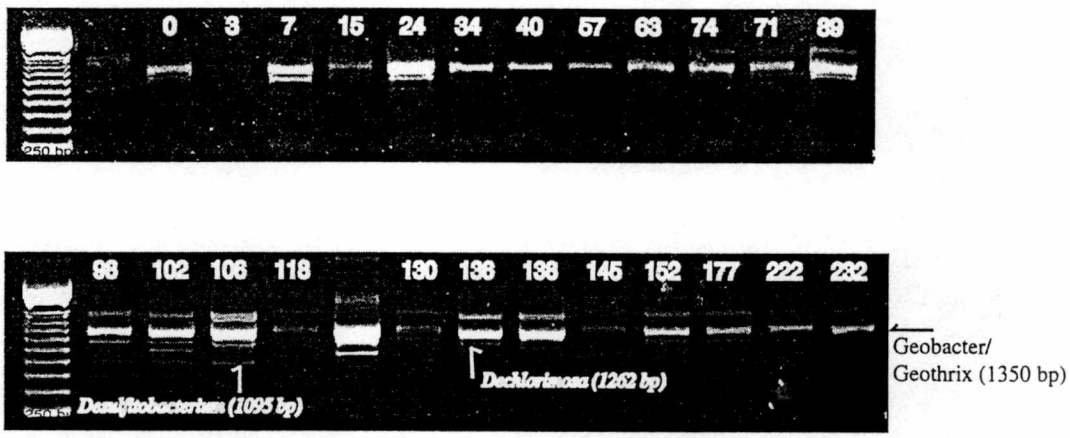


Figure 4-6. RISA gel with 250bp ladders with lanes labelled with elapsed time.

Clonal libraries were constructed to identify bacteria in the influent and effluent water samples. Clonal libraries in this experiment were constructed using universal primers (530f & 1392r) for 16S rDNA and RISA primers (1055f & 23Sr) for the intergenic region between the 16S and the 23S rDNA. Eight rDNA clonal libraries (Table 4-2) were prepared from bulk DNA extracted from influent and effluent samples and analyzed to determine microbial diversity. Tables 4-3 and 4-4 summarize the distribution by phylogenetic divisions for libraries obtained prior to TCE injection (and influent water) and after TCE injection, respectively. The size of the ribosomal interspace spacer region was determined for five of the RISA clones. It was determined that *Geobacter, Geothrix* and *Trichlorobacter* had a fragment size of 1350 bp, *Dechlorimosa* was 1262 bp and *Desulfitobacterium* was 1095 bp (Figure 4-6).

Based on the clonal sequences, a broad spectrum of bacterial and archaeal diversity was observed prior to TCE injection and in the influent water, with a shift towards diminished diversity occurring after the injection of TCE. Prior to TCE injection 2 clones were archaeal and 40 clones were bacterial. 26 clones were ≥94% identical to rDNA sequences available in GenBank as of August 2000 (Table 4-4). Phylogenetic groups represented included: Cytophaga (19%), Plantomycetales (9.5%), Proteobacteria (α) (21%), Proteobacteria (β) (12%), Proteobacteria (γ) (14%), Proteobacteria (δ) (4.7%), Firmicutes (9.5%), and Archeaon (4.7%). Approximately 1.8% of the clone sequences could not be placed into known phylogenetic groups and 1 clone was affiliated with a candidate division with no cultivated representatives (TM7 division) (Dojka et al., 1998).

Table 4-2. Clone libraries constructed from influent and effluent water.

Clone Library	Primers	Source of water	Number of Clones Sequenced	Elapsed time	Influent Concentration of TCE (ppm)
778	530f-1392r	influent	25	-40	0
716	530f-1392r	influent	7	-40	0
5	530f-1392r	effluent	10	-6	0
16	530f-1392r	effluent	12	34	0.1
22	530f-1392r	effluent	11	74	13
40	530f-1392r	effluent	15	136	0
40b	530f-1392r	effluent	16	136	0
40R	1055f-23S	effluent	11	136	0

Table 4-3: Clone library of influent and effluent water prior to TCE injection.

Clone Number[a]	Putative Division	Database Match[b]
778-18, 778-29	*Crenarchaeota*	99%, 94% uncultured archaeon WCHA1-38 (AF050613)
778-26	Unidentified bacterium	
5-11	Candidate division TM7	
778-17,778-31,778-37,716-15, 716-115	*Cytophagales*	
778-1, 778-24	*Cytophagales*	94% uncultured soil bacteria DgEP16 (AF59759)
778-39	*Cytophagales*	95% uncultured bacterium BURTON-31 (AF142849)
5-13	*Firmicutes*	99% *Rhodococcus erythropolis* hydrocarbon contaminated soil (AF2309876)
778-40	*Firmicutes*	98% High mol%G+C Gram positive bacterial (AJ225339)
716-13	*Firmicutes*	
716-12	*Firmicutes*	95% unidentified eubacterium clone BSV76 (AJ229220)
778-21	*Plantomycetales*	
778-33, 778-36;716-16	*Plantomycetales*	
778-25	*Proteobacteria* δ Subdivision	
5-10	*Proteobacteria* δ Subdivision	
778-5	Proteobacteria γ Subdivision	96% *Pseudomonas* sp. CAFB-JP4-6 fuel-hydrocarbon contaminated aquifers (AF210800)
778-3	Proteobacteria γ Subdivision	95% Uncultred gamma proteobacteria Sva0091 (UGA240987)
716-14	Proteobacteria γ Subdivision	95% iron-oxidizing lithotroph ES-1 (AF012541)
778-27	Proteobacteria γ Subdivision	95% *Xanthomonas axonopodis* (AF123091)
778-6	Proteobacteria γ Subdivision	97% *Pseudomonas* sp. J1 isolated from creosote contaminated soil (AF195877)
778-32	Proteobacteria γ Subdivision	
778-38	Proteobacteria β Subdivision	98% uncultured proteobacterium 1405-9 (UPR7650)
716-114	Proteobacteria β Subdivision	
778-8	Proteobacteria β Subdivision	97% *Ralstonia pickettii* aerobic, toluene-degrading bacteria (L37367)
5-12	Proteobacteria β Subdivision	98% *Herbaspirillum seropedicae* (AJ238361)
5-04	Proteobacteria β Subdivision	99% *Ferribacterium limneticum* Fe(III)-reducing mining-impacted freshwater lake (Y17060)
778-19	Proteobacteria α Subdivision	97% *Caulobacter* sp. (AJ227773)
778-20, 778-34	Proteobacteria α Subdivision	97-99% *Caulobacter crescentus* (AF125194)

09-May	Proteobacteria α Subdivision	98% uncultured eubacteria (AJ224988)
778-41	Proteobacteria α Subdivision	97% *Afipia* genosp. 12 (U87783)
5-08	Proteobacteria α Subdivision	100% *Methylobacterium mesophilicum* (D32225)
5-06	Proteobacteria α Subdivision	98% *Rhodobacter capsulatus* (D16427)
5-03,5-07	Proteobacteria α Subdivision	100% *Sphingomonas* (X89909)

[a]Clone number=clone library-clone number

[b]Database matches greater than or equal to 94%

Table 4-4: Clone library of effluent water after to TCE injection.

Clone Number[a]	Putative Division	Database Match[b]
40-37	Unidentified bacterium	
16-30	*Firmicutes*	99% *Microbacterium keratanolyticum* (Y17233)
40R-9	*Firmicutes*	96% *Desulfitobacterium chlororespirans* reductive dechlorination of 3-chloro-4-hydroxybenzoate (U68528)
40-47	*Cytophagales*	95% uncultured eubacterium WCHB1-69 from chlorinated-solvent-contaminated aquifer (AF050545)
40b-16	*Cytophagales*	
22-02; 40R-1, 40R-6, 40R-11	*Fibrobacter/Acidobacteria* group	99% *Geothrix fermentans* dissimilatory Fe(III)-reducing bacteria (U41563)
16-6,16-7,16-8,16-14,16-23,16-25,16-27,16-28;22-3,22-4,22-6;40-49,40b-1,40b-4,40b-10,40b-11,40b-13,40b-14,40b-15,40b-20,40b-21	*Proteobacteria* δ Subdivision	96-99% *Trichlorobacter thiogenes* Reductive dehalogenation of trichloroacetic acid (AF223382)
22-1	*Proteobacteria* δ Subdivision	
1612	*Proteobacteria* δ Subdivision	
16-18; 40-23,40-33,40-42,40-46, 40b-8,40b-9	*Proteobacteria* δ Subdivision	97% uncultured bacterium SJA-113 anaerobic,trichlorobenzene-transforming microbial consortium (AJ009487)
22-18, 40R-4	*Proteobacteria* δ Subdivision	96-98% *Geobacter arculus* (U96917)
40b-3	Proteobacteria β Subdivision	94% unidentified beta proteobacterium (AB013258)
40b-12, 40R-8,40R-12	Proteobacteria β Subdivision	97% uncultured bacterium S28 (AF072922)
40b-19, 40R-10	Proteobacteria β Subdivision	95% *Dechlorisoma* dissimilatory (per)chlorate-reducing (AF170349)
40b-24	Proteobacteria β Subdivision	98% nitrogen-fixing bacterium COL (AF214642)
40R-2	Proteobacteria β Subdivision	99% *Duganella zoogloeoides* (D14256)
40R-7	Proteobacteria α Subdivision	96% *Hyphomonas* sp (AJ224047)

[a]Clone number=clone library-clone number

[b]Database matches greater than or equal to 94%.

This profile of 16S rDNA sequences is consistent with a soil and groundwater environment.

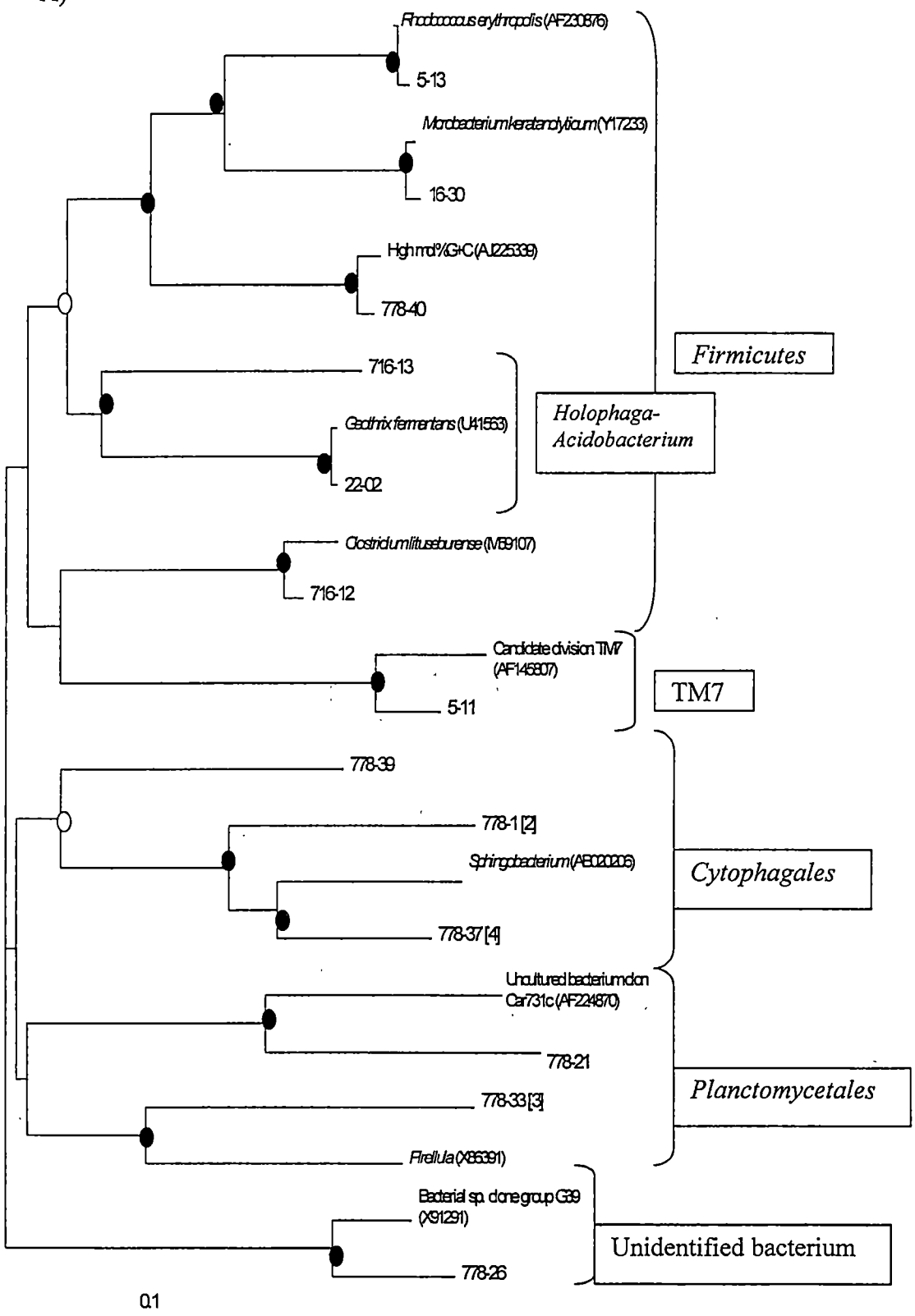
An analysis of the 16S rDNA sequences from effluent water after TCE exposure indicates a decline in microbial diversity population. Of the 65 clones sequenced, 0 clones were archaeal and 65 clones were bacterial. 61 clones were ≥ 94% identical to rDNA sequences available in GenBank as of August 2000. Many of the closest matches in Table 4-5 were anaerobic organisms. The phylogenetic groups represented include: Cytophaga (3%), Plantomycetales (0%), Proteobacteria (α) (1.5%), Proteobacteria (β) (16.6%), Proteobacteria (δ) (68%), Firmicutes (3%), and Fibrobacter/Acidobacteria (7.7%). Approximately 1.5% of the clone sequences could not be placed into known phylogenetic groups. The profile of 16S rDNA sequences is consistent with other reported Fe(III) reducing environments with organic contaminant degradation (Snoeyenbos-West et al., 2000; DeWever et al., 2000; Lovely and Anderson, 2000). The diversity of the microbial community prior to and after TCE injection is shown in an evolutionary distance tree of the bacterial domain (Figure 4-7).

4.4 Discussion and Conclusions

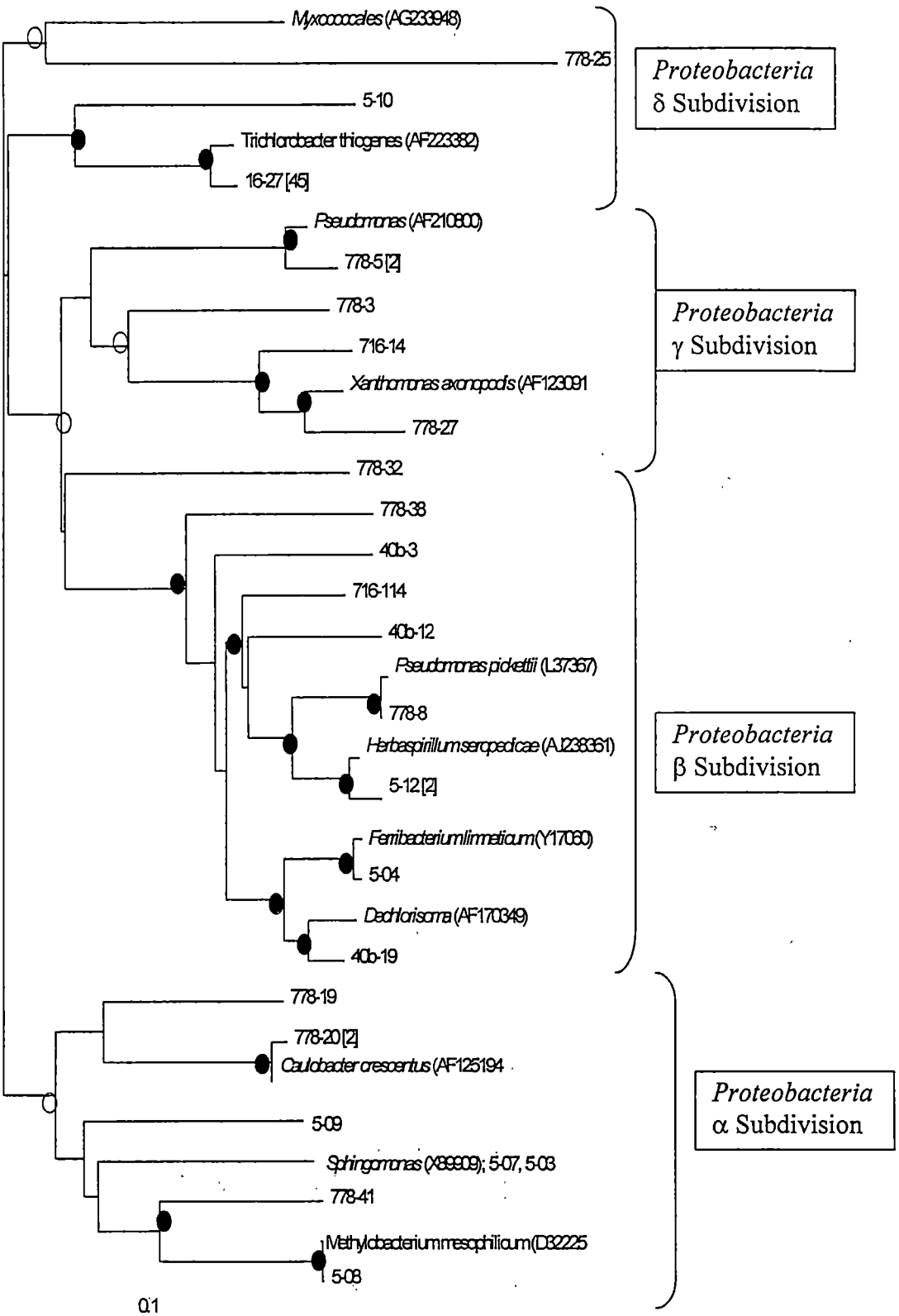
Results from this preliminary investigation suggest that microbial and geochemical conditions in the uncontaminated saprolite were favorable for anaerobic biodegradation of TCE. Changes in the influent TCE concentrations prohibited determination of the amount of mass loss of TCE due to biodegradation. Reductive dechlorination of TCE by microorganisms results in the formation of the daughter product (cDCE). Thus the

Figure 4-7: Distance matrix trees showing phylogenetic relationships of 16S rDNA clones from prior and after TCE injection. Putative divisions are listed outside the brackets for panels A, B, and C. A) *Cytophaga, Firmucutes, Plantomycetales, Holophaga/Acidobacterium*, and TM7. B) *Proteobacteria*; and C) *Archea*. Numbers in brackets indicated number of nearly identical clones found in the same library. Brach points supported by bootstrap values (number tree with same branch order per 1000 tree generated) >75% are indicated by filled circles and those supported by >50% are indicated by open circles. Genbank accession numbers are in parenthesis.

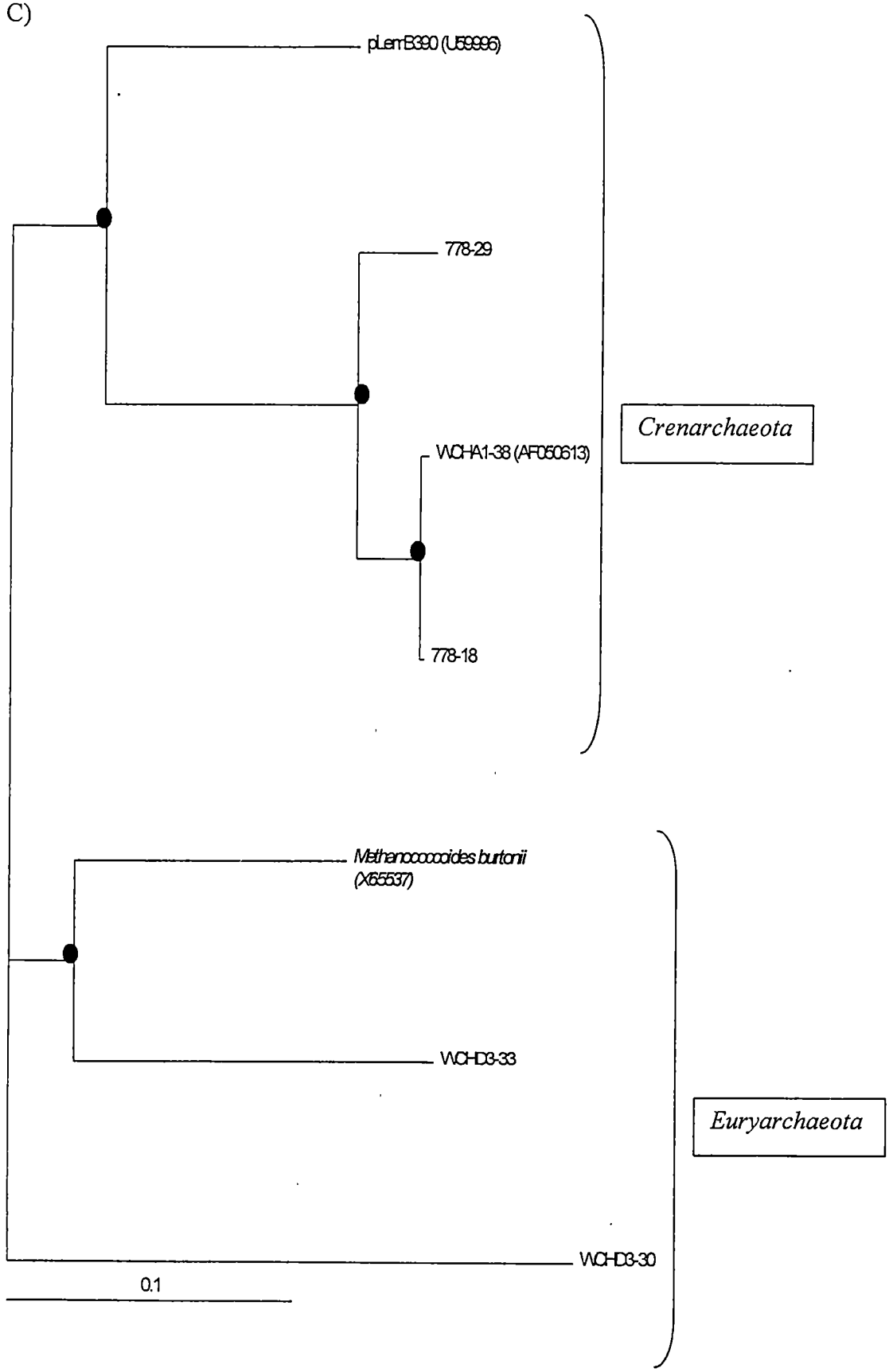
A)


Rhodococcus erythropolis (AF230876)
5-13
Microbacterium keratanolyticum (Y17233)
16-30
High mol%G+C (AJ225339)
778-40
716-13
Geothrix fermentans (U41563)
22-02
Holophaga-Acidobacterium
Firmicutes
Clostridium litusburense (M59107)
716-12
Candidate division TM7 (AF145807)
5-11
TM7
778-39
778-1 [2]
Sphingobacterium (AB020206)
778-37 [4]
Cytophagales
Uncultured bacterium clone Car731c (AF224870)
778-21
778-33 [3]
Planctomycetales
Firellula (X85391)
Bacterial sp. clone group G39 (X91291)
778-26
Unidentified bacterium
0.1

B)


Myxococcales (AG233948)
778-25
5-10
Trichlorobacter thiogenes (AF223382)
16-27 [45]
Pseudomonas (AF210800)
778-5 [2]
778-3
716-14
Xanthomonas axonopodis (AF123091
778-27
778-32
778-38
40b-3
716-114
40b-12
Pseudomonas pickettii (L37367)
778-8
Herbaspirillum serpedicae (AJ238361)
5-12 [2]
Ferribacterium limneticum (Y17060)
5-04
Dechlorisoma (AF170349)
40b-19
778-19
778-20 [2]
Caulobacter crescentus (AF125194
5-09
Sphingomonas (X89909); 5-07, 5-03
778-41
Methylobacterium mesophilicum (D32225
5-08
0.1
Proteobacteria δ Subdivision
Proteobacteria γ Subdivision
Proteobacteria β Subdivision
Proteobacteria α Subdivision

C)


pLemB390 (U59996)
778-29
WCHA1-38 (AF050613)
778-18
Crenarchaeota
Methanococcoides burtonii
(X65537)
WCHD3-33
Euryarchaeota
WCHD3-30
0.1

presence of cDCE in the effluent indicates that anaerobic biodegradation of TCE had occurred in the saprolite.

Redox conditions in the column effluent indicated that iron reducing conditions developed after exposure to TCE. Fe(II) was not observed for the 48 days prior to injection of TCE but only after 4 days the start of 0.1 ppm TCE injection into the column. The switching of the influent water from the high concentration to a TCE-free influent water caused an increase in the concentration of Fe(II) in the effluent. This suggests that the high concentration of TCE was semi-inhibitory to the iron reducing bacteria, and once the concentration of TCE decreased, bacterial activity increased leading to enhanced production of Fe(II). Alternatively, it could also signify a time dependent reaction not influenced by changing TCE concentrations. The presence of Fe(II) does indicate that reducing conditions favoring TCE biodegradation existed in the saprolite, however this does not provide conclusive evidence of a link between iron reductions and biodegradation of TCE.

