Evaluation of Anaerobic Biodegradation of Organic Carbon Extracted from Aquifer Sediment

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Thesis submitted to the Faculty of
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

Master of Science
in
Environmental Science and Engineering

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6 September 2006
Blacksburg, Virginia

Keywords: bioremediation, biodegradable, organic carbon, TOC

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(ABSTRACT)

In conjunction with ongoing studies to develop a method for quantifying potentially biodegradable organic carbon (Rectanus et al 2005), this research was conducted to evaluate the extent to which organic carbon extracted using this method will biodegrade in anaerobic environments. The ultimate goal is to use this method for the evaluation of chloroethene contaminated sites in order to estimate the long-term sustainability of monitored natural attenuation (MNA) as a remediation strategy. Although relatively recalcitrant under aerobic conditions, the breakdown of chlorinated solvents primarily occurs through the anaerobic process of reductive dechlorination. The biodegradation of organic carbon in these anaerobic environments drives the system to reducing conditions conducive for reductive dechlorination. The extraction procedure developed by Rectanus *et al.* (2005) has been tested in several series of aerobic bioassays to determine the biodegradable fraction of carbon extracted. This study seeks to show that the carbon removed from the sediment by this extraction process will also degrade in anaerobic environments.

Three aquifer sediment samples characterized by low, medium, and high carbon concentrations were taken from Naval Submarine Base Kings Bay, Georgia. Two sites were also sampled from Naval Amphibious Base Little Creek, Virginia. MLS20 is a site located inside of a chloroethene plume, and MLS10 is located outside of the plume. For approximately 12 weeks aqueous total organic carbon (TOC), headspace carbon dioxide (CO_2), volatile fatty acids (VFAs), and headspace hydrogen concentrations were monitored for evidence of the biodegradation of organic carbon.

Although few VFAs were observed throughout the experiments, their presence as early as 8 days after inoculation indicated that the bioassays were anaerobic. The fewest VFAs were seen in the MLS20 bioassays, while the most VFAs were observed in the MLS10 bioassays. MLS20 exhibited low levels of TOC loss and the low VFA levels indicate that complex organic matter was not highly degraded in these bioassays. The higher level of VFAs observed in MLS10 bioassays corresponded with little TOC degradation, indicating that although more complex organics were being broken down, conditions were not reduced enough to further oxidize the organic carbon. As much as 50% TOC loss was observed in the Kings Bay bioassays with few VFAs detected.

Loss of TOC was accompanied by CO_2 generation which provides supporting evidence that organic carbon was being oxidized. Hydrogen was observed in the bioassays, suggesting that VFAs resulting from organic carbon breakdown were being oxidized. This indicates that organic carbon removed from sediment using the extraction process is biodegraded anaerobically and could lead to conditions capable of sustaining reductive dechlorination.

Acknowledgements

I would like to thank Dr. Mark Widdowson and Dr. John Novak for their advice and guidance throughout my time researching at Virginia Tech. They allowed me to explore ideas on my own but were always available to provide recommendations and further insights. I would also like to thank Dr. Peter Vikesland for being part of my committee and offering advice that led to the completion of my thesis. I extend a very special thanks to Heather Rectanus for her incredible support and training throughout my research. I thoroughly enjoyed my time working with her and could not have completed this without her help.

I would also like to thank Jody Smiley and Julie Petruska for keeping things running smoothly, for their technical assistance, and for advice in analytical procedures. They made many days in the lab more efficient and productive for me. I am grateful to my colleagues for creating the supportive and enjoyable work environment and particularly to Chris Wilson for his advice in the lab along the way. I would like to thank Lauren Raup for her help with preparing and analyzing samples.

I would like to acknowledge the Strategic Environmental Research and Development Program (SERDP) for the financial support I received to study and conduct this research at Virginia Tech.

I am so grateful for my family; a sincere thanks to Mom, Dad, JJ, Kristy, Charlynda, Jacqui, Julie, David, and Hans for their continual encouragement and prayers and for always being there to kick back with and have fun.

All glory to God Whom in Jesus Christ makes all things possible.

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Introduction

The chlorinated solvents tetrachloroethene (PCE) and trichloroethene (TCE) have commonly been used since the 1940s in dry cleaning and as degreasing agents. Years of improper storage and handling have made these compounds and their daughter products among the most common contaminants in aquifer sediments and groundwater (Wiedemeier et al. 1999). PCE and its daughter products are regulated under the Safe Drinking Water Act Amendments of 1986 with an established maximum contaminant level (MCL) for PCE and TCE of 5µg/L, for *cis*-DCE of 70µg/L, and for VC of 2µg/L (Bradley 2000; EPA 1995; Freedman and Gossett 1989). Efforts to remediate groundwater polluted with these chemicals largely consisted of pump-and-treat methods until the 1990s and more recently when researchers began describing the ability of microorganisms to degrade chloroethene compounds (Bradley 2000).

Although relatively recalcitrant under aerobic conditions, the breakdown of PCE primarily occurs through the anaerobic process of reductive dechlorination, in which a chlorine atom is replaced by a hydrogen atom (Vogel and McCarty 1985). Dechlorination of PCE yields TCE, which degrades to primarily form *cis*-DCE, followed by the formation of VC, which finally breaks down to the harmless compound, ethene. Although this microbial process appears to be occurring in most sites that are contaminated with chloroethenes and where the redox condition in the groundwater

system is favorable, the extent to which reductive dechlorination occurs varies greatly. Incomplete reductive dechlorination is of great concern because PCE, TCE, and DCE are currently suspected carcinogens, and VC is a confirmed carcinogen. Each subsequent step of dechlorination requires increasing highly reducing conditions. While PCE is readily transformed to TCE, the conversion of VC to ethene requires either sulfate-reducing or methanogenic conditions. However, because studies have shown that these compounds can be completely oxidized under anaerobic redox conditions, the sustainability of natural attenuation as a remediation technology for these contaminated sites is now being investigated (Bradley 2000).

During reductive dechlorination, chlorinated ethene-respiring bacteria utilize hydrogen as the electron donor and the chlorinated solvent as the electron acceptor. Hydrogen is a byproduct of volatile fatty acid (VFA) fermentation, and VFAs are generated from the breakdown of organic carbon. The source of organic carbon at chlorinated ethene contaminated sites can be either derived from either anthropogenic sources (e.g., petroleum hydrocarbon compounds, landfill waste, etc.) or naturally-occurring sources. By ultimately supplying hydrogen, the breakdown of carbon leads to increasingly reduced conditions, and therefore is believed to have substantial control over the extent of reductive dechlorination. A link between the amount of biodegradable carbon and the reduction potential at a site can enable a greater understanding as to extent that indigenous microorganisms can remediate a contaminated site (Wiedemeier 1996; Wiedemeier 1998).

Objectives

Current research is being conducted to develop a method for quantifying potentially biodegradable natural organic carbon (NOC) in aquifer sediment (Rectanus et al. 2005). The first stage of this method development was to establish a procedure for extracting carbon from the surface of aquifer and stream bed sediment. The extent of biodegradation of this NOC was then tested in a series of aerobic bioassays. Rectanus *et al*. (2005) have demonstrated that the organic carbon extracted from sediment using this method biodegrades under aerobic conditions; however, reductive dechlorination is an

anaerobic process. As a companion project, this research seeks to show that the organic material extracted from the sediment is capable of supporting anaerobic decomposition of chlorinated solvents. Three research objectives were identified:

- to determine the extent to which extracted organic carbon is biodegraded under anaerobic conditions
- to establish whether VFAs are produced from the carbon degradation, therefore providing a link between carbon and hydrogen generation
- to determine the extent of hydrogen production as the extracted carbon is degraded

Using the recently developed method (Rectanus et al. 2005), bioassays were constructed using carbon extracted from aquifer sediment samples obtained from three different sites. Anaerobic carbon biodegradation was monitored over time by observing the loss of total organic carbon in bioassays that were maintained in an oxygen-free environment. Supplemental evidence of organic carbon loss was provided by monitoring the headspace carbon dioxide concentrations in the bioassays. Aqueous samples were taken from the bioassays over time and tested for the presence of VFA's. Hydrogen generation was examined in separate anaerobic bioassays by analyzing headspace samples for the presence of hydrogen.

Literature Review

Chlorinated ethenes are a class of solvents used in industry that were first produced over 100 years ago and are now among the most widespread contaminants in aquifer sediment and groundwater. The parent compounds, tetrachloroethene (perchloroethene or PCE) and trichloroethene (TCE) are most frequently used in dry cleaning and as degreasing agents and are preferred because of their nonflammable and non-corrosive nature. They were commonly used by the 1940's and introduced into aquifers through improper storage, disposal, and handling (Wiedemeier et al. 1999). Although they were first believed to be recalcitrant in groundwater systems, research has since shown that under appropriate conditions, certain microorganisms will degrade chloroethenes. In the natural environment, this is primarily accomplished through a process called reductive dechlorination. A major concern, however, is that if not completely dechlorinated to harmless ethene (ETH), the other daughter products, *cis*-1,2-dichloroethene (*cis*-DCE) and vinyl chloride (VC), are actually more harmful to human health than the source compounds, PCE and TCE.

Chloroethenes are alkenes, which are unsaturated hydrocarbons characterized by at least two carbon atoms joined by a double bond. A chlorinated ethene has one to three chlorine atoms single-bonded to either of the carbon atoms. PCE contains four chlorine atoms bonded to the carbon atoms, TCE contains three chlorine atoms, DCE contains two chlorines, and VC has only one chlorine atom bonded to a carbon atom. While the other compounds are suspected carcinogens, VC is a priority pollutant listed by the EPA and

the only known carcinogen of the group (Bradley 2000; Vogel et al. 1987). Upon losing its one chlorine substituent, VC becomes the harmless compound, ETH.

Although not very efficient, pump-and-treat methods were used starting in the 1980s in an effort to restore chloroethene-contaminated sites. The discovery of microbial degradation of chloroethenes during the 1980s spurred research investigating the potential for implementing bioremediation as a strategy at these locations. One of the primary concerns was that the concentration of chloroethenes would be too low to serve as a primary substrate supporting growth and metabolism of microorganisms. Since this time, five different process have been described that degrade some or all of the chloroethene compounds: aerobic cometabolism, aerobic oxidation, anaerobic cometabolism, anaerobic oxidation, and halorespiration or reductive dechlorination (Wiedemeier et al. 1999).

Aerobic Biodegradation

Aerobic Oxidation

Aerobic oxidation is a process that has been observed to transform *cis*-DCE and VC. The chlorinated compound serves as the primary substrate and is oxidized to CO_2 and water, while oxygen, sulfate or other compounds act as the electron acceptor. With VC serving as the primary substrate, microbial growth has been observed as a result of aerobic oxidation (Hartmans and de Bont 1992). Although not appearing to support microbial growth, aerobic oxidation of *cis*-DCE in which it served as the primary substrate for energy production has been seen (Bradley and Chapelle 2000). These two compounds are typically only present as a result of the reductive dechlorination of PCE and TCE, a process that occurs only in strictly anaerobic environments. Therefore, the environment is typically not suitable for aerobic oxidation to occur and so this process is of little importance in evaluating the potential for natural attenuation of the parent compounds at a PCE- or TCE-contaminated site. However, this process can be significant in removing *cis*-DCE and VC on the fringe of a plume or in surface water environments (Bradley 2000).

Aerobic Cometabolism

Aerobic cometabolism is a process in which the chloroethenes TCE, *cis*-DCE, and VC, are oxidized to form carbon dioxide (CO_2). The likelihood for these compounds to undergo oxidation increases with decreasing number of associated chlorine atoms (Vogel 1994). This degradation is purely accidental and provides no known benefit to the microorganisms. The occasional conversion is accomplished by non-specific oxygenases that are produced in the presence of oxygen and a primary substrate. Although these conditions are not often seen in the middle of a chloroethene plume, this process can be significant on the fringes of a plume, at the interface between groundwater and surface water, in surface water sediments, and it has been successfully engineered in the field (Bradley 2000). Significant cometabolic VC transformation was observed in an experiment when methane was added to a methane-utilizing population. The study showed that the extent of the transformation of VC was related to the amount of methane utilized, not the incoming concentration of VC. The removal of VC in this experiment showed that aerobic cometabolism could be responsible for considerable removal of VC (Dolan and McCarty 1995). However, complete transformation of VC in a plume is usually not possible through this process, and therefore cometabolic oxidation could not be relied upon as a primary remediation strategy (Bradley 2000).

Anaerobic Biodegradation

Anaerobic Oxidation

The 1996 discovery of anaerobic oxidation of VC opened the door and eventually led to the acceptance of in situ bioremediation as a strategy for cleaning up chloroethenes in aquifers. Prior to this, it appeared that reductive dechlorination resulted in the accumulation of *cis*-DCE and VC, and there existed no known reliable mechanism for further breaking these contaminants down to ETH. Serving as the electron donor, VC and *cis*-DCE transformed to CO_2 via this pathway. In an experiment examining the anaerobic oxidation of VC by Bradley and Chapelle (1996), this process occurred at rates comparable to aerobic mineralization under Fe(III)-reducing conditions, and the availability of Fe(III) largely governed the extent and rate of mineralization. The percent recovery of CO_2 closely matched the loss of VC, thereby indicating that VC is converted

straight to CO_2 instead of undergoing an intermediate reductive step (Bradley and Chapelle 1996). Further research demonstrated that while VC oxidation decreased in more highly reduced conditions, the rate of *cis*-DCE oxidation was not significantly different between Fe(III)-reduced, sulfate-reduced, or methanogenic conditions. More strongly reducing conditions, such as Mn(IV)-reducing, were required for *cis*-DCE to be oxidized directly to CO_2 without the accumulation of intermediates. The extent and rate of mineralization of *cis*-DCE was strongly linked to the bioavailability of Mn(IV) (Bradley et al. 1998b). Therefore, a likely pathway for complete degradation involves a reduction of *cis*-DCE to VC, and subsequent oxidation of VC to CO_2.

Humic acids may enable anaerobic mineralization of *cis*-DCE and VC to CO_2 to occur at faster rates without the accumulation of toxic products. Bradley et al. (1998a) found that the rate of VC mineralization was increased by the addition of humic acid. Held under anaerobic conditions, the humic acid was reduced, while VC was oxidized. Under anaerobic conditions, *cis*-DCE was mineralized both with and without the addition of humic acid. However, when humic acid was not added, low mineralization was observed with the accumulation of trace amounts of VC, ethene and ethane. Upon the addition of humic acid, the rate of mineralization was significantly faster and did not result in the accumulation of VC, ethene or ethane. This indicates that humic acid addition could potentially reduce risk associated with *cis*-DCE degradation (Bradley et al. 1998a).

Reductive Dechlorination via Anaerobic Cometabolism

Anaerobic cometabolism results in dehalogenation that provides no benefit to the microorganisms. As with aerobic cometabolism, this process results from enzymes present that inadvertently reduce the chlorinated solvent. Although many bacterial strains are known to accomplish this activity, the rate at which this mechanism proceeds is several orders of magnitude slower than metabolic dehalogenation, which also proceeds under anaerobic conditions. It is therefore not considered as a reliable mechanism for *in situ* bioremediation (Fetzner 1998).

Reductive Dechlorination via Respiration

Reductive dechlorination, also known as halorespiration, is the primary process understood to be responsible for the degradation of chloroethenes in groundwater. This occurs in environments depleted of oxygen and nitrate, and the chlorinated solvent serves as the electron acceptor while dissolved hydrogen is typically the electron donor. A chlorine atom is replaced by a hydrogen atom and in sequence chlorinated ethenes degrade from PCE to TCE, to DCE, to VC, to ETH. Dechlorination of TCE may form either *cis*-DCE or *trans*-DCE, but the formation of *cis*-DCE is much more commonly observed (Bradley 2000). Figure 1 shows the pathway of reductive dechlorination.

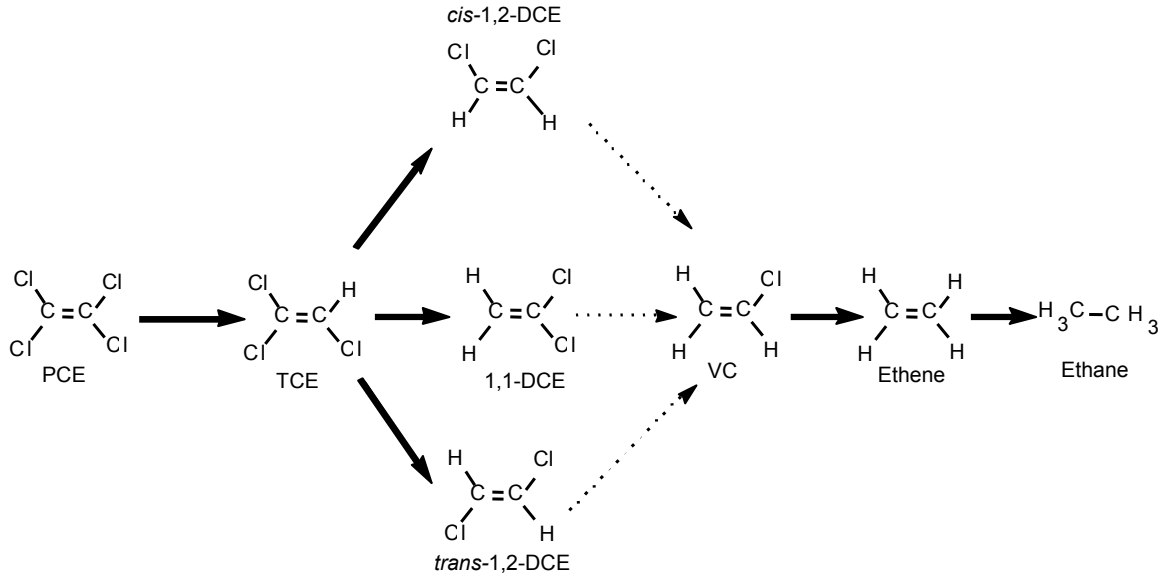

Figure 1. Pathway of the sequential reduction of PCE to Ethane.

Increasing halogenation signifies a more oxidized compound and with each additional chlorine atom associated, the compound is more prone to undergo reduction. Every step of dehalogenation results in a more stable alkene that is less likely to be reduced than the parent compound (Vogel et al. 1987). While PCE readily transforms to form TCE in anaerobic environments, each subsequent step requires more strongly reduced conditions and occurs at a slower rate. The conversion of TCE to *cis*-DCE requires Fe(III)-reducing

environments; *cis*-DCE to VC requires at least sulfate-reducing environments; and the conversion of VC to ETH requires strongly reducing, methanogenic conditions (Bradley 2000; Freedman and Gossett 1989; Vogel et al. 1987). Because the last two steps in this sequence require such a specialized environment, the accumulation of *cis*-DCE and VC is a concern. Complete dechlorination to ETH demands sufficient electron donor concentrations, suitable redox conditions, and the presence of the dechlorinating organisms (Bradley 2000).

Only one bacterial strain, *Dehalococcus ethenogenes*, is known to be capable of dechlorinating PCE completely to ETH. These microorganisms utilize the chloroethene as the sole electron acceptor and hydrogen as the electron donor, but in laboratory experiments they grew only when both PCE and H_2 were present (Maymo-Gatell et al. 1997). The highly reduced nature of hydrogen makes it a high energy electron donor, but it is suspected that this bacterial strain may also be capable of using formic and acetic acid in this capacity. Because they are not able to use a variety of electron donors, activity of these organisms may be hindered by competition for the limited donors available to them (Bradley 2000; Smatlak et al. 1996; Wiedemeier et al. 1999).

Reductive dechlorination consumes one mole of hydrogen for every chloride removed. However, because of the competition between subsurface microorganisms, the concentration of hydrogen in the subsurface does not directly relate to the amount of chloride that can be removed. The use of hydrogen is not 100% efficient (Bradley 2000). Different studies have shown that reductive dechlorination utilized only 5-10% of the hydrogen available (Ballapragada et al. 1997; Sewell and Gibson 1991). The environmental hydrogen concentration is a significant factor governing competition among microorganisms, but the process by which the hydrogen becomes available does not affect whether the organisms are active or not (Carr and Hughes 1998; Yang and McCarty 1998).

The presence of hydrogen is usually a result of the fermentation of natural organic carbon (NOC) in the subsurface which begins when dissolved oxygen levels decrease below

approximately .5 mg/L (Wiedemeier et al. 1999). This fermentation produces volatile fatty acids (VFAs) that are β-oxidized to yield hydrogen. Reductive dechlorination removes hydrogen from the system, thus maintaining the fermentation of VFAs as an energetically favorable process. Meanwhile, the β-oxidation of VFAs continues to supply hydrogen to the system, allowing dechlorination to continue (Wiedemeier et al. 1999). Organic carbon should be 25-100X the concentration of the chlorinated solvent in order for the contaminants to be removed. This necessity is due in part to the competition between the various microbes, as well as the fact that organic carbon ultimately drives the system to a more reduced state, which is required for the last steps of reductive dechlorination (Bradley 2000).

Competition for Electron Donor

Subsurface competition with dechlorinators for electron donor is primarily among sulfate reducers and methanogens (Fennell et al. 1997). This competition appears to be related to threshold concentrations of hydrogen necessary for different hydrogen-accepting microbial processes to occur. Electron acceptors involved in higher energy reactions typically require lower hydrogen concentrations than do lower energy hydrogen oxidation reactions (Yang and McCarty 1998). A study by Fennell et al. (1997) found that reductive dechlorination occurs at low hydrogen concentrations, while methanogenic activity requires greater concentrations of hydrogen (Fennell et al. 1997). A batch reactor study by Yang and McCarty (1998) determined a threshold hydrogen concentration of 2.2 +/- 0.9 nM H_2 for reductive dechlorination to occur, and a concentration threshold of 10.9 +/- 3.3 nM H_2 for methanogenesis to occur (Yang and McCarty 1998). Although the competition for electron donor may impact the activity of the microorganisms, it appears that when sufficient donor is present, dechlorination and methanogenesis do not inhibit each other (Carr and Hughes 1998). Fennell et al. observed equal dechlorination over the long-term regardless of whether methanogenic activity was occurring or not (Fennell et al. 1997). Further, at high H2 partial pressures, dechlorination was not inhibited by other organisms using H2 (Carr and Hughes 1998).

Appropriate Hydrogen Concentrations for Reductive Dechlorination

The rates of VFA fermentation are very different depending on the fatty acid, and these varying rates affect the concentration of hydrogen produced. The fermentation of butyric and proprionic acids give a selective advantage to dechlorinators over methanogens because the reaction rates for these VFAs are slower than for other VFAs, such as lactic acid. Therefore these reactions yield lower hydrogen concentrations over a longer period of time. However, Fennell et al. qualifies this finding with the statement that the pathway from VFA to H_2 is complex, and therefore "adding ethanol or lactic acid may be almost the equivalent of adding proprionic acid—a slow release H_2 donor" (Fennell et al. 1997). In another experiment, reductive dechlorination was maintained in ethanol-fed, lactate-fed, and butyrate-fed systems, but the butyrate-fed system resulted in the most complete dechlorination (Smatlak et al. 1996). Carr and Hughes (1998) compared the rates and extent of dechlorination with the addition of methanol, lactic acid, or H_2 serving as donors. They found that although H_2 initially resulted in the most rapid dechlorination, the rates and extent were not different over the long term, therefore indicating that the type of donor was not significant (Carr and Hughes 1998). This was further confirmed in comparing butyric acid, lactic acid, propionic acid, and ethanol. Although there were initial differences in the rates of dechlorination, the long-term effect was the same between the different electron donors (Fennell et al. 1997).

It is not necessarily the rate at which hydrogen is produced, but rather the organisms present that determine the steady-state hydrogen concentration. Fe(III) reducers need low hydrogen concentrations and will use hydrogen present regardless of the rate at which it is supplied until they have reduced the Fe(III) available. At this point, their activity will cease and the dissolved hydrogen concentration will increase until adequate for sulfate-reducing bacteria to utilize it. These microorganisms will consume the hydrogen until sulfate is no longer present and then hydrogen will again increase until methanogens are able to commence activity (Bradley 2000). Table 1 shows hydrogen concentrations necessary for these microbial processes to occur. Dechlorinators require hydrogen concentrations of approximately 1nM and therefore most of their activity is predicted to take place in sulfate-reducing or methanogenic conditions (Smatlak et al. 1996).

Table 1. ***Hydrogen concentrations necessary for microbial processes.***

Hydrogen Concentrations (nM)	Microbial Process
< .1	Denitrification
0.2 - 0.8	Iron (III) Reduction
1 - 4	Sulfate Reduction
5 - 20	Methanogenesis

Table adapted from (Chapelle 1995).

Results from laboratory experiments support that the organisms present determine the steady-state hydrogen concentration. Ballapragada et al. (1997) conducted an experiment in which different electron donor loading rates were applied to a dechlorinating population. The dechlorination kinetics were different for only a few hours before populations responded and grew, therefore adjusting donor concentrations to a typical steady-state concentration (Ballapragada et al. 1997). In examining the effects of increased hydrogen addition to a laboratory column, Carr and Hughes (1998) saw an increase in dechlorination. It appears that the different strains of bacteria increased their activity without inhibiting other populations by competition for the available hydrogen (Carr and Hughes 1998). However, dechlorinators hold a competitive advantage over methanogens at low hydrogen concentrations and can successfully compete at up to 100ppm H_2. Since hydrogen concentrations seldom surpass this in methanogenic environments, dechlorinators should be able to successfully compete for hydrogen in these conditions. In cases where electron donor is limited, dechlorinators should be able to out-compete methanogens, therefore maintaining dechlorination with little production of methane (Ballapragada et al. 1997).

Complete dechlorination to ethene under methanogenic conditions has been observed. Various potential electron donors were supplied in a laboratory experiment, and results showed that methanol was most successful in sustaining reductive dechlorination. Hydrogen, formic acid, acetic acid and glucose were also effective electron donors. Daughter products of PCE and TCE were seen, but significant quantities of CO_2 and CH_4 were not observed. This indicates that reductive dechlorination was the pathway for elimination of the parent compounds (Freedman and Gossett 1989).

It may be beneficial to supply limited donor, therefore allowing dechlorination to occur without excess growth of methanogenic bacteria. (Ballapragada et al. 1997). However, increased rates of halorespiration has been observed many times when larger quantities of hydrogen were supplied to a dechlorinating system (Ballapragada et al. 1997; Carr and Hughes 1998; Fennell et al. 1997). In an experiment by Ballapragada et al. (1997), dechlorination did not occur or happened at very slow rates when either acetate or no electron donor was supplied. Increasing the loading of proprionic acid, lactic acid, and H_2 always resulted in rapid dechlorination (Ballapragada et al. 1997). These results indicate that supplying lower levels of hydrogen to a system may needlessly limit the capability of the organisms to dechlorinate the solvents (Wiedemeier et al. 1999).

Site Characterization

Study Sites

Aquifer sediment was sampled from two different locations: the Naval Amphibious Base Little Creek, located in Norfolk, Virginia (sampled at two sites); and the Naval Submarine Base in Kings Bay, Georgia (sampled at three sites).

Naval Amphibious Base Little Creek, Virginia Beach, Virginia

Site Description

The Naval Amphibious Base Little Creek (NABLC) is a 2147 acre site located on the coast in Virginia Beach, Virginia. It was commissioned in 1945 to provide support services for amphibious warfare training. An on-site laundry facility improperly disposed of dry cleaning wastes into a storm sewer from 1973-1978 and consequently contaminated the unconfined Columbia Aquifer with over 200 gallons of PCE and 1000 gallons of other dry cleaning wastes. The dry cleaning facility was demolished in 1987 and replaced by a commissary in 1992. Over 12,500 employees work at NABLC, and the base houses approximately 3,600 people (http://epa.gov/reg3hwmd/npl/VA5170022482.htm, CH2MHill, 2000).

Hydrogeology

The Columbia Aquifer is the upper-most water-bearing unit in the Atlantic Coastal Plain aquifer systems and is underlain by a clay aquitard. It has a coarse sand and shell bed interspersed with clay, silt and gravel lenses. Groundwater flows at a rate of 0.47 ft/day and discharges to several lakes and streams, which eventually empty into the Chesapeake Bay. The Yorktown Aquifer is 30-40 feet thick and is confined below the Columbia Aquifer by a clay layer 19-24 feet below the surface. It extends to a depth of 200 ft and serves as the drinking water source for the region. At this time, the Yorktown Aquifer is uncontaminated. Vertical hydraulic conductivity is very minor and therefore little threat exists of contamination spreading to the Yorktown Aquifer (CH2MHill, 2000).

Current Status and Remediation Strategy

PCE and its daughter products are present in the Columbia Aquifer with substantial spatial variability and with concentrations exceeding the MCL's established by the EPA. NABLC was added to the National Priorities List (NPL) in 1999. A Federal Facilities Agreement (FFA) was signed in 2003 in which the Navy, EPA, and Virginia Department of Environmental Quality (VDEQ) cooperated to lay out specific requirements for the Site Management Plan (SMP). A background study of the soil and groundwater was conducted by the Navy, who continues to monitor the groundwater. The SMP summarizes the problem, ranks sites in order of priority, and presents a timeline for taking remedial action. A remediation strategy has not been developed for all of the 9 sites prioritized at this location as research is still underway (http://epa.gov/reg3hwmd/npl/VA5170022482.htm, CH2MHill, 2000).

Naval Submarine Base, Kings Bay, Georgia

Site Description

Sediment samples were also provided by the U.S. Geological Survey which were collected at the Naval Submarine Base (NSB) Kings Bay. The facility is comprised of over 16,000 acres on flat marshland located in Kings Bay on the southern coast of

Georgia. Construction of the base was completed in 1958 with further additions made throughout the 1980's and completed in the early 1990's. It is used as a homeport and training facility for naval submarines. (http://www.globalsecurity.org/wmd/facility/kings_bay.htm, https://www.denix.osd.mil/denix/Public/News/Earthday99/Awards99/NAKingsBay/kingsbay.html). The Old Camden Road Landfill is a 35 acre abandoned site on the base that was in use from 1974-1981 (http://www.epa.gov/correctiveaction/gw-app.htm, http://toxics.usgs.gov/topics/rem_act/solvent_plume.html). Two sources within the landfill have been identified as leaching PCE into the groundwater which flows toward Porcupine Lake (http://toxics.usgs.gov/topics/rem_act/solvent_plume.html).

Hydrogeology

Groundwater flows at a rate of approximately 0.23 m/day. The most permeable portion of the aquifer is 10-11 meters thick with a hydraulic conductivity of up to 10 m/day. This permeable layer is surrounded by finer-grain sands overlain by organic-rich sediments approximately 3-5 meters below the surface. This organic matter from the landfill is largely responsible for reducing conditions in the aquifer. The area immediately near the source zone has been measured to be sulfate-reducing, while iron (III)-reducing conditions exist further from the source. While PCE and TCE are the only detectible solvents near the source, TCE, cis-DCE, and VC all appear in sequence down gradient. By 150 meters from the source, VC is essentially the only chlorinated solvent present (Chapelle and Bradley 1999).

Current Status and Remediation Strategy

The remediation strategy implemented combines source area removal using in-situ chemical oxidation with Fenton's reagent, and monitored natural attenuation. The most concentrated portion of the plume was drastically reduced during the first three years of treatment from 120,000 ppb in 1999 to 120 ppb in 2002. Chlorinated solvent concentrations in part of the off-site plume have dropped below drinking water standards (http://www.epa.gov/correctiveaction/gw-app.htm).

Collection of Aquifer Sediment

Samples were collected from the aquifers using a Geoprobe unit and were stored in aseptic acetate liners at 4°C until analyzed. Two sites were sampled at NAB Little Creek and these are shown in Figure 2. MLS20 is located outside of the chlorinated ethene plume upgradient of the source areas. Concentrations of total chlorinated ethenes have historically exceeded 1 mg/L within the plume. MLS10 is located just outside of the plume. Two sample sets at MLS 10 were collected at depths of 8-10 feet and 20.5-22 feet below land surface. Samples at MLS 20 were obtained at depths of 8-10 feet and 22 feet. Kings Bay samples were taken from three different locations denoted as KBA, 5/11/02, and Outcrop, and they are characterized by low, medium, and high carbon concentrations respectively.

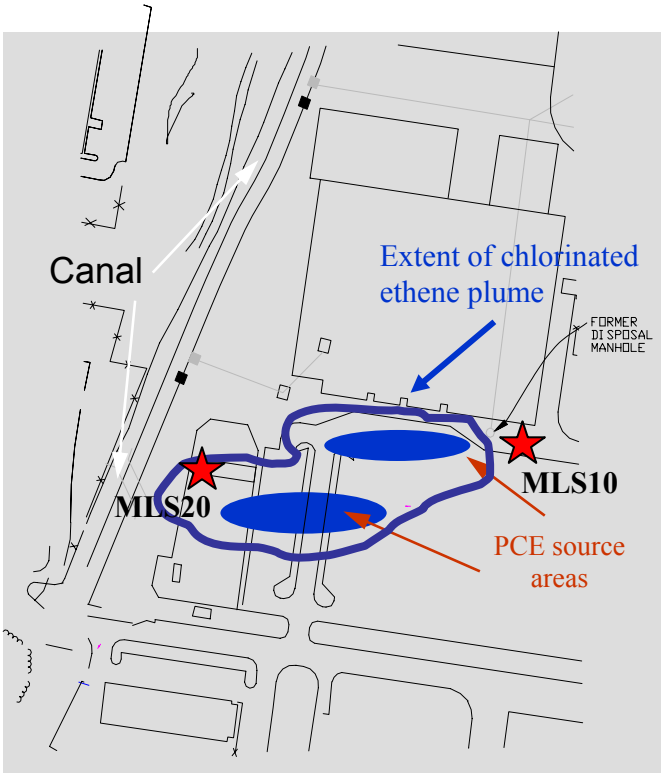

Figure 2. Naval Amphibious Base Little Creek, Virginia Beach, Virginia. Locations of the chloroethene plume, the source areas, and the sampling sites: MLS10 and MLS20. ***Figure adapted from (CH2MHill, 2000).***

Materials and Methods

Extractions

The extent to which carbon was biodegraded from aquifer sediment samples was tested using material extracted from aquifer sediment. Sediment was baked over night at 70° C and then ground and sieved through 2mm pores. A sequential five day extraction procedure was used in which sediment was continuously mixed with an extracting solution for 24 hours on a rotary tumbler. The first three 24-hour extractions were performed using a 0.1% sodium pyrophosphate solution. The fourth extraction was conducted with 0.5N sodium hydroxide, and the fifth extraction again used 0.1% sodium pyrophosphate. Fifteen grams of sediment and 30mL of extractant were added to 50 mL centrifuge tubes. Weights of the tube, the tube with sediment, and the tube with sediment and extractant were recorded. Triplicate extractions were run for the sediment from each location. After the addition of sediment and extractant, the solution was homogenized on a vortex mixer and placed on a rotary tumbler for 24 hours. They were then centrifuged for 25 minutes at 2000 rpm to separate dissolved species from the solid material. The supernatant was decanted and stored at 4 degrees centigrade in 40 mL glass EPA vials. The remaining pellet was weighed before adding 30 mL of the extractant and repeating the process. The pH of each centrate was recorded and a 2 mL sample was taken for measuring total organic carbon (TOC) in the solution. Control tubes containing only the

extracting solution were run in order to determine if any carbon was being extracted from the centrifuge tube itself.