Microbial community diversity decreased after the TCE injection. A larger number of clones of *Geothrix* and *Proteobacteria* δ Subdivision (*Geobacter* sp. and *Trichlorobacter*) were after exposure to TCE. These organisms have been have been implicated in iron reduction. The role of iron reducing bacteria, such as *Geobacter* sp., in the oxidation of TCE has been observed (Krumholz et al., 1996; Lovely and Anderson, 2000). These organisms have been shown to dominate 16S rDNA sequences in laboratory studies in which Fe(III) reduction was stimulated with introduction of various

organic electron donors (Snoeyenbos-West et al., 2000). *Trichlorobacter* was also found to be important in reductive dechlorination of organic compounds like trichloroacetic acid, but experiments with TCE were found to be inconclusive (De Wever et al., 2000). Molecular analysis in both field and laboratory studies have demonstrated that microorganisms of the genus *Geobacter* become dominant members of the microbial community when Fe(III) reducing conditions develop as the result of the presence of organic contaminants such as hydrocarbons (Lovely and Anderson, 2000). The injection of TCE may have initiated a shift in the microbial community to one dominated by iron reducing bacteria known to be involved in the biodegradation of organic compounds (Snoeyenbos-West et al., 2000; Lovely and Anderson, 2000; De Wever et al., 2000).

Comparative analysis of 16S rDNA sequences does not allow for definitive determination of which microorganisms are responsible for TCE biodegradation (Rooney-Varga et al., 1999). Based on these results, it is proposed that iron reducing bacteria like *Geobacter* spp. and *Geothrix* are associated with reductive dechlorination of TCE. The results from this study supporting this finding include: 1) the significant increase in iron reducing bacteria upon the addition of TCE to the column, 2) the specific enrichment of a tight phylogenetic cluster of *Geobacter* spp. and *Geothrix* not found in the uncontaminated groundwater, 3) the presence of anaerobic daughter product (cDCE), and 4) the fact that the genus *Geobacter* and *Geothrix* contains organisms known to be able to perform reductive dechlorination of organic compounds using Fe(III) as the electron acceptor. This appears to be the first study to have a potential link between these organisms and biodegradation of TCE.

In conclusion, this preliminary study tested the adequacy of the experimental design and methodologies, and provided preliminary indications that biodegradation of TCE could occur in the saprolite. The experience gained from this led to improvements in the subsequent experiment, Chapter 3, including addition of an inhibited control column. The concentration of TCE in this preliminary study ranged from 0.1 to 13 ppm and showed that even at the highest level there was some evidence of biodegradation (appearance of cDCE). Therefore in the later experiment (Chapter 3) a midrange level of 1 ppm was used. It was also determined that the Shimadzu GC-14A GC was not effective in detection of low levels of cDCE and could not detect VC. A different GC located at ORR was used for the experiment described in Chapter 3 because it has a better detection limit (10 ppb) and could analyze larger sample volumes (5 mL). The methods for determination of redox conditions were found to be adequate and were not changed for the latter experiment. Methods for determination of microbial community structure were developed during this experiment and were applied to the latter experiments. The clonal libraries indicated the dominant microorganism after exposure to TCE to be *Geobacter*, *Geothrix* and *Trichlorobacter*. This information was used to find primers for these organisms and will later be used to examine and develop probes for these important organisms. Overall, this preliminary study indicated that biodegradation of TCE can occur in fractured saprolite and aided in development of methodologies for construction of a latter study in biodegradation of TCE (Chapter 3).

Chapter 5

Conclusions and Implications

This research demonstrated that anaerobic biodegradation of TCE can occur in both fractured shale (Chapter 2) and saprolite (Chapters 3 and 4) at the Oak Ridge Reservation. In both materials, the presence or appearance of daughter products of reductive dechlorination of TCE coincide with the existence or development of redox conditions and microbial communities thought to be related to anaerobic biodegradation of TCE. Similar trends were observed in both the fractured shale and saprolite, thus indicating that biodegradation pathways in both materials are similar. This study also demonstrated that a combination of geochemical and microbiological techniques could be used effectively to determine whether or not biodegradation of TCE was occurring. This is the first scientific study into biodegradation of TCE in fractured shale and saprolite that the author is aware of, and the findings should be of interest to many regulators, consultants and researchers working in this field.

At the WAG5 site, located in fractured shale, it appears that all of the TCE and most of daughter products are removed by biodegradation within 50 m of the suspected source area (Chapter 2). Because the contaminant source history and the background conditions are uncertain, it is not clear whether biodegrading conditions developed rapidly, or only developed after many years of exposure to contamination. The laboratory experiments (Chapters 3 and 4) indicate that biodegradation of TCE is efficient in saprolite. Within 30-230 days of introducing TCE to the previously uncontaminated biotic column,

different indicators of biodegradation appeared and by the end of the experiment (250 days) the effluent concentration of TCE had been reduced by 50%. This suggests that in the shale, which is geochemically and microbiologically similar to the saprolite, biodegradation conditions can also develop rapidly. These studies imply that for TCE in shale and saprolite, natural attenuation via biodegradation will likely be an effective remediation strategy.

There is a tendency to ascribe a single dominant dechlorination pathway to an aquifer based on bulk parameters such as the presence of Fe(II), or the isolation of a microorganism (Lee et al., 1998). However many aquifers, especially in fractured shale and saprolite, can be complex heterogeneous mixtures of aerobic and anaerobic microenvironments that result from fluctuations in the water table and differences in permeability, lithology, channeling of water flow, and frequently, proximity to sources of contamination. Multiple dechlorination pathways are likely to operate in heterogeneous aquifers at the same time as indicated by the types of microorganisms and the redox conditions that were identified in this research. Thus in the natural environment TCE biodegradation is likely to be a completed by different processes.

Data obtained from this research will be applied towards creating a conceptual model for TCE biodegradation in fractured clay-rich materials. This model can then be used to predict the potential for TCE degradation in bedrock and saprolite at ORR, and in similar materials at other sites. A better understanding of the fate and transport of DNAPL in fractured clay-rich materials may allow for development of more efficient treatment or

containment strategies. Heterogeneities in the geologic materials (especially fractured materials) strongly influence DNAPL distribution and can severely limit the performance of remediation technologies. Hence, the first steps in the clean up of a contaminated site are to characterizing the geological and hydrogeological aspects of the site and determine the extent of the DNAPL contamination. Biological characterization is also needed to determine if natural attenuation via biodegradation is occurring. Techniques developed in this study could be used to help characterize sites and better understand biodegradation processes occurring in fractured shale and saprolite.

Chapter 6

Future Research

6.1 Investigations of Microbial Community Structure in Groundwater

Preliminary investigations into biodegradation of TCE (Chapter 4) developed strategies to determine the microbial communities structure via clonal libraries. The clonal library from the preliminary investigation indicated the types of microorganisms that potentially develop after exposure of saprolite to TCE. Following completion of this dissertation, clonal libraries will be constructed following methods in Chapter 4 on influent and effluent samples from the biotic column in Chapter 3. Influent water samples prior to addition of TCE and one week after exposure to TCE will be done to determine if exposing the groundwater to TCE will cause a shift in the microbiological community. Effluent water sample prior to TCE injection and 16, 64, and 141 days after exposure to TCE will also be analyzed. It is expected that the microbial community will shift and develop into a community dominated by organisms able to biodegrade TCE and will be similar to clonal libraries from the preliminary investigation.

6.2 Investigations of Microbial Community Structure in Saprolite

Additional research on the biotic column from Chapter 3 will also be done to determine if microbial communities in the column effluent (Chapter 3 and Section 6.1 above) are representative of communities in the saprolite. As well, we will examine the distribution of different microbial communities to determine the influence of fractures and geological materials on microbial community structure. This work will be conducted utilizing the

biotic column from Chapter 3. The column will be disassembled and soil lithologies mapped. The saprolite will then be sampled using microcores on a grid-like fashion (Pitner, 2000) to determine microbial community structure throughout the saprolite. Methods for determining microbial community will be done by screening samples with probes and specific primers. Clonal libraries will be used to analyze selected samples. Areas adjacent to the microcore samples will be prepared for thin sections to determine mineralogy, lithology, and fracture distribution so that correlations between microbial community and lithology can be made. It is expected that there will be systematic differences in the microbial communities, according to the type of saprolite (sandstone, siltstone, or shale). Identification of microorganisms responsible for biodegradation of TCE and correlations with effluent water, lithology, mineralogy, and fracture distribution are to aid in the remediation efforts of contaminated sites in similar geologic materials. This future research, plus clonal libraries from the preliminary experiment (Chapter 4), will lead to an additional publication from this dissertation research.

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Appendices

Appendix 1: VOC and redox data from WAG5 collected during 1998 and 1999.

Well	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17a	17b
Date	10/01/1997																	
O2 (ppm)	0.582	0.016	0.02	0.74	0.02	1.2	474.8	1.54	0.7957	0.03	4.3	0.016	0.564	0.02	0.02	0.02	0.614	
NO3	0.3	0.3	0.4	0.4	0.3	0.4	0.4	0.3	0.4	.4.	0.4	0.3	0.4	0.4	0.5	0.5	0.4	
Fe(II)	2.12	1.88	1.07	1.47	0.99	1.2	2.96	0.76	4.08	3.16	2.05	10.75	2.96	6.1	0.24	1.02	5.12	
SO4																		
S	0.003	0.027	0.002	0.015	0.004	0.017	0.007	0.006	0.01	0.005	0.104	0.035	0.14	0.101	0.015	0 011	0.092	
CH4																		
Eh																		
Temp																		
Cl																		
BTEX																		
TCE ppb	2.49	4.04	0.8	1.43	0 82	0.83	0.55	0.52	0.32	0.32	5.97	1.78	3.48	1.44	1.6	0	0	0.7
DCE ppb	9.48	16.35	1 97	7.67	2.14	7.92	5.88	6.32	2.96	3.02	24.17	8.71	17.8	11.29	13.56	4.41	13.57	8.6
VC ppb	0	5.15	1.51	4.05	2.46	5.27	3	3.84	4.01	4.08	5.47	5.1	4.65	5.39	5.19	4.36	5.45	6.26
CA																		
Date	12/01/1997																	
O2 (ppm)																		
NO3																		
Fe(II)																		
SO4																		
S																		
CH4																		
Eh																		
Temp																		
Cl																		
BTEX																		
TCE ppb	1.73	4.22	0.68	1.9	0	1.23	0.7	0	0	0	6.95	2.87	3.66	1.82	1.92	0	0	0
DCE ppb	7.32	15.74	1.81	9.6	3 49	11.09	8.26	7.03	3.97	3.58	25.97	12.23	18.89	11.05	16.56	6.09	7.51	0
VC ppb	5.08	17.78	4 82	13.66	8.54	15.85	12.85	11.62	10.83	9.84	13.95	14.52	16 01	14.52	15.55	13.82	7.3	0
CA																		
Date	2&3/98																	
O2 (ppm)	0.744	0.02	0.016	0.5563	0.02	0.505	0.548	0.832	0.5487	0.392	0.02	1.22	0.02	1.62	0.016	1.41	0.012	5.4
NO3	0.143	0.3	0.3	0.4	0.4	0.5	0.5	0.5	0.6	0 5	0.4	0.4	0.4	0.4	0.4	0.4	0.9	0.5
Fe(II)	2	1.81	1.04	1.61	0.99	1.06	3.68	0.64	7.04	6.08	1.82	4.26	3.66	8.28	0.24	1.42	4.26	9.2
SO4(3/99)	3.1449	3.3498	1.6188	2.2533	3.3372	2.7236	3.171	3.9075	3.407	3.7536	4.4853	2.763	4.23	4.508	4.4191	3.1901	5.6053	
S	0.01	0.04	0	0.01	0.01	0.04	0.02	0	0.01	0	0.05	0.05	0.04	0	0.03	0.01	0.05	0.01
CH4	6.267	4.857	3.853	2.926	5.227	1.78	1.245	1.367	3.17	2.781	1.613	7.843	3.46	1.046	0.916	0.649	0.2	1.758
Eh (not te	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Temp	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Cl (3/99)	11.4539	12.3896	10.0531	10.2603	8.166	12.207	12.1442	10.2204	9.9066	9.8765	7.5845	11.5907	11.3626	11.6062	11.2221	10.6352	10.09	
BTEX	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TCE ppb	3.51	7.31	1.67	3.22	1.65	2.22	1.33	1.4	0	0	13.16	4.74	6.62	3.18	3.77	0	0	0
DCE ppb	12.5	24.37	3.26	13.58	4.58	20.17	10.61	15.64	5.87	5.88	49.14	25.69	34.006	26.15	29.15	10.05	23.94	0
VC ppb	13.76	19.09	5.43	11.57	7.79	18.04	13.12	16.32	10.62	10.14	20.71	19.74	20.52	19.2	19.36	13.37	17.4	0
CA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Date	May-98																	
O2 (ppm)																		
NO3																		
Fe(II)																		
SO4																		
S																		
CH4	4.176	3.908	3 319	2.46	3.098	1.08	1.014	0.909	3.481	3.609	1.909	6.289	4.136	1.835	1.028	0.884	0.227	
Eh																		
Temp																		
Cl																		
BTEX																		
TCE ppb	0	0	0	0	0	0	0	0	0	0	5.49	0	0	0	0	0	0	0
DCE ppb	22.77	16.09	0.66	3.02	0.71	6.09	2.96	4.74	0.53	0.5	15.94	7.83	10.7	9.39	10.16	2.75	8.67	0
VC ppb	2.38	2.99	0.83	1.54	1.08	3.03	2	2.58	1.93	1.68	3.77	3.86	4.01	3.71	3.97	1.43	3.51	1.07
CA																		
Date	Jun-98																	
O2 (ppm)																		
NO3																		
Fe(II)																		
SO4																		
S																		
CH4	3.194	3.758	2.203	1.818	3.214	0.464	0.647	0.552	1.978	1.266	1.741	6.173	1.683	1.101	0.671	0.243	0.193	
Eh																		
Temp																		
Cl																		
BTEX																		
TCE ppb	0	2.41	0	0.9	0	0.76	0	0	0	0	5.15	1.08	1.97	1.06	1.22	0	0	
DCE ppb	4.56	23.37	1.12	3.74	1.5	6.91	4.04	5.67	2 12	2.23	18.46	5.06	11.1	9.45	10.34	3.23	9 94	
VC ppb	3.15	4.52	1.14	2.3	1.73	2.59	2.45	2.81	2.21	2.27	4.94	3.94	4.3	3.74	3.85	2.2	3.93	
CA																		
Date	6&8/99																	
O2 (ppm)	2	3	1.62	1.68	2	4	1.51	3	0.98	1.21	1.96	2.05	1.37	3	1.33	1.62	1.46	
NO3	3 3	4	3.6	3.6	3	3.6	2.6	3 6	4.3	4.6	5	3.6	4.3	4.3	4.3	3	4	
Fe(II)	2.06	1.53	1.07	1.53	1.11	1.27	2.34	0.72	2.55	2.6	1.59	3.84	3.29	1.93	0.2	0.53	3.2	
SO4	1.9967	3.3464	0.5517	2.2514	1.3653	2.5359	2.5537	2.2843	2.5604	2.7881	3.5406	1.7664	3.2905	3.0606	3.5779	2.3105	4.3644	
S	0.005	0.047	0.004	0.003	0.008	0.061	0.138	0.002	0.01	0.004	0.143	0.0055	0.018	0.046	0.077	0.012	0.047	
CH4	1.9535	1.6027	2.7065	1.2451	1 9906	0.4264	1.5789	0.2009	3.3838	1.9968	1.0322	7.0727	1.3865	0.905	0.9835	0.5771	0.2487	
Eh (not te	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Temp	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Cl	16.4458	18.0375	14.1441	16.52	15.9829	15.8961	16.6559	17.4767	16 9112	17.2007	17.6604	18.6878	19.133	17.1442	17.7166	17.1291	15.6532	
BTEX	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TCE ppb	0.4609	1.7304	0	0.7615	0	0.7147	0	0	0	0	34.2779	2.0347	2.4479	1.5066	1.1355	0	0	
DCE ppb	5.0257	9.8961	1.4858	5.3906	2.4557	9.4054	5.902	6.4994	3.2138	3.2214	28.8889	11.2921	17.0343	15.4537	13.4261	5.5522	14.0499	
VC ppb	4.1513	5.0777	0	2.4454	0	3.4971	2.648	1.0697	1.989	2.932	5.5584	4.0119	4.3063	3.5697	2.8572	2.1448	3.3173	
CA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Well	18a	19a	19b	19c	2-4	2-5	4-3	4-4	A	B	C	D	E	F	G	H	I	J	K
Date																			
O2 (ppm)	1 34	0.018		0.02	0.4205		0.487	0.016											
NO3	0.9	0.5		0.7	0.5	0.4	0.4	0.6											
Fe(II)	1.23	7.55		5.25	1.34	3.68	2.24	2.66											
SO4																			
S	0.031	0.015		0.052	0 347	0.072	0.002	0.049											
CH4																			
Eh																			
Temp																			
Cl																			
BTEX																			
TCE ppb	0	0																	
DCE ppb	0.68	0.74																	
VC ppb	3.93	2.48																	
CA																			
Date																			
O2 (ppm)																			
NO3																			
Fe(II)																			
SO4																			
S																			
CH4																			
Eh																			
Temp																			
Cl																			
BTEX																			
TCE ppb	0		0	0	0	0	0	0											
DCE ppb	4.28		0	0.27	0	0	0	0											
VC ppb	14.41	9.68	0	6.31	0	0	0	0											
CA																			
Date																			
O2 (ppm)	1.33	0.28	0.016	0.012	0.873	1.49	1.18	4.72											
NO3	0.5	0.5	0.5	0.5	0.5	0.4	0.4	0 6											
Fe(II)	5	8.56	5.27	5.88	1.18	2.48	4.89	4.35											
SO4(3/99)	4.0386	6.2601		5 5503	2.8574	2.2377	3.3021	2.2109											
S	0.04	0.01		0.09	0.3	0.04	0	0.01											
CH4	1.875	0.923	0.989	1.426					0.133	1.709	1.59	3.72	2.377	2.227	2.466	1.153	4.145	7.66	2.081
Eh (not te	0	0	0	0	0	0	0	0											
Temp	10	10	10	10	10	10	10	10											
Cl (3/99)	10.917	11.1628		11.3479	10.638	10.0209	9 8885	9.5373											
BTEX	0	0	0	0	0	0	0	0											
TCE ppb	0	0	0	0	0	0	0	0	0	0	0	0	0.8	0	0.8	25.31	1.8	0	0
DCE ppb	14.76	0	0	0	0	0	6.16	3.82	0	0	0	0.48	1.94	0	0.3	25.89	10.05	0	1.92
VC ppb	16	12.14	7.79	6.83	0	3.18	8.48	9.65	0	0	1.2	2.42	6.82	0	1.8	13.01	9.64	0	1.04
CA	0	0	0	0	0	0	0	0											

Date																			
O2 (ppm)																			
NO3																			
Fe(II)																			
SO4																			
S																			
CH4	1.123	0.635		1.162		3.536	1.439	5.939	0.607	0.758	0.55	2.839	1.595	1.52	1.896	2.626	1.699	11.115	2.815
Eh																			
Temp																			
Cl																			
BTEX																			
TCE ppb	0	0	0	0		0	0	0											
DCE ppb	4.66	0	0	0		0	1.55	0.66											
VC ppb	3.45	2.54	1.27	1.48		1.36	1.59	1.59											
CA																			
Date																			
O2 (ppm)																			
NO3																			
Fe(II)																			
SO4																			
S																			
CH4	0.942	0.504	0.665	0.712	3.142	2.671	2.847	3.817	0.736	0.658	3.93	3.718	1.676	1.203	1.708	3.892	2.542	19.032	5.51
Eh																			
Temp																			
Cl																			
BTEX																			
TCE ppb	0	0	0	0	0		0	4.05	0	0	0	0	0	0	0	0	5.13	0	0
DCE ppb	5.57	0	0	0	0.64		1.98	7.9	1.59	0	0	0	3.07	0.7	0	44.1	2301	2.36	2.99
VC ppb	3.98	2.97	1.91	1.71	1.54		2.06	2.07	0	0	0.87	1.65	5.15	2.59	1.11	23.75	12.28	2.56	2.61
CA																			
Date																			
O2 (ppm)	4	3		1.81	1.74	4	0.85	4		2.05	4	4	3	3	3	4	4		3
NO3	2.3	1		1	5.6	4.6	3.6	1		1	5	0	5.4	4.4	1.9	0	4		1.9
Fe(II)	3.54	5.94		4.1	0.99	2.15	4.16	3.1		13.68	7.68	13.68	15.04	13.92	4	12.52	6.36		6.56
SO4	1.8163	6.135		4.9822	3.3808	0.195	2.1312	0 6907		0.366	0.2815	0	2.1009	1.8098	0.5268	0.6713	3.1267		0.999
S	0.033	0.011		0.02	0.384	0.082	0.027	0.017		0.012	0.005	0.021	0.074	0.006	0.022	0.047	0.752		0.224
CH4	1.8247	0.9878		0.3211	13.8628	3.3899	2.4738	1.3276		1.2892	0.8627	2.1461	2.1055	2.725	1.4369	5.0916	2.3286		1.9298
Eh (not te	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Temp	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Cl	16.3869	17.589		17.2855	10.5921	15.4847	16.6846	17.4717		4.1551	3.1841	2.0567	11.0711	8.9815	18.4727	5.4138	15.1128		23.1757
BTEX	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TCE ppb	0	0			0	0	0	0		0	0	0	0	0	0.3613	3.4832	7.391	0	0 4517
DCE ppb	7.7609	0.8015			1.2988	0	3.4994	2.9742		1.028	0	0	5.2956	4.3572	2.6643	31.8239	34.5786	3 2631	2.6759
VC ppb	2.5421	1.731			0	0	3.3013	3.1476		0	0	0	2.3893	3 9239	0.9125	14.8222	16.9276	1 8997	0 8656
CA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix 2: WAG5 ICAP analysis for samples collected 9/29/99.

ICAP Analysis for samples collected 9/2/99
**** All concentrations are in ppm**

Well	Al	Ca	Cd	Co	Fe	K	Mg	Mn	Na	Si
1	0.0487	98.45	0.0539	0.3470	2.711	3.702	13.49	9.133	30.75	11.30
2	0.0516	112.5	0.0434	0.2026	1.329	3.567	14.34	5.958	35.31	10.29
3	0.0611	123.6	0.0398	0.1765	0.9785	2.933	12.41	1.270	25.96	11.87
4										
5	0.0506	129.3	0.0420	0.1565	0.8765	3.232	12.35	8.370	29.29	11.93
6	0.0497	129.9	0.0421	0.0800	0.0000	3.450	14.18	8.752	34.90	10.55
7	0.0344	135.2	0.0521	0.3524	2.324	2.601	13.54	5.042	33.26	11.92
8	0.0626	131.1	0.1061	0.1691	0.1864	3.539	14.25	11.01	36.09	9.985
9	0.0397	131.2	0.0541	0.4290	2.647	3.729	14.06	5.323	33.94	12.39
10	0.0311	134.8	0.2809	0.4502	2.686	3.931	14.29	5.375	34.54	12.56
11	0.0740	124.0	0.0470	0.1380	0.5281	2.864	14.17	16.83	30.85	12.19
12	0.0392	117.2	0.0451	1.5970	13.73	2.477	14.58	5.140	36.45	9.951
13	0.0358	127.5	0.0414	0.9569	8.091	2.347	14.50	5.863	34.95	11.38
14	0.0406	134.4	0.0406	0.0791	0.0339	3.091	14.88	8.428	36.82	10.10
15	0.0483	134.8	0.0372	0.0807	0.0481	3.056	14.81	8.625	34.72	10.28
16	0.0702	135.8	0.1571	0.2851	0.6979	2.836	14.17	6.303	36.13	11.79
17a	0.0239	162.2	0.0430	0.4509	2.135	3.701	13.99	1.093	28.65	8.731
18a	0.0420	144.1	0.0442	0.3574	1.446	4.293	14.03	5.220	26.18	7.622
19a	0.0339	155.5	0.0423	0.3010	2.186	4.699	22.12	0.3866	16.01	11.62
19c	0.0568	156.4	0.0424	0.3953	2.982	4.722	22.14	0.3954	17.20	11.61
2d4	0.0368	165.8	0.0745	0.6038	2.936	3.927	16.71	1.306	23.82	9.769
2d5	0.0387	178.2	0.0420	0.3120	2.208	3.897	16.04	1.385	18.25	9.853
4d3	0.0210	137.5	0.1360	0.3930	2.760	2.959	13.85	7.121	30.33	13.49
4d4	0.0487	152.7	0.0700	0.5150	3.715	4.811	13.64	4.807	26.87	12.95
A	*	D	R	Y	*	W	E	L	L	*
B	0.1112	43.97	0.0472	3.209	28.86	8.258	13.85	12.39	4.208	3.449
C	0.052	41.98	0.0457	1.82	16.02	9.597	16.28	15.41	3.806	3.710
D	0.3518	32.45	0.0503	2.706	24.88	20.90	14.04	6.80	1.946	4.516
E	0.0774	73.79	0.0495	3.562	32.94	5.304	15.59	8.247	17.61	5.556
F	0.074	74.94	0.0467	1.473	13.57	6.767	15.00	13.63	18.62	8.769
G	0.3399	37.29	0.0452	8.2210	8.991	3.703	16.97	18.60	33.58	4.529
H	0.0263	95.58	0.0434	0.7687	7.061	7.426	51.23	0.5368	18.48	13.25
I	0.0258	146.7	0.0421	0.4101	3.549	3.786	12.71	1.507	25.37	7.081
J	*	D	R	Y	*	W	E	L	L	*
K	0.0225	105.5	0.0456	1.52	15.05	5.948	15.65	1.506	8.164	3.507

Appendix 3: WAG5 sequence alignment from Archae clone library from well 10 and 11. Reference strains are listed with accession numbers.