Bioassay Set-Up

Bioassays were constructed using the supernatant combined from the first three days of extractions and using the supernatant combined from the last two extractions. Sodium pyrophosphate and sodium hydroxide are believed to remove different pools of carbon. While sodium pyrophosphate is a gentler extraction and removes the softer carbon, sodium hydroxide acts much more harshly on the sediment, removing carbon that is more strongly held in the sediment matrix. By combining the decanted liquid into these two solutions, the biodegradability of the different pools could be compared.

Bioassays were prepared in sterilized 40mL glass EPA vials that had been acid washed and baked at 350 °C for one hour in order to remove any carbon. Throughout the experiment, they were stored in an anaerobic glovebox containing 95% ultra high purity nitrogen and 5% hydrogen. A mixed culture was created using 10g of A horizon soil added to 90 mL of minimal salts media (MSM). The MSM contained 0.1% pyrophosphate which served to extract microorganisms from the soil (Zuberer 1994). Microcosms were supplemented with a MSM that was prepared by combining: 3.4g KH_2PO_4, 4.35g K_2HPO_4, 1.0g NH_4Cl, 186mg EDTA, 150mg $MgSO_4 \bullet H_2O$, 4.5mg $MnSO_4 \bullet 4H_2O$, 0.5mg $NaMoO_4 \bullet 2H_2O$, 0.15mg H_3BO_3, 20mg $CaCl_2$, 1.5mg $ZnCl_2$, 0.5mg $CuCl_2 \bullet 2H_2O$, 1.5mg $CoCl_2 \bullet 6H_2O$, and 11mg $FeCl_2 \bullet 4H_2O$ per 1L. Bioassays were constructed using 30mL of centrate from the extractions, 2 mL of the soil culture, and 2 mL MSM (Figure 3). Bioassays were brought to a neutral pH using H_3PO_4 and NaOH. Triplicates of the two pools of centrate were constructed for each site. Bioassays were capped and then the headspace was purged for ten minutes with nitrogen filtered through a .22um filter in order to remove CO_2 and O_2 from the headspace. Controls were prepared with 30mL of centrate and 4mL of FSMS. These were sterilized in an autoclave cycle run at 121°C for 15 minutes. In order to better ensure that bacterial growth did not occur, 131 uL of sodium azide (NaN_3) were added to the controls for a NaN_3 concentration of 25 mg/kg. The headspace was subsequently flushed with sterile

nitrogen for ten minutes and all bioassays were placed in the anaerobic glovebox. Controls were constructed in order to show that carbon did not degrade in the absence of the bacterial culture. Table 2, Table 3, and Table 4 provide the matrix used to construct the bioassays for Kings Bay, MLS 10, and MLS 20 respectively.

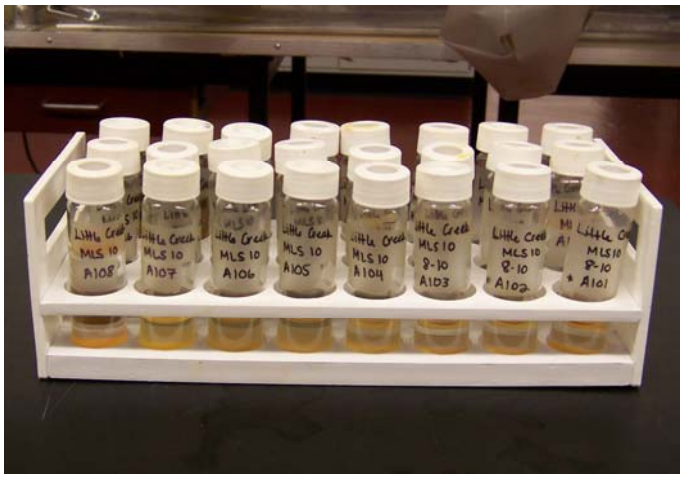

Figure 3. Photograph of the bioassays constructed for biodegradation analysis. After the microcosms were inoculated, they were stored in an anaerobic glovebox consisting of 95% ultra high purity nitrogen gas and 5% hydrogen gas.

Table 2. ***Kings Bay Microcosm Matrix.***

	Combined Centrate	# of Duplicates	Extract (mL)	MSM (mL)	Culture (mL)	NaN3 (mL)
KBA	Days 1-3	3	30	2	2	0
	Days 4-5	3	30	2	2	0
5/11/2002	Days 1-3	3	30	2	2	0
	Days 4-5	3	30	2	2	0
Outcrop	Days 1-3	3	30	2	2	0
	Days 4-5	3	30	2	2	0
KBA Control	Days 1-3	3	30	4	0	0.131
	Days 4-5	3	30	4	0	0.131
5/11/02 Control	Days 1-3	3	30	4	0	0.131
	Days 4-5	3	30	4	0	0.131
Outcrop Control	Days 1-3	3	30	4	0	0.131
	Days 4-5	3	30	4	0	0.131

Due to the low amount of sediment available for extractions, the 5/11/02 and Outcrop extracts were diluted to approximately ½ concentration in order to have enough extract

solution to create the microcosms. The extremely high carbon content in these sediments allowed for dilutions without resulting in carbon concentrations that resembled low carbon sediments. Samples were taken after these dilutions to have an accurate concentration of the extracts incorporated into the bioassays. These are the concentrations reported as the extract concentrations.

Table 3. ***MLS 10 Microcosm Matrix.***

	Combined Centrate	# of Duplicates	Extract (mL)	MSM (mL)	Culture (mL)	NaN3 (mL)
8-10 Feet	Days 1-3	3	30	2	2	0
	Days 4-5	3	30	2	2	0
20.5-22 Feet	Days 1-3	3	30	2	2	0
	Days 4-5	3	30	2	2	0
8-10 Feet Control	Days 1-3	3	30	2	0	0.131
	Days 4-5	3	30	2	0	0.131
20.5-22 Feet Control	Days 1-3	3	30	4	0	0.131
	Days 4-5	3	30	4	0	0.131

Table 4. ***MLS 20 Microcosm Matrix.***

	Combined Centrate	# of Duplicates	Extract (mL)	MSM (mL)	Culture (mL)	NaN3 (mL)
8-10 Feet	Days 1-3	3	29	3	2	0
	Days 4-5	3	29	3	2	0
22 Feet	Days 1-3	3	29	3	2	0
	Days 4-5	3	29	3	2	0
8-10 Feet Control	Days 1-3	3	20	2.655	0	0.092
	Days 4-5	1	29	5	0	0.131
	Days 4-5	2	20	2.655	0	0.092
22 Feet Control	Days 1-3	3	20	2.655	0	0.092
	Days 4-5	1	29	5	0	0.131
	Days 4-5	2	20	2.655	0	0.092

Continuous Sampling

Sampling was conducted in the glovebox and included measuring headspace CO_2, aqueous TOC concentration, and VFA concentrations. Samples were taken every four days during the first two weeks, weekly for the next two weeks, and then every two

weeks until the final sampling event. Bioassays remained in the glovebox throughout the experiment in order to prevent the introduction of oxygen. Only one of the triplicate controls was sampled throughout the experiment in order to better ensure a sterile environment in the two unsampled bioassays. These two controls were sampled only at the beginning and end of the experiment. Bioassays were monitored for approximately twelve weeks.

Carbon Dioxide Analysis

Measuring CO_2 generation involved using a gas tight syringe to take a one milliliter sample from the headspace. The sample was injected into a GOW-MAC (Bridgewater, N.J.) gas chromatograph Series 580 with a thermal conductivity detector. The sample was run for 8.5 minutes through a column maintained at 50°C. The detector and injector were set at 90°C and 70°C respectively with the detector current held at 200 mV. Helium was the carrier gas flowing at a rate of 20 mL/min. A standard curve was created using a 15% CO_2 standard. Sample peak areas were related back to the standard curve to obtain headspace CO_2 concentrations.

Total Organic Carbon Analysis

After taking the headspace sample, the caps were removed in order to obtain a sacrificial 2 mL liquid sample for measuring aqueous TOC, and a .99 mL liquid sample was taken for VFA analysis. The TOC sample was acidified with 0.1 mL H_2SO_4 and stored in a 4 mL glass EPA vial at 4°C until analyzed. TOC analysis was achieved using a Shimadzu TOC-V CSN Total Organic Carbon Analyzer. The furnace temperature was kept at 680 C and ultra zero grade air (carrier gas) flowed at 150 mL/min through the combustion tube with TOC standard catalyst. Samples were sparged with ultra zero grade air for 1 minute at a flow rate of 100 mL/min before analysis. Injection volume was 150 µL.

Volatile Fatty Acid Analysis

The VFA samples were analyzed for the presence of acetate, propionate, isobutyrate, butyrate, isovalerate, caproate, hexanoate, and heptanoate. Each sample was prepared with 0.99mL sample and acidified with 0.01 mL H_3PO_4. It was then filtered through a 0.45µM presterilized filter and crimp capped. During the first part of the research,

samples were analyzed using a Shimadzu Gas Chromatograph (GC) 14A (Shimadzu Scientific Instruments, Columbia, MD) with flame ionization detector (FID). Helium was the carrier gas and nitrogen gas was the make-up gas. The column temperature started at 80°C and increased to 140°C over a 3 minute time period. The detector and injection temperatures were 250 and 200°C respectively. The second half of the VFA samples were analyzed on a Hewlett-Packard 5890 GC with FID. Helium was the carrier gas and nitrogen was the make-up gas. The program started at a temperature of 100 C and increased to 154 C over a 12 minute period.

Data Analysis

Live Anaerobic Bioassays

Data from this research was analyzed to better understand the extent to which organic carbon extracted from aquifer sediment is degradable under anaerobic conditions. Anaerobic bioassays were constructed using extracts derived using sediments from three different sites. These were maintained and sampled for a total of 79-93 days looking at TOC concentrations, as well as the production of VFAs and headspace CO_2. TOC concentrations were monitored in order to determine the anaerobic biodegradability of the carbon, with the generation of carbon dioxide as supporting evidence for the breakdown of carbon and subsequent conversion to CO_2. VFA production demonstrates that the bioassays were anaerobic and that fermentation was occurring within the microcosms. Carbon breakdown, VFA production, and hydrogen generation in this anaerobic environment are preliminary evidence that the extracts could be capable of supporting reductive dechlorination in contaminated aquifers.

Killed Controls

Triplicate controls were monitored with the expectation that there would be negligible carbon loss from the initial timepoint to the end of the experiment. This would confirm whether the loss of carbon in the live bioassays was due to the biotic activity of the microorganisms present. Of the triplicate bioassays, one was sampled throughout the experiment while two remained sealed until the final timepoint. This minimized the

opportunity for contamination of two of the controls, while allowing observation of what was occurring in one of the controls throughout the experiment.

Data Reporting

Bioassays constructed from the extract combined from the first three days of pyrophosphate extractions are denoted as "Pyro", while the bioassays constructed from days 4 and 5 of extractions are denoted as "Base". The "Base" bioassays for the Kings Bay sediment were constructed 3 days after the "Pyro" bioassays and the difference in days for the timepoints on the CO_2 graphs reflects this.

Microcosms were run as triplicates, and one sample was taken from each of these triplicates for carbon dioxide, volatile fatty acid, and TOC analysis. The values for these parameters are reported as averages of the triplicate samples calculated after analyzing one sample per microcosm. Dilutions of the TOC samples were factored into the reported averages: 100 μL acid addition to a 2mL TOC sample for preservation; 3mL water addition to a 1mL sample for TOC analysis; a 1.133X dilution for the addition of the MSM and soil culture in the bioassays; and a 1.1377X dilution in the controls for addition of MSM, soil culture, and sodium azide. Extraction data incorporates the acid dilution of 100 uL into a 2mL sample and the 3 mL water dilution into 1 mL sample for TOC analysis. External check standards of a known concentration were analyzed at the beginning and end of sample analysis runs in order to account for machine drift over time. Drift calculations were incorporated for all Kings Bay TOC data and for some of the MLS10 and MLS20 data. This procedure is described in Appendix G. Most of the MLS10 and MLS20 did not have appropriate data in order to factor drift into the reported values.

TOC Utilization Calculation

The activity of the microorganisms was determined as the time in which CO_2 production was sustained. The TOC measurements after CO_2 production ceased were not included in this analysis. The TOC utilization was calculated as the difference in TOC from the initial timepoint sample to the timepoint at which CO_2 was no longer being produced.

VFA Analysis

VFA samples were analyzed on 2 different machines due to technical problems with one of the instruments. Initially, results were analyzed using a Shimadzu 14A GC and later using a Hewlett-Packard 5890 GC. Some sample concentrations were below the lowest standard concentration in the standard curve, and therefore these reported values may not be accurate, but they can be compared in relative amounts. The lowest concentration detected for the standard curve run on each machine is shown in Table 5. Sample concentrations presented are shown as a reference to see relative quantities. Few VFAs were observed throughout the experiment.

Table 5. ***VFA concentrations in the lowest standard used to construct a standard curve and the concentration detected by two different GCs.***

VFA	Standard Concentration	Shimadzu 14A Concentration Detected	Hewlett-Packard 5890 Concentration Detected
Acetic	1.500	-	0.732
Acetic	3.000	3.417	
Proprionic	1.850	2.369	2.667
Isobutyric	2.205	2.636	3.188
Butyric	2.205	2.727	3.079
Isovaleric	2.555	3.196	3.394
n-Valeric	2.555	3.245	3.379
Isocaproic	2.905	3.715	3.782
Hexanoic	2.905	3.689	3.988
Heptanoic	3.255	4.387	4.306

Comparison to Aerobic Data

Comparisons were made with TOC data derived from aerobic bioassays (Rectanus 2006). The two data sets were evaluated in order to derive any possible links between the extent of biodegradability in aerobic and anaerobic environments. The aerobic bioassays were constructed using extract from the same extraction procedure used for the anaerobic bioassays. TOC data for the aerobic experiment was gathered using a Dohrmann DC-80 Total Organic Carbon Analyzer, while data for the anaerobic bioassays was collected using the Shimadzu TOC-V CSN Total Organic Carbon Analyzer. The TOC concentrations reported from analysis by the two different machines appeared to be inconsistent and consequently eliminated the ability to make direct comparisons.

Hydrogen Measurement

Separate bioassays were constructed with the purpose of confirming H_2 production. They contained a total volume of 60 mL with the same ratio of components as that added in the other bioassays. With solution from the MLS 10 extractions, 53 mL of extract, 3.5 mL MSM, and 3.5 mL of soil culture were added. The bioassays were prepared in 160 mL bottles, flushed with filtered nitrogen for approximately 10 minutes, sealed with a rubber stopper and crimp cap, and stored in a glovebox containing 100% ultra high purity grade nitrogen (Figure 4). These were sampled for hydrogen production on the same schedule as the other bioassays. Hydrogen was measured using a reduced gas detector (RGD, Trace Analytical, Menlo Park, CA) with a detection limit of 0.05uL/L. Ten mL of headspace sample was injected using a gas tight syringe.

Figure 4. Photograph of the bioassays constructed for headspace hydrogen measurement using MLS10 sediment extractions. These were stored in a glovebox containing 100% ultra high purity nitrogen gas.

Positive Controls

The activity of the mixed culture used was tested by constructing positive control bioassays (Figure 5). The microcosms contained a total of 60 mL, and the volumes of constituents added were calculated based on the same ratios as used in the bioassays. The

positive controls consisted of 53 mL MSM, 3.5 mL of a 5% yeast extract solution, and 3.5 mL of the soil culture. The yeast extract was filtered through a 0.22 um Stericup vacuum driven presterilized filter and added to produce a concentration of 50 mg/L in the bioassays. They were sealed with rubber stoppers and crimp caps, flushed with filtered N_2 for approximately 10 minutes, and maintained in 160 mL bottles in an anaerobic glovebox containing 100% ultra high purity grade nitrogen. These microcosms were sampled on the same time schedule as the extract bioassays and analysis included measurement of headspace CO_2, aqueous TOC, VFA production, and H_2 generation in the headspace.

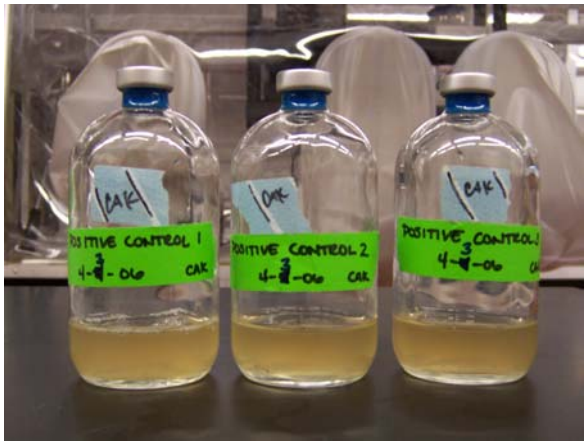

Figure 5. Photograph of the positive control bioassays constructed for ensuring the quality of the mixed culture used in the live bioassays. These were stored in glovebox containing 100% ultra high purity nitrogen gas.

Results and Discussion

Aquifer sediments from two different locations and at different depths were used in order to compare the anaerobic biodegradability of NOC extracted from shallow and deep sediments, sediments found inside and outside a chlorinated solvent plume, and sediments containing carbon concentrations ranging from low to high. Biodegradability was examined by looking at the loss of TOC over time and subsequent conversion to CO_2, breakdown of more complex organics to VFAs, and the generation of hydrogen in the headspace. The production of VFAs was evidence of an anaerobic environment within the bioassays and that the system was reduced. This increasingly reduced environment would be more conducive for reductive dechlorination.

Positive Controls

Positive controls constructed with yeast extract as a carbon source and MSM were monitored in order to ensure the quality of the soil culture used in the bioassays. These were sampled in the same manner and time frame as the bioassays. TOC was shown to degrade over time, reaching its lowest concentration after 20 days. After day 30, an upward trend of TOC concentration was observed. An increase in organic carbon after a period of biodegradation has been observed in other experiments as well (Kalbitz 2003; Schnabel et al. 2002). Two of the triplicates behaved in the same way, while the other control responded differently. Figure 6 shows the TOC concentrations over time for

Positive 1, and Figure 7 shows the TOC concentrations over time for the average of Positives 2 and 3.

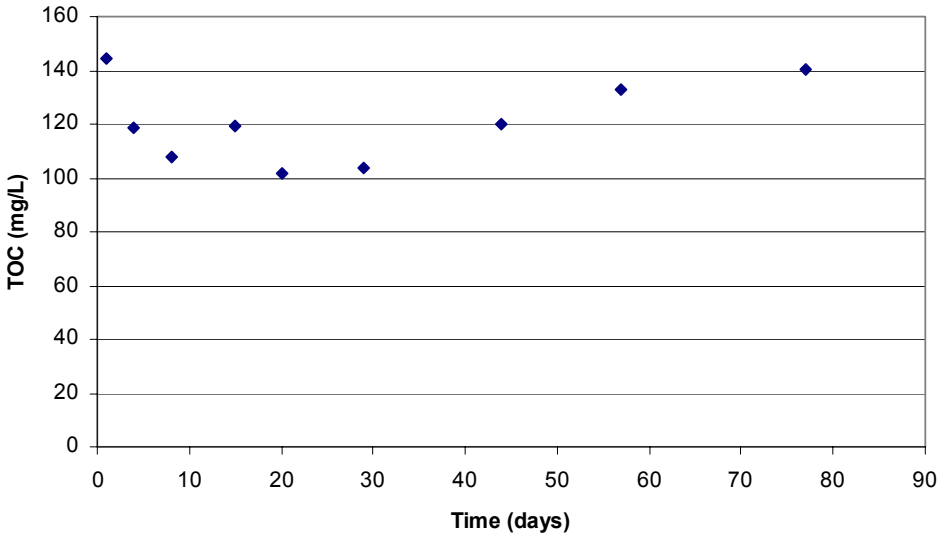

Figure 6. TOC concentrations (mg/L) over time for Positive Control 1.

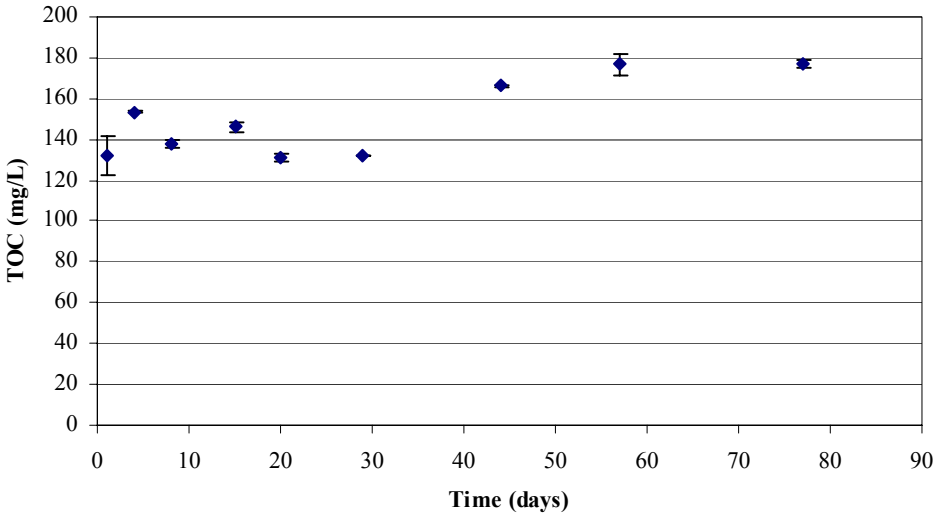

Figure 7. TOC concentrations (mg/L) over time for the average of Positive Controls 2 and 3.

Positive 1 experienced two times more loss of TOC that the other two positive bioassays experienced and produced over twice as much CO_2 as the other controls (Figure 8).

However, the other two controls on average generated twice as many VFA acetic acid equivalents (Figure 9).

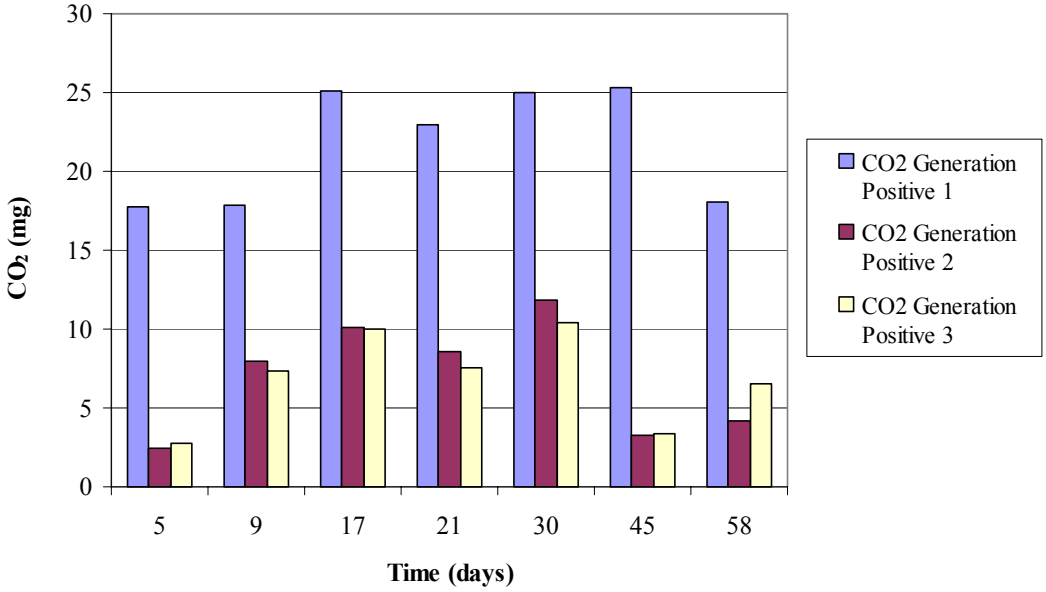

Figure 8. CO_2 Generation over time in the headspace of the positive controls.

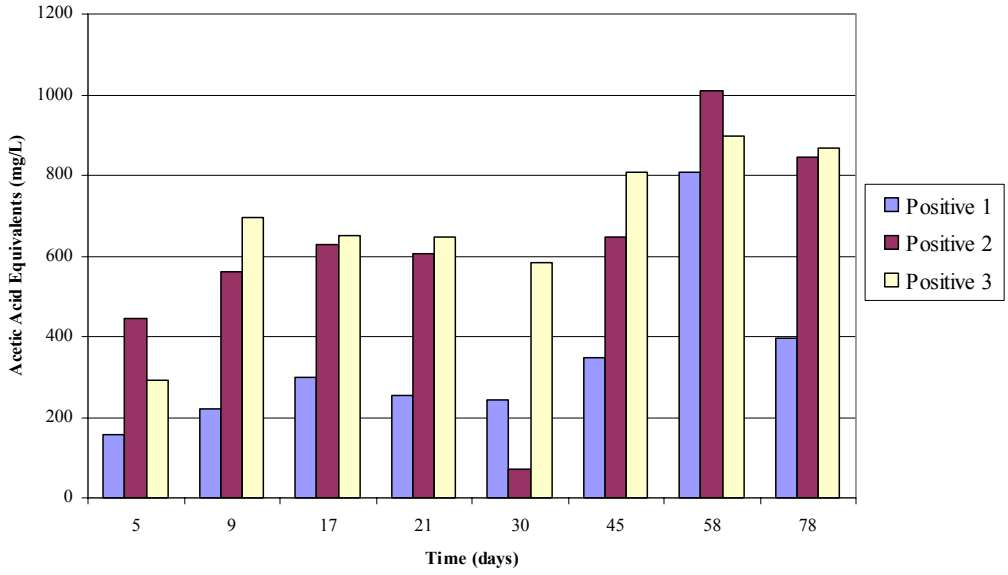

Figure 9. Total VFAs as acetic acid equivalents produced in the positive controls over time.

It is possible that the VFAs in the first control were quickly oxidized to form CO_2 thus explaining the high CO_2 levels maintained in this bioassay throughout the experiment.

The other two bioassays also produced VFAs but did not act as quickly in oxidizing the VFAs, thus resulting in lower levels of CO_2 and higher quantities of VFAs. These higher quantities of VFAs would factor into the TOC measurement and therefore show a smaller loss of TOC. Due to discrepancies in using different machines for VFA analysis, a mass balance throughout the experiment was not possible. All positive control bioassays produced more hydrogen than the analytical procedure was able to determine.

The loss of TOC within these positive controls shows that the culture used in the bioassays is active in the presence of a suitable carbon source. Both hydrogen and VFAs were produced in conjunction with this biodegradation of TOC and where more VFAs were detected, less CO_2 was observed. While Positive Control #1 had the greatest amount of carbon degradation and CO_2 production, it produced the lowest concentration of VFAs. VFAs, predominantly acetic acid, were being produced within 5 days of setting up the positive control bioassays. The quality of the culture was appropriate for the purpose of this experiment seeing that when given the MSM and a suitable organic carbon source, the organisms actively biodegraded the carbon to yield CO_2, VFAs, and H_2.

Bioassays for Hydrogen Measurement

Bioassays were constructed for measuring headspace hydrogen with the same procedure and ratios of components as the live bioassays. A total of 60 mL of solution was combined in 160-mL glass bottles and sealed with a rubber stopper and crimp cap. Although the bottles were not flushed between sampling, the sampling technique resulted in some gas leakage from the headspace, and therefore a cumulative concentration of H_2 could not be calculated. MLS10 extractions were used to construct pyro and base bioassays for both the shallow and the deep sediment extracts. The deeper sediment bioassays produced approximately twice as much hydrogen gas as did the more shallow sediment bioassays, and the base bioassays much greater quantities of hydrogen gas than did the pyro bioassays. By day 23, the shallow pyro bioassay was producing little to no hydrogen gas, and the deep pyro bioassay no longer showed hydrogen production on day 52 (Figure 10). However, the base bioassays continued to produce hydrogen gas for the

entire 101 days that samples were taken (Figure 11). Concentrations continued to build as time went on. However, because the headspace was not flushed but some headspace gas was lost during sampling, it is unclear how much H_2 is due to accumulation and how much is new hydrogen production.

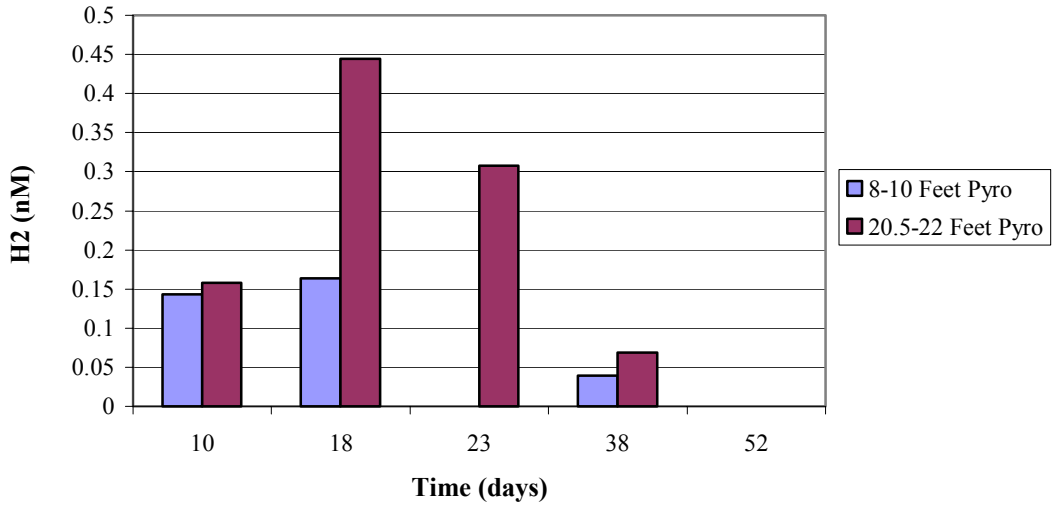

Figure 10. Headspace hydrogen production (nM) in the MLS10 8-10 feet pyro bioassays and 20.5-22 feet pyro bioassays.

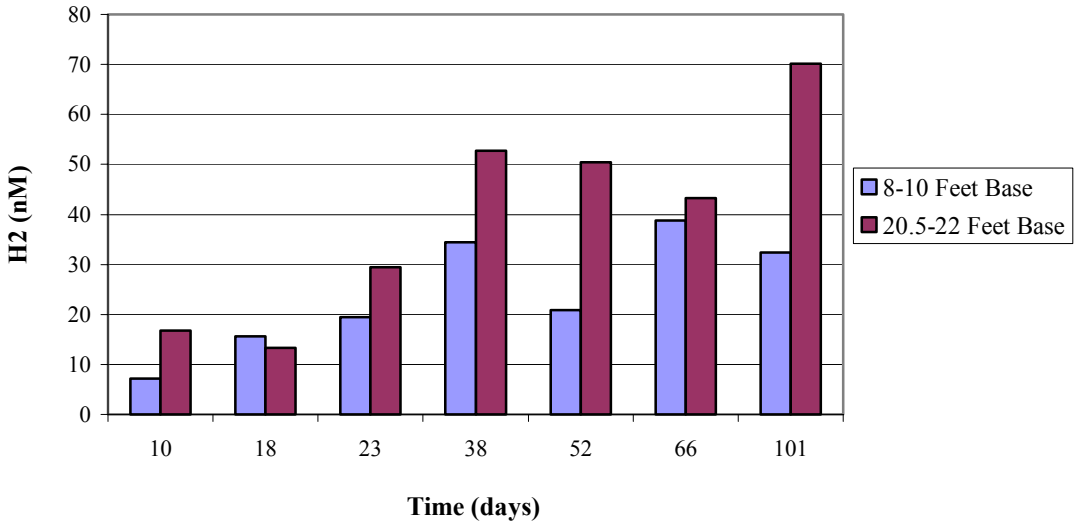

Figure 11. Headspace hydrogen production (nM) in the MLS10 8-10 feet base bioassays and 20.5-22 feet base bioassays.

NSB Kings Bay

Sediment from three different sampling locations were provided by the U.S. Geological Survey from NSB Kings Bay. These samples were labeled KBA, 5/11/02 and Outcrop. The KBA and 5/11/02 samples were collected from the semi-confined aquifer which is the focus of an extensive study of biodegradation of chlorinated ethenes. The Outcrop sample was derived from high-carbon sediment that overlies the semi-confined aquifer.

Initial TOC

In looking at the initial live bioassay TOC concentrations and the rest of the timepoint data, the initial concentrations are quite different than the rest of timepoint data. After examining the data for the timepoint samples, the extract TOC concentrations correspond more closely to the values expected as the starting bioassay concentrations. A comparison of the initial live bioassay concentrations to the extract concentrations shows that the KBA bioassays TOC concentrations more closely matched the TOC concentrations of those extractions than did the 5/11/02 and Outcrop bioassay concentrations (Table 6). While the extraction TOC concentrations for KBA are 7-14% lower than the time 0 concentrations, the initial bioassay concentrations for 5/11/02 are approximately 40% lower than the extraction concentrations. The Outcrop time 0 concentrations are approximately 20-80% lower than the extraction concentrations. In examining the rest of the bioassay timepoint TOC data, the time 0 values are inconsistent as a starting concentration implying some sort of error in these samples. For this reason, the extraction carbon data was used as the initial concentration for determining carbon loss in the live bioassays.