CLUSTAL X (1.64b) multiple sequence alignment

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Dp11u5                          CACAAGTGGTAGTCGGGTTTTATTGGGCCTAAAGCGTTCGTAGCCGGGCA
Dp10u34                         CACAAGTGGTAGTCGG-TTTTATTGGGCCTAAAGCGTTCGTAGCCGGGCA
Dp11u25                         CACTAGTAGT-GTCCGTGAATATTGCGTTTAAAGCGTTCGTAGCCGGCTA
pGrfc26_U59986_                 TCCGAGT-GGTGTGGATGTTTATTGGGCCTAAAGCATCCGTAGCTGGCTA
pLemB390_U59996_                TCCGAGT-GGTGTGGATGTTTATTGGGCCTAAAGCATCCGTAGCTGGCTA
WCHA1-38_AF050613_              CTCAAGT-GGTCAGGATGATTATTGGGCCTAAAGCATCCGTAGCTCGTTT
WCHD3-30_AF050612_              TCCAAGT-CGCAGCCATCATTATTGGGTCTAAAACATCCGTAGCTTGCTT
Dp11u27                         TCCAAGT-CGCAGCCATCATTATTGGGTCTAAAACATCCGTAGCTTGCTT
Dp10U1                          CATAAGT-TGTGTCCACTAATATTGGGCCTAAAGCGTCTGTAGCCTGTCC
Dp11u2                          CTCTAGT-GGTA-CCATTTTTATTGGGCCTAAAGCGTTCGTAGCCGGTTT
Dp10u12                         -GCCGAT-GGTAACCGTTTTTATTGGGTTTAAAGGGTCCGTAGCCGGCTT
Methanoccoidesburtonii_X65537_  CCCGAGT-GGTAATCACTTTTATTGGGTCTAAAGGGTCCGTAGCCGGTTT
Methanosarani_L48408_           CTCAAGT-GGTGGCCGCTATTATTGGGCTTAAAGGGTCCGTAGCCGGACC
Soyang1Af-1100Ar_AF056368_      CTCGAGTTGGTGGCCACTATTACTGGGCTTAAAGCGTTCGTAGCTGGTCT
Methanobrevibacter_AB009827_    CTCTAGT-GGTAGCCATTTTTATTGGGCCTAAAGCGTTCGTAGCCGGTTT
Dp11u31                         CACGAGT-GGCAACCGATATTATTGGGCCTAAAGCGTTCGTAGCTGGCCT
WCHD3-33_AF050619_              CCCGAGT-GGTGGTCGATATTATTGAGCCTAAAACGTTCGTAGCCGGTCT
Dp11u20                         CCCAAGT-GGTGATCACGTATATTGGGTCTAAAGCATTCGTAGCCGGTTT
                                      *              ** ** *  ****   *  *****  *

Dp11u5                          TTCAAGTCCTTGGGTAAATTCGGCAGCTCAACTG--TCGGAATTCCGAGG
Dp10u34                         TTCAAGTCCTTGGGTAAATTCGGCAGCTCAACTG--TCGGAATTCCGAGG
Dp11u25                         AGCAAGTTTTCTGTGAAATCTTTCGGCTCAACCGAATAGGCTTGCAGAAA
pGrfc26_U59986_                 GGTCAGTCCCTTGTTAAATCCACCGAATTAATCGT-T-GGATTGCGGGGG
pLemB390_U59996_                GGTCAGTCCCTTGTTAAATCCACCGAATTAATCGT-T-GGATTGCGGGGG
WCHA1-38_AF050613_              TGTAAGTTTTCGGTTAAATCCATGCGCTTAACGTA-T-GGGCTGCCGGGA
WCHD3-30_AF050612_              ATTAAGTTTCTTGTGAAATCTTATCTCTTAAGGAT-AAGGCGTGCAAGAA
Dp11u27                         AATAAGTTCCTTGTGAAATCTCATCTCTTAAGGAT-GAGGCGTGCAAGGA
Dp10U1                          AAAAAGTTTTTGGTGAAATCTACAAGCCTAACTTG-TAGGCGAGCCAAAA
Dp11u2                          GATAAGTCTCTGGTGAAATCCTATAGCTTAACTGT-GGGACTTGCTGGAG
Dp10u12                         ACTAAGTCTCTTGGGAAATCTGGCGGCTCAATCGT-CAGGCGGCCAAGAG
Methanoccoidesburtonii_X65537_  GATCAGTTCTTCGGGAAATCTGACAGCTCAACTGT-TAGGCTTCCGGGGA
Methanosarani_L48408_           AGTTAGTCCATTGGGAAATCTTACGGCTTAACCGT-AAGGCTGCCAGTGG
Soyang1Af-1100Ar_AF056368_      GTTAAGTCTCTGGGGAAATCTACTGGCTTAACCDA-TAGGCGTCTCAGGG
Methanobrevibacter_AB009827_    AATAAGTCTCTGGTGAAATCCTACAGCTTAACTGT-GGGAATTGCTGGAG
Dp11u31                         TGTAAATCCTCTGTGAAATCGTTTTGCTTAACATA-ACGGCGCGCAGGGG
WCHD3-33_AF050619_              TGTAAATCCTTGGGTAAATCGGCCAGCTTAACTGT-CCGAAGTCC-GGGG
Dp11u20                         GTTAAGTCTTCTGTGAAATCTGATAGAA-AACTAT-CAGGCGTGCAGGAG
                                    * *     *  ****          **       *

Dp11u5                          ATACTGTTTGTCTTGAGGTCGGGTGAAGGTGTGGGCACTTCTGGAGTAGG
Dp10u34                         ATACTGTTTGTCTTGAGGTCGGGTGAAGGTGTGGGTACTTCTGGAGTAGG
Dp11u25                         ATACTACTTGGCTCGAGAGTGGGGGAAGCTAAAGGTACTGTANGGGGAGC
pGrfc26_U59986_                 ATACTGCTTGGCTAGGGGACGAGAGAGGCAGACGGTATTTTCGGGGTAGG
pLemB390_U59996_                ATACTGCTTGGCTAGGGGGCGAGAGAGGCAGACGGTATTTTCGGGGTAGG
WCHA1-38_AF050613_              ATACTGCATAACTAGGAAGTGGGAGAGGTAGACGGTACTCGGTAGGAAGG
WCHD3-30_AF050612_              ATACTGTTAAGCTAGAGACTGGAAGACGTAGAAAGTATGTCTAAAGTAGC
Dp11u27                         GTACTGCTAAGCTAGAGACTGGAAGACGTAGAAAGTATGTATAAAGTAGC
Dp10U1                          ATACTCTTGGACTCGAGGCCGGGAGAAGTCAAAGGAATTCCTGAGGTAGC
Dp11u2                          ATACTATTAGACTTGAGGTCGGGAGAGGCCCGCGGTACTCCCAGGGTAGG
Dp10u12                         ATACTGGTAGGCTTGGGACCGGGAGAGGTGGGAGGTACTCCAGGGGTAGG
Methanoccoidesburtonii_X65537_  ATACTGTCAGACTTGGGACCGGGAGAGGTAAGAGGTACTACAGGGGTAGG
Methanosarani_L48408_           ATACTGCTGGTCTTGGGACCGGGAGAGGCAAGAGGTACTTCAGGGGTAGG
Soyang1Af-1100Ar_AF056368_      ATACTGGCAGACTAGGGACCGGGAGAGGTGAGGGGTACTCCAGGGGTAGG
Methanobrevibacter_AB009827_    ATACTATTAGACTTGAGGTCGGGAGAGGTTAGAGGTACTCCCAGGGTAGG
Dp11u31                         ACACTGCTTGGCTTGGGACCGGGAGAGGTAGGGGGTATTTCTTGGGGAGC
WCHD3-33_AF050619_              AGACTGCAAGACTTGGGATCGGGAGAGGTCAGAGGTACTTCTGGGGTAGG
Dp11u20                         GTACTGGCAAGCTTGGAACCGGGAGGAGCCTGGAGTACTTTTAGGGTAGG
                                  ***      ** *     *   *  *      * *        * **

Dp11u5                          GGTGAAATCTTCTGATTCCAGGGGGACCGCCTGTGGTGAAAACGCACACT
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Dp10u34                                 GGTGAAATCTTCTGATTCCAGGGGGACCGCCTGTGGTGAAAACGCACACC
Dp11u25                                 GGTAAAATGCTGTAATCCTTGCAGGACCACCAGTGGCGAAGGCGTTTATC
pGrfc26_U59986_                         GGTGAAATCCTATAATCCCGGGAAGACCACCAGTGGCGAAGGCTGTCTGC
pLemB390_U59996_                        GGTGAAATCCTATAATCCCGGGAAGACCACCAGTGGCGAAGGCTGTCTGC
WCHA1-38_AF050613_                      GGTAAAATCCTTTGATCTATCGATGACCACCTGTGGCGAAAGCGGTCTAC
WCHD3-30_AF050612_                      GGTAAAATGTGTTAATCTTAGGCAGACTCACAACAGCGAAGGCATTCTAC
Dp11u27                                 GGTAAAATGTGTTAATCTTATGCAGACTCACAACAGCGAAGGCATTCTAC
Dp10U1                                  GGTGAAATGCTATGATCTTAGGAGGACCACCAGTAGCGAAGGCGTTTGAC
Dp11u2                                  GGTGAAATCCCGTAATCCTGGGAGGACCACCTATGGCGAAGGCGGCTCAC
Dp10u12                                 GGTGAAATCTCGTAACCCTTGGGGGACCACCGATGGCGAAGGCATCCCAC
Methanoccoidesburtonii_X65537_          AGTGAAATCTTGTAATCCCTGTGGGACCACCAGTGGCGAAGGCGTCTTAC
Methanosarani_L48408_                   AGTGAAATCCTGTAATCCTTGAGGGACCGCCAGTGGCGAAGGCGTCTTGC
Soyang1Af-1100Ar_AF056368_              AGTGAAATCCTGTAATCCTTGGGGGACCACCTGTGGCGAAGGCGCCTCAC
Methanobrevibacter_AB009827_            GGTGAAATCCTGTAATCCTGGGAGGACCACCTGTGGCGAAGGCGTCTAAC
Dp11u31                                 GGTAAAATGTTATAATCCAAGAAGGACCACCTGTGGCGAAGGCGCCCTAC
WCHD3-33_AF050619_                      GGTAAAATCCTGTAATCCTAGAAGGACCACCGGTGGCGAAGGCGTCTGAC
Dp11u20                                 GGTAAAATCCTGTAATCCTAAAGGGACTACCTGTGGCGAAGGCGCCAGGC
                                         ** ****    * *         ***   *    * ***  *

Dp11u5                                  TAGAACGAGCCTGACGGTGAGGAACGAAACCCAGGGGAGCGAACGGGATT
Dp10u34                                 TAGAACGAGCCTGACGGTGAGGAACGAAACCCAGGGGAGCGAACGGGATT
Dp11u25                                 TAAAACACGTCTGACGGTGAGGGACGAAAGCTATGGGAGCAAACGGGATT
pGrfc26_U59986_                         TAGAACGCGCCCGACGGTGAGGGATGAAAGCTGGGGGAGCGAACCGGATT
pLemB390_U59996_                        TAGAACGCGCCCGACGGTGAGGGATGAAAGCTGGGGGAGCGAACCGGATT
WCHA1-38_AF050613_                      CAGAACACGTTCGACGGTGAGGGATGAAAGCTGGGGGAGCAAACCGGATT
WCHD3-30_AF050612_                      GAGGACAGTTCTGACAGTAAAGGATGAAGGCTAGGGGCGCAAAGTGGATT
Dp11u27                                 GAGGACAGTTCTGACAGTAAAGGATGAAGGCTAGGGGCGCAAAATGGATT
Dp10U1                                  TAGAACGGACCTGACGGTGAGAGACGAAAGCTGGGGGATCGACCCGGATT
Dp11u2                                  TGGAACGAGTCCGACGGTGAGGGACGAAAGCCGGGGGCGCGAACCGGATT
Dp10u12                                 CAGAACGGGTCCGACGGTGAGGGACGAAAGCTGGGGGCACGAACCGGATT
Methanoccoidesburtonii_X65537_          CAGAACGGGTCCGACGGTGAGGGACGAAAGCTGGGGGCACGAACCGGATT
Methanosarani_L48408_                   TAGAACGGGTCCGACGGTGAGGGACGAAAGCTAGGGGCACGAACCGGATT
Soyang1Af-1100Ar_AF056368_              CAGAACGGCTCCGACAGTGAGGGACGAAAGCTGGGGGAGCAAACCGGATT
Methanobrevibacter_AB009827_            TGGAACGAACCTGACGGTGAGGGACGAAAGCTAGGGGCGCGAACCGGATT
Dp11u31                                 TAGAACGGATCCGACAGTGAGGAACGAAAGCCAGGGGAGCAAAATGGATT
WCHD3-33_AF050619_                      TAGAACGAATTCGACGGTGAGGAACGAAGCCCTGGGGCGCAAACGGGATT
Dp11u20                                 TAGAACGGATTCGACGGTGAGGAATGAAAGCGAGGGGAGCAAAGGGGATT
                                            **       *** ** *   * ***  *   ***  * *   *****

Dp11u5                                  AGATACCCCGGTAGTCCTGGGC----GTAAACGATGTCCGTTTGGTGTTG
Dp10u34                                 AGATACCCCGGTAGTCCTGGGC----GTAAACGATGTCCGTTTGGTGATG
Dp11u25                                 AGATACCCCGGTAGTCCTGGGC----GTNAACGATGTCCGTTTGGTGTTG
pGrfc26_U59986_                         AGATACCCGGGTAGTCCCAGCT----GTAAACGATGCAGACTAGGTGTTT
pLemB390_U59996_                        AGATACCCGGGTAGTCCCAGCT----GTAAACGATGCAGACTAGGTGTTT
WCHA1-38_AF050613_                      AGATACCCGGGTAGTCCCAGCT----GTAAACTATGCAAACTCAGTGATG
WCHD3-30_AF050612_                      AGATACCCATGTAGTCCTAGCA----GTAAACACTGCACACTAAACATTA
Dp11u27                                 AGATACCCATGTAGTCCTAGCA----GTAAACACTGCACACTAAACATTA
Dp10U1                                  AGATACCCGGTTAGTCCCAGCT----GTAAACGATGCAGACTAGGTGTCC
Dp11u2                                  AGATACCCGGGTAGTCCCAGCT----GTAAACGATGCTCGCTAGGTGTCA
Dp10u12                                 AGATACCCGGGTAGTCCCAGCT----GTAAACGATGCTCGCTAGGTGTCA
Methanoccoidesburtonii_X65537_          AGATACCCGGGTAGTCCCAGCC----GTAAACGATGTTCGCTAGGTGTCA
Methanosarani_L48408_                   AGATACCCGGGTAGTCCTAGCC----GTAAACGATGCGAGCTAGGTGTCA
Soyang1Af-1100Ar_AF056368_              AGATACCCGGGTAGTCCCAGCT----GTAAACGATGTGCGTTAGGTGTAT
Methanobrevibacter_AB009827_            AGATACCCGGGTAGTCCTAGCC----GTAAACGATGCGGACTTGGTGTTA
Dp11u31                                 AGATACCCACGTAGTCCTGGCC----GTAAACGCTGTGAGCTAGGTGTTG
WCHD3-33_AF050619_                      AGATACCCCGGTAGTCAGGGT----GTAAACGCTGCGGGCTTGGTGTTG
Dp11u20                                 AGATTGAAGAGAAGTCGCGGAAAATTTTTAAGTCTTATCACTAATAAGTG
                                        ****         ****   *       * **   *       *

Dp11u5                                  CACACTCTACGTGGGTGTGCAGTGCCGTAGCGTANGCGTTA-AACGGACC
Dp10u34                                 CACACTCTACGTGGGTGTGCAGTGCCGTAGCGTAAGCGTTA-AACGGACC
Dp11u25                                 CACACTCTACGTGGGTGTGCAGTGCCGTANCGTANGCGT-A-AACGGACC
pGrfc26_U59986_                         GGACGGCCACGTGCCGTTCTAGTGCCGCAGGGAAGCTGTTA-AGTCTGCC
pLemB390_U59996_                        GGACGGCCACGTGCCGTTCTAGTGCCGCAGGGAAGCTGTTA-AGTCTGCC
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WCHA1-38_AF050613_                  CATTGGCTTGTGGCCAATGCAGTGCTGCAGGGAAGCCGTTA-AGTTTGCC
WCHD3-30_AF050612_                  GTACCTCTTCGAGAGGTATTAGTGCTGTAGAGAAGTCGAAG-AGTGTGCT
Dp11u27                             GTACCTCTTCGAGAGGTATTAGTGCTGTAGAGAAGTCGA-G-AGTGTGCT
Dp10U1                              TATCAGCTATAAGCTGTAGGGGTGCCGAAGGGAAGCCATTA-ATTCTGCC
Dp11u2                              GGTGCGGTGCGACCGCATCTGGTGCCGCAGTGAAAACTTGA-AGCGAGTC
Dp10u12                             GGTGCGGTGCGACCGCATCTGGTGCCGCAGTGAAAACTTGA-AGCGAGTC
Methanoccoidesburtonii_X65537_      GGGGCGGTGCGACCGCTTCTGGTGCCGTAGGGAAGCCGTGA-AGCGAACC
Methanosarani_L48408_               CGTGGATTGCGAATCCATGTGGTGCCGTAGGGAAACCGTGA-AGCTCGCC
Soyang1Af-1100Ar_AF056368_          CGGTGACCACGAGTCACCGAGGTGCCGAAGAGAAATCGTGA-AACGTACC
Methanobrevibacter_AB009827_        GGATGGCTTTGAGCCGCCCTAGTACCGAAGGGAAGCTGTTA-AGTCCGCC
Dp11u31                             CATATTCTATATGAATGTGCAGTGTCGGAGTGAAGATGTTA-AGCTCACC
WCHD3-33_AF050619_                  GGGGTCCTTAGTGGGCGCCCAGTGCCGGAGAGAAGTTGTTA-AGCCTGCT
Dp11u20                             ATATGGCTAAATTTCAAACGTACATTGGAGGAACACCCAGAGAATTCAAC
                                                          * *             *

Dp11u5                              GCCTGGGGAGT--ATGACTGCAAGGTTGAAACTTAAAGGAATTGACGGGG
Dp10u34                             GCCTGGGGAGT--ATGACTGCAAGGTTGAAACTTAAAGGAATTGACGGGG
Dp11u25                             GCCTGGGGAGT--ATGACTGCNAGGCTGAAACTCCNAGGAATTGACGGGG
pGrfc26_U59986_                     GCCTGGGGAGT--ACGATCGCAAGATTGAAACTTAAAGGAATTGGCGGGG
pLemB390_U59996_                    GCCTGGGGAGT--ACGATCGCAAGATTGAAACTTAAAGGAATTGGCGGGG
WCHA1-38_AF050613_                  GCCTGGGAAGT--ACGTACGCAAGTATGAAACTTAAAGGAATTGGCGGGG
WCHD3-30_AF050612_                  ACCTGGGAAGT--ATAGCCGCAAGGCCGAAACTTAAAGGAATTGGCGGGG
Dp11u27                             ACCTGGGAAGT--ATAGCCGCAAGGCCGAAACTTAAAGGAATTGGCGGGG
Dp10U1                              GCCTGGGAAGT--ACGGCCGCAAGACTGAAACTTAATGGAATTGGCGGGG
Dp11u2                              ACCTGGGAAGT--ACGGTCGCAAGGCTGAAACTTAAAGGAATTGGCGGGG
Dp10u12                             ACCTGGGAAGTT-ACGGTCGCAAGGCTGAAACTTAAAGGAATTGGCGGGG
Methanoccoidesburtonii_X65537_      ACCTGGGAAGT--ACGGCCGCAAGGCTGAAACTTAAAGGAATTGGCGGGG
Methanosarani_L48408_               GCCTGGGAAGT--ACGGCCGCAAGGCTGAAACTTAAAGGAATTGGCGGGG
Soyang1Af-1100Ar_AF056368_          GCCTGGGAAGT--ACGGTCGCAAGGCTGAAACTTAAAGGAATTGGCGGGG
Methanobrevibacter_AB009827_        GCCTGGGAAGT--ACGGTCGCAAGACTGAAACTTAAAGGAATTGGCGGGG
Dp11u31                             GCCTGGGAAGT--ACNGTCGCAAGACTGAAACTTAAAGGAATTGGCGGGG
WCHD3-33_AF050619_                  GCTTGGGGAGT--ATGTCCGCAAGGATGAAACTTAAAGGAATTGGCGGGG
Dp11u20                             ACAGTGAATGTATATGAATTCGATAA-GAAATTTACAG--ATTGGTACAA
                                     *   *   **  *      * *    **** *    *  ****

Dp11u5                              GAGCACCNCAAGGGGTTGGAGGCTGCGGTTTAATTGGATT-CAACGCCGG
Dp10u34                             GAGCACCACAAGGGGT-GGAGGCTGCGGTTTAATTGGATT-CAACGCCGG
Dp11u25                             GCTCGC-ACAAGCGGT-GGAGTATGTGGTTTTATTCCAAAACAACGCGCA
pGrfc26_U59986_                     GAGCACCACAAGGGGT-GAAGCTTGCGGTTTAATTGGAGT-CAACGCCGG
pLemB390_U59996_                    GAGCACCACAAGGGGT-GAAGCTTGCGGTTTAATTGGAGT-CAACGCCGG
WCHA1-38_AF050613_                  GAGCACCACAAGGGGT-GAAGCCTGCGGTTCAATTGGAGT-CAACGCCAG
WCHD3-30_AF050612_                  AGACACTACAACAGGT-GACGCGTGCGGTTCAATTAGATT-CTACACCGT
Dp11u27                             AGACACTACAACAGGT-GACGCGTGCGGTTCAATTAGATT-CTACACCGT
Dp10U1                              GCGCACTACAAGGGTG-GATGC-TGCCGTTTAATTGGATA-CGACGCCAG
Dp11u2                              GAGCACTACAACGGGT-GGAGCCTGCGGTTCAATTGGATT-CAACGCCGG
Dp10u12                             GAGCACTACAACCGGTTGGACCCTGCGGTTCAATTGGATT-CAACGCCCG
Methanoccoidesburtonii_X65537_      GAGCACTACAACGGGT-GGAGCCTGCGGTTTAATTGGACT-CAACGCCGG
Methanosarani_L48408_               GAGCACCACAACGGGT-GGAGCCTGCGGTTTAATTGGACT-CAACGCCGG
Soyang1Af-1100Ar_AF056368_          GAGCACCACAACGGGT-GGAGCCTGCGGTTTAATCGGACT-CAACGCCGG
Methanobrevibacter_AB009827_        -AGCACCACAACGCGT-GGAGCCTGCGGTTTAATTGGATT-CAACGCCGG
Dp11u31                             GAGCACCACAA-GGGT-GCGGCGTGCGGTTTAATCCAACT-CAACGCGGA
WCHD3-33_AF050619_                  GAGCACCGCAACGGGA-GGAGCGTGCGGTTTAATTGGATT-CAACACCGG
Dp11u20                             TAATAC--CTATCTGATGGTCTGTTCTTCTGCAACAGAAT---TTGCAGG
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Dp11u5                              AA-ATCTCACTGGGCGTTGACAGCAGTATGATGTTCAAGCTGACGACTT
Dp10u34                             AA-ATCTCACTGGGCGT-GACAGCAGTATGATGTTCAAGCTGACGACTT
Dp11u25                             GA-ANCTTACCTGGGCTTGACATGCTTGA-ATTAACCATCTGAAAAGAT
pGrfc26_U59986_                     AA-ATCTCACCGGGAGCG-ACAGCAGTATGAAGGCCAGATTAAAGGTCT
pLemB390_U59996_                    AA-ATCTCACCGGGAGCG-ACAGCAGTATGAAGGCCCGATTAAAGGTCT
WCHA1-38_AF050613_                  AA-ATCTTACCCGGAGAG-ACAGCAGAATGAAGGTCAAGCTGAAGACTT
WCHD3-30_AF050612_                  GA-ACCTCACCAGGAGCG-ACAGCAGGATGAAGGTCAGTCTGAAGGGCT
Dp11u27                             GA-ACCTCACCAGGAGCG-ACAGCAGGATGAAGGTCAGTCTGAAGGGCT
Dp10U1                              AT-AATTTACCAAGGGTTTACAGCCGAATGATGA-CA-TCTGAAGGGCT
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Dp11u2                          AA-AGCTTACCGGGTCAG-ACAGCAATATGAAGT-CAAGCTAAAACTT-
Dp10u12                         GA-AACTTACCGGACTCG-ACAGCACTATGAAATTCAGGCTAAAACCT-
Methanoccoidesburtonii_X65537_  AA-AACTCACCGGGGGCG-ACAGCAAAATGTAGGTCAAGCTAAAGACTT
Methanosarani_L48408_           AA-AGCTCACCGGAGACG-ACAGCGGGATGAGGGCCAGGCTGATGACCT
Soyang1Af-1100Ar_AF056368_      AA-ATCTCACCGGATAAG-ACAGCGGAATGATAGCCGGGCTGAAGACTC
Methanobrevibacter_AB009827_    AC-ATCTCACCAGGGGCG-ACAGCAGTATGATGGCCAGGTTGATGGTCT
Dp11u31                         GA-ATCTCACCATGGGCG-ACCGCAGGATGAAAGTCCAGCTAATGACTT
WCHD3-33_AF050619_              AC-AACTCACCAGGAGCG-ACTATTACATGAAGACCAGGCTGATGACCT
Dp11u20                         ATTATTTGAGGCGCCAATTTCAGCACTTTTAGCATCAAAGGGCGTCCA-
                                   *  * *          *               *
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Appendix 4: WAG5 sequence alignment from Cytophaga and Firmucutes sequence clone library from well 10 and 11. Reference strains are listed with accession numbers.

CLUSTAL X (1.64b) multiple sequence alignment

```
Dp10u22                 TGCTAGCGTTGTCCGG--AATTACTGGG-TGTAAAGGGAGCGCAGGCGGATTATTAAGTC
uncultured              CCCAAGCGTTGTCCGG--ATTTACTGGG-TGTAAAGGGAGCGCAGGCGGATTATTAAGTC
Dp10e4                  ------CGTTGTCCGG--AATCACTGGG-TGTAA-GGGAGCGCAGGCGGGTCAGCAAGTC
Bsv40_AJ229196_         GGTTAGCGTTGTCCGG--ATTTACTGGG-TGTAAAGGGCGCGTAGGCGGGTTTGTAAGTC
Dp10u3                  GGCAGGCGTTACTCGGGGATTGATTGGG-TGTAAAGGGCGTGTAGGTGGCGGATTAAGTC
a17o                    ----------------------------------------TGTAGGTGGTGAATTAAGTC
dP11U37                 -GGCGACGTTACTCGG--ATTTACTAGG-CGTAAAGCGCATGTAGGTGGTTGAATAAGTC
Dp10e15                 GGCAAGCGTTACTCGG--ATTTATTGGG-TGTAAAGGGCAGGTAGGCGTTCCACCAAGTT
Dp11e7                  GGCAAGCGTTACTCGG--ATTTATTGGGGTGTAAAGGGCAGGTANGCGTTCCACCAAGTT
Dp10u20                 GGCGAGCGTTACTCGG--ATTTATTGGG-TGTAAAGGGCAGGTAGGTGTTTCATCAAGTT
Dp10u5                  GGCGAGCGTTACTCGG--ATTTATTGGG-TGTAAAGGGCAGGTAGGTGTCCTATCAAGTT
Dp10e2                  GGCGAGCGTTACTCGG--ATTTATTGGG-TGTAAAGGGCAGGTAGGCGTCTTAACAAGTT
Dp10u26                 GGCGAG-GTTACTCGG--ATTTATTGGG-TGTAAAGGGCAGGTAGGCGTCTTAACAAGTT
S23                     TGCNNNNATTACTCG---ATTTATTGGG-TGTAAAGGGCAAGTAGGCGTCTTGACAAGTT
Dp10u32                 GGCGAC-GTTACTCGG--TTTGACTGGG-TGTAAAGGGTTCGCAGGCGGTCTTACAAGTG
Dp10u25                 GGCGAGCGTTGTCCGG--AATTATTGGG-CGTAAAGGGCGTGTAGGCGGCCTTTTAAGTC
OPB54_Af027087.1_       GGCGAGCGTTGTCCGG--AATTACTGGG-CGTAAAGGGCGTGTAGGCGGCCCTTTAAGTC
DP10e16                 GGGCTACGTTATCCGG--AATTACTGGG-CGTAAAGGGTGCGTAGGTGGTTTTTTAAGTC
Dp11e13                 -----CCGTTA-CCGG--AATTACTGGG-CGTAAAGGGTGCGTANGTGGTTTTTTAAGTC
Clostiridium            GGCTAACGTTATCCGG--AATTA--GGG-CGTAAAGGGTGCGTAGGTGGTTTTTTAAGTC
Dp10e5                  GGCGAGCGTTGTTCGG--AATTACTGGG-CTTAAAGGGCGCGTAGGCGGTGAGATAAGTC
Dp10u27                 GGCAA-CGTTGTTCGG--AATTACTGGG-CTTAAAGGGCGCGTAGGCGGTGAGATAAGTC
MUG4_AB011296_          GGCGAGCGTTGTTCGG--AATTACTGGG-CTTAAAGGGCGCGTAGGCGGTGGTGTAAGTC
m62798                  TGCNAGCGTTATCCGG--ATTCACTGGG-TTTAAAGGGAGCGTAGGTGGGTTGATAAGTC
dp11e8                  TGCAA-CGTTATCCGGG-ATTCACTGGG-TTTAAAGGGTGCGTANGTGGGTTGGTAAGTC
Dp11u32                 TGCAAGCGTTATCCGG--ATTTATTGGG-TTTAAAGGGTCCGTAGGCGGACTAGTAAGTC
Dp10e1                  TGCAAGCGTTATCCGG--ATTTATTGGG-TTTAAAGGGTGCGTAGGCGGTAGTTTAAGTC
Dp11u18                 TGCAA-CGTTATCCGG--ATTTATTGGG-TTTAAAGGGTGCGTAGGCGGTAGTTTAAGTC
clone                   TGCAAGCGTTATCCGG--ATTTATTGGG-TTTAAAGGGTGCGTAGGCGGTATTTTAAGTC
Dp10u8                  -CCAAGCGTTATCCGG--ATTTATTGGG-TTTAAAGGGTGCGTAGGCGGCTTGCTAAGTC
Dp10u21                 -CCAAGCGTTATCCGGG-ATTCACTGGG-TTTAAAGGGTGTGTAGGCGGGATATTAAGTC
                                                      * * * *       ****

Dp10u22                 GATTGTGAAATCTTGCGGCTCAACCGCAAAA-CAGCAGTCGATACTGATGATCTTGAGTG
uncultured              GATTGTGAAATCTTGCGGCTCAACCGCAAAA-CAGCAGTCGATACTGTTAATCTTGAGTG
Dp10e4                  ATTGGTGAAATCCGGAGGCTTAACCTCAGGA-CTGCCAATGATACTGCCGATCTTGAGTA
Bsv40_AJ229196_         AGAGGTGAAATCCTGCAGCTTAACTGCAGAG-CTGCCTTTGATACTGCAAATCTTGAGTT
Dp10u3                  GGGTGTGAAATCCCTTGGCTCAACCAAGGAAACTGCATTCGATACTGATCTGCTTGAGTG
a17o                    GATTGTGAAATCCCTTGGCTTAACCAAGGAA-CTGCATTCGAAACTGATTCGCTTGAGTG
dP11U37                 CGTGGTGAAATTTCTTGGCTTAACTGAAAAATGTCCATG-GAAACTATTCGGCTTGAGTG
Dp10e15                 AGAAGTGAAATCCTTTGGCTCAACCAAAGAA-CTGCTTCTAAAACTGGCGGAATTGAGGC
Dp11e7                  AGAAGTGAAATCCTTTGGCTCAACCAAAGAA-CTGCTTCTAAAACTGGCGGAATTGAGGC
Dp10u20                 AGAAGTGAAATCCTTTGGCTCAACCAAAGAA-CTGCTTCTAAAACTGATGAAATTGAGGC
Dp10u5                  AGAAGTGAAATCCTGTGGCTTAACCACAGAA-CTGCTTCTAAAACTGATGGGATTGAGGC
Dp10e2                  AGAAGTGAAATCCTGCAGCTCAACTGCAGAA-CTGCTTTTAAAACTGTTGAGATTGAGGC
Dp10u26                 AGAAGTGAAATCCTGCAGCTCAACTGCAGAA-CTGCTTTTAAAACTGTTGAGATTGAGGC
S23                     AGGAGAGAAATCCTGCAGCTCAACTGCAGAA-CTGCTTTTAAAACTGTTAAGATTGAGAC
Dp10u32                 TAAGGTGAAATCCTTCGGCTCAACCGGAGAA-TTGCCTTACAAACTGTTTGACTAGAGGC
Dp10u25                 AGACGTGAAAACCCCGGGCTCAACCCGGGAA-CTGCGTTTGAGACTGGAGGGCTTGAGGA
OPB54_Af027087.1_       AGGCGTGGAAGCCCTGGGCTTAACCCAGGAA-CTGCGCTTGAGACTGGGGGGCTTGAGGG
DP10e16                 AGAAGTGAAAGGCTACGGCTCAACCGTAGTA-A-GCTTTTGAAACTAGAGAACTTGAGTG
Dp11e13                 AGAAGTGAAAGGCTACGGCTCAACCGTAGTA-A-GCTTTTGAAACTAGAGAACTTGAGTG
Clostiridium            AGAAGTGAAAGGCTACGGCTCAACCGTAGTA-A-GCTTTTGAAACTAGAGAACTCGAGTG
Dp10e5                  CGTGGTGAAATCCTATGGCTTAACCATAGAA-TTGCCTCGGAAACTGTCTTACTTGAGTC
Dp10u27                 TGAGGTGAAATCCTATGGCTTAACCATAGAA-TTGCCTTGGAAACTGTTTTACTTGAGTC
MUG4_AB011296_          CGGGGTGGAATGCTACAGCTCAACTGTAGAG-CTGCCTTGGAAACTGCATTACTTGAGTC
m62798                  AGTGGTGAAATCTTCGAGCTTAACTCGGAAA-CTGCCATTGATACTATCAGTCTTGAATA
dp11e8                  AGTGGTGAAATCTCCGAGCTTAACTTGGAAA-CTGCCATTGATACTATCAGTCTTGAATA
Dp11u32                 AGTGGTGAAATCCTGCAGCTCAACTGTAGAA-CTGCCATTGATACTGCTAGTCTTGAATT
Dp10e1                  AGTGGTGAAATCCTGCAGCTCAACTGTAGAA-CCGCCATTGAAACTGAATTACTTGAGTN
Dp11u18                 AGTGGTGAAATCCTGCAGCTCAACTGTAGAA-CCGCCATTGAAACTGAATTACTTGAGTA
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clone                AGTGGTGAAAACTTGCAGCTTAACTGTAAGA-CTGCCATTGAAACTGAAGTACTTGAGTT
Dp10u8               AGTGGTGAAAGTTTGCAGCTTAACTGTAAAA-TTGCCATTGATACTGACGAGCTTGAATA
Dp10u21              AGTGGTGAAATCCTACAGCTTAACTGTAGAA-CTGCCATAGAAACTGATGTTCTTGAATG
                        * * **       *** ***          *     * ***       * **

Dp10u22              TGGAAGAGAGTGGCGGAATTCATGGTGTAGCAGTGAAATGCGTAGATATCATGAAGAACA
uncultured           TGGAAGAGAGTGGCGGAATTCATGGTGTAGCAGTGAAATGCGTAGATATCATGAAGAACA
Dp10e4               CAGAAGAGGAAGACGGAATTCCTGGTGTAGCGGTGAAATGCGTAGATATCAGGAAGAACA
Bsv40_AJ229196_      CAGAAGAGAGAGATGGAATTCCAGGTGTAGTGGTGAAATACGTAGATATCTGGAAGAACA
Dp10u3               ATAGAGAGGTAAGCGGAATTCCCGGTGTAACAGTGAAATGTGTAGATATCGGGAGGAACA
a17o                 ATAGAGAGGTAAGTGGAATTCCCGGTGTAACAGTGAAATGTGTAGATATCGGGAGGAACA
dP11U37              TGGCAGGGGGAGACGGAATTCCCGGTGTAGCGGTGAAATGCGTAGATATCGGGAGGAACA
Dp10e15              AAAAAGAGGAGAGCGGAATTCCCGGTGTAAGGGTGAAATCTGTAGATATCGGGAGGAACA
Dp11e7               AAAAAGAGGAGAGCGGAATTCCCGGTGTAAGGGTGAAATCTGTAGATATCGGGAGGAACA
Dp10u20              TAAAAGAGGAGAGCGGAATTCCCGGTGTAAGGGTGAAATCTGTAGATATCGGGAGGAACA
Dp10u5               TAGAAGAGGAGAGCGGAATTCCCGGTGTAAGGGTGAAATCTGTAGATATCGGGAGGAACA
Dp10e2               TGGGAGAGGAGAGCGGAATTCTCGGTGTAAGAGTGAAATCTGTAGATATCGAGAGGAACA
Dp10u26              TGGGAGAGGAGAGCGGAATTCTCGGTGTAAGAGTGAAATCTGTAGATATCGAGAGGAACA
S23                  TGGGAGAGGAGAGTGGAATTCTCGGTGTAAGAGTGAAATCTGTAGATATCGAGAGGAACA
Dp10u32              TAGGAGAGGAGAACGGAACTGCCGGTGTAAGGGTGAAATCTGTGGATATCGGCAGGAACG
Dp10u25              CGGGAGAGGAAAGTGGAATTCCCGGTGTAGCGGTGAAATGCGTAGATATCGGGAGGAACA
OPB54_Af027087.1_    CAGGAGAGGGAAGTGGAATTCCCGGTGTAGCGGTGAAATGCGTAGATATCGGGAGGAACA
DP10e16              CAGGAGAGGAGAGTAGAATTCCTAGTGTAGCGGTGAAATGCGTAGATATTAGGAGGAATA
Dp11e13              CAGGAGAGGAGAGTATATTTCCTAGTGTAGCGGTGAAATGCGTAGATATTAGGAGGAATA
Clostiridium         CAGGAGAGGAGAGTAGAATTCCTAGTGTAGCGGTGAAATGCGTAGATATTAGGAGGAATA
Dp10e5               AGCGAGGGGATGGTGGAATTCCTGGTGTAGCAGTGAAATGCGTAGATATCAGGAGGAAGG
Dp10u27              AGCGAGGGGATGGTGGAATTCCCGGTGTAGCGGTGAAATGCGTAGATATCGGGAGGAAGG
MUG4_AB011296_       GGTGAGGGGAAGGCGGAATTCCTGGTGTAGCGGTGAAATGCGTAGATATCAGGAGGAAGG
m62798               TTGTGGAGGTTAGCGGAATATGTCATGTAGCGGTGAAATGCTTAGATATGACATAGAACA
dp11e8               TTGTGGAGGTTAGCGGAATATGTCATGTAGCGGTGAAATGCTTAGATATGACATAGAACA
Dp11u32              CGGTTGAAGTGGGCGGAATATGGCATGTAGCGGTGAAATGCTTAGATATGCCATAGAACA
Dp10e1               TTCTTGAGGTAGGCGGAATGTGTAGTGTAGCGGTGAAATGCTTAGATATTACACAGAACA
Dp11u18              ATCTTGAGGTAGGCGGAATGTGTAGTGTAGCGGTGAAATGCTTAGATATTACACAGAACA
clone                ATCTTGAGGTAGGCGGAATGTGTAGTGTAGCGGTGAAATGCTTAGATATTACACAGAACA
Dp10u8               TAGTTGAGGTAGGCGGAATGAGTCAAGTAGCGGTGAAATGCATAGATATGACTCAGAACA
Dp10u21              TGGTTGAGGTAAGCGGAATGTGTAGTGTAGCGGTGAAATGCTTAGATATTACACAGAACA
                        *          *         ***   *******   * *****       ***

Dp10u22              CCGGTTGCGAAGGCGGCCACTTAGTCCATTACTGACGCTCATGCTCGAAAGCGTGGGGAT
uncultured           CCGGTTGCGAAGGCGGCCACTTAGTCCATTACTGACGCTCATGCTCGAAAGCGTGGGGAT
Dp10e4               CCGGTGGCGAAGGCGGTCTTCTGGTCTGTAACTGACGCTCATGCTCGAAAGCGTGGGGAG
Bsv40_AJ229196_      CCAGTGGCGAAGGCGGTCTCTTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGTAG
Dp10u3               CCAGTGGCGAAGGCGGCTTACTGGCTATCTACTGACACTGAAACGCGAGAGCTAGGGGAG
a17o                 CCAGTGGCGAAGGCGACTTACTGGCTGTCTACTGACACTGAAACGCGAGAGCTAGGGGAG
dP11U37              CCAATGGCGAAAACAGTCTCCTGGGCCAATACTGACACTGAGGTGCGAAAGCTAGGGGAG
Dp10e15              CCAGTGGCGAAAGCGGCTCTCTAGTTTTGTCCTGACGCTGAGCTGCGAAAGCTAGGGGAG
Dp11e7               CCAGTGGCGAAAGCGGCTCTCTAGTTTTGTCCTGACGCTGAGCTGCGAAAGCTAGGGGAG
Dp10u20              CCAGTGGCGAAAGCGGCTCTCTGGTTTAGTCCTGACACTGAGCTGCGAAAGCTAGGGGAG
Dp10u5               CCAGTAGCGAAAGCGGCTCTCTGGTCTGGCCCTGACGCTGAACTGCGAAAGCTAGGGGAG
Dp10e2               CCAGTGGCGAAGGCGGCTCTCTGGTCCAGCTCTGACGCTGAACTGCGAAAGCTAGGGGAG
Dp10u26              CCAGTGGCGAAGGCGGCTCTCTGGTCCAGCTCTGACGCTGAACTGCGAAAGCTAGGGGAG
S23                  CCAGTGGCGAAGGCGGCTCTCTGGTCCAGTTCTGACGCTGAATTGCGAAAGCTAGGGGAG
Dp10u32              CCCGTGGAATAATCGGTTCTCTGGTCCAGTCCTGACGCTCAGGAACGAAAGCTAGGGGAG
Dp10u25              CCAGTGGCGAAGGCGGCTTTCTGGACCGTCCCTGACGCTGAGGCGCGAAAGCCAGGGGAG
OPB54_Af027087.1_    CCAGTGGCGAAGGCGGCTTCCTGGACTGTTCCTGACGCTGAGGCGCGAAAGCCAGGGGAG
DP10e16              CCAGTAGCGAAGGCGGCTCTCTGGACTGTAACTGACACTGAGGCACGAAAGCGTGGGGAG
Dp11e13              CCAGTAGCGAAGGCGGCTCTCTGGACTGTAACTGACACTGAGGCACGAAAGCGTGGGGAG
Clostiridium         CCAGTAACGAAGGCGGCTCTCTGGACTGTAACTGACACTGAGGCACGAAAGCGTGGGGAG
Dp10e5               CCGGTGGCGAAGGCGACCATCTGGCGCTGAACTGACGCTGAGGCGCGAAAGCGTGGGTAG
Dp10u27              CCGGTGGCGAAGGCGGCCATCTGGCGCTGAACTGACGCTGAGGCGCGAAAGCGTGGGTAG
MUG4_AB011296_       TCTGTGGCGAAGGCGGCCTTCTGGCACTTGACTGACGCTGAGGCGCGAAAGCGTGGGGAG
m62798               CCAATTGCGAAGGCAGCTGGCTACACATATATTGACACTGAGGCTCGAAAGCGTGGGGAT
dp11e8               CCAATTGCGAAGGCAGCTGGCTACACATATATTGACACTGAGGCACGAAAGCGTGGGGAT
Dp11u32              CCGATTGCGTAGGCAGCTCACTAAGCCTGGATTGACGCTGAGGGACGAAAGCGTGGGGAG
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Dp10e1                  CCGATTGCGAAGGCAGCTTACTAAGGGTTTACTGACGCTGATGCACGAAAGCGTGGGGAT
Dp11u18                 CCGATTGCGAAGGCAGCTTACTAAGGGTTTACTGACGCTGATGCACGAAAGCGTGGGGAT
clone                   CCGATTGCGAAGGCAGCTTACTAAGGATACACTGACGCTCAGGCACGAAAGCGTGGGGAT
Dp10u8                  CCGATTGCGAAGGCAGCTTACTAAGCTATTATTGACGCTGAGGCACGAAAGCGTGGGGAT
Dp10u21                 CCTATTGCGAAGGCAGCTTGCTAAGCCATGATTGACGCTGAGACACGAAAGCGTGGGGAT
                         *  *     *  *       *          **** ** *    *** ***  *** *

Dp10u22                 CAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGAATACTCGGTGT--CGG
uncultured              CAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGAATACTCGGTGT--CGG
Dp10e4                  CAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGGATACTCGATGT--CGG
Bsv40_AJ229196_         CAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGATGAATACTAGATGT--TGG
Dp10u3                  CAAACAGGATTAGATACCCTGGTAGTCCTAGCCGTAAACGATGTTCACTAGGTGT--CGG
a17o                    CAAACAGGATTAGATACCCTGGTAGTCCTAGCCGTAAACGATGTTCACTAGGTGT--CGG
dP11U37                 CAAACAGGATTAGATACCCTGGTAGTCCTGGCCGTAAACGGTGAACACTAGGTGTATCCT
Dp10e15                 CAAACAGGATTAGATACCCTGGTAGTCCTAGCCGTAAACGATGAGCACTAGGTGT--TGG
Dp11e7                  CAAACAGGATTAGATACCCTGGTAGTCCTAGCCGTAAACGATGAGCACTAGGTGT--TGG
Dp10u20                 CAAACAGGATTAGATACCCTGGTAGTCCTAGCCGTAAACGATGAGCACTAGGTGT--TGG
Dp10u5                  CAAACAGGATTAGATACCCTGGTAGTCCTAGCCGTAAACTATGAGCACTAGGTGT--TGG
Dp10e2                  CAAACAGGATTAGATACCCTGGTAGTCCTAGCCGTAAACGATGAGCACTAGGTGT--TGG
Dp10u26                 CAAACAGGATTAGATACCCTGGTAGTCCTAGCCGTAAACTATGAGCACTAGGTGT--TGG
S23                     CAAACAGGATTAGATACCCTGGTAGTCCTAGCCGTAAACTATGAGMACTAGGTGT--TGG
Dp10u32                 CAAACAGAATTAGATACTCTGGTAGTCCTAGCCGTAAACGATGGGCACTAGGTGT--TTG
Dp10u25                 CGAACAGGATTAGATACCCCGGTAGTCCTGGCCGTAAACGATGGGTACTANGTGT--GGG
OPB54_Af027087.1_       CGAACGGGATTAGATACCCCGGTAGTCCTGGCTGTAAACGATGGGTACTAGGTGT--GGG
DP10e16                 CAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTACTAGGTGT--CGG
Dp11e13                 CAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTACTAGGTGT--CGG
Clostiridium            CAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTACTAGGTGT--CGG
Dp10e5                  CAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGGTGGGCATTAGGTGT--AGG
Dp10u27                 CAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGGTGGGTATTAGGTGT--AGG
MUG4_AB011296_          CAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGGTGGGTATTAGGTGT--GGG
m62798                  CAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACTATGGATACTCGACAT---AC
dp11e8                  CAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACTATGGATACTCGACAT---AC
Dp11u32                 CGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGATTACTCGACAT---TA
Dp10e1                  CAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGATGACTCGCTGA---TG
Dp11u18                 CAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGATGACTCGCTGT---TG
clone                   CGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGATGACTCGCTGT---TG
Dp10u8                  CGAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGATTACTCGATGT---CG
Dp10u21                 CAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGATAACTAGTTGT---TG
                        * *** * ********* * ********  **  *****  **   * *

Dp10u22                 GTCGCAA----GATTCGGCGCCT-AAGCAAACGCATTAAGT-ATTCCACCTG------
uncultured              GTCGCAA----GATTCGGCGCCT-AAGCAAACGCATTAAGT-ATTCCACCTG------
Dp10e4                  GCCGCAA----GGCCCGGTGTCC-AAGCTAACGCGTTAAGT-ATCCCACCTG------
Bsv40_AJ229196_         GCCTGAG----GGCTCAGTGTCG-CAGCTAACGCATTAAGT-ATTCCACCTG------
Dp10u3                  GCCCTCT---GGGTTCGGTGCCG-CAGTAAACACATTAAGT-GAGCCACCTG------
a17o                    GCCCTCT---GGGTTCGGTGCCG-CCGTTAACACATTAAGT-GAACCGCCTG------
dP11U37                 GAGTTAT---CGGGATG-TGCCG-AAGCTAACGCATTAAGT-GTTCCGCCTG------
Dp10e15                 GGGGTTT---CCCTTCAGCGCCGCAAGATAACTCGTTAAGT-GCTCCACCTG------
Dp11e7                  GGGGTTT---CCCTTCAGCGCCGCAAGATAACTCGTTAAGT-GCTCCACCTG------
Dp10u20                 GGGGTTA---CC-TTCAGCGCCGTAAGATAACTCGTTAAGT-GCTCCACCTG------
Dp10u5                  GGG-TAA---CCCTTCAGCGCCGTAAGATAACTCGTTAAGTTGCTCCACCTG------
Dp10e2                  AGGTTTA---CC-TTCAGCGCCGTAAGTTAACACGTTAAGT-GCTCCACCTG------
Dp10u26                 GGGTTTA---CC-TTCAGCGCCGTAAGTTAACACGTTAAGT-GCTCCACCTG------
S23                     GGGTTTA---CC-TTCAGCGCCGCAAGTTAACACGTTAAGT-GCTCCACC--------
Dp10u32                 TCCGTAA---GG-ATGGGTGCCG-AAGCTAACGCGTTAAGT-GCCCCACCTG------
Dp10u25                 GGGTATCGACCCCCTCCGTGCCG-GAGTTAACACAATAAGT-ACCCCGCCTG------
OPB54_Af027087.1_       GGGTATCGACCCCTCCCGTGCCG-GAGTTAACACAATAAGT-ACCCCGCCTGGGGAGT
DP10e16                 GGGTTAC--CCCCCTCGGTGCCG-CAGCTAACGCATTAAGT-ACTCCGCCTG------
Dp11e13                 GGGTTAC--CCCCCTCGGTGCTG-CAGCTAACGCATTAAGT-ACTCCGCCTG------
Clostiridium            GGGTTAC--CCCCCTCGGTGCCG-CAGCTAACGCATTAAGT-ACTCCGCCTG------
Dp10e5                  GCTCGTAA--GGGTTCTGTGCCG-TAGGGCAACCATTAAAT-GCCCCGCCTG------
Dp10u27                 GCTCGTAA--GGGTTCTGTGCCG-AAGGGAAACCATTAAAT-ACCCCGCCTG------
MUG4_AB011296_          GCCTTCAT--GGGTTCCGTGCCG-TAGCGAAAGCATTAAAT-ACCCCGCCTG------
m62798                  GCGATACA---CTGTGTGTGTC-TGAGCGAAAGCATTAAGTAT-CCCACCTG------
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dp11e8              GCGATAAA---CTGTGTGTGTC-TGAGCGAAAGCATTAGGTAT-CCCACCTG------
Dp11u32             GCGATACA---CGGTTAGTGTCATAAGCGAAAGCATTAAGTAA-TCCACCTG------
Dp10e1              GCGATATA---CAGTCAGCGGC-TTAGCGAAAGCGTTAAGTCA-TCCACCTG------
Dp11u18             GCGATATA---CAGTCCNCCGC-TTAGCGAAAGCGTTAAGTCA-TCCACCTG------
clone               GCGATACA---CAGTCAGCGGC-TTAGCGAAAGCGTTAAGTCA-TCCACCTG------
Dp10u8              GCGATATA---CAGTCGGCGTC-AAAGCGAAAGCGTTAAGTTAATCCACCTG------
Dp10u21             GCGATATA---CAGTCAGCGAC-AAAGCGAAAGCATTAAGTTA-TCCACCTG------
                                           *   *  *  **  *    ** **
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Appendix 5: WAG5 sequence alignment from Proteobacteria and OP11 clone library from well 10 and 11. Reference strains are listed with accession numbers.

CLUSTAL X (1.64b) multiple sequence alignment