Table 6*: Comparison of Time 0 TOC concentrations (mg/L) to extraction TOC concentrations (mg/L) for Kings Bay bioassays.*

	Total Organic Carbon (mg/L)		
	Extraction	**Time 0**	**% Difference**
KBA Pyro	48.9	56.8	13.9
KBA Base	70.9	76.7	7.5
5/11/02 Pyro	133.2	95.9	-38.8
5/11/02 Base	65.8	46.8	-40.4
Outcrop Pyro	260.2	146.1	-78.1
Outcrop Base	408.4	345.8	-18.1

The combination of extracts from the first three days of extractions contained less organic carbon than the combination of extracts from days 4 and 5. The 5/11/02 sediment is the only exception to this in which the extractions from days 1-3 was 58% higher than that of the combined extracts from days 4-5. Figure 12 illustrates the differences observed in the time 0 and extraction TOC concentrations, as well as the differences between the TOC concentrations in the first three days of extractions as compared with days 4 and 5.

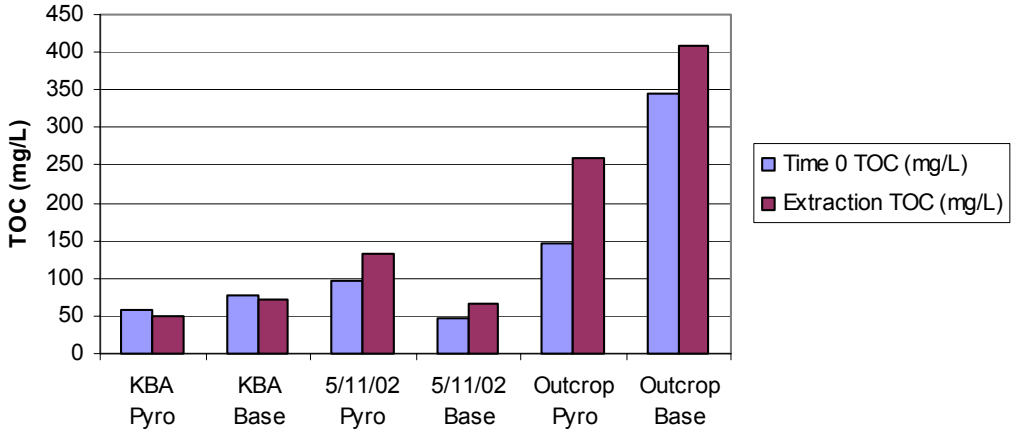

Figure 12. Comparison of Kings Bay live bioassays time 0 TOC concentrations with extraction TOC concentrations. "Pyro" indicates extracts combined from days 1-3, and "base" indicates extracts combined from days 4-5.

Kings Bay Bioassay Controls

Some of the Kings Bay controls did show a loss of TOC despite the measures taken to eliminate microbial growth in the bioassays; however an overall steady concentration of TOC through time was observed, thus showing that the loss of carbon in the live bioassays is largely due to the biotic activity of the soil culture. The losses in the controls were less than those seen in the bioassays, with the exception of that observed in the KBA bioassays (Figure 15). These controls experienced losses equal to or greater than those seen in the live bioassays. This may be due to regrowth of microorganisms within this microcosm, or it may reflect variation in the samples or analysis. The data fluctuated throughout the experiment, and no steady decrease of TOC concentration is apparent (data shown in Appendix C). Variation in TOC concentrations over time is seen in the

timepoint data for some of the controls. With few exceptions in all bioassays, the controls that were not sampled until the last timepoint showed less loss of TOC than the sampled controls. The additional loss of carbon in the sampled controls is likely due to incidental inoculation during opening of the bioassays for sampling.

KBA Sediment Sample

Headspace CO_2

Carbon dioxide in the headspace was monitored for supplemental evidence of biodegradation of organic carbon showing that as carbon was degraded, part of it was oxidized to form CO_2. Steady CO_2 production was sustained through day 22 in the pyro bioassays maintaining 0.018 to 0.027 mg CO_2 in the headspace before decreasing below .01 mg CO_2. The base bioassays steadily decreased in CO_2 production with each sampling, but maintained 0.082 to 0.166 mg CO_2 through day 33 before decreasing to 0.012 to 0.045 mg CO_2. Headspace CO_2 in one of the triplicate controls was monitored, while the other two remained capped throughout the experiment. Both pyro and base bioassays were showing negligible CO_2 production after day 50 (Figure 13 and Figure 14). The pyro control produced negligible CO_2 levels, while the base control CO_2 levels were comparable to those seen in the bioassays. However, both of the controls that remained capped throughout the experiment contained only 0.001 mg CO2 in the headspace at the end of the experiment. No methane was detected.

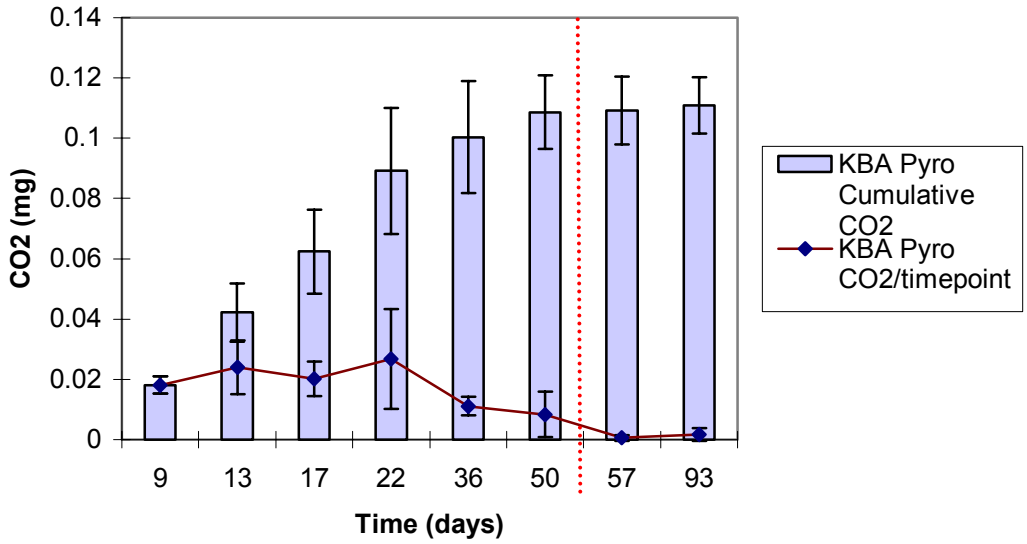

Figure 13. Generation of headspace CO_2 (mg) in the KBA pyro live bioassays.

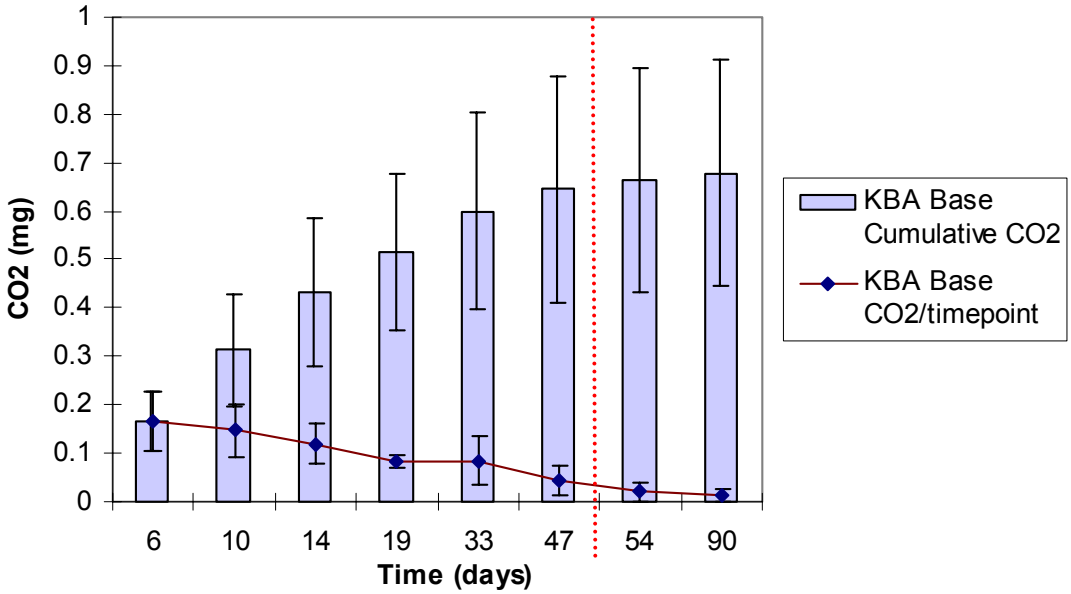

Figure 14. Generation of headspace CO_2 (mg) in the KBA base live bioassays

Anaerobic Live Bioassays TOC

TOC loss was calculated as the loss occurring during the time that CO_2 was observed in the headspace. KBA pyro and base bioassays showed 20% and 7% loss of TOC, respectively. This corresponds to losses of 11.4 and 5.0 mg/L TOC. Figure 15 shows

that an overall downward trend is observed over time in the KBA bioassays and on the last sampling day, TOC decreases indicating that continued degradation may still be occurring; however CO_2 production was not detected at this time. Larger scatter was present in the data during the first 18 days of sampling, after which triplicates closely replicated.

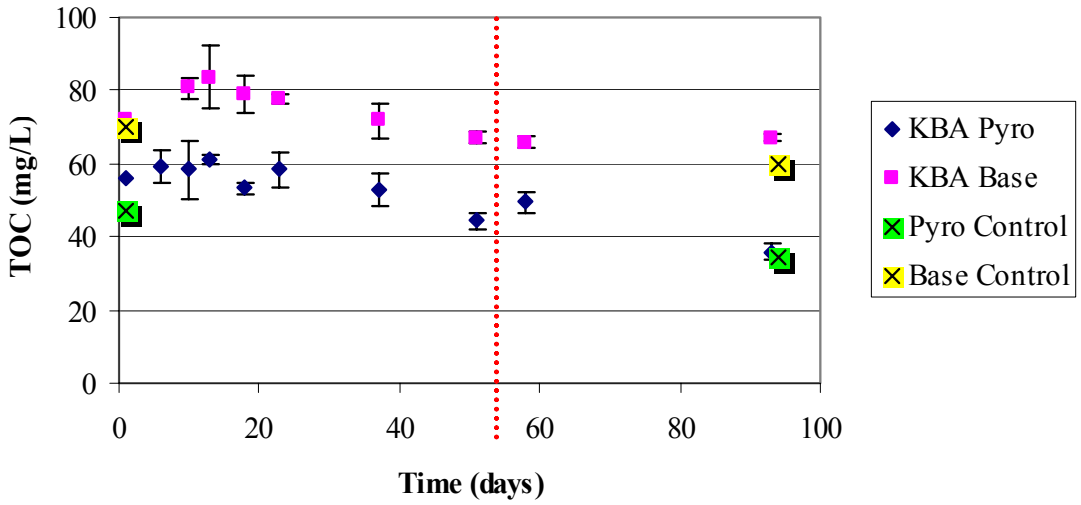

Figure 15. KBA live bioassays aqueous TOC concentrations (mg/L) monitored for a period of 93 days. The initial and final TOC concentrations are shown for the control bioassays. The red line indicates the time at which headspace CO_2 was no longer generated.

Conversion of Aqueous TOC to Headspace C-CO_2

Carbon dioxide mass in the headspace was converted to mass of C-CO_2/headspace and compared to the loss of TOC in the bioassays for purposes of determining % conversion from aqueous TOC to C-CO_2. The pyro bioassays demonstrated a conversion of 4 to 25%, and the base bioassays showed a conversion of 18 to 142% TOC to C-CO_2. The high number on the base conversion calculation indicates that more C-CO_2 was produced than there was loss of TOC in the bioassays.

VFA Analysis

Acetic acid was detected in the base bioassays only on days 18 and 30. The VFAs observed in the KBA bioassays are listed in Table 7. The levels on day 30 were

approximately 1/5 the amount detected on day 9. The base control contained approximately ½ the amount of acetic acid as the live bioassays on day 9. One of the pyro bioassays contained proprionic acid on day 18.

Table 7. ***Summary of VFAs (mg/L) in Kings Bay Bioassays.***

	KBA Pyro	KBA Base	KBA Base Control	Outcrop Pyro Control	Outcrop Base Control
Acetic Acid		24.204	5.817	0.192	22.465
Proprionic Acid	21.541				
Isobutyric Acid					
Butyric Acid					
Isovaleric Acid					
Caproic Acid					
Isocaproic Acid					
Hexanoic Acid					
Heptanoic Acid					
Total as equivalents of Acetic Acid (mg/L)	30.565	24.204	5.817	0.192	22.465

Conclusions from KBA Sediment

The base and pyrophosphate extractions conducted on days 4 and 5 removed more carbon from the sediment than the first three days of pyrophosphate extractions; however the pyro bioassays showed greater % TOC degradation under anaerobic conditions. This suggests that the carbon extracted during the first three days of extractions removes a higher percentage of biodegradable material.

The same trends of TOC concentration over time were seen in comparing the data for the pyro and base bioassays; there was a steady decline of aqueous TOC over time. Despite the high C-CO_2 production, the % TOC converted to C-CO_2 was lower on day 23 than on other days. This increase in CO_2 production could partly be a result of fermentation of the VFAs detected in the previous timepoint. More VFAs were detected in both pyro and base bioassays on day 18 than on any other day in all Kings Bay sediments examined. It is not totally clear if TOC concentrations were still decreasing at the conclusion of the

sampling. Although the last sample taken contained lower TOC than the previous timepoint samples, it was not as low as the concentration observed on day 23. However, the overall trend points toward continued degradation at the conclusion of the experiment. Negligible CO_2 is generated after day 50 indicating that TOC no longer continues to degrade.

Despite these trends observed in the live bioassays, the control bioassays experienced as great a TOC loss as did the live bioassays. This is likely due to regrowth of microorganisms; however no tests were conducted to confirm this. Therefore, the control data does not allow for conclusive evidence of anaerobic biodegradation in the live bioassays.

5/11/02 Sediment Sample

Headspace CO_2

Steady CO_2 production was sustained through day 22 maintaining 0.01mg CO_2 in the headspace of the pyro bioassays whereas the base bioassays decrease in CO_2 production by day 19. The base bioassays produced 0.082 to 0.147mg CO_2 before this substantial decline. Generation of CO_2 was maintained throughout the experiment in the pyro bioassays, but ceased production after day 47 in the base bioassays (Figure 16 and Figure 17). The pyro control produced CO_2 levels comparable to that of those seen in the live bioassays of approximately 0.01 mg until day 20 when production decreased. The base control CO_2 levels were 23 to 40% lower than those seen in the bioassays until day 48 when a spike of CO_2 was observed. Controls that remained capped throughout the experiment showed only .006 mg CO_2 in the pyro control and 0.010 mg CO_2 in the base control. No methane was detected.

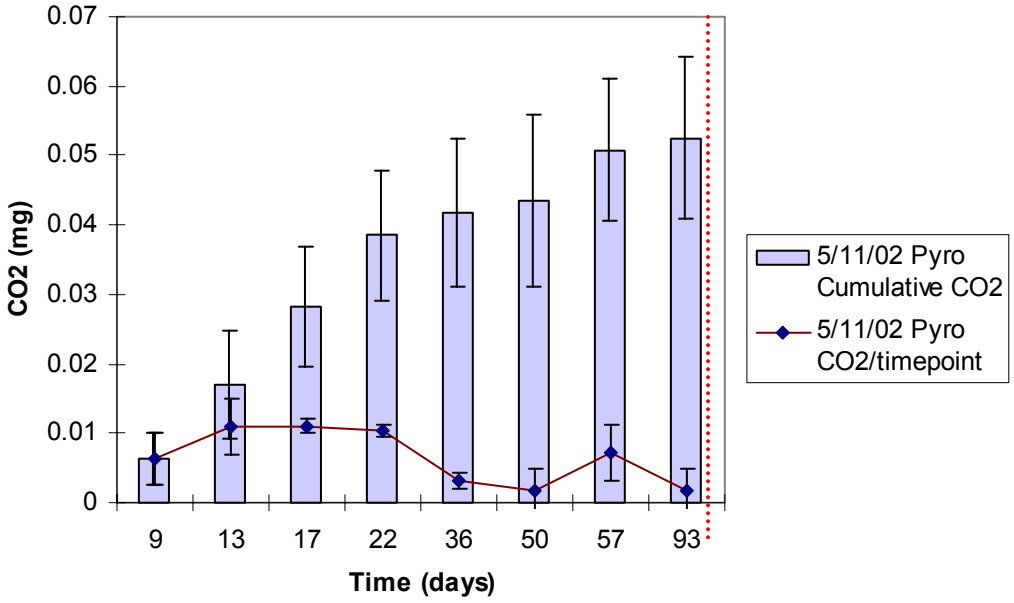

Figure 16. Generation of headspace CO_2 (mg) in the 5/11/02 pyro live bioassays.