```
Dp11e12         CCGTTGTTCGGAATCACTGGGCTTAAAGGGC-ATGTAGGCGGTGCGCCAAGTGTCTTGTG
Sva0503         GCGTTATTCGGAATCACTGGGCTTAAAGAGT-ACGTAGGCGGATGGCCAAGTATCTTGTG
Dp10u35         GCGTTATCCGGATTTATTGGGCGTAAAGGGT-CCGTAGCCGGTCTTGTAAGTCTTTGGTT
dp10u16         GCGTTATCCGGATTTATTGGGCGTAAAGCGC-ATGTAGGAGGTTTTGTGCGTCCTTGGTT
Dp10u13         GCGTTATCCGGATTTATTGGGCGTAAAGGGT-GTGTAGGTGGTTTTGTTAGTCTTTCGTT
Dp11u4          GCGTTATCCGGAAC-ATTGGGCGTAAAGGGT-GTGTANGCGGTTTCGTTAGTCTTCCGTT
Dp10e3          GCGTTATCCGGATTTATTGGGCGTAAAGCGT-CTGCAGACGGTAAGGCATGTTCGGGGTT
Dp11e14         ACGTTACTCGGAATTACTGGGCGTAAAGCGT-CTGCAGGCGTCCTAAAAAGTCTGGTGTA
OPd3             GCATTATCCGGATTTACTGGGCGTAAAGCGT-CCGCAGGCGGTTTTGAAAGTCATTCGCC
Dp11u3          --------CGGATTTACTGGGTGTAAAGCGT-CTCTAGGCGGCTTAATAAATTTTTAGTT
Dp11u10         --CGTTACCGGATTTA-TGGGCGTAAAGCGT-ATGTAGGCAGTTTATNATGTCAATGGTT
LgD8            GCGTTATCCGGATTTATTGGGCGTAAAGCGT-TCGTAGGCGGTTTTGTAAGTCGTTTGTT
uncultured      GCGTTGTTCGGAATCACTGGGCGTAAAGCGC-GTGCAGGCGGCCTATTAAGTCAGATGTG
dp10e17         ACGTTGTTCGGAATTATTGGGCGTAAAGGGC-GCGCAGGCGGTAAGATAAGTCAGACGTG
WCHB1-12        GCGTTGTTCGGAATTATTGGGCGTAAAGAGC-GTGTAGGCGGCTGAATAAGTCAGATGTG
Dp11u38         GCGTTGTTCGGAATTATTGGGCGTAAAGAGC-GTGTAGGCGGCTGAATAAGTCAGATGTG
Syntrophus      GCGTTGTTCGGAATCATTGGGCGTAAAGAGC-GTGTAGGCGGCTGGATAAGTCAGATGTG
DP10e14         ACGTTGTTCGGAATTATTGGGCGTAAAGAGC-GTGTAGGCGGCCTGATGTGTCAGATGTG
dp10u10         GCGTTGTTCGGAATTATTGGGCGTAAAGCGC-GTGTAGGCGGTCTGATTAGTCTGATGTG
Geobacter       GCGTTGTTCGGATTTATTGGGCGTAAAGCGC-GTGTAGGCGGTTTCTTAAGTCTGATGTG
Dp11e16         ACGTTGTTCGGAATTATTGGGCGTAAAGGGC-GCGTAGGCGGCCTTATAAGTCAGATGTG
Dp11u7          ACGTTGTTCGGAATTATTGGGCGTAAAGCGC-GTGTAGGCGGTCCTGTGTGTCAGATGTG
Dp11u28         AAGTTGTTCGGAATTACTGGGCGTAAAGGGC-GTGCAGGCGGTCCTGTAAGAGAGTTGTG
Dp10u25         GCGTTGTCCGGAATTATTGGGCGTAAAGGGC-GTGTAGGCGGCCTTTTAAGTCAGACGTG
Dp11u8          ACATTGTTCGGATTTACTAGGCGTAAAGGGA-GCGTAGGTGGCTTTGTAAGTTGGAAGTG
Dp11u33         GCGTTAATCGGAATTATTGGGCGTAAAGGGC-ACGTAGGTGGTTGAATAAGTTAGGTGTA
BPC023          GCGTTAATCGGAATTACTGGGCGTAAAGCGC-TCGTAGGTGGATGTTTAAGTCGATTGTG
Dp10u23         GCGTTAATCGGAATTACTGGGCGTAAAGCGT-GCGCAGGCGGTTGTGCAAGACCGATGTG
Ralstonia       GCGTTAATCGGAATTACTGGGCGTAAAGCGT-GCGCAGGCGGTTGTGCAAGACCGATGTG
Dp11u29         GCGTTAATCGGAATTACTGGGCGTAAAGCGT-GCGCAGGCGGTTGTGCAAGACCGATGTG
Dp10u30         GCGTTAATCGGAATTACTGGGCGTAAAGCGT-GCGCAGGCGGTTGTGCAAGACCGATGTG
dp10e13         ACGATAATCGGAATTACTGGGCGTAAAGCGT-GCGCAGGCGGTTTTGTAAGACAGATGTG
Dp10u29         GCGTTAATCGGAATTACTGGGCNTAAAGCGT-GCGCAGGCGGTTTTGTAAGACAGGCGTG
SBR1001         GCGTTAATCGGAATTACTGGGCGTAAAGCGT-GCGCAGGCGGCTTTGTAAGACAGACGTG
Azoarcus        GCGTTAATCGGAATTACTGGGCGTAAAGCGT-GCGCAGGCGGCTTTGTAAGACAGGTGTG
Dp11u25         GTAGTGTCCGTGAATATTGCGTTTAAAGCGT-TCGTAGCCGGCTAAGCAAGTTTTCTGTG
Lgd10           GCATTATCCGGTTTTATTGGGCGTAAAGGGTCGTGTAGGCGGTTTGGCAAGTTATCTTTG
DP10u18         GCGTTATCCGGATTCACTGGGCGTAAAGTGTCGTGTAGGCGGTCTACCGCATCATACCTG
Dp10u19         GCGTTATCCGGATTCACTGGGCGTAAAGA-TCGTGTAGGCGGTTCACCGCATCGCACTTG
                        **      * *  *  *****        *

Dp11e12         AAA-TCCCTCGGCTCAACCGAGGAA--TTGCTGGGCAAA-CTGGCGCACT--TGAGGCAG
Sva0503         AAA-TCCCTCGGCTCAACCGAGGAA--TTGCAGGGTAAA-CTGGTCGTCT--TGAGGCAG
Dp10u35         AAATGCCAGAAGCTCAACTTTTGGA--ATGCCAGGGNAAACTGCAGGACT--TGAGGGTG
dp10u16         AAA-GCCCACCGCTCAACGGTGGAA--NTGCCCGGGATA-CGGCAGAACT--AGAGGGAG
Dp10u13         AAA-GCTCTCGGCTTAACCGGGAAA--GTGCGAAGGAAA-CGGCAAGACT--AGAGGGTG
Dp11u4          AAA-TTCTTCGGCTCAACCGGGGGC--ATGCGGAGGAAA-CGGCGAAACTT-AGAGGACG
Dp10e3          AAA-GACCCAGGCTCAACCTGGGGA--AAGCGTCGAAAA-CTACCTTGCTT-AGAGGACA
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OPd3            AAA-TCCCGAAGCTCAACTTCGGAA--CCGCGAATGATA-CTTCAAAACT--AGAGGCCG
Dp11u3          AAA-TCTTTGGGCTTAACCTAAAGT--TTGCTAAAAACA-CTGTTAAGCT--AGAGACCG
Dp11u10         AAA-TCCTTCGGCTCAACCGAGGAA--CCGCTGTTGAAA-CTGGTAAACT--AGAGTATG
LgD8            AAA-TCTTCAGGCTTAACCTGGAGG--CTGCAGGTGATA-CTGCAAGACT--TGAGTGTG
uncultured      AAA-GCCCTCGGCTCAACCAAGGAA--CTGCATCTGAAA-CTGGCAGGCT--TGAGTACG
dp10e17         AAA-GCCCTCGGCTTAACTGAGGAA--TTGCGTTTGAAA-CTGTTTTGCT--TGAGTACA
WCHB1-12        AAA-TCCCTGGGCTTAACCCAGGAC--GTGCATTTGAAA-CTATTCAGCT--TGAGTAGG
Dp11u38         AAA-TCCCTGGGCTTAACTCAGGAA--GTGCATTTGAAA-CTATTCAGCT--TGAGTAGG
Syntrophus      AAA-GCCCTGGGCTTAACCCAGGAA--GTGCATTTGAAA-CTGTTCAGCT--TGAGTAAG
DP10e14         AAA-GCCCTGGGCTTAACCCAGGAA--GTGCATTTGAAA-CTGTCAGGCT--TGAGTAGG
dp10u10         AAA-GCCCTGGGCTCAACCCAGGAA--GTGCATTGGATA-CTGTCAGACT--TGAATACG
Geobacter       AAA-GCCCTGGGCTCAACCCAGGAA--GTGCATTGGATA-CTGGGAGACT--TGAATACG
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Dp11e16         AAA-TCCCTCGGCTTAATCGAGGAA--GTGCATTTGAAA-CTGTGAGGCT--TGAGTATG
Dp11u7          AAA-TCCTCGGGCTCAACCCGGGAC--GGGCATTTGAAA-CTGCAGGACT--TGAGTACG
Dp11u28         AAA-TGCCCTGGCTCAACCAGGGAA--ATGCACCTCTGA-CTGCAGGACT--TGAGTATG
Dp10u25         AAA-ACCCCGGGCTCAACCCGGGAA--CTGCGTTTGAGA-CTGGAGGGCT--TGAGGACG
Dp11u8          AAA-TTCCATGGCTTAACCATGGAA--CTGCTTCCAAGA-CTGCTTAGCT--TGAGTATA
Dp11u33         AAA-TTCCCGGGCTTAACCCGGGGT--GGTCGCCTGATA-CTGTTTAACT--AGAGTAGG
BPC023          AAA-GCCCCGGGCTTAACCTGGGAA--CTGCAGTCGAAA-CTGGGCATCT--AGAGTATG
Dp10u23         AAA-TCCCCGAGCTTAACTTGGGAA--TTGCATTGGTGA-CTGCACGGCT--AGAGTGTG
Ralstonia       AAA-TCCCCGAGCTTAACTTGGGAA--TTGCATTGGTGA-CTGCACGGCT--AGAGTGTG
Dp11u29         AAA-TCCCCGAGCTTAACTTGGGAA--TTGCATTGGTGA-CTGCACGGCT--AGAGTGTG
Dp10u30         AAA-TCCCCGGGCTTAACCTGGGNAA-CTGCATTTGTGA-CTGCAAGGCT--AGAGTATG
dp10e13         AAA-TCCCCGGGCTTAACCTGGGAA--CTGCGTTTGTGA-CTGCAAGGCT--AGAGTATG
Dp10u29         AAA-TCCCCGGGCTTAACCTGGGAA--CTGCGTTTGTGA-CTGCAAGGCT--AGAGTGCG
SBR1001         AAA-TCCCCGGGCTTAACCTGGGAA--CTGCGCTTGTGA-CTGCAAGGCT--CGAGTGCG
Azoarcus        AAA-TCCCCGGGCTTAACCTGGGAA--CTGCGCTTGTGA-CTGGAAGACT--AGAGTATG
Dp11u25         AAA-TCTTTCGGCTCAACCGAATAGGCTTGCAGAAAATA-CTACTTGGCT--CGAGAGTG
Lgd10           AAA-TTCCAATGCCTAACATTGGAA--TTGGAGATAAAA-CTGTCATACT--AGAGTTTT
DP10u18         AAA-GCCCGAGGCTTAACCTCGGCG--TTGGGTATGAGA-TGGGTAGACTC--GAGGGAG
Dp10u19         AAA-GCACGAGGCTTAACCTCGTCG--TTGGGTGCGAGA-TGGGTAGACTTCTGAGGGCA
                ***         **  **                      *          *   **

Dp11e12         GTATG-GGCGAGCG-AGCTCTTGGTGGAGCGGTGAAATGCGTAGATATCAAGAGG-AACG
Sva0503         GTAGA-GGTAACTGGAACTCATGGTGGAGCGGTGGAATGCGTAGATATCATGGGGGAACG
Dp10u35         TTAGG-GGCTGATGGAACGCACGGTGGAGGGGTGAAATCCGTTGATATCGTGCGG-AACA
dp10u16         TTAGA-GGTGAATGGAACCCACGGTGTAGGGGTGAAATCCGTTGATATCGTGGGG-AACA
Dp10u13         CAAGA-GGCTTATGGAACTCATGGTGTAGGGGTGAAATCCGTTAATATCATGGGG-AACA
Dp11u4          GAAGATGGNNNCTGGAACTCATGGTGTAGCGGTGAAATGCGTTGATATCATGGGG-AACA
Dp10e3          TAAGA-GGCCAATAGAACTCATGGTGTAGGGGTGAAATCCGTTGATACCATGGGG-AATA
Dp11e14         TCAGA--GGCCCCGGAATGTCGTGTGTAGGGGTAAAATCCGTTGATCCACGATGG-AACG
OPd3            GGAGA-GGCAAGTGGAACTACCGGTGTAGCGGTAAAATGCGTTAATATCGGTAGG-AACA
Dp11u3          GGAGA-GGCCAGTGGANNAGCCGGTGTAGTAGTTAAATGCGTTAATATCGGCTAG-AACA
Dp11u10         GGAGA-GGCAAGCGGAATTGCCGGTGTAGGGGTCAAATCCGTTAATATCGGCAGG-AACA
LgD8            GGAGA-GGCAAGCGGAATGCCCGGTGTAGCGGTAGAATGTGTTAATATCGGGTAG-AACA
uncultured      GGAGA-GGAGAGTGGAATTCCCGGTGTAGAGGTGAAATTCGTAGATATCGGGAAG-AACA
dp10e17         GGAGA-GGAGAGTGGAATTCCCAGTGTAGCGGTGAAATGCGTAGATATTGGGAGG-AACA
WCHB1-12        GGAGA-GGAAAGTGGAATTCCTGGTGTAGAGGTGAAATTCGTAGATATCAGGAGG-AACA
Dp11u38         GGAGA-GGAAAGTGGAATTCCTGGTGTAGAGGTGAAATTCGTAGATATCAGGAGG-AACA
Syntrophus      GGAGA-GGAAAGTGGAATTCCTGGTGTAGAGGTGAAATTCGTAGATATCAGGAGG-AACA
DP10e14         GGAGA-GGGAAGTGGAATTCCTGGTGTAGAGGTGAAATTCGTAGATATCAGGAGG-AACA
dp10u10         GGAGA-GGGTAGTGGAATTCCTAGTGTAGGAGTGAAATCCGTAGATATTAGGAGG-AACA
Geobacter       GGAGA-GGGTAGTGGAATTCCTAGTGTAGGAGTGAAATCCGTAGATATTAGGAGG-AACA
Dp11e16         GGAGA-GGAAAGTCGGANNCCTAGTGTAGAGGTGAAATTCGTAGATATTAGGAAG-AACA
Dp11u7          AGAGA-GGGAAGCGGAATTCCCGGTGTAGCAGTGAAATGCGTAGATATCGGGAGG-AACA
Dp11u28         GGAGA-GGATGGGGGAATTCCCGGTGTAGCGGTGAAATGCATTGATATCGGGAGG-AACA
Dp10u25         GGAGA-GGAAAGTGGAATTCCCGGTGTAGCGGTGAAATGCGTAGATATCGGGAGG-AACA
Dp11u8          GGAGA-GGGAAATGGAATTCCTGGTGTAGCGGTGAAATGCGTAGATATCAGGAAG-AACA
Dp11u33         GAAGA-GGAA-GTGGAATTCTCGGTGTAGCGGTGAAATGCGTAGATATCGGGAGG-AACA
BPC023          GTAGA-GGAAAGTGGAATTCCCGGTGTAGCGGTGAAATGCGTAGATATCGGGAGG-AACA
Dp10u23         TCAGA-GGGGGGTAGAATTCCACGTGTAGCAGTGAAATGCGTAGAGATGTGGAGG-AATA
Ralstonia       TCAGA-GGGGGGTAGAATTCCACGTGTAGCAGTGAAATGCGTAGAGATGTGGAGG-AATA
Dp11u29         TCAGA-GGGGGGTAGAATTCCACGTGTAGCAGTGAAATGCGTAGAGATGTGGAGG-AATA
Dp10u30         GCAGA-GGGGGGTAGAATTCCACGTGTAGCAGTGAAATGCGTAGATATGTGGAGG-AATA
dp10e13         GCAGA-GGGGGGTAGAATTCCACGTGTAGCAGTGAAATGCGTAGAGATGTGGAGG-AATA
Dp10u29         GCAGA-GGGGGGTAGAATTCCGCGTGTAGCAGTGAAACGCGTAGATATGCGGAGG-AATA
SBR1001         GCAGA-GGGGGGTGGAATTCCACGTGTAGCAGTGAAATGCGTAGAGATGTGGAGG-AACA
Azoarcus        GCAGA-GGGGGGTGGAATTCCACGTGTAGCAGTGAAATGCGTAGAGATGTGGAGG-AACA
Dp11u25         GGGGA-AGCTAAAGGTACTGTAGGGGGAGCGGTAAAATGCTGTAATCCTTGCAGG-ACCA
Lgd10           CTTTAGTGGGACTGGAACTCAAGGAGGAGTAGTGAAATGCGTTGATACCTCGGGG-AACA
DP10u18         GTAGA-GGAAAGGGGAACTGACGGTGGAGCAGTGAAATGCGTTGATATCGTCAGG-AACA
Dp10u19         GTAGA-GGAAAGGGGAACTGATGGTGGAGCAGTGAAATGCGTTGATATCATCAGG-AACA
                      *               * * **  **  **       *         * *

Dp11e12         CCGGTGGTGAAGACGACTCACTGGGCCTGTCCTGACGCTGAGG-TGCGAAAGCCAGGGGA
Sva0503         CCAAAGGTGANGACAAGTTACTGGGCCTGTCCTGACGCTGAGG-TACGAAAGCGTGGGTA
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Dp10u35         CCAAAAGCGAAAGCATTCAGCTGGGGCAACCCTGACGGTGAGG-GACGAAAGCGTGGGGA
dp10u16         CCAAAGGCGAAGGCAGTTCACTGGGACTTTCCTGACTCTGANA-TGCGAAAGCGTGGGGA
Dp10u13         CCGAAGGCGAAGGCAATAAGCTGGTGCATTTCTGACACTGAAA-CACGAAAGCGTGGGTN
Dp11u4          CCGAAAGCGAAGGCAAGAAACTGGTCCGCTCCTGACGCTGAAA-CACGAAAGCGTGGGTC
Dp10e3          CCAAGGGCGAAGGCATTTGGCTGGTGTGTTCCTGACGTTCAGA-GACGAAAGCGTGGGTC
Dp11e14         CCAAAAGCGAAGGCAGGGTGCTGGGGGTGTACTGACGCTCAGA-GACGAAAGCGTGGGGA
OPd3             CCAAAAGCGAAGGCAGCTTGCTAGAACGGTCCTGACGCTCAGG-GACGAAAGCGTGGGGA
Dp11u3          CCAAAAGCGAAGGCAACTGGCTAGAACGGTTCTGACGCTCATAAGACGAAAGCGTGGGTA
Dp11u10         CCAAATGCGAAGGCAGCTTGCTACAACATCACTGACGCTGAGA-TACGAAAGCGTGGGGA
LgD8            CCAAAGGCGAAGGCAGCTTGCTGGAACACAACTGACGCTCAGTGAACGAAAGCGTGGGGA
uncultured      CCGGTGGCGAAGGCGGCTCTCTGGACCGATACTGACGCTGAGA-CGCGAAAGCGTGGGGA
dp10e17         CCGGTGGCGAAGGCGGCTCTCTGGACTGTTACTGACGCTGAGG-CGCGAAAGCATGGGGA
WCHB1-12        CCGGTGGCGAAGGCGACTTTCTGGCCCTATACTGACGCTGAGA-CGCGAGAGCGTGGGTA
Dp11u38         CCGGTGGCGAAGGCGACTTTCTGGCCCTATACTGACGCTGAGA-CGCGAGAGCGTGGGTA
Syntrophus      CCGGTGGCGAAGGCGACTTTCTGGCCCTATACTGACGCTGAGA-CGCGAGAGCGTGGGTA
DP10e14         CCGGTGGCGAAGGCGACTTCCTGGCCCTATACTGACGCTGAGA-CGCGAGAGCGTGGGTA
dp10u10         CCGGTGGCGAAGGCGGCTACCTGGACCGATATTGACGCTGAGA-CGCGAAAGCGTGGGTA
Geobacter       CCGGTGGCGAAGGCGGCTACCTGGACCGATATTGACGCTGAGA-CGCGAAAGCGTGGGGA
Dp11e16         TCGGTGGCGAAGGCGGCTTTCTGGACCAATACTGACGCTAAGG-CGCGAAAGCATGGGGA
Dp11u7          CCAGTGGCGAAGGCGGCTTCCTGGATCGCAACTGACGCTGAGA-CGCGAAAGCGTGGGTA
Dp11u28         CCAGTAGCGAAGGCGGCTCTCTGGACTGATACTGACGCTCAAG-CGCGAAGGCTTGGGGA
Dp10u25         CCAGTGGCGAAGGCGGCTTTCTGGACCGTCCCTGACGCTGAGG-CGCGAAAGCCAGGGGA
Dp11u8          CCGGTGGCGAAGGCGATTTCCTGGCCTAACACTGACACTGAGG-CTCGAAAGCTAGGGGA
Dp11u33         CCAGTGGCGAAGGCGGCTTCCTGGTCCACTACTGACACTCAAG-TGCGAGAGCGTGGGGA
BPC023          CCAGTGGCGAAGGCGGCTTTCTGGACCAATACTGACACTGAGG-AGCGAAAGCGTGGGGA
Dp10u23         CCGATGGCGAAGGCAGCCCCCTGGGATAACACTGACGCTCATG-CACGAAAGCGTGGGGA
Ralstonia       CCGATGGCGAAGGCAGCCCCCTGGGATAACACTGACGCTCATG-CACGAAAGCGTGGGGA
Dp11u29         CCGATGGCGAAGGCAGCCCCCTGGGATAACACTGACGCTCATG-CACGAAAGCGTGGGGA
Dp10u30         CCGATGGCGAAGGCAGCCCCCTGGGTCAATACTGACGCTCATG-CACGAAAGCGTGGGGA
dp10e13         CCGATGGCGAAGGCAGCCCCCTGGGTCAATACTGACGCTCATG-CACGAAAGCGTGGGGA
Dp10u29         CCGATGGCGAAGGCAGCCCCCTGGGTCGACACTGACGCTCATG-CACGAAAGCGTGGGGA
SBR1001         CCGATGGCGAAGGCAGCCCCCTGGGTCGACACTGACGCTCATG-CACGAAAGCGTGGGTA
Azoarcus        CCGATGGCGAAGGCAGCCCCCTGGGCCAATACTGACGCTCATG-CACGAAAGCGTGGGGA
Dp11u25         CCAGTGGCGAAGGCGTTTATCTAAAACACGTCTGACGGTGAGG-GACGAAAGCTATGGGA
Lgd10           CCAATGGTGAAGACAAGTCCCTGGGAGATAACTGACGCTGAGA-CACGAAAGCTAGGGGA
DP10u18         CCAAAGGCGAAGGCACCTTTCTGGACCTCTCCTAACGCTGAGA-CACGAAAGCTAGGGGG
Dp10u19         CCAAAGGCGAAGGCACCTTTCTGGACTGTACCTGACGCTGAGA-CACGAAAGCTAGGGGA
                 *    * **   *       **          * **  * *     ***  **   **

Dp11e12         GCAAACGGGATTAGATACCCCGGTAGTCCTGGCCCTAAACGA-TGTCTACTAG--ATCGG
Sva0503         GCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGA-TGTATACTAG--TCCGG
Dp10u35         GCAAAAAGGATTAGATACCCTTGTAGTCCACGCTGTAAACTA-TGGATGCTAGCTATGTG
dp10u16         GCAAAAAGGATTAGATACCCTTGTAGTCCACGCCCTAAACGA-TGCCTGTTAGCTGTTTC
Dp10u13         GCGAATGGGATTAGATACCCCAGTAGTCCACGCCCTAAACGA-TGTTCTCTAGTTTTTCG
Dp11u4          GCGAATGGGATTAGATACCCCAGTAGTCCACGCCCTAAACGA-TGATCTCTAGCTTTGAG
Dp10e3          GCGAATGGGATTAGATACCCCAGTAGTCCACGCCCTAAACTA-TGGATGCTAGCTGTGGG
Dp11e14         GCAAAGGGGATTAGATACCCCCGTAGTCCACGCCCTAAACGA-TGCGTGCTCGGTGTAGG
OPd3            GCGAATAGGATTAGATACCCTAGTAGTCCACGCCCTAAACGA-TGAGTGNTAGGCATTGG
Dp11u3          GCGAATGGGATTAGATACCCCAGTAGTCCACGCTGTAAACGA-AGGGCACTAAGCATTGG
Dp11u10         GCGAAGCGGATTAGATACCCGCGTAGTCCACGCCGTAAACGA-TGGATACTANGCATTGA
LgD8            GCGAAAGGGATTAGATACCCCTGTAGTCCACGCTGTAAGCTA-TGGCTACTAGATTTTGG
uncultured      GCAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGA-TGGGCACTAGGTGTGGA
dp10e17         GCAAACGGGATTAGATACCCCGGTAGTCCATGCTGTAAACGA-TGGGCACTAGGTGTAGA
WCHB1-12        GCAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGA-TGTTCACTAGGTGTTGG
Dp11u38         GCAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGA-TGTTCACTAGGTGTTGG
Syntrophus      GCANACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGA-TGTTCACTAGGTGTTGG
DP10e14         GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGA-TGTTCACTAGGTGTTGG
dp10u10         GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGA-TGAGAACTAGGTGTTGC
Geobacter       GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGA-TGAGAACTAGGTGTTGC
Dp11e16         GCAAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACTA-TGAACACTAGGTGTTGA
Dp11u7          GTAAACAGGATTAGATACCCTGGTAATCCACGCCCTAAACGA-TGGGTACTAGGTGTTGT
Dp11u28         GCAAACAGGACTAGATACCCTGGTAGTCCAAGCGGTAAACTA-TGGGTACTAGGTGTCGG
Dp10u25         GCGAACAGGATTAGATACCCCGGTAGTCCTGGCCGTAAACGA-TGGGTACTANGTGTGGG
Dp11u8          GCAAACAGGATTAGATACCCTGGTAGTCCTAGCTGTAAACGA-TGATCATTAGGTGTAGG
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Dp11u33         GCAAACAGGATTAGATACCCTGGTAGTCCACGCTGTGAACGA-TGAAAACTAGACGTTGG
BPC023          GCAAACAGGATTAGAGACCCTGGTAGTCCACGCCGTAAACGA-TGAGAACTAGGCGTTGG
Dp10u23         GCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGA-TGTCAACTAGTTGTTGG
Ralstonia       GCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGA-TGTCAACTAGTTGTTGG
Dp11u29         GCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGA-TGTCAACTAGTTGTTGG
Dp10u30         GCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGA-TGTCAACTAGGTGTTGG
dp10e13         GCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGA-TGTCAACTAGGTGTTGG
Dp10u29         GCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGA-TGTCAACTAGGTGTTGG
SBR1001         GCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGA-TGTCAACTAGGTGTTGG
Azoarcus        GCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGA-TGCCAACTAGGTGTTGG
Dp11u25         GCAAACGGGATTAGATACCCCGGTAGTCCTGGGCGTAAACGA-TGTCCGTTTGGTGTTGC
Lgd10           GCGAAGCAGATTAGAGACCTGCGTAGTCCTAGCCCTAAACGAATGTCTGCTACCTGCATG
DP10u18         GCGAAGCAGATTAGAGACCTGCGTAGTCCTAGCCCTAAACGCATGTCTGCTAGATGTCCC
Dp10u19         GCGAAGCAGATTAGAGACCTGCGTAGTCCTAGCCCTAAACGCATGTCTGCTAGTTGCCCC
                *   *   ** **** ***   *** ***  *   * * *   *     *

Dp11e12         AGTCACTCTGACGTGTTTCCG-GCCGA-------AATGAAAACGGTAAGT
Sva0503         AGGAACTCTGACGTTTTTTCG-GACGA-------AATGAAAACGTTAAGT
Dp10u35         A-AGTGTC-GACCCTTC-ACGTGGCGA-------AGCTAACGCGTTAAGC
dp10u16         G-AGTATC-GACCCTCG-GGGTGGCGTT------AGCTAACGCGTTAAAC
Dp10u13         G-AGTATC-GACCCTCT-GAGANACAA-------AGCTAACGCNTTAAGA
Dp11u4          G-AGTATC-GACCCTCT-TCGAGGCTA-------AGCTAACGCGATAAGA
Dp10e3          G-AGTATC-GACCCTCT-CCGCGGCGA-------AGCTAACGCATTAAGC
Dp11e14         A-GTTTTC-AATTGCTC-CTGTGCCCA-------AGCTAACGCGGTAAGC
OPd3            A-AGTATC-GACCCTTT-CAGTGCCGTT-----AAGGTAACCCGTTAAGC
Dp11u3          A-AGTATC-GACCCTTA-CAGTGCTGTTTAACTAAGCTAACGCCTTAAGT
Dp11u10         G-AGTATC-GACCCTCT-CTGTGTCGTTCTAAAAAGCTAACGCGTTAAGT
LgD8            G-AGTTTC-GACCCTCT-CAGAGTCGACGAAACAAGCTAACGCGTTAAGT
uncultured      G-GGTATT-GACCCCTT-CTGTGCCGG-------AGCTAACGCATTAAGT
dp10e17         G-GGTTTC-AACCCCTT-CTGTGCCGA-------AGCTAACGCATTAAGT
WCHB1-12        G-GGTATT-GACCCTCT-CAGTGCCGC-------AGCTAACGCATTAAGT
Dp11u38         G-GGTATT-GACCCTCT-CAGTGCCGA-------AGCTAACGCATTAAGT
Syntrophus      G-GGTATT-GACCCTCT-CAGTGCCGC-------AGCTAACGCATTAAGT
DP10e14         G-GGTATT-GACCCCCT-CAGTGCCGG-------AGCTAACGCGTTAAGT
dp10u10         G-GGTATT-GACCCCTG-CAGTGCCGC-------AGCTAACGCATTAA-T
Geobacter       G-GGTATT-GACCCCTG-CAGTGCCGC-------AGCTAACGCATTAAGT
Dp11e16         G-GGAGTT-AAACCCTT-CAGTGCCGA-------AGCTAACGCATTAAGT
Dp11u7          G-GGTGTTTGACCCCCG-CAGTGCCGA-------AGCTAACGCGATAAGT
Dp11u28         G-GGTATC-GACCCCCT-CGGTGCCGA-------AGCTAACGCATTAAGT
Dp10u25         G-GGTATC-GACCCCCT-CCGTGCCGG-------AGTTAACACAATAAGT
Dp11u8          A-GGTATC-GACCCCTT-CTGTGCCGC-------AGCTAACGCATTAAAT
Dp11u33         G-GGGCTT-GT-CCCCT-TTGTGTCTT-------AGCTAACGCGATAAGT
BPC023          G-AAGCTT-GA-CTTCT-TAGTGCCGT-------AGCTAACGCGATAAGT
Dp10u23         G-GATTCA-T--TTCCT-TAGTAACGT-------AGCTAACGCGTGAAGT
Ralstonia       G-GATTCA-T--TTCCT-TAGTAACGT-------AGCTAACGCGTGAAGT
Dp11u29         G-GATTCA-T--TTCCT-TAGTAACGT-------AGCTAACGCGTGAAGT
Dp10u30         G-GGAGGA-GACTTCCT-TAGTACCGC-------AGCTAACGCGTGAAGT
dp10e13         G-GGAGGA-GACTTCCT-TAGTACCGC-------AGCTAACGCGTGAAGT
Dp10u29         G-GGAGGA-GACTTCCT-TAGTACCGC-------AGCTAACGCGTGAAGT
SBR1001         G-GAAGGA-GACTTCCT-TAGTACCGT-------AGCTAACGCGTGAAGT
Azoarcus        G-TGGGTA-AAACCATT-TAGTACCGT-------AGCTAACGCGTGAAGT
Dp11u25         ACACTCTACGTGGGTGTGCAGTGCCGT-------AGCGTANGCGTTAAAC
Lgd10           A---ATCATG-CTTTTGCATGACTCGTGTGGGAAAGGTAACCCGTTAAGC
DP10u18         G---------CGCAAGTGGGGTGTCGTAAG-CTAA-----CGCGTTAAGC
Dp10u19         GGA-TCAAAGCGCACACGCTTTGAGAAATT-CGAAGC--ACGAAATACGA
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Appendix 6: VOC and redox conditions from influent for the biotic and inhibited columns.

date	date	Elapsed time (days)	DO (ppm)	Fe(II) (ppm)	Sulfate (ppm)	pH	eH (mV)	Chloride (ppm)	Bromide (ppm)	sulfide (ppm)	TCE (aer ppb)	TCE µM	TCE (an2 ppb)	TCE (µM)
11/03/1999	36467	-82												
11/09/1999	36473	-76		0	67	7.72	166	0.16						
11/17/1999	36481	-68	8.59	0	65	6.7	213	0.109		0				
11/22/1999	36486	-63												
12/01/1999	36495	-54												
12/15/1999	36509	-40	3.23	0		7.7	-48	0.376						
12/17/1999	36511	-38									0	0		
01/06/2000	36531	-18										0		
01/13/2000	36538	-11										0		
01/24/2000	36549	0									61	0.4636	1000	7.6
01/28/2000	36553	4	5.72	0	64.6	6.63	170		0.02	0		0		
01/31/2000	36556	7										0		
02/02/2000	36558	9							1.84			0		
02/04/2000	36560	11									937.69	7.126444	2103	15.9828
02/09/2000	36565	16	3.96	0	65.4	7.27	282		0.028			0		0
02/14/2000	36570	21									2219	16.8644		0
02/25/2000	36581	32										0		0
03/29/2000	36614	65	4.91	0	58.7	7.19	179	0.9		0		0		0
04/08/2000	36624	75	2.83		68.7	6.78	238	0.03		0		0		0
04/09/2000	36625	76	1.36	0				0.01				0		0
05/10/2000	36656	107	3.25	0								0		0
05/15/2000	36661	112										0	1859	14.1284
06/05/2000	36682	133										0	1320	10.032
06/08/2000	36685	136	3.2	0	63.74	6.38	197	0.01		0		0		0
06/14/2000	36691	142		0	58.5	7.05		0.012		0	816	6.2016	952	7.2352
06/21/2000	36698	149		0	61.9	7.35	72	0.05		0		0	1121	8 5196
06/29/2000	36706	157										0	2690	20.444
07/21/2000	36728	179									2968	22.5568	3192	24.2592
09/28/2000	36797	248	3.95	0	68.7	7	-12	1.46		0		22.5		24.2

Appendix 7: VOC and redox results from the inhibited column.

date	date	Elapsed time (days)	Flow rate	DO (ppm)	Fe(II) (ppm)	Sulfate (ppm)	pH	eH (mV)	Chloride (ppm)	Bromide (ppm)	sulfide (ppm)	TCE (ppb)	TCE (µM)	cDCE (ppb)	VC (ppb)
11/01/1999	36465	-84	set for 0.2 mL/min												
11/09/1999	36473	-76	0.2												
11/16/1999	36480	-69													
11/18/1999	36482	-67													
11/22/1999	36486	-63	0.22												
12/15/1999	36509	-40		5.7	0		5.26	32.3	0.7						
12/17/1999	36511	-38										0.018	0.000137		
12/20/1999	36514	-35	0.2												
12/22/1999	36516	-33		5.23	0	26	5.7		52						
01/13/2000	36538	-11										35.6	0.27056		
01/19/2000	36544	-5		5.56	0		5.12	216	0.272						
01/24/2000	36549	0	0.16	6.65								0			
01/25/2000	36550	1										92	0.6992	0	0
01/27/2000	36552	3										114	0.8664		
01/28/2000	36553	4										150	1.14	0	0
01/29/2000	36554	5		5.72	0	58 7	5.98	207		0.627	0	81	0.6156	0	0
02/02/2000	36558	9	0.24	5.38	0	63	5.37	294		1.19					
02/04/2000	36560	11										328	2.4928	0	0
02/09/2000	36565	16	0.155	4.78	0	61.05	5.53	241		0.581					
02/11/2000	36567	18										244	1.8544		
02/14/2000	36570	21										309	2.3484	0	0
02/16/2000	36572	23		4.74	0	59.35	5.4	300		0.284					
02/17/2000	36573	24										318	2.4168		
02/25/2000	36581	32	0.199												
02/28/2000	36584	35										1597	12.1372	0	0
03/01/2000	36586	37		3.5	0	62.75	5.11			0.123					
03/08/2000	36593	44										657	4.9932		
03/19/2000	36604	55										673	5.1148		
03/21/2000	36606	57										1017	7.7292	0	0
03/27/2000	36612	63										2395	18.202		
03/29/2000	36614	65		4.91	0	58.7	7.19	286	0.7						
03/30/2000	36615	66										1696	12.8896	0	0
04/03/2000	36619	70										1654	12.5704	0	0
04/06/2000	36622	73		4.96	0		5.87	181	0.442						
04/09/2000	36625	76	0.11												
04/14/2000	36630	81										413	3.1388	0	0
04/17/2000	36633	84										420	3.192	0	0
04/20/2000	36636	87		5.4	0	50	6.38	162	0.03						
04/24/2000	36640	91	0.35												
05/01/2000	36647	98	0.4												
05/04/2000	36650	101		4.57	0	49 04	6.91		0.227						
05/08/2000	36654	105	0.25												

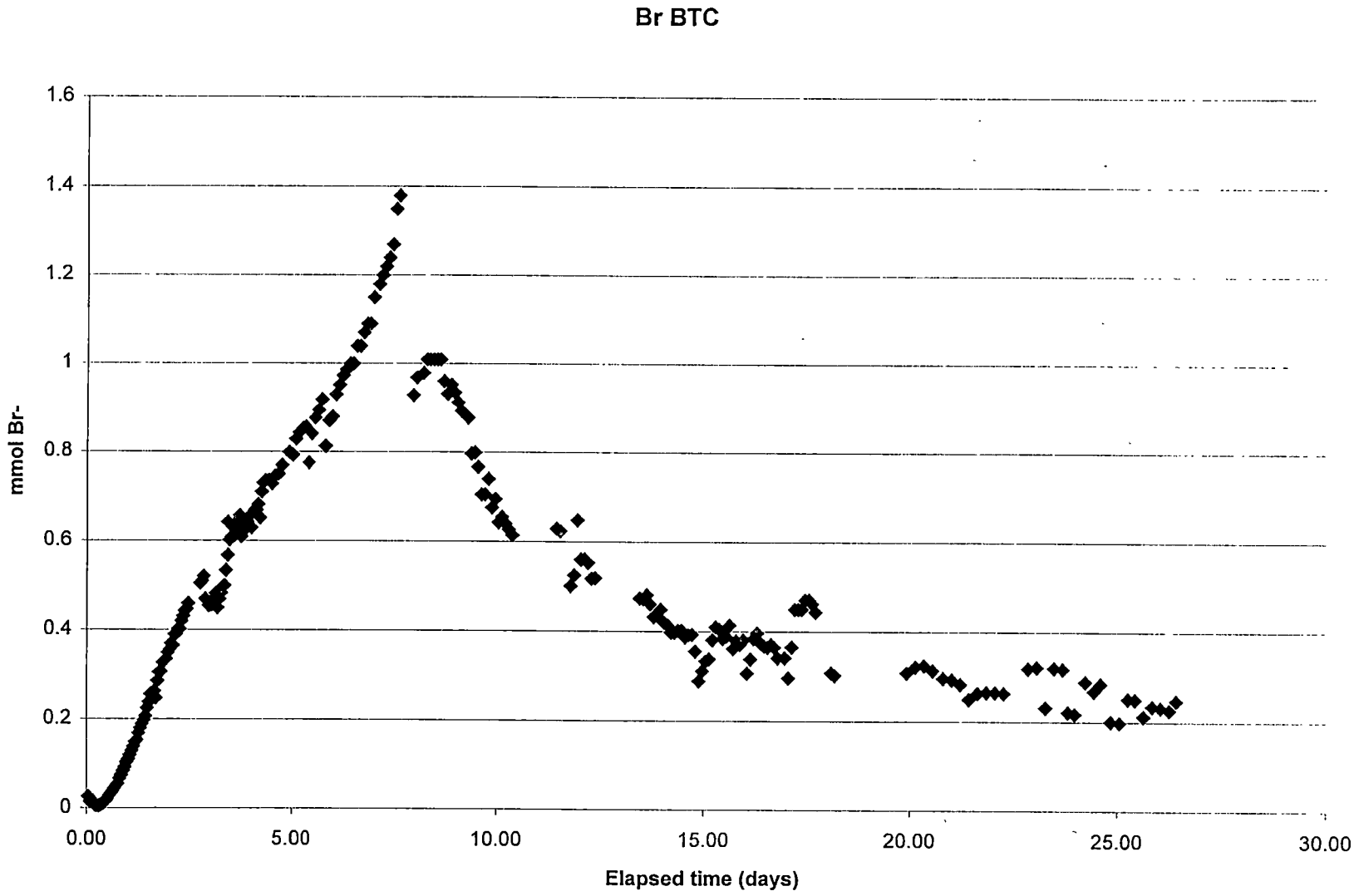
date	date	Elapsed time (days)	Flow rate	DO (ppm)	Fe(II) (ppm)	Sulfate (ppm)	pH	eH (mV)	Chloride (ppm)	Bromide (ppm)	sulfide (ppm)	TCE (ppb)	TCE (µM)	cDCE (ppb)	VC (ppb)
05/10/2000	36656	107		3.25	0	52.82	7.25	-8.5							
05/17/2000	36663	114		6.66	0	53.6	6.28	128							
05/22/2000	36668	119										2109	16.0284	0	0
05/23/2000	36669	120	0.15												
05/24/2000	36670	121		3.8	0	57.02	6.07	130							
06/05/2000	36682	133										840	6.384		
06/08/2000	36685	136	0.22	4.14		63.74	6.43	104	1.42						
06/14/2000	36691	142	0.28	4.26	0	65.42	6.52	72	1.8						
06/15/2000	36692	143										872	6.6272	0	0
06/21/2000	36698	149	0.3	4.5	0	75.4	6.64	148	8.1						
06/27/2000	36704	155	0.209		0	60.38	6.58	104	0.627						
06/29/2000	36706	157										1417	10.7692	0	0
07/05/2000	36712	163			0	60.38	6.48	87							
07/07/2000	36714	165	0.24												
07/18/2000	36725	176		5.45	0	61.33	6.23	29.3	0.06						
07/24/2000	36731	182										1644	12.4944	0	0
07/26/2000	36733	184		3.16	0	57.8	6.31	34	0.222						
07/28/2000	36735	186													
07/29/2000	36736	187	0.25												
08/02/2000	36740	191		3.48	0	62.9	6.31	28							
08/04/2000	36742	193	0.27												
08/14/2000	36752	203	0.18												
08/16/2000	36754	205		3.92	0	67.1	6.3	32.7							
09/14/2000	36783	234										1611	12.2436	0	0
09/15/2000	36784	235										1590	12 084	0	0
09/19/2000	36788	239										1350	10.26	0	0
09/28/2000	36797	248		5.31	0	67	5.68	61	0.255						
12/14/2000	36875	326										1669	12.6844		

Appendix 8: VOC and redox results from the biotic column.

date	date	elapsed time (days)	AFCEE Score	Flow rate (mL/min)	DO (ppm)	Fe(II) (ppm)	Sulfate (ppm)	pH	eH (mV)	Chloride (ppm)	Bromide (ppm)	sulfide (ppm)	TCE (ppb)	TCE (µM)	cDCE (ppb)	cDCE (µM)	TCE (µM)	VC (ppb)
11/29/1999	36493	-56																
12/04/1999	36498	-51																
12/24/1999	36518	-31																
12/15/1999	36509	-40	-1		3.41	0		5.92	56.2	0.7								
12/22/1999	36516	-33			3.74	0		6.4	280	16.1		0						
01/11/2000	36536	-13											11.8	0.08968	0.05	0.000515	0.08968	
01/19/2000	36544	-5			2.3	0		5 8	172	0.677				0				
01/24/2000	36549	0		0.16	1.57								0	0	0	0	0	
01/25/2000	36550	1											3 8	0.02888	0	0	0.02888	0
01/27/2000	36552	3											97	0.7372	0	0	0 7372	
01/28/2000	36553	4											263	1.9988	0	0	1.9988	0
01/29/2000	36554	5	1		2.25	0.2	53.66	6.77	152		0.445	0		0				
01/31/2000	36556	7												0				
02/02/2000	36558	9	1	0.22	1.54	0.4	53.66	6.37	187		1.24			0				
02/04/2000	36560	11											344	2.6144	0	0	2.6144	0
02/09/2000	36565	16	1	0.196	3.11	0.4	60	6.23	140		0.25	0		0				
02/11/2000	36567	18											950	7.22	0	0	7.22	
02/13/2000	36569	20											1381	10.4956	0	0	10 4956	
02/16/2000	36572	23	1		2.3	0.5	62.75	6.85	123		0.149		1818	13.8168	0	0	13.8168	
02/17/2000	36573	24											1179	8.9604	0	0	8.9604	0
02/21/2000	36577	28												0				
02/22/2000	36578	29											1272	9.6672	0	0	9 6672	
02/24/2000	36580	31											1818	13.8168	10	0.103	13.8168	0
02/25/2000	36581	32		0.19										0				
02/28/2000	36584	35											1746	13.2696	0	0	13.2696	0
03/01/2000	36586	37	4		0.76	0.6	60 2	6.03	186					0				
03/08/2000	36593	44											1522	11.5672	0	0	11 5672	
03/19/2000	36604	55											1752	13.3152	0	0	13.3152	
03/21/2000	36606	57											2122	16.1272	54	0.5562	16.1272	
03/27/2000	36612	63											2173	16.5148	66	0.6798	16.5148	0
03/29/2000	36614	65	3		1.5	0.6	53.6	6.45	96	7		0.02		0				
03/30/2000	36615	66											2285	17.366	62	0 6386	17.366	
04/03/2000	36619	70											2164	16.4464	74	0.7622	16.4464	
04/09/2000	36625	76	3	0.19	3.18	0.8	59.54	5.96	55	0.8		0 01		0				
04/14/2000	36630	81											1603	12.1828	18	0.1854	12.1828	0
04/17/2000	36633	84		0.214									1565	11.894	25	0.2575	11.894	
04/20/2000	36636	87	6		2.3	1.2	56.18	6.12	90	0.138		0.02		0				
04/24/2000	36640	91		0.18										0				
05/01/2000	36647	98		0.15										0				
05/04/2000	36650	101			1 86	0.8	49.04	6.76		0.631				0				
05/10/2000	36656	107	4		1.75	0.8	48.62	6.39	28.4	0.862		0		0				
05/15/2000	36661	112											1386	10.5336	24	0 2472	10.5336	0
05/16/2000	36662	113		0.176										0				
05/17/2000	36663	114	4		1.88	0.9	50.3	6 02	-82			0		0				
05/18/2000	36664	115											1273	9.6748	22	0.2266	9.6748	0
05/23/2000	36669	120											1486	11.2936	16	0.1648	11.2936	
05/24/2000	36670	121	5	0.19	1.75	1	54.5	6.11	-120			0		0				
06/05/2000	36682	133											840	6.384	58	0.5974	6.384	0
06/08/2000	36685	136	8	0.17	1.5	1.2	58.7	6.23	-200	5 14		0.02		0				

date	date	elapsed time (days)	AFCEE Score	Flow rate (mL/min)	DO (ppm)	Fe(II) (ppm)	Sulfate (ppm)	pH	eH (mV)	Chloride (ppm)	Bromide (ppm)	sulfide (ppm)	TCE (ppb)	TCE (µM)	cDCE (ppb)	cDCE (µM)	TCE (µM)	VC (ppb)
06/13/2000	36690	141												0				
06/15/2000	36692	143	8	0.16	1.54	1.2	49.15	6.37	-143	0.992		0 04	744	5.6544	72	0.7416	5.6544	0
06/21/2000	36698	149	11	0.16	1	1.3	58.5	6.32	-220	0.194		0 05		0				
06/23/2000	36700	151											989	7.5164	84	0 8652	7.5164	
06/26/2000	36703	154											1266	9.6216	97	0.9991	9.6216	0
06/27/2000	36704	155	8	0.18		1.3	52	6.06	-22	1.39		0.1		0				
06/29/2000	36706	157											1404	10.6704	80	0.824	10.6704	0
07/05/2000	36712	163	11			1.4	54	6.31	-17	0.537		0.15		0				
07/07/2000	36714	165		0.19										0				
07/18/2000	36725	176			0.73	1.4	49.46	6				0.06		0				
07/21/2000	36728	179		0.12										0				
07/27/2000	36734	185	7		1.7	1.3	53.66	6.04	-60	0.211		0.04		0				
07/28/2000	36735	186												0				
07/24/2000	36731	182											2133	16.2108	129	1.3287	16.2108	0
07/29/2000	36736	187		0.18										0				
08/02/2000	36740	191	10		0.91	1 5	54.5	6.26	-13.3	2.98		0.09		0				
08/04/2000	36742	193		0.18										0				
08/14/2000	36752	203		0.15										0				
08/16/2000	36754	205	10		1.06	1.5	60 38	5 92		0.17		0.2		0				
08/28/2000	36766	217											2326	17.6776	65	0.6695	17.6776	0
09/14/2000	36783	234											1929	14.6604	36	0 3708	14.6604	9
09/15/2000	36784	235											1884	14.3184	25	0.2575	14.3184	10.2
09/19/2000	36788	239											1536	11.6736	91	0.9373	11.6736	8.5
09/25/2000	36794	245											1703	12.9428	112	1.1536	12.9428	10
09/28/2000	36797	248	15		1.42	1.6	62	6.77	-24	2.98		0.17		0		0		
12/14/2000	36875	326											1105	8.398	44	0.4532		16

Appendix 9: Bromide breakthrough for the preliminary column.