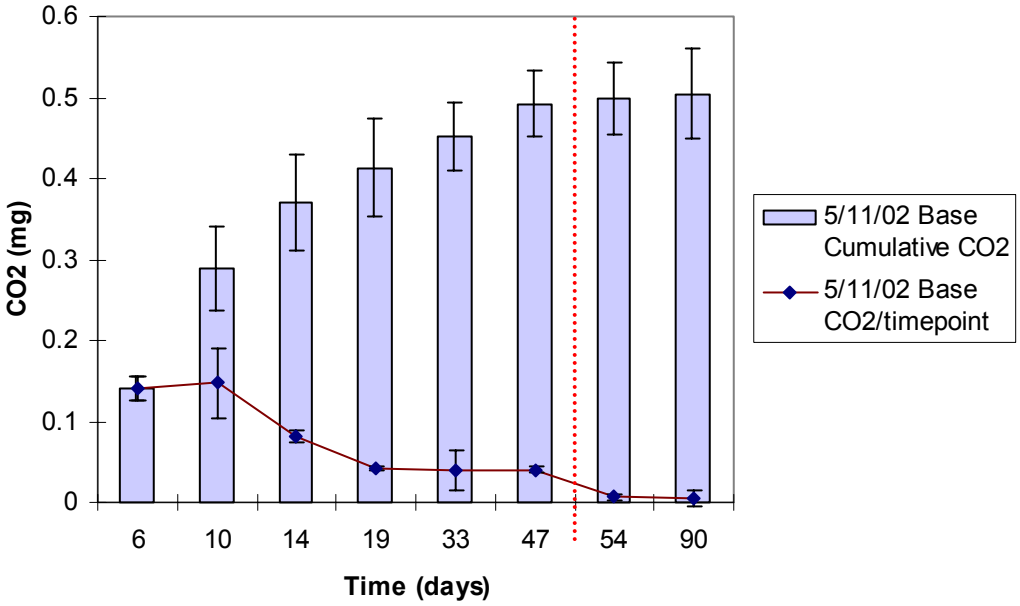

Figure 17. Generation of headspace CO_2 (mg) in the 5/11/02 base live bioassays.

Anaerobic Live Bioassays TOC

The 5/11/02 pyro and base bioassays showed 28% and 18% loss of TOC, respectively. This corresponds to a loss of 44.8 and 12.0 mg/L TOC. Figure 18 shows that the bioassays experienced a steady decline of TOC over time. It appears that concentrations

are steady at the conclusion of the experiment. The base bioassays show a slow but steady decrease in TOC throughout the experiment (Figure 18). Controls maintained steady TOC concentrations throughout the experiment.

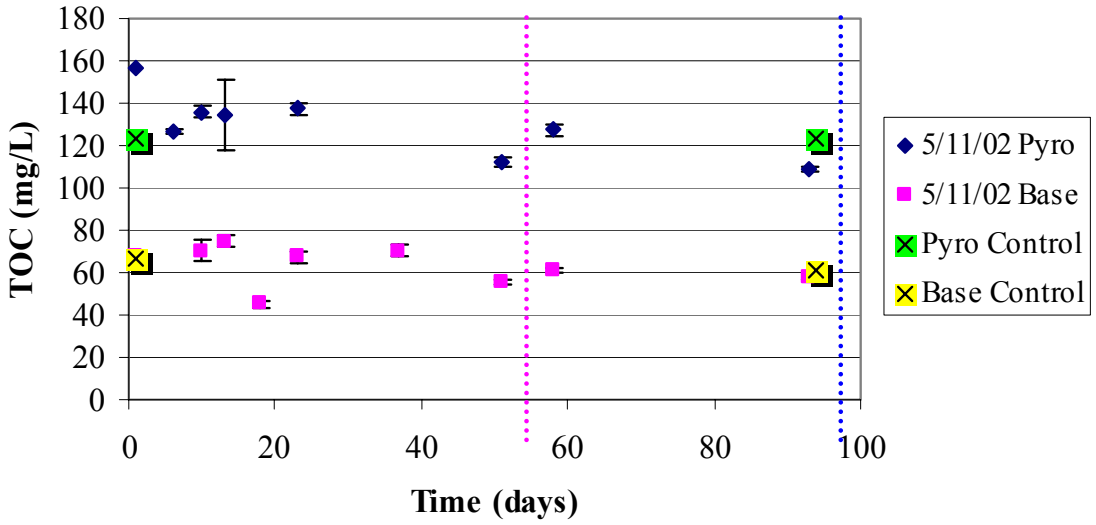

Figure 18. 5/11/02 live bioassays aqueous TOC (mg/L) monitored for 93 days. The initial and final TOC concentrations are shown for the control bioassays. The blue line indicates the time at which CO_2 was no longer generated in the pyro bioassays, and the pink line indicates when CO_2 was no longer generated in the base bioassays.

Conversion of Aqueous TOC to Headspace C-CO_2

Very low levels of CO_2 were produced in the pyro bioassays, converting only 0-7% aqueous TOC to C-CO_2. The highest percent conversion occurred on day 58. The base bioassays however experienced 31-132% TOC conversion to C-CO_2. Day 20 was the only day on which more CO_2 production was observed than there was loss of TOC. High TOC to C-CO_2 conversion rates were seen throughout the time that these bioassays were monitored.

VFA Analysis

No VFAs were detected in the 5/11/02 sediment throughout the experiment.

Conclusions from 5/11/02 Sediment

The pyro bioassays degraded roughly 11% more TOC than did the base bioassays, and unlike any of the other sediments from Kings Bay, the pyrophosphate extractions pulled more carbon off of the sediment than the final base and pyrophosphate extractions. The initial TOC level in the pyro bioassays was higher than the following four timepoints which are close in value. It is unclear if this initial timepoint is not representative of the actual TOC levels in the bioassays at the start of the experiment. Even if the TOC utilized was not as great as the numbers calculated, anaerobic biodegradation was observed in these bioassays.

The base bioassays showed a steady decrease in TOC for the duration of the experiment. No sharp decreases occurred, and the lowest TOC concentration was observed on the last day of sampling, however because CO_2 generation was no longer seen after day 47, any TOC loss after this point was not taken into account.

Outcrop Sediment Sample

Headspace CO_2

Figure 19 displays the headspace CO_2 observed in the pyro bioassays over time. Steady carbon dioxide production was sustained in the pyro bioassays through day 22 maintaining 0.013 mg to 0.021 mg CO_2 in the headspace. Generation of CO_2 increased through day 22 before decreasing and approaching zero after approximately 50 days. The base bioassays maintained consistent headspace CO_2 levels through day 47 at approximately 0.11 to 0.21 mg CO_2 (Figure 20). Only at the very end of the sampling did CO_2 levels decrease significantly. The pyro and base controls produced carbon dioxide levels comparable to or even higher than those seen in the bioassays. The base control CO_2 levels were 27 to 57% higher throughout the experiment than those observed in the bioassays. However, controls that remained capped throughout the experiment contained only 0.001 mg CO_2 in the pyro control and 0.020 mg CO_2 in the base control, levels which are almost exactly the same as those seen in the live bioassays in the final sampling event. No methane was detected.

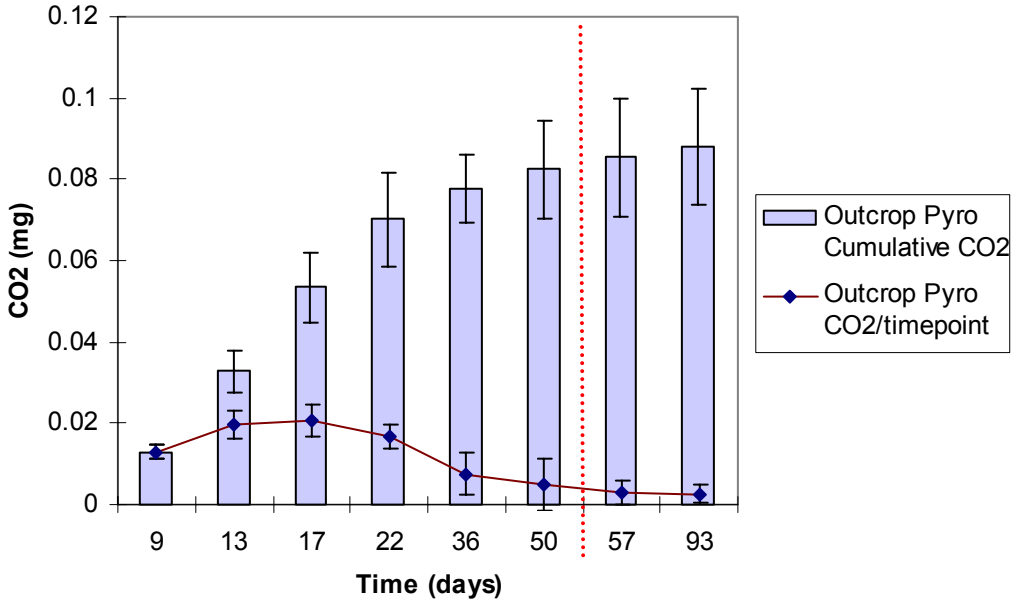

Figure 19. Generation of headspace CO_2 (mg) in the Outcrop pyro live bioassays.

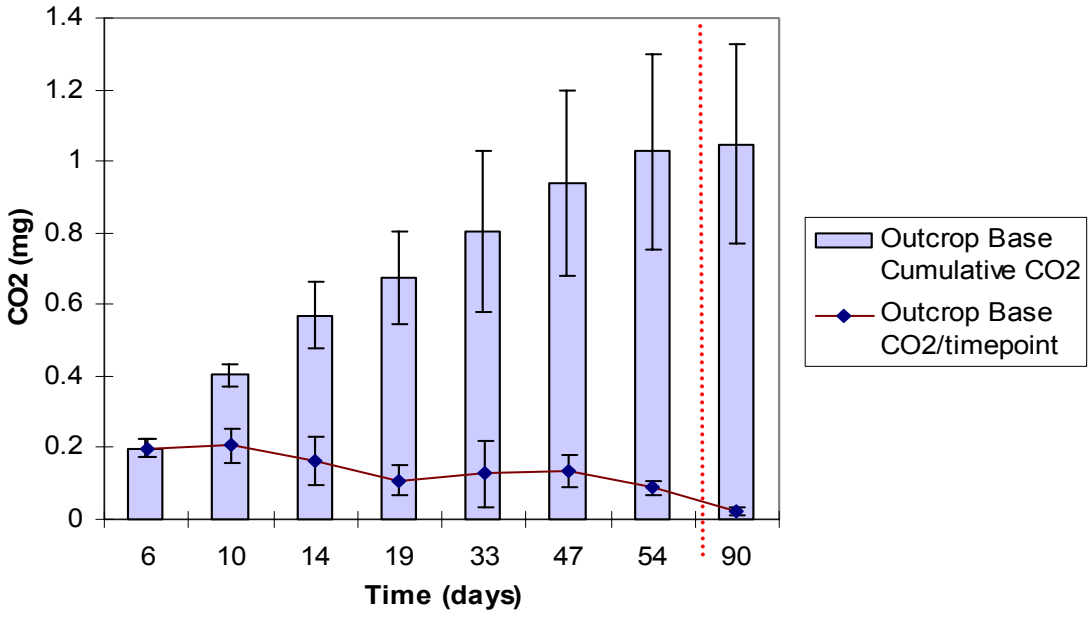

Figure 20. Generation of headspace CO_2 in the Outcrop base live bioassays.

Anaerobic Live Bioassays TOC

The Outcrop pyro and base bioassays showed 50% and 0% loss of TOC respectively. This corresponds to a loss of 129.9 and 0.00 mg/L TOC. The pyro bioassays display a sharp decrease of TOC concentration through day 18 and then mostly level off for the

remainder of the sampling days (Figure 21). No upward or downward trend is detected in the data in the base bioassay time sequence. The controls show no loss of TOC over time therefore indicating that the loss seen in the pyro bioassays is due to biotic activity.

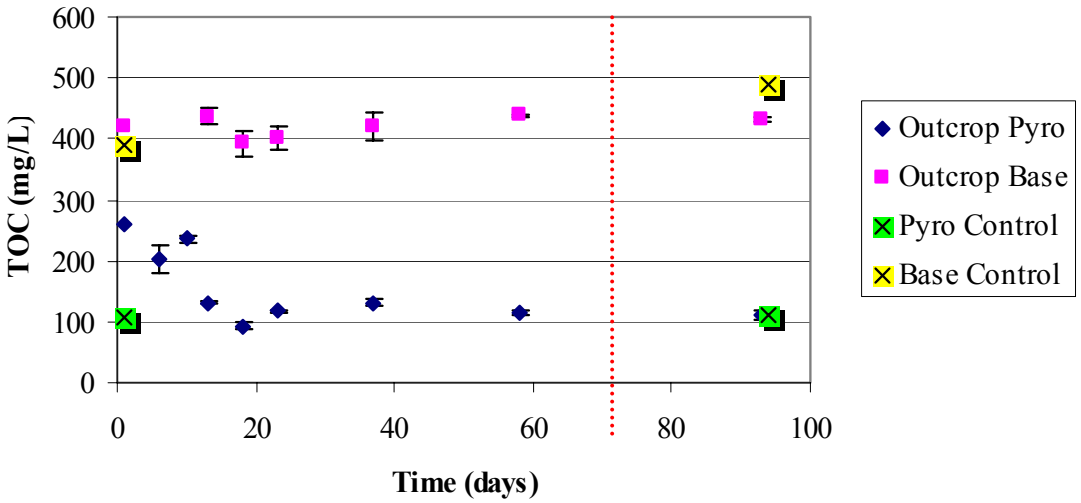

Figure 21. Outcrop live bioassay aqueous TOC concentrations (mg/L) for a period of 93 days. The initial and final TOC concentrations are shown for the control bioassays. The red line indicates the time at which headspace CO_2 was no longer generated.

Conversion of Aqueous TOC to Headspace C-CO_2

Very little of the observed TOC degraded in the pyro bioassays was detected as C-CO_2. Only 0.22 to 0.70% TOC lost was converted to C-CO_2. However, the base bioassays experienced a conversion of 15.0 to 64.3% TOC to C-CO_2.

VFA Analysis

The outcrop pyro control contained acetic acid on day 93 and the base control contained acetic acid on day 9 (Table 7). The pyro control contained levels just at detection limit, while the base control contained relatively high amounts of acetic acid. No VFAs were seen in any of the outcrop live bioassays.

Conclusions from Outcrop Sediment

The outcrop sediment contained much more carbon than did the other sediments, with the base and final pyrophosphate extractions pulling off 38% more TOC than the first three

pyrophosphate extractions. However, the material extracted by the pyrophosphate extractions was much more biodegradable seeing a loss of 50% TOC in the pyro bioassays vs. 0% TOC in the base bioassays. Despite the high carbon concentrations in the base bioassays, no carbon was degraded. The pyro bioassays showed degradation through the first 18 days before reaching a steady concentration for the remainder of the timepoints.

Generation of CO_2 in the pyro bioassays is consistent with the timeframe of the loss of TOC. Sustained CO_2 production through day 22 is observed as carbon is degraded, followed by a sharp drop in CO_2 levels, also consistent with the steady concentration of TOC observed at this time. There is not a great change of CO_2 production in the base bioassays until a decline begins on day 54. However, the control bioassays exhibited higher CO_2 production than did the live bioassays, indicating that CO_2 production in the live bioassays was not a result of biotic activity.

Comparison to Aerobic Live Bioassays

In both the aerobic and anaerobic experiments, the pyro bioassays exhibited higher biodegradability than did the base bioassays Table 8). With the exception of one of the 5/11/02 extractions, the base extractions removed more organic carbon from the sediment. This indicates that the pyrophosphate extractions remove a higher percentage of biodegradable material than did the base and final pyrophosphate extractions. The aerobic bioassays did display higher amounts of TOC utilization both in terms of percent utilized and amount lost as mg/L.

Table 8. ***Comparison of % TOC utilized in aerobic and anaerobic bioassays and the day on which the lowest TOC concentration occurred.***

		Aerobic				Anaerobic			
		Initial TOC	Final TOC	TOC Utilization (mg/L)	% TOC Utilization	Initial TOC	Final TOC	TOC Utilization (mg/L)	% TOC Utilization
King's Bay									
KBA	**Pyro**	70	21	49	70	56	44	11	20
	Base	40	23	17	42	72	67	5	7
5/11/2002	**Pyro**	185	55	130	70	157	112	45	29
	Base	401	144	257	64	67	55	12	18
Outcrop	**Pyro**	618	232	386	62	261	131	130	50
	Base	3187	1844	1343	42	419	422	-3	-1
MLS 10									
8-10 Feet	**Pyro**	9	8	1	12	16	13	2	15
	Base	6	5	1	24	17	13	3	19
20.5-22 Feet	**Pyro**	64	44	21	32	20	13	6	32
	Base	33	15	18	54	32	29	3	10
MLS20									
8-10 Feet	**Pyro**	2	increase	-	-	15	14	1	9
	Base	2	increase	-	-	13	11	2	18
22 Feet	**Pyro**	37	26	10	28	17	15	2	14
	Base	16	9	7	43	19	21	increase	-

Aerobic data provided by (Rectanus 2006).