Br BTC
mmol Br-
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0.00
5.00
10.00
15.00
20.00
25.00
30.00
Elapsed time (days)

Appendix 10: VOC results from the preliminary column.

Sample ID	Julain Time	Time elapsed (days)	TCE (ppm)	DCE(ppm)
04/02/1999	36252	1	0.0036	0
04/04/1999	36254	3	0.026	0
04/05/1999	36255	4	0.028	0
04/07/1999	36257	6	0.029	0
04/12/1999	36262	11	0.03	0
04/14/1999	36264	13	0.025	0
04/15/1999	36265	14	0.043	0
04/18/1999	36268	17	0.054	0
04/19/1999	36269	18	0.046	0
04/21/1999	36271	20	0.04	0
04/22/1999	36272	21	0.039	0
04/26/1999	36276	25	0.036	0
04/27/1999	36277	26	0.036	0
04/28/1999	36278	27	0.035	0
04/30/1999	36280	29	0.035	0
05/03/1999	36283	32	0.06	0
05/10/1999	36290	39	0.117	0
05/14/1999	36294	43	0.13	0
05/18/1999	36298	47	0.104	0
05/19/1999	36299	48	0.13	0
05/21/1999	36301	50	0.304	0
05/25/1999	36305	54	0.08	0
05/26/1999	36306	55	0.11	0
05/27/1999	36307	56	0.57	0
06/02/1999	36313	62	5.98	0
06/07/1999	36318	67	8.3	0
06/11/1999	36322	71	11.6	0
06/15/1999	36326	75	11.7	0
06/24/1999	36335	84	9.15	0
06/27/1999	36338	87	10.61	0
06/29/1999	36341	90	7.805	0
06/30/1999	36341	90	8.58	0
07/06/1999	36347	96	8.88	0
07/12/1999	36353	102	9.9	1.2
07/13/1999	36354	103	8.965	
07/14/1999	36355	104	9.4	
07/19/1999	36360	109	11.5	
07/25/1999	36366	115	8.41	
07/26/1999	36367	116	10.75	0.12
07/29/1999	36370	119	11.7	
07/28/1999	36369	118	9.56	0.1
08/08/1999	36380	129	10.4	
08/09/1999	36381	130	8.99	
08/20/1999	36392	141	3.16	
09/13/1999	36416	165	4.54	
09/15/1999	36418	167	5.4	
09/28/1999	36431	180	0.26092	0.06615
11/05/1999	36469	218	0.18556	
11/08/1999	36472	221	0.10899	
11/16/1999	36480	229	0.34419	
11/22/1999	36486	235	0.4845	
12/20/1999	36514	263	0.097	
12/23/1999	36517	266	0.0724	
12/31/1999	36525	274	0.0538	

Appendix 11: Redox results from the influent and effluent from the preliminary column.

Source	Date	Date	Elapsed Time days	DO	Eh (mV)	total Fe (ppm)	Fe(II) (ppm)	SO4 (ppm)	S- (ppm)	pH	Cl- (mmol at Cl-)	Temp °C	Depth to water (m)	Ammonia ppm	Flow rate ml/min
E	04/05/1999	36255	4			0.4	0.2	30	0						
E	04/27/1999	36277	26	2.6		1.2	1.2	36	0						
E	05/11/1999	36291	40		63		1		0	5.66		16		0	
E	05/17/1999	36297	46	3		1.3	1	20	0	5.88					
E	05/21/1999	36301	50	4		1	0.6	38.54		6.16					
E	05/24/1999	36304	53	2.4	58.8		1		0	5.75					
E	06/03/1999	36314	63	5.46		1.2	1.2	33.5	0	5.47					
E	06/14/1999	36325	74	1.74			1.4		0						
E	06/23/1999	36334	83	3.61		1	1	50.3	0	5.66					
E	08/01/1999	36373	122	1.8		1.2	1.2	35	0	5.5	3.94				
E	08/09/1999	36381	130	1.8		1.2	1.2	48	0	5.8					
E	08/15/1999	36387	136	2.03			1.2		0						
E	08/17/1999	36389	138	1.85			1.3		0						
E	11/03/1999	36467	216		91.4	2	2	58.7	0.1	5.86	0.05				0.156
E	11/10/1999	36474	223	0.06		2	1.7	53	0	5.27	0.187	12			
E	11/15/1999	36479	228												0.25
E	11/17/1999	36481	230		132	2	2	48		5.86	1.36				
E	12/13/1999	36507	256												0.2
E	12/15/1999	36509	258	4.2	48	2.8	2.8		0		1.17				
E	12/23/1999	36517	266	3.33	58.7	3.5	2.8	50	0	5.87	2.74				
E	01/10/2000	36535	284	2.7	112	3.5	3.4	45	0	5.57	0.153				
I	02/18/1999	36209	-42		37	0	0		0			12	>55 ft	0	
I	03/04/1999	36223	-28	0.8	110					6.98					
I	03/11/1999	36230	-21		32	0	0	present	0	6.86		11.3	16.48	0	
I	04/01/1999	36251	0		24	0	0		0	6.87		13.8	16.72	0	
I	04/08/1999	36258	7	1									16.78		
I	04/19/1999	36269	18	0.8									16.78		0.2
I	04/28/1999	36278	27	1.25		0	0	40		7.12		14.9			
I	05/05/1999	36285	34										16.3		
I	05/11/1999	36291	40	0.61											
I	05/21/1999	36301	50	1.2											0.2
I	05/28/1999	36308	57												
I	06/03/1999	36314	63	0.73									17.12		
I	06/18/1999	36329	78												
I	06/21/1999	36332	81												
I	07/01/1999	36342	91	4.31											
I	07/26/1999	36367	116	1.2											
I	08/01/1999	36373	122	1.5		0	0	35	0	6.8	0.0803				
I	11/09/1999	36473	222	0.06	166.7	0	0	67	0	7.72	0.16	17.5			
I	4/1/99 (test	########	7			0	0	0	0						0.2
								47.33333							

Appendix 12: Preliminary column sequence alignment for Archae clone library with reference strains are listed with accession numbers.

CLUSTAL X (1.64b) multiple sequence alignment

```
WCHA1-38              C-CTCAAGTGGTCAGGATGATTATTGGGCCTAAAGCATCCGTAGCTCGTTTTGTAAGTTT
778u18530f            CACTCAAGTGGTCAGGATGATTATTGGGCCTAAAGCATCCGTAGCTCGTTTTGTAAGTTT
778u29                -ACTCGAGTGGTCAGGAGGTTTATTGGGCCTAAAGCATCCGTAGCCGGCTGCTCAAGTTT
pLemB390              --TCCGAGTGGTGTGGATGTTTATTGGGCCTAAAGCATCCGTAGCTGGCTAGGTCAGTCC
Methanococcoides      GGCCCGAGTGGTAATCACTTTTATTGGGTCTAAAGGGTCCGTAGCCGGTTTGATCAGTTC
WCHD3-33              GGCCCGAGTGGTGGTCGATATTATTGAGCCTAAAACGTTCGTAGCCGGTCTTGTAAATCC
WCHD3-30              GGTCCAAGTCGCAGCCATCATTATTGGGTCTAAAACATCCGTAGCTTGCTTATTAAGTTT

WCHA1-38              TCGGTTAAATCCATGCGCTTAACGTATGGGCTGCCGGGAAT-ACTGCATAACTAGGAAGT
778u18530f            TCGGTTAAATCCATGCGCTTAACGTATGGGCTGCCGGGAAT-ACTGCATAACTAGGAAGT
778u29                CCGGTTAAATCCACACGCTTAACGTATGGGCTGCCGGAAAT-ACTGTTCAGCTAGGGAGT
pLemB390              CTTGTTAAATCCACCGAATTAATCGTTGGATTGCGGGGGAT-ACTGCTTGGCTAGGGGGC
Methanococcoides      TTCGGGAAATCTGACAGCTCAACTGTTAGGCTTCCGGGGAATACTGTCAGACTTGGGACC
WCHD3-33              TTGGGTAAATCGGCCAGCTTAACTGTCCGAAGTCCGGGGAG-ACTGCAAGACTTGGGATC
WCHD3-30              CTTGTGAAATCTTATCTCTTAAGGATAAGGCGTGCAAGAAATACTGTTAAGCTAGAGACT

WCHA1-38              GGGAGAGGTAGACGGTACTCGGTAGGAAGGGGTAAAATCCTTTGATCTATCGATGACCAC
778u18530f            GGGAGAGGTAGACGGTACTCGGTAGGAAGGGGTAAAATCCTTTGATCTATTGATGACCAC
778u29                GGGAGAGGTAGACGGTACTCNATAGGAAGGGGTAAAATCCTTTGATCTATTGATGACCAC
pLemB390              GAGAGAGGCAGACGGTATTTTCGGGGTAGGGGTGAAATCCTATAATCCCGGGAAGACCAC
Methanococcoides      GGGAGAGGTAAGAGGTACTACAGGGGTAGGAGTGAAATCTTGTAATCCCTGTGGGACCAC
WCHD3-33              GGGAGAGGTCAGAGGTACTTCTGGGGTAGGGGTAAAATCCTGTAATCCTAGAAGGACCAC
WCHD3-30              GGAAGACGTAGAAAGTATGTCTAAAGTAGCGGTAAAATGTGTTAATCTTAGGCAGACTCA

WCHA1-38              CTGTGGCGAAAGCGGTCTACCAGAACACGTTCGACGGTGAGGGATGAAAGCTGGGGGAGC
778u18530f            CTGTGGCGAAGGCGGTCTACCAGAACACGTTCGACGGTGAGGGATGAAAGCTGGGGGAGC
778u29                CTGTGGCGAAGGCGGTCTACCAGAACACGTTCGACGGTGAGGGATGAAAGCTGGGGGAGC
pLemB390              CAGTGGCGAAGGCTGTCTGCTAGAACGCGCCCGACGGTGAGGGATGAAAGCTGGGGGAGC
Methanococcoides      CAGTGGCGAAGGCGTCTTACCAGAACGGGTCCGACGGTGAGGGACGAAAGCTGGGGGCAC
WCHD3-33              CGGTGGCGAAGGCGTCTGACTAGAACGAATTCGACGGTGAGGAACGAAGCCCTGGGGCGC
WCHD3-30              CAACAGCGAAGGCATTCTACGAGGACAGTTCTGACAGTAAAGGATGAAGGCTAGGGGCGC

WCHA1-38              AAACCGGATTAGATACCCGGGTAGTCCCAGCTGTAAACTATGCAAACTCAGTGATGCATT
778u18530f            AAACCGGATTAGATACCCGGGTAGTCCCAGCTGTAAACTATGCAAACTCAGTGATGCATT
778u29                AAACCGGATTAGATACCCGGGTAGTCCCAGCTGTAAACTATGCAAACTCAGTGATGCATT
pLemB390              GAACCGGATTAGATACCCGGGTAGTCCCAGCTGTAAACGATGCAGACTAGGTGTTTGGAC
Methanococcoides      GAACCGGATTAGATACCCGGGTAGTCCCAGCCGTAAACGATGTTCGCTAGGTGTCAGGGG
WCHD3-33              AAACGGGATTAGATACCCCGGTAGTCAGGGT-GTAAACGCTGCGGGCTTGGTGTTGGGGG
WCHD3-30              AAAGTGGATTAGATACCCATGTAGTCCTAGCAGTAAACACTGCACACTAAACATTAGTAC

WCHA1-38              GGCTTGTGGCCAATGCAGTGCTGCAGGGAAGCCGTTAAGTTTGCCGCCTGGGAAGTACGT
778u18530f            GGCTTGTGGCCAATGCAGTGCTGCAGGGAAGCCGTTAAGTTTGCCGCCTGGGAAGTACGT
778u29                GGCTTGTGGCCAATGCAGTGCTGCAGGGAAGCCGTTAAGTTTGCCGCCTGGGAAGTACGT
pLemB390              GGCCACGTGCCGTTCTAGTGCCGCAGGGAAGCTGTTAAGTCTGCCGCCTGGGGAGTACGA
Methanococcoides      CGGTGCGACCGCTTCTGGTGCCGTAGGGAAGCCGTGAAGCGAACCACCTGGGAAGTACGG
WCHD3-33              TCCTTAGTGGGCGCCCAGTGCCGGAGAGAAGTTGTTAAGCCTGCTGCTTGGGGAGTATGT
WCHD3-30              CTCTTCGAGAGGTATTAGTGCTGTAGAGAAGTCGAAGAGTGTGCTACCTGGGAAGTATAG

WCHA1-38              ACGCAAGT-ATGAAACTTAAAGGAATTGGCGGGGGAGCACCACAAGGGGT-GAAGCCTGC
778u18530f            ACGCAAGTTATGAAACTTAAAGGAATTGGCGGGGGAGCACCACAAGGGGTTGAAGCCTGC
778u29                ACGCAAGT-ATGAAACTTAAAGGAATTGGCGGGGGAGCACCACAAGGGGT-GAAGCCTGC
pLemB390              TCGCAAGA-TTGAAACTTAAAGGAATTGGCGGGGGAGCACCACAAGGGGT-GAAGCTTGC
Methanococcoides      CCGCAAGG-CTGAAACTTAAAGGAATTGGCGGGGGAGCACTACAACGGGT-GGAGCCTGC
WCHD3-33              CCGCAAGG-ATGAAACTTAAAGGAATTGGCGGGGGAGCACCGCAACGGGA-GGAGCGTGC
WCHD3-30              CCGCAAGG-CCGAAACTTAAAGGAATTGGCGGGGAGACACTACAACAGGT-GACGCGTGC
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WCHA1-38              GGTTCAATTGGAGTCAACGCCAGAAATCTTACCCGGAGAGA
778u18530f            GGTTCAATTGGAGTCAACGCCAGAAATCTTACCCGGA----
778u29                GGTTCAATTGGAGTCNACGCCANAAATCTTACCCGGAGAGA
pLemB390              GGTTTAATTGGAGTCAACGCCGGAAATCTCACCGGGAGCGA
Methanococcoides      GGTTTAATTGGACTCAACGCCGGAAAACTCACCGGGGGCGA
WCHD3-33              GGTTTAATTGGATTCAACACCGGACAACTCACCAGGAGCGA
WCHD3-30              GGTTCAATTAGATTCTACACCGTGAACCTCACCAGGAGCGA
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Appendix 13: Preliminary column sequence alignment CFB clone library with reference strains are listed with accession numbers.

CLUSTAL X (1.64b) multiple sequence alignment

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Rhodococcus             GTAATACGTAGGGTGCAAGCGTTGTCCGGAATTACTGGGCGTAAAGAGT-TCGTAGGCGG
513                     ----------------------GTCCGGAATTACTGGGCGTAAAGAGT-TCGTAGGCGG
Microbacterium          GTAATACGTAGGGCGCAAGCGTTATCCGGAATTATTGGGCGTAAAGAGC-TCGTAGGCGG
1630                    -----------------AGCGTTGTTCGGATTTATTGGGCGTAAAGAGC-TCGTAGGCGG
High                    -------------------CGTTGTCCGGATTTATTGGGCGTAAAGAGC-TCGTAGGTGG
778u40                  ------------GGGGCAACGTTGTCCGGATTTATTGGGCGTAAAGAGC-TCGTAGGTGG
71613                   ------TACGGGGGGGCAACGTTGTTCGGAATTACTGGGCGTAAAGGGC-GCGTANGCGG
WCHA2-13                -----TACGGGGGGGCAAGCGTTGTTCGGAATTATTGGGCGTAAAGGGC-GCGTAGGCGG
Sphingobacterium        GTAATACGGAGGATCCAAGCGTTATCCGGATTTATTGGGTTTAAAGGGT-GCGTAGGCGG
778u37                  --------------CCAAGCGTTATCCGGATTTATTGGGTTTAAAGGGT-GCGTAGGCGG
778u1                   ------CGGAAGGTGCAAGCGTTATCCGGATTCACTGGGTTTAAAGGGT-GCGTAGGCGG
778u39                  -------AAGAGTGGCGAGCGTTGTTCGGAATTACTGGGCTTAAAGGGC-GCGTAGGCGG
uncultured              GTAATACGGAAGGTGCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGT-CCGCAGGCTT
778u21530f              ---------GGGATGCAA-CGTTACTCGGAATCACTGGGCGTAAAGCGT-CTGTAGGCGT
778u33                  ------------AGGCCGACGTTAATCGGAATCACTGGGCTTAAAGCGT-GCGTAGGCGG
Pirellula               GTAAGACGAACCGACCGAACGTTATTCGGAATTACTGGGCTTAAAGGGT-GCGTAGGCGG
X91291                  -------AGAGGTGGCGAGCGTTACTCGGATTAATTGGGTGTAAAGGGC-AAGTAGGCGT
778u26                  ---------ANGTGGCGAGCGTTACTCGGATTTATTGGGTGTAAAGGGC-ANGTANGCGT
Clostridium             GTAATACGTAGGGGGCNNGCGTTATCCGGAATTACTGGGCGTAAAGGGT-GCGTAGGTGG
71612                   --------TANGGGGCTA-CGTTATCCGGAATTACTGGGCGTAAAGGGT-GCGTAGGTGG
Geothrix                GTAATACAGAGGGGGCAAGCGTTATTCGGAATTATTGGGCGTAAAGGGC-GCGTAGGCGG
2202                    ------------------------------------------------------------
SJA-87                  GTAATACAGAGGGGGCAAGCGTTATTCGGAATTATTGGGCGTAAAGGGC-GCGTAGGCGG
AJ241004                GTAATACGGAGGGGGCTAGCGTTGTTCGGAATTATTGGGCGTAAAGGGC-GCGTAGGCGG
candidate               GTCATACGGAGGATCCAAGCGTTATCCGGAATTACTGGGCGTAAAGAGTTGCGTAGGTGG
511                     ----------------------ATCCGGAATTACTGGGCGTAAAGAGTTGCGTAGGTGG

Rhodococcus             TTTGTCGCGTCGT-TTGTGAAAA-CCAGCAGCTCAACTGCT-GGCTTGCAGGCGATACGG
513                     TTTGTCGCGTCGT-TTGTGAAAA-CCAGCAGCTCAACTGCT-GGCTTGCAGGCGATACGG
Microbacterium          TTTGTCGCGTCTG-CTGTGAAAT-CTGGGGGCTCAACCCCC-AGCCTGCAGTGGGTACGG
1630                    TTTGTCGCGTCTG-CTGTGAAAT-CTGGGGGCTCAACCCCC-AGCCTGCAGTGGGTACGG
High                    CTCGGTAAGTCGG-GTGTGAAAT-TTCGAGGCTCAACCTCG-AGACGCCACCTGATACTG
778u40                  CTCGGTAAGTCGG-GTGTGAAAT-TTCGAGGCTCAACCTCG-AGACGCCACCTGATACTG
71613                   TCCGCTAAGTTGG-ATGTGAAAA-CTCTGGGCTTAACCCAG-AGCCTGCATTCAAAACTG
WCHA2-13                TGCGGTAAGTCTT-CTGTGAAAC-CCCTGGGCTCAACCCAG-GGCCTGCAGGGGAAACTG
Sphingobacterium        CTTTTTAAGTCAG-GGGTGAAAG-ACGGTAGCTCAACTATC-GCAGTGCCCTTGATACTG
778u37                  CGTTGTAAGTCAG-TGGTGAAAG-TTTGCAGCTTAACTGTA-AAATTGCCATTGATACTG
778u1                   GTAGGTAAGTCAGTGGGTGAAAT-CCTGGAGCTCAACTCCA-GAACTGCCATTGATACTA
778u39                  TGAGATAAGTCCG-TGGTGAAAT-CCTATGGCTTAACCATAGNAATTGCCTCGGAAACTG
uncultured              TCTTCCAAGTCTG-GTGTAAAAG-CACGGAGCTCAACTCCG-TGTACGTACCGGAAACTA
778u21530f              TTTGGAAAGTCTG-AGGTCAAAT-GTCGGGGCCTAACCCCG-TCAACGTCTTGGAAACTT
778u33                  ATCTTCAGGCCTG-TTGTGAAAT-CCCACGGCTCAACCGTG-GAATTGCGATGGGAACCG
Pirellula               CCATGCAAGTCAG-ATGTGAAAT-CCCACGGCTCAACCGTG-GAACTGCGTTTGAAACTG
X91291                  CTTGACAAGTTAG-GAGTGAAATTCCTGCAGCTCAACTGCA-GAACTGCTTTTAAAACTG
778u26                  CTTAACAAGTTAG-AAGTGAAAT-CCTGCAGCTCAACTGCA-GAACTGCTTTTAAAACTG
Clostridium             TTTCTTAAGTCAG-AAGTGAAAG-GCTACGGCTCAACCGTA--GTAAGCTTTTGAAACTA
71612                   TTTTTTAAGTCAG-AAGTGAAAG-GCTACGGCTCAACCGTA--GTAAGCTTTTGAAACTG
Geothrix                TTTTTTAAGTCAG-ATGTGTAAT-CCCCGAGCTCAACTTGG-GAACTGCATCTGAGACTG
2202                    ------------------------------------------------GCATCTGAGACTG
SJA-87                  TGTCTTAAGTGGG-ATGTGCAAT-CCCCGGGCTTAACCTGG-GAACTGCATCCCAGACTG
AJ241004                CTAAGCAAGTCAA-AGGTGAAAT-CCCTCGGCTCAACCGAG-GAACTGCCCCTGAAACTG
candidate               CAGAGTAAGTTGA-TAGTGAAAG-CGTCCGGCTCAACCGGA-TATACATTATCAAAACTG
511                     CATTGTAAGTCAA-TAGTGAAAG-CGTTCGGCTCAACCGAA-TATCCATTATTGAAACTG

Rhodococcus             GCA-GACTTGAGTACTGCAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGA
513                     GCA-GACTTGAGTACTGCAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGA
Microbacterium          GCA-GACTAGAGTGCGGTAGGGGAGATTGGAATTCCTGGTGTAGCGGTGGAATGCGCAGA
1630                    GCA-GACTAGAGTGCGGTAGGGGAGATTGGAATTCCTGGTGTAGCGGTGGAATGCGCAGA
High                    CTGTGGCTTGAGTCCGGTAGGGGAGCGTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGA
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778u40                  CTGTGGCTAGAGTCCGGTAGGGGAGCGTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGA
71613                   ACG-GGCTAGAGTTCTGGAGGGGATAGCGGAATTCCTGGTGTAGCGGTGAAATGCGTAGA
WCHA2-13                CCG-TGCTGGAGTGTGGGAGAGATGCGTGGAATTCCCGGTGTAGCGGTGAAATGCGTAGA
Sphingobacterium        AAG-AGCTTGAATGAACTAGAGGTAGGCGGAATGTGACAAGTAGCGGTGAAATGCATAGA
778u37                  CAG-TGCTTGAGTACAGATGAGGTGGGCGGAATGTGTCATGTAGCGGTGAAATGCATAGA
778u1                   TCT-ATCTTGAATATTGTGGAGGTAAGCGGAATATGTCATGTAGCGGTGAAATGCTTAGA
778u39                  TCT-TACTTGAGTCCAGTAGGGGAGCGTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGA
uncultured              GAA-GGATAGAGTCATACAGAGGCATCTGGAATTCCATGTGTAGGGGTAAAATCCGTTGA
778u21530f              CCA-GAATTGAGCAATGGAGAGGCACCTGGAATGCCATGAGTAGGAGTAAAATCCGTAGA
778u33                  GAG-ATCTTGAGTCAGGTAGAGGCGGGTGGAACGATAGGTGGAGCGGTGAAATGCGTAGA
Pirellula               CAT-GGCTTGAGGGAGATAGGGGTGAGCGGAACTGATGGTGGAGCGGTGAAATGCGTTGA
X91291                  TCA-AGATTGAGACTGGGAGAGGAAAGCGGAATTCTCGGTGTAAGAGTGAAATCTGTAGA
778u26                  TTG-AGATTGAGGCTGGGAGATNTNTTNTNAATTCTCGGTGTAAGAGTGAAATCTGTAGA
Clostridium             AGA-GACTTGAGTGCAGGAGAGGAGAGTAGAATTCCTAGTGTAGCGGTGAAATGCGTAGA
71612                   AGA-AACTTGAGTGCAGGAGAGGAGAGTAGAATTCCTAGTGTAGCGGTGAAATGCGTAGA
Geothrix                GAA-GGCTAGAGTACTGGAGAGGGTGGTGGAATTCCTCGTGTAGCGGTGAAATGCGTAGA
2202                    GAA-GGCTAGAGTACTGGAGAGGGTGGTGGAATTCCTCGTGTAGCGGTGAAATGCGTAGA
SJA-87                  GGA-CGCTGGAGTACTGGAGAGGGTGGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGA
AJ241004                CTT-GGCTTGAGTCCCGGAGAGGGTAGTGGAATTCCCAGTGTAGCGGTGAAATGCGTAGA
candidate               CTC-AGCTAGAGGATGAGAGAGGTTATTGGAATTCCTAGTGTAGGAGTGAAATCCGTAGA
511                     CAA-AGCTAGAGGACAAGAGAGGTTATTGGAATTCCTAGTGTAGGAGTGAAATCCGTAGA

Rhodococcus             TATCAGGAGGAACACCGGTGGCGAAGGCGGGTCTCTGGGCAGTAACTGACGCTGAG-GAA
513                     TATCAGGAGGAACACCGGTGGCGAAGGCGGGTCTCTGGGCAGTAACTGACGTTGAG-GAA
Microbacterium          TATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTAACTGACGCTGAG-GAG
1630                    TATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTAACTGACGCTGAG-GAG
High                    TATCAGGAGGAACACCAGCGGCGAAGGCGGCGCTCTGGGCCGGTACTGACACTGAG-GAG
778u40                  TATCAGGAGGAACACCAGCGGCGAAGGCGGCGCTCTGGGCCGGTACTGACACTGAG-GAG
71613                   TATCAGGAGGAACACCGGTGGCGAAGGCGGCTATCTGGACAGAGTCTGACGCTGAG-GCG
WCHA2-13                TGTCGGGAGGAACACCTGCGGAGAAGACGGCGCACTGGACCACTACTGACGCTGAGAGCG
Sphingobacterium        TATGTCACAGAACACCGATTGCGAAGGCAGCTTACTATGGTTTTATTGACGCTGAG-GCA
778u37                  TATGACACAGAACACCGATTGCGAAGGCAGCTCACTAAACTGTAACTGACGCTGAG-GCA
778u1                   TATGACATAGAACACCCATTGCGAAGGCAGCTTACTACGCATATATTGACGCTGAG-GCA
778u39                  TATCAGGAGGAACACCAGCGGCGAAGGCGGCGCTCTGGGCTGGCACTGACGCTGAG-GAG
uncultured              TCCATGGAGGAACGCCAAAAGCGAAGGCAGGATGCTGGGTATGTACTGACGCTCAG-GGA
778u21530f              TACATGGTAGAACGCCAAAAGCGAAGGCAGGGTGCTAGACATTCGCTGACGCTGAG-AGA
778u33                  TATCTATCGGAACGCCAAAGGAGAAATCAGCCCGCTGGGCCTGTTCTGACGCTGAG-GCA
Pirellula               TATCATCAGGAACACCGGTGGCGAAGGCGGCTCACTGGGTCTCTTCTGACGCTGAG-GCA
X91291                  TATCGAGAGGAACACCAGTGGCGAAGGCGGCTTTCTGGTCCAGTACTGACGCTGAA-TTG
778u26                  TATCGAGAGGAACACCAGTGGCGAAGGCGGCTCTCTGGTCCAGCTCTGACGCTGAA-CTG
Clostridium             TATTAGGAGGAATACCAGTTGCGAAAGCGGCTCTCTGGACTGTAACTGACACTGAG-GCA
71612                   TATTAGGAGGAATACCAGTTGCGAAGGCGGCTCTCTGGACTGTAACTGACACTGAG-GCA
Geothrix                GATGAGGAGGAACACCAGTGGCGAAGGCGGCCACCTGGACAGTAACTGACGCTGAG-GCG
2202                    GATGAGGAGGAACACCAGTGGCGAAGGCGGCCACCTGGACAGTAACTGACGCTGAG-GCG
SJA-87                  GATGTGGAGGAACACCAGTGGCGAAGGCGGCCACCTGGACAGTAACTGACGCTGAG-GCG
AJ241004                TACTGGGAGGAACACCGGTGGCGAAGGCGGCTACCTGGACGGGTACTGACGCTGAG-GCG
candidate               TATTAGGAGGAACACCGATGGCGTAGGCAGATAACTGGCTCATTCCTGACACTAAG-GCA
511                     TATTAGGAGGAACACCGATGGCGTAGGCAGATAACTGGCTTGTTCCTGACACTAAG-GCA

Rhodococcus             CGAAAGCGTGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGG
513                     CGAAAGCGTGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGG
Microbacterium          CGAAAGGGTGGGGAGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGG
1630                    CGAAAGGGTGGGGAGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGG
High                    CGAAAGCGTGGGGATCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGTTGGG
778u40                  CGAAAGCGTGGGGATCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGTTGGG
71613                   CGAAAGCTAGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCTAGCCCTAAACGATGGA
WCHA2-13                CGAAAGCTAGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCTAGCCTTAAACGATGAA
Sphingobacterium        CGAAAGCGTGGGGATCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGAA
778u37                  CGAAAGCGTGGGGATCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGAT
778u1                   CGAAAGCGTGGGGATCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACTATGGA
778u39                  CGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACTTTGAT
uncultured              CGAAAGCGTGGGGAGCGAACGGGATTAGATACCCCGGTAGTCCACGCCCTAAACTATGCG
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778u21530f      CGAAAGCGTGGGGAGCGAAGGGGATTAGATACCCCCGTAGTCCACGCCCTAAACGATGCG
778u33          CGAAAGCGTGGGTAGCAAACGGGATTAGATACCCCGGTAGTCCGCGCCGTAAACGATGCG
Pirellula       CGAAAGCTAGGGGAGCGAACGGGATTAGATACCCCGGTAGTCCTAGCTGTAAACGATGAG
X91291          CGAAAGCTAGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCTAGCCGTAAACGATGAG
778u26          CGAAAGCTAGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCTAGCCGTAAACGATGAG
Clostridium     CGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCTAGCCGTAAACGATGAG
71612           CGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAG
Geothrix        CGAAAGTGTGGGTAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAG
2202            CGAAAGTGTGGGTAGCAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGATGAA
SJA-87          CGAAAGTGTGGGTAGCAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGATGAA
AJ241004        CGAAAGTGTGGGTAGCAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGATGAA
candidate       CGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGATGGG
511             CGAAAGCATGGGTAGCAAACGGGATTAGATACCCCGGTAGTCCATGCCGTAAACGATGGA
                CGAAAGCATGGGTAGCAAACGGGATTAGATACCCCGGTAGTCCATGCTGTAAACGATGGA

Rhodococcus     CGCTAGGTGTGGGTTCC-TTCCACGGAATCC--GTGCCG-TAGCTAACGCATTAAGCGCC
513             CGCTAGGTGTGGGTTCC-TTCCACGGAATCC--GTGCCG-TAGCTAACGCATTAAGCGCC
Microbacterium  AACTAGTTGTGGGGTCCATTCCACGGATTCC--GTGACG-CAGCTAACGCATTAAGTTCC
1630            AACTAGTTGTGGGGTCCATTCCACGGATTCC--GTGACG-CAGCTAACGCATTAAGTTCC
High            CACTAGGTGTGGGGTTCTATCAACGGACTCC--GTGCCG-AAGCTAACGCATNAAGTGCC
778u40          CACTAGGTGTGGGGCTCTATCAACGGGCTCC--GTGCCG-AAGCTAACGCATTAAGTGCC
71613           TACTTGGTGTGACTGGGATTGAATCCAGTC---GTGCCG-AAGCTAACGCATTAAGTATC
WCHA2-13        TGCTTGGTGTGACGGGTACCCAATCCCGCC---GTGCCG-GAGCTAACGCGATAAGCATT
Sphingobacterium TACTCGCTGTTAGCGAT-----ACACAGTTA--GCGGCT-AAGCGAAAGCGTTAAGTATT
778u37          TACTCGATGTTAGCGAT-----ATACAGTTA--GCGTCA-AAGCGAAAGCGTTAAGTAAT
778u1           TACTCGACATACGCGAT-----ACACAGTGT--GTGTCT-GAGCGAAAGCATTAAGTATC
778u39          TACTCGCTGTTGGCGAT-----ACACTGTCA--GCGGCT-AAGCGAAAGCGTTAAGTAAT
uncultured      TGCTCGGTGTTGG-GAGTT--CAATCTTTCCCAGTGCCC-AAGCTAACGCGGTAAGCACG
778u21530f      TGCTCGATGTAGGAGATTTT-CAATTGTCTCCTGTGTCC-AAGCTAACGCGGTAAGCACG
778u33          CACTAGACTGAGG-GAGCTT-GACGCTTTCTCAGT--CG-TAGCAAAAGTGCTAAGTGCG
Pirellula       CACTGGATCGAGG-GACCTCCCACAGTTTCTCGGT--CG-TAGCGAAAGTGTTAAGTGCT
X91291          CACTAGGTGTTGGGGGTTT-----ACCCTTA--GCGCCGTAAGTTAACACGTTAAGTGCT
778u26          CACTAGGTGTTGGAGGTTT-----ACCTTCA--GCGCCGTAAGTTAACACGTTAAGTGCT
Clostridium     TACTAGCTGTCGGNNNG--TTACCCCCCTCG--GTGGCG-CAGCTAACGCATTAAGTACT
71612           TACTAGGTGTCGGGGG---TTACCCCCCTCG--GTGCCG-CAGCTAACGCATTAAGTACT
Geothrix        CACTTGGTGTGGAGGGAGTT-GACCCCTTCC--GTGCCG-GAGCTAACGCGTTAAGTGTT
2202            CACTTGGTGTGGAGGGAGTT-GACCCCTTCC--GTGCCG-GAGCTAACGCGTTAAGTGTT
SJA-87          CACTTGGTGTGGCGGGAGTT-GACCCCTGCC--ATGCCG-TAGCTAACGCGATAAGTGTT
AJ241004        CACTTGGTGTTGCGGGTATC-GACCCCTGCA--GTGCCG-AAGCTAACGCATTAAGTGCC
candidate       TGCTAGCTGTTATCGGTATC-GACCCGG-TA--GTAGCG-AAGCTAACGCGT--------
511             TGCTAGCTGTTAGAGGTATC-GACCCCCCTA--GTAGCG-AAGCTAACGCGTTAAGCATC

Rhodococcus     C
513             C
Microbacterium  C
1630            C
High            C
778u40          C
71613           C
WCHA2-13        C
Sphingobacterium C
778u37          C
778u1           C
778u39          C
uncultured      C
778u21530f      C
778u33          C
Pirellula       C
X91291          C
778u26          C
Clostridium     C
71612           C
Geothrix        C
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2202	C
SJA-87	C
AJ241004	C
candidate	-
511	C

Appendix 14: Preliminary column sequence alignment for Proteobacteria clone library with reference strains are listed with accession numbers.

CLUSTAL X (1.64b) multiple sequence alignment

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778u8                ---------GTCCA-GCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTTGT
Pseudomonas          ATACGTAGGGTCCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTTGT
512                  --------------------AATCGGAATTACTGGGCATAAAGCGTGCGCAGGCGGTTAT
Herbaspirillum       ATACGTAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTTGT
716114               ---------------GCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTCTT
504                  --------GGTGCGAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTTTG
Ferribacterium       ATACGTAGGGTGCGAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTTTG
40b19                ----------------CGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTTTT
Dechlorisoma         ATACGTAGGGTGCGAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTTTC
40b3                 --------GGTGCGAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTTTA
40b12                --------GGTGCGAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTTCT
778u38               --------GGTGCG-ACGTTAATCGGAATTACTGGGCGTAAAGGGTGCGCAGGCGGTTGC
778u27               ----CAAAGGTGCAA-CGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTAGGTGGTGGT
Xanthomonas          ATACGAAGGGTGCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTAGGTGGTGGT
71614                ----AAANGGTGCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTAGGTGGTTCG
778u3                ----------GCAA-CGTTAATCGGAATTACTGGGCGTAAAGCGAGTGTAGGTGGCCGC
778u5                ---------GTGCAA-CGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTANGTGGTTTG
PsAF210800           ATACAGAAGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTTG
2202                 ------ANGGGGCGN-CGTTATTCGGAATTATTGGGCGTAAAGGGCGCGTATGCGGTTTT
Geothrix             ATACAGAGGGGGCAAGCGTTATTCGGAATTATTGGGCGTAAAGGGCGCGTAGGCGGTTTT
Sphingomonas         ATACGGAGGGAGCTAGCGTTATTCGGAATTACTGGGCGTAAAGCGCACGTAGGCGGCTTT
508                  --------------------GCTCGGAATCACTGGGCGTAAAGGGCGCGTAGGCGGCGTT
Methylobacterium     ATACGAAGGGGGCTAGCGTTGCTCGGAATCACTGGGCGTAAAGGGCGCGTAGGCGGCGTT
778u41               ------AAGGGGCTA-CGTTGCTCGGAATCACTGGGCGTAAAGGGCGCGTAGGCGGCGTT
778u34               ------AAGGGGCTA-CGTTGCTCGGAATTACTGGGCGTAAAGCGCACGTAGGCGGACTC
778u20530f           -------AGGGGGTA-CGTTGCTCGGAATTACTGGGCGTAAAGGGAGCGTAGGCGGACTG
Caulobacter          ATACGAAGGGGGCTAGCGTTGCTCGGAATTACTGGGCGTAAAGGGAGCGTANGCGGACTG
778u19530f           ------AAGGTGCAAGCGTTAATCGGATTTACTGGGCGTAAAGCGCGCGTANGCGGCTAA
509                  -----------------TTATTCGGAATTACTGGGCGTAAAGCGCACGTAGGCGGATTA
510                  --------------------GTTCGGAATTATTGGGCGTAAAGCGGGTGTAGGTGGGCTT
Bdellovibrio         AGACGAGGGATCCTAGCGTTGTTCGGAATTATTGGGCGTAAAGCGGATGTAGGTGGCTTT
1627                 --------------AGCGTTGTTCGGATTTATTGGGCGTAAAGCGCGTGTAGGCGGTCTT
Trichlorobacter      ATACGGAGGGTGCAAGCGTTGTTCGGATTTATTGGGCGTAAAGCGCGTGTAGGCGGTTTG
Myxococcales         AGACAGAGGGTGCAAACGTTGTTCGGAATTACTGGGCGTAAAGCGCGTGTAGGCGGCTCG
778u32               ---CTTANGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGCTGC
778u25530f           -------GGATGCAAGCGTTACTCGGAATCACTGGGCGTAAAGCGTCTGTAGGCGTCTTG

778u8                GCAAGACCGATGTGAAATCCCCGAGCTTAACTTG-GGAATTGCATTGGTGACTGCACGGC
Pseudomonas          GCAAGACCGATGTGAAATCCCCGAGCTTAACTTG-GGAATTGCATTGGTGACTGCACGGC
512                  GTAAGACAGATGTGAAATGCCCGGGCTCAACCTG-GGAACTGCATTTGTGACTGCACGGC
Herbaspirillum       GTAAGACAGATGTGAAATCCCCGGGCTCAACCTG-GGAATTGCATTTGTGACTGCACGGC
716114               TTAAGTCAGATGTGAAATCCCCGGGCTTAACCTG-GGAACTGCGTTTGAAACTGGAAGGC
504                  TTAAGATAGGCGTGAAATCCCCGGGCTCAACCTG-GGAACTGCGTTTATGACTGGCAGGC
Ferribacterium       TTAAGATAGGCGTGAAATCCCCGGGCTCAACCTG-GGAACTGCGTTTATGACTGGCAGGC
40b19                GTAAGACAGACGTGAAATCCCCGGGCTTAACCTG-GGAACTGCGTTTGTGACTGCAAGGC
Dechlorisoma         GTAAGACAGACGTGAAATCCCCGGGCTCAACCTG-GGAACTGCGTTTGTGACTGCGAGGC
40b3                 TCTAGTCTGATGTGAAAGCCCCGGGCTTAACCTG-GGAACTGCGTAGGAAACTGATAAAC
40b12                ATAAGACAGATGTGAAATCCCCGGGCTTAACCTG-GGAACTGCGTTTGTGACTGTAGGAC
778u38               GCAAGTCAGGCGTGAAATCCCCGGGCTTAACCTG-GGAATGGCGCTTGAAACTACGTGAC
778u27               TTAAGTCTGCTGTGAAAGCCCTGGGCTCAACCTGGGAG-TTGCAGTGGATACTGGATCAC
Xanthomonas          TTAAGTCTGTTGTGAAAGCCCTGGGCTCAACCTGGGAA-TTGCAGTGGATACTGGATCAC
71614                TTAAGTCTGATGTGAAAGCCCTGGGCTCAACCTGGGAA-CTGCATTGGATACTGGCGATC
778u3                ATAAGTCGGATGTGAAAGCCCTGGGCTTAACCTGGGAA-TGGCATTCGATACTGTGTGGC
778u5                TTNAGTTGGATGTGAAAGCCCCGGGCTCGNCCTGNGAAACTGCATTCAAAACTGACAAGC
PsAF210800           TTAAGTTGAATGTGAAATCCCCGGGCTCAACCTGGGAAACTGCATCCAAAACTGGCAAGC
2202                 TTAAGTCAGATGTGTAATCNCCNGGCTCNCCTAAAGGAAAAGCATCTGAGACTGGAAGGC
Geothrix             TTAAGTCAGATGTGTAATCCCCGAGCTCAACTTG-GGAACTGCATCTGAGACTGGAAGGC
Sphingomonas         GTAAGTTAGAGGTGAAAGCCTGGAGCTCAACTCC-AGAATTGCCTTTAAGACTGCATCGC
508                  TTAAGTCGGGGGTGAAAGCCTGTGGCTCAACCAC-AGAATGGCCTTCGATACTGGGACGC
Methylobacterium     TTAAGTCGGGGGTGAAAGCCTGTGGCTCAACCAC-AGAATGGCCTTCGATACTGGGACGC
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778u41          TTAAGTCGGGGGTGAAATCCTGGAGCTCAACTCC-AGAACTGCCTTCGATACTGGGAGTC
778u34          TTTAGTCAGAGGTGAAAGCCCAGGGCTCAACCTT-GGAATTGCCTTTGATACTGGCAGTC
778u20530f      TTTAGTCAGAGGTGAAAGCCCAGGGCTCAACCTT-GGAATTGCCTTTGATACTGGCAGTC
Caulobacter     TTTAGTCAGAGGTGAAAGCCCAGGGCTCAACCTT-GGAATTGCCTTTGATACTGGCAGTC
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Bdellovibrio    GTAAGTCAGATGTGAAAGCCCAGGGCTCAACCCT-GGAAGTGCATTTGATACTGCGAAGC
1627            TTAAGTCTGATGTGAAAGCCCCGGGCTCAACCTG-GGAAGTGCATTGGATACTGGGAGAC
Trichlorobacter TTAAGTCTGATGTGAAAGCCCTGGGCTCAACCTG-GGAAGTGCATTGGAAACTGGCAGAC
Myxococcales    GAAAGTCGGATGTGAAAGCCCAGGGCTCAACCCT-GGAAGTGCACTCGAAACTCCCGAGC
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Pseudomonas     TA-GAGTGTGTCAGAGGGGGGTAGAATTCCACGTGTAGCAGTGAAATGCGTAGAGATGTG
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Herbaspirillum  TA-GAGTGTGTCAGAGGGGGGTAGAATTCCACGTGTAGCAGTGAAATGCGTAGATATGTG
716114          TA-GAGTGTGGCAGAGGGGGGTGGAATTCCACGTGTAGCAGTGAAATGCGTAGATATGTG
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Ferribacterium  TA-GAGTATGGCAGAGGGGGGTGGAATTCCACGTGTAGCAGTGAAATGCGTAGAGATGTG
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Xanthomonas     TA-GAGTGTGGTAGAGGGTAGCGGAATTCCCGGTGTAGCAGTGAAATGCGTAGAGATCGG
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Geothrix        TA-GAGTACTGGAGAGGGTGGTGGAATTCCTCGTGTAGCGGTGAAATGCGTAGAGATGAG
Sphingomonas    TT-GAATCCAGGAGAGGTGAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGATATTCG
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Myxococcales    TT-GAGTCCCGGAGAGGAAGGCGGAATTCTCGGTGTAGAGGTGAAATTCGTAGATATCGA
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Trichlorobacter     GAGGAACACCGGTGGCGAAGGCGGCTACCTGGACCGATATTGACGCTGAGACGCGAAAGC
Myxococcales        GAGGAACACCTGTGGCGAAGGCGGCCTTCTGGACGGTGACTGACGCTGAGACGCGAAAGC
778u32              GAGGAACATCAGTGGCGAAGGCGGCTGTCTGGCCAAACACTGACGCTCAGGTGCGAAAGC
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Pseudomonas         GTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACTAGT
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Herbaspirillum      GTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCTACTAGT
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Trichlorobacter     GTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTACTAGG
Myxococcales        GTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGGGTGCTAGG
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Pseudomonas          TGTTGGGGA--TTCA--TTTCCTTAGTAACGTAGCTAACGCGT-GAAGTTGACCGCCTGG
512                  TGTCGGGTC--TTAA--TTGACTTGGTAACGCAGCTAACGCGT-GAAGTAGACCGCCTGG
Herbaspirillum       TGTCGGGTC--TTAA--TTGACTTGGTAACGCAGCTAACGCGT-GAAGTAGACCGCCTGG
716114               TGTCGGGGA--AGCA--ATTCCTTGGTAACGAAGCTAACGCGTTGAAGTTGACCGCCTGG
504                  TGTTGGGTGGGTAAA--ACCATTTAGTACCGGAGCTAACGCGT-GAAGTTGACCGCCTGG
Ferribacterium       TGTTGGGAGGGTAAA--ACCTTTTAGTACCGGAGCTAACGCGT-GAAGTTGACCGCCTGG
40b19                TGTTGGGAGGGTTAA--ACCTTTTAGTACCGTAGCTAACGCGTTGAAGTTGACCGCCTG-
Dechlorisoma         TGTTGGAAGGGTTAA--ACCTTTTAGTACCGCAGCTAACGCGT-GAAGTTGACCGCCTGG
40b3                 TGTTGGGGA-GGAGA--CCTCCTTAGTAACGCAGCTAACGCGT----------------
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778u38               TGTTGGAGGAGTTAA--ATCCTTTAGTAACGCAGCTAACGCGA-GAAGTCATCCGCCTGG
778u27               TGTTGGGTGCAACTT--GGCACCCAGTATCGAAGCTAACGCGT-TAAGTTCGCCGCCTGG
Xanthomonas          TGTTGGGTGCAATTT--GGCACGCAGTATCGAAGCTAACGCGT-TAAGTTCGCCGCCTGG
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Geothrix             TGTGGAGGGAGTTGA--CCCCTTCCGTGCCGGAGCTAACGCGT-TAAGTGTTCCGCCTGG
Sphingomonas         TGTCGGGGCTCTTA---GAGCTTCGGTGGCGCAGCTAACGCAT-TAAGTTATCCGCCTGG
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Methylobacterium     TGTTGGGGTGCTTG---CA-CCTCAGTAGCGCAGCTAACGCTT-TGAGCATTCCGCCTGG
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Caulobacter          TGTCGGCATGCATG---CA-TGTCGGTGACGCAGCTAACGCAT-TAAGCACTCCGCCTGG
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Bdellovibrio         TGTTAGAGGTATTGA--CCCCTTTAGTGACGAAGCTAACGCGT-TAAGTATCCCGCCTGG
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Trichlorobacter      TGTTGCGGGTATTGA--CCCCTGCAGTGCCGCAGCTAACGCAT-TAAGTACTCCGCCTGG
Myxococcales         TGTCGCGGGCTTTGA--CCCCTGCGGTGCCGTAGCTAACGCCT-TAAGCACCCCGCCTGG
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778u8                G
Pseudomonas          G
512                  G
Herbaspirillum       G
716114               G
504                  G
Ferribacterium       G
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Dechlorisoma         G
40b3                 -
40b12                G
778u38               G
778u27               G
Xanthomonas          G
71614                G
778u3                G
778u5                G
PsAF210800           G
2202                 G
Geothrix             G
Sphingomonas         G
508                  G
Methylobacterium     G
778u41               G
778u34               G
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Caulobacter	G
778u19530f	G
509	G
510	G
Bdellovibrio	G
1627	G
Trichlorobacter	G
Myxococcales	G
778u32	G
778u25530f	G

Appendix 15: Nomenclature and abbreviations

AFCEE	air force center for environmental excellence
bp	base pair
DCE	dichloroethene
DNAPL	dense nonaqueous phase liquid
DO	dissolved oxygen
GC	gas chromatograph
HPLC	high pressure liquid chromatograph
ICP	inductively coupled plasma
IRB	iron reducing bacteria
MNA	monitored natural attenuation
MPN	most probable number
ORR	Oak Ridge reservation
PCR	polymerase chain reaction
ppb	parts per billion
ppm	parts per million
rDNA	ribosomal deoxyribonucleic acid
sp.	species
SRB	sulfate reducing bacteria
SWSA7	solid waste storage area 7
TCE	trichloroethylene
TOC	total organic carbon
VC	vinyl chloride
VOC	volatile organic carbon
WAG5	waste area grouping 5

Vita

Melissa E. Lenczewski was born in Garden City, Michigan. She became interested in biology and science while attending Northeast Middle School in Midland, MI. During the advance biology class at Midland High School, Mrs. Shields introduced her to microbiology. After finishing High School at Peoria High School, Peoria, Arizona decided to major in Microbiology at the University of Arizona. During her junior year got a job in an environmental microbiology laboratory under Dr. Charles Gerba. This experience gave her the opportunity to realize that a combination of microbiology and environment was for her. She graduated with a B.S. in 1991 and decided to stay with Dr. Gerba for a Master's degree but in the Soil, Water and Environmental Science program. The decision proved to be invaluable for learning more about the environment and how this related to microbiology. She spent two summers at Borden Air Force Base, Borden, Ontario, Canada investigating virus transport in sandy aquifers. During the second summer was asked to go to Denmark to aid in virus transport research with the Danish Geological Survey and Dr. Larry McKay. After graduating in 1993, worked for Amway Corporation as a Research Scientist examining personal and homecare products for microorganisms. This experience made her realize that industry was not for her and that a life in academia was her calling. In 1997, started a Ph.D. in Geological Sciences at the University of Tennessee under Dr. Larry McKay. She finished in December of 2000 with a job as an Assistant Professor at Northern Illinois University, DeKalb, IL to start in January of 2001. Her research goal is to always combine her love of the environment with microbiology.