NAB Little Creek

Samples from two different sampling locations were collected with the assistance of CH2M Hill (Virginia Beach, VA office) NAB Little Creek. Samples were collected from shallow and deep locations in the surficial aquifer which is known to be contaminated with chlorinated ethenes in which reductive dechlorination has been demonstrated as a natural attenuation process. MLS10 was derived from a location upgradient of the chlorinated ethene plume and MLS20 is inside the plume.

MLS10

Initial TOC

The Time 0 TOC concentrations for the MLS10 8-10 feet extract live bioassays were 37 to 54% higher than the extract concentrations, and the 20.5-22 feet live bioassays contained 10 to 26% more TOC than the extracts. However the controls were only 0.04 to 14% different than the extract concentrations. Table 9 lists the differences between the

time 0 TOC concentrations and the extract concentrations for the live bioassays as well as the controls. Given the similarity between the extract TOC concentrations and the controls, the difference in the live bioassays and the extraction TOC concentrations is likely attributed to the carbon present in addition of the soil culture that was added to the live bioassays; however, this is still a larger increase than would have been expected.

Table 9. ***Comparison of MLS10 bioassays time 0 TOC concentrations to extraction concentrations.***

	Total Organic Carbon (mg/L)		
	Extraction	**Time 0**	**% Difference**
8-10 Pyro	10.0	15.8	36.6
8-10 Base	7.6	16.6	54.2
20.5-22 Pyro	14.4	19.6	26.5
20.5-22 Base	28.7	32.0	10.4
8-10 Pyro Control	10.0	9.8	-2.0
8-10 Base Control	7.6	8.1	6.4
20.5 -22 Pyro Control	14.4	16.8	14.1
20.5-22 Base Control	28.7	28.7	0.0

The combination of extracts from the first three days of extractions contained more organic carbon in the shallow soil than the combination of extracts from days 4 and 5 for the shallow soil. However, the combination of extracts from the first three days of extractions contained less carbon than the last two days of extractions for the deep soil (Figure 22Figure 22).

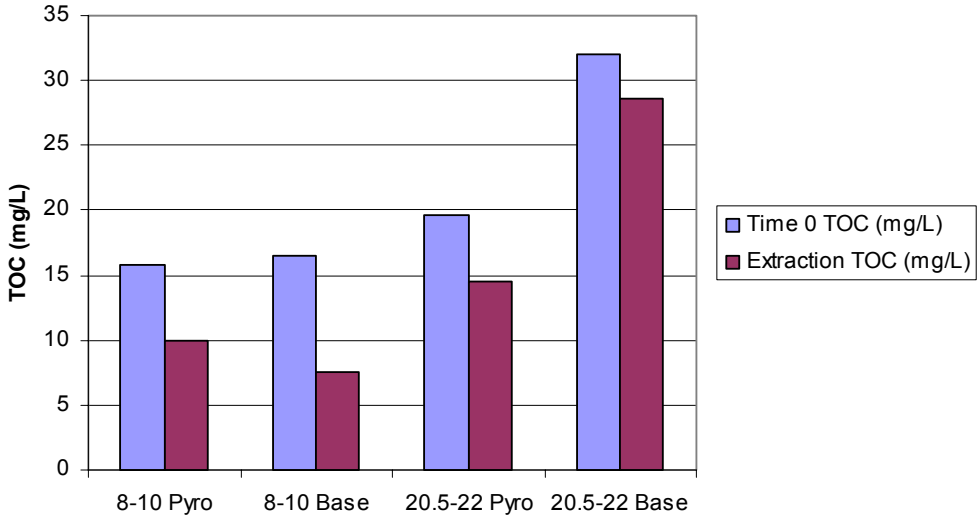

Figure 22. Comparison of time 0 TOC concentrations with extraction TOC concentrations for MLS10 live bioassays. "Pyro" indicates extracts combined from days 1-3, and "base" indicates extracts combined from days 4-5.

MLS10 Bioassay Controls

The MLS10 bioassay controls showed negligible change (Figure 25 and Figure 28). The deep controls did show an increase in TOC during the last sampling but the observed concentrations were within ranges of reasonable sample variation.

MLS10 (8-10 ft)

Headspace CO_2

Steady carbon dioxide production was sustained throughout the experiment in the pyro bioassays ranging from 0.019 mg to 0.043 mg in the headspace (Figure 23)Figure 23. Headspace CO_2 (mg) in MLS10 8-10 sediment pyro bioassays over time (days).. Production of CO_2 increased very little through day 28 before decreasing to slightly lower concentrations. The base bioassays produced 0.06 to 0.09 mg CO_2, never showing a decreasing trend (Figure 24). The pyro controls contained negligible headspace CO_2. The base control maintained CO_2 levels 21.9 to 54.5% that seen in the live bioassays. The unsampled pyro controls contained an average of 0.067 mg headspace $C0_2$, and the unsampled base controls averaged 0.09 mg CO_2 in the headspace. Both of these sets of unsampled controls are the same or higher in carbon dioxide levels than the bioassays. No methane was detected.

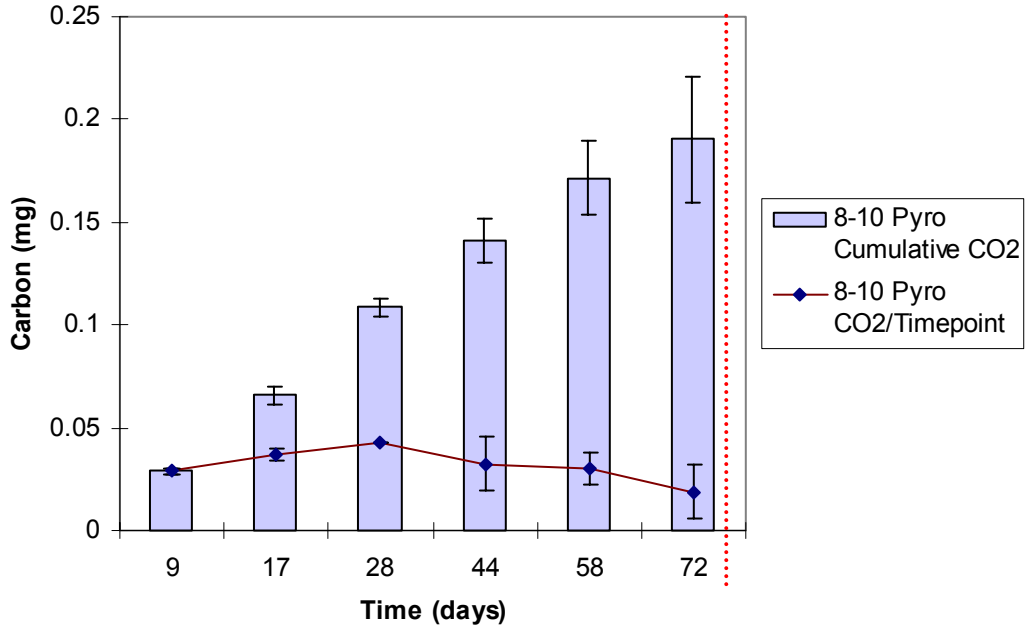

Figure 23. Headspace CO_2 (mg) in MLS10 8-10 sediment pyro bioassays over time (days).

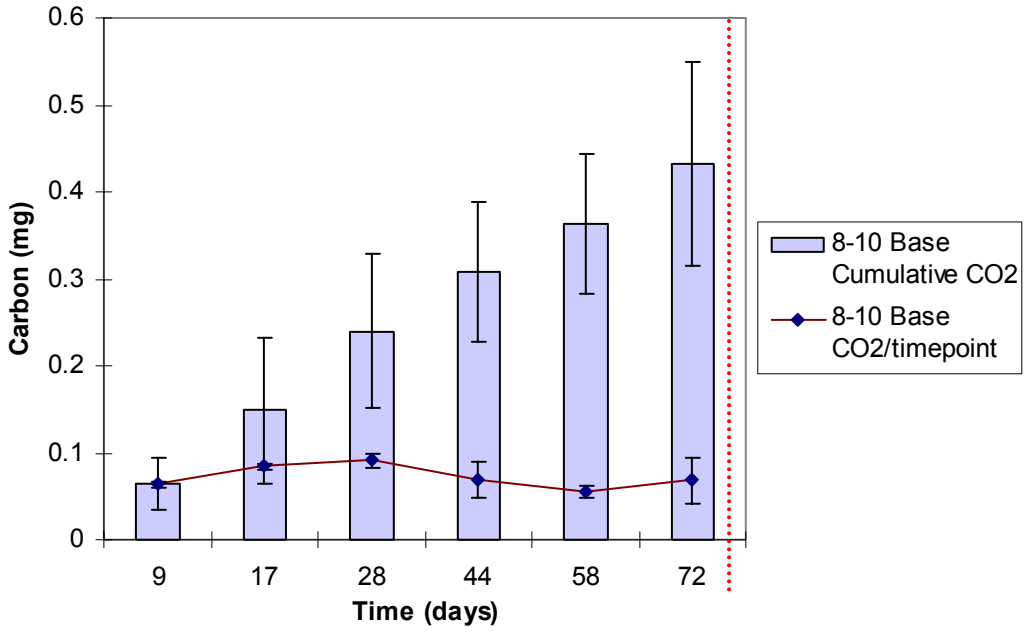

Figure 24. Headspace CO_2 (mg) in MLS10 8-10 sediment base bioassays over time (days).

Anaerobic Live Bioassays TOC

The 8-10 feet pyro and base bioassays showed a 15% and 19% loss of TOC respectively. This corresponds to losses of 2.4 mg/L and 3.2 mg/L TOC. A gradual loss of TOC occurs over time and TOC appears to be degrading slowly at the last time point. This is supported by the sustained CO_2 production observed in the headspace. No loss of TOC was observed in the control bioassays.

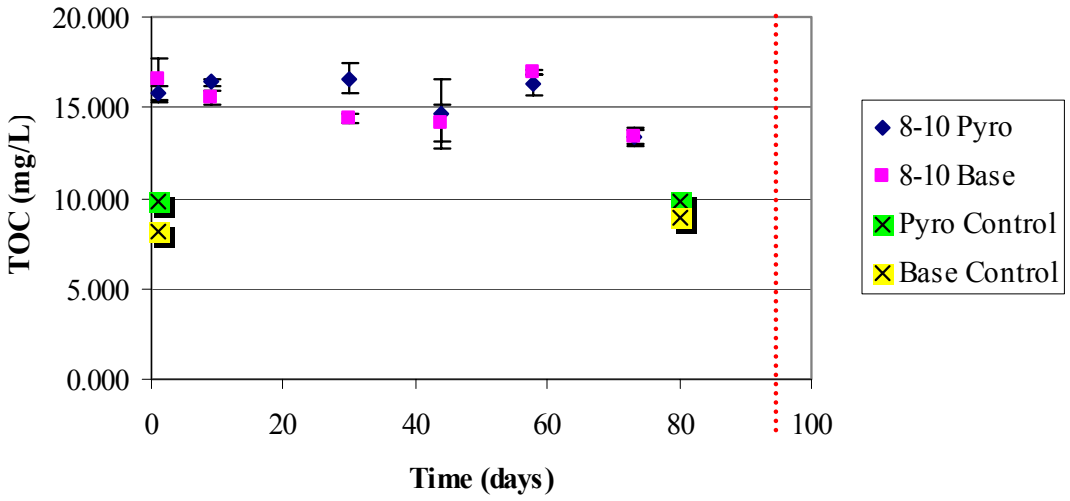

Figure 25. MLS 10 TOC concentrations over time monitored for a period of 73 days. The initial and final TOC concentrations are shown for the control bioassays. The red line indicates when headspace CO_2 was no longer being generated.

Conversion of Aqueous TOC to Headspace C-CO_2

The pyro bioassays experienced 13 to 62% conversion of aqueous TOC to headspace C-CO_2, with peak conversion occurring on day 44. Every other sample contained more C-CO_2 than loss of TOC. The base bioassays had 26 to 74% conversion of aqueous TOC to C-CO_2. More C-CO_2 was generated during days 21-49 than loss of TOC was observed.

VFA Analysis

More VFAs were produced in the 8-10 sediments than in any of the other bioassays. A list of VFAs observed can be seen in Table 10. Acetic acid was observed on day 8 in one of the pyro bioassays. Two of the pyro bioassays produced acetic acid on day 45, one of which also contained isobutyric acid. Two of the three shallow pyro bioassays contained

acetic acid on day 58, and the other one contained butyric, isocaproic, and isovaleric acid. However, it seems likely that part of the VFAs detected were carry over in the machine from previously run samples. The pyro control contained lower levels of isocaproic acid on day 58 than was observed in the live bioassay. A shallow base bioassay contained isobutyric acid on day 45, and two shallow base bioassays contained low levels of isocaproic acid on day 58. The shallow base control contained near detection limit levels of isovaleric acid.

Table 10. ***Summary of VFAs (mg/L) detected in the MLS 10 bioassays***

	8-10 Pyro	8-10 Base	8-10 Pyro Control	8-10 Base Control	20.5-22 Base	20.5-22 Pyro Control
Acetic Acid	17				44	21
Proprionic Acid						
Isobutyric Acid	1	1				
Butyric Acid	1					
Isovaleric Acid	1			0.2		
Caproic Acid						
Isocaproic Acid	1	1	1			
Hexanoic Acid						
Heptanoic Acid						
Total as equivalents of Acetic Acid (mg/L)	22	2	1	0.2	44	21

Conclusions from MLS10 (8-10 ft) Sediment

Although the pyrophosphate extractions pulled more carbon off the shallow sediment, the base and pyrophosphate extractions appear to have pulled off carbon that is slightly more biodegradable.

The shallow MLS10 sediment bioassays contained VFA's at points throughout the experiment, primarily in the form of acetic acid. VFA concentrations on day 8 were much higher than those seen on subsequent days. The presence of VFAs in these bioassays signifies that complex organics were broken down, but it may be that reducing conditions were such that the VFAs produced from this break down were not further oxidized. Since VFAs factor into the TOC concentration, their existence is consistent

with the fact that little loss of TOC was observed. The presence of VFAs in this set of bioassays may correspond with the behavior exhibited in positive controls 2 and 3.

MLS10 (20.5-22 ft)

Headspace CO_2

Both base and pyro bioassays maintained steady CO_2 production through day 28, after which levels decreased during all subsequent headspace sampling (Figure 26 and Figure 27). Through day 28, the pyro bioassays generated 0.040 to 0.052 mg C-CO_2 in the headspace, while the base bioassays produced 0.093 to 0.123 mg C-CO_2 in the headspace. Generation of CO_2 began to decrease after day 17. This corresponds to the decrease in the loss of TOC observed at this time. Almost no C-CO_2 was detected in the pyro control throughout the experiment, while the base controls produced 43% the amount observed in the live bioassays during the first sampling, and increasing from there. By day 58 the control was producing more CO_2 than the live bioassay. No methane was detected.

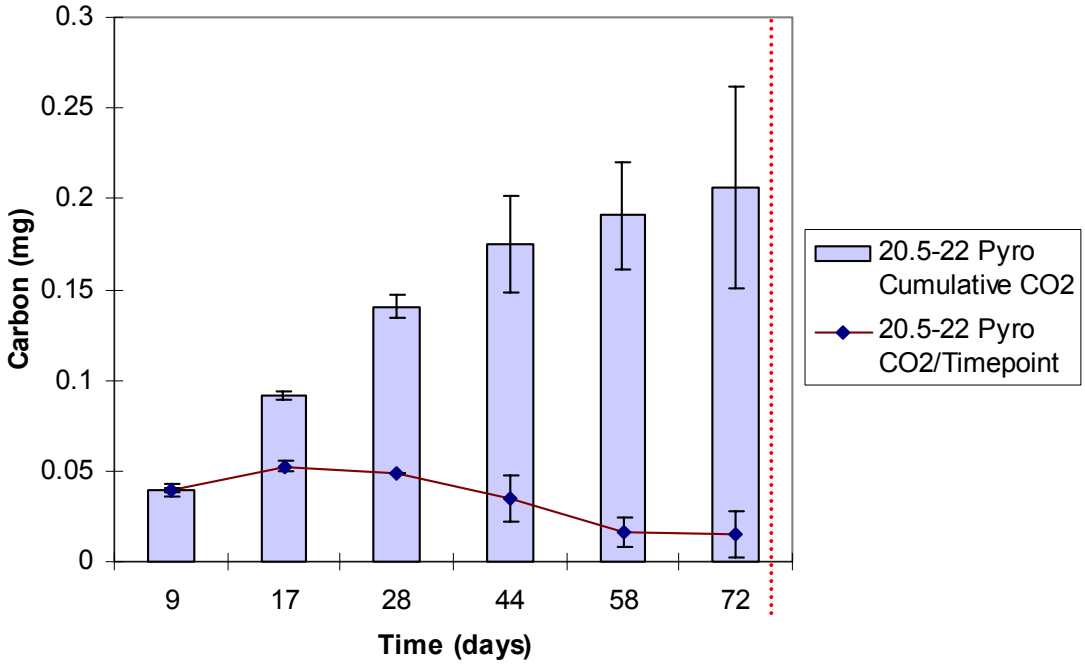

Figure 26. Headspace CO_2 (mg) in MLS10 20.5-22 sediment pyro bioassays over time (days).

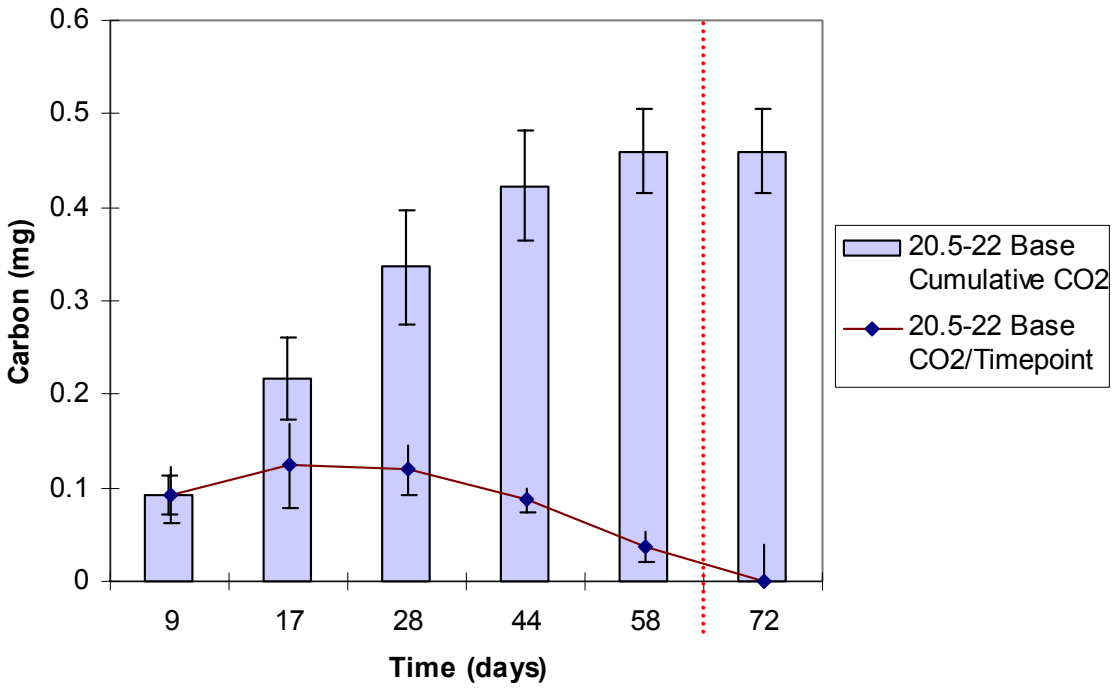

Figure 27. Headspace CO_2 generated in the MLS10 20.5-22 base bioassays over time (days).

Anaerobic Live Bioassays TOC

The 20.5-22 feet pyro and base bioassays showed 32% and 10% loss of TOC, respectively. This corresponds to a loss of 6.2 mg/L and 3.2 mg/L TOC. Both sets decrease initially through day 17 and then level out for the remainder of the experiment showing with a slight increase like what was seen in the positive controls (Figure 28). The base bioassays experience a decrease during in the last timepoint, but without further timepoint data, it is difficult to distinguish if this is further degradation or variation in the sample.

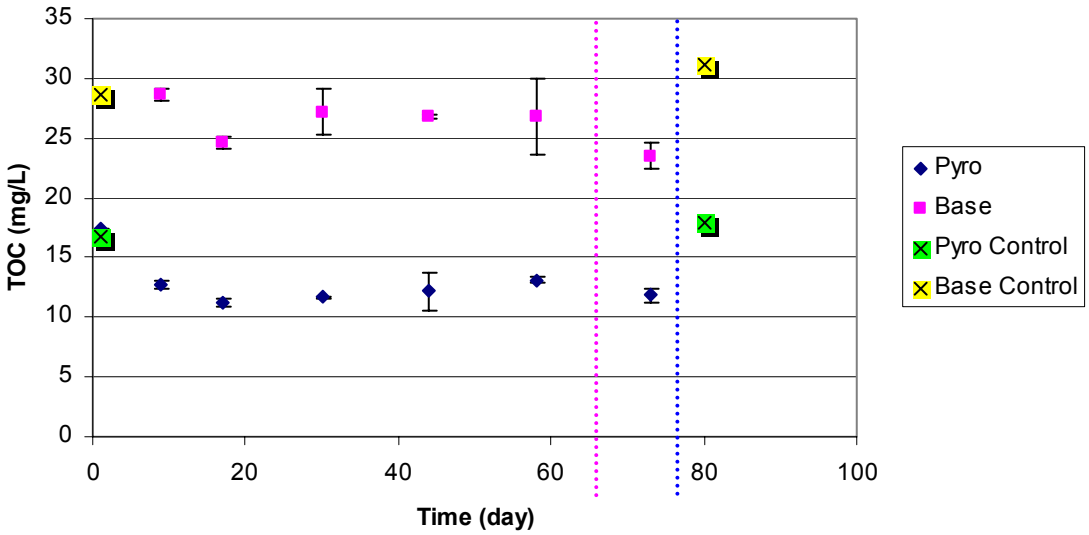

Figure 28. MLS10 20.5-22 sediment live bioassays aqueous TOC concentrations (mg/L) monitored for a duration of 73 days. The initial and final TOC concentrations are shown for the control bioassays.

Conversion of Aqueous TOC to Headspace C-CO_2

The deep pyro bioassays showed a conversion of 6.0% and 43.0% aqueous TOC to C-CO_2 on the 9th and 17th days of the experiment. However, for the rest of the time, more C-CO_2 was produced than loss of TOC observed. The base bioassays experienced 32 to 37% conversion during the middle of the experiment; however the last sample revealed a 96% conversion.

VFA Analysis

Two of the deep base live bioassays contained high levels of acetic acid on day 8, and the other deep base bioassay contained a little acetic acid on day 58. The deep pyro control contained high levels of acetic acid on day 8 and much lower levels on day 45. These concentrations are listed in Table 10.

Conclusions from MLS10 (20.5-22 ft) Sediment

The opposite trend was seen in the extractions of the deeper sediment than was seen in the shallow sediment. The pyrophosphate extractions pulled off less carbon, but quite a

bit more biodegradation was observed in the pyro bioassays. Again, the control TOC concentrations resembled that seen in the extracts, but the live bioassays initial concentrations were considerably higher. This can be attributed to the addition of a soil culture.

The deep sediment shows clear trends of CO_2 production and aqueous TOC degradation. Degradation appears to occur through day 17, with CO_2 production beginning to decrease after day 28. The C-CO_2 concentrations decrease for the remainder of the time while no further TOC is degraded. Of all the MLS10 sediments, the deep pyrophosphate bioassays experienced the greatest percent degradation, while the deep base bioassays experienced the least % degradation.

No VFAs were detected in the deep live bioassays until day 58, and even then, the concentration was very low. Given that a significant loss of TOC was observed, this is consistent with the theory discussed for the 8-10 sediment where more VFAs were observed but little loss of TOC was seen.

Comparison to MLS10 Sediment Aerobic Live Bioassays

The deeper sediment bioassays experienced greater TOC utilization in both the aerobic and anaerobic bioassays, and the shallow sediment exhibited very similar degradability in both experiments. Although degradability greater in the deeper sediment, the aerobic bioassays experienced greater TOC loss than did the anaerobic bioassays.

MLS20

Initial TOC

Large differences exist between the MLS20 extractions and the Time 0 bioassay data (Table 11). The extraction TOC values were 51-86% lower than the shallow live bioassay time 0 TOC concentrations, while the deep extractions were 18-55% lower in TOC than the initial live bioassay concentrations. With the exception of the shallow pyro control, all control bioassays were higher in TOC concentration than the extracts. It is

unclear why such large differences exist in TOC concentrations between the bioassays and the extracts.

Table 11. ***Comparison of time 0 and extraction TOC concentrations (mg) for MLS20.***

	Total Organic Carbon (mg/L)		
	Extractions	**Time 0**	**% Difference**
8-10 Pyro	6.20	12.79	51.50
8-10 Base	2.15	15.30	85.98
8-10 Control	6.20	5.19	-19.51
8-10 Control	2.15	6.76	68.24
22 Pyro	13.28	16.25	18.25
22 Base	8.43	18.79	55.12
22 Control	13.28	15.44	13.98
22 Control	8.43	16.22	48.01

Figure 29 depicts that the combination of extracts from the first three days of extractions contained more organic carbon for both shallow and deep soil than the combination of extracts from days 4 and 5.

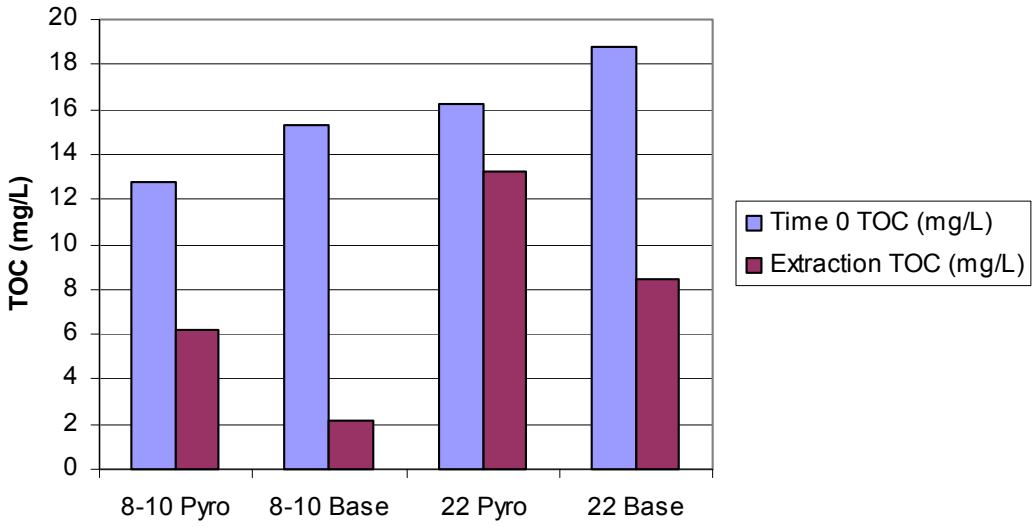

Figure 29. Comparison of time 0 TOC concentrations (mg/L) and extraction TOC concentrations (mg/L) for MLS20 live bioassays. "Pyro" indicates extracts combined from days 1-3, and "base" indicates extracts combined from days 4-5.

MLS20 Bioassay Controls

Bioassay controls showed negligible TOC decrease (Figure 32 and Figure 35). The controls confirmed that degradation seen in the live bioassays was a result of an active biodegrading culture.

MLS20 (8-10 ft)

Headspace CO_2

Carbon dioxide production in the shallow pyro bioassays peaked at 0.047mg CO_2 on day 9 but after this point, there was fluctuation between 0.01 to 0.03 mg CO_2 in the headspace (Figure 30Figure 30). The shallow base bioassays generated a large quantity of CO_2 on day 9 and then decreased exponentially through the rest of the sampling points (Figure 31). While the pyro bioassays generated CO_2 for the entirety of the experiment, the base bioassays produced negligible amounts of CO_2 after day 47. The controls produced comparable CO_2 levels with the live bioassays thru day 51, and then dropped off to almost no CO_2 production the remainder of the time. The unsampled controls contained 0.01 mg headspace CO_2 during the final sampling. No methane was detected.

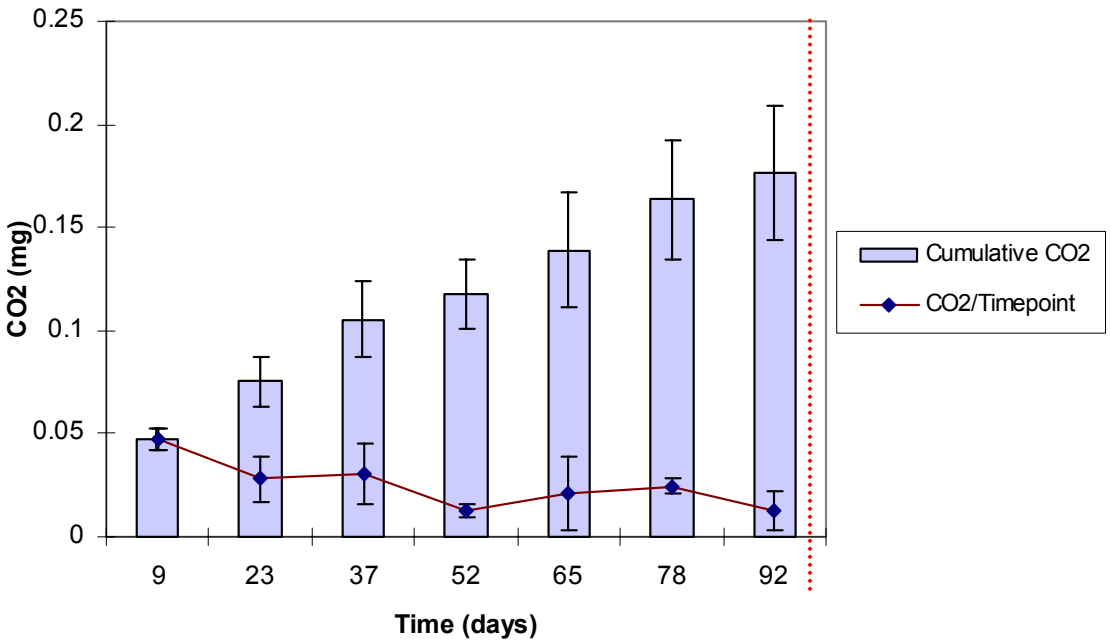

Figure 30. Headspace CO_2 (mg) over time in the MLS20 8-10 pyro bioassays.

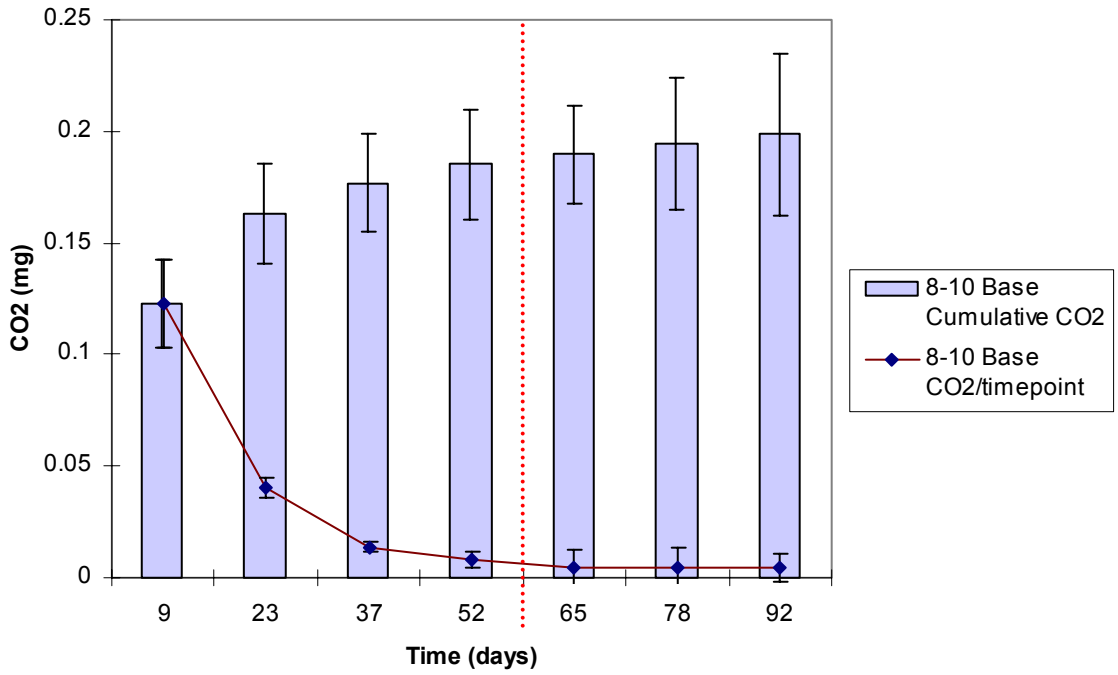

Figure 31. Headspace CO_2 generated in the MLS20 8-10 base bioassays.

Anaerobic Live Bioassays TOC

The 8-10 feet pyro and base bioassays showed a decrease of 9% and 18% TOC respectively. This corresponds to a loss of 1.4 mg/L and 2.4 mg/L TOC. Figure 32 illustrates that the TOC concentrations in the base bioassays show no strong trend of decreasing TOC, and it is difficult to conclude if biodegradation has occurred. Although fluctuation occurs in the data, the pyro bioassays show more conclusive evidence that some TOC has been utilized in the bioassays. The controls do not show a loss of TOC thus supporting that the loss of TOC is due to biotic activity.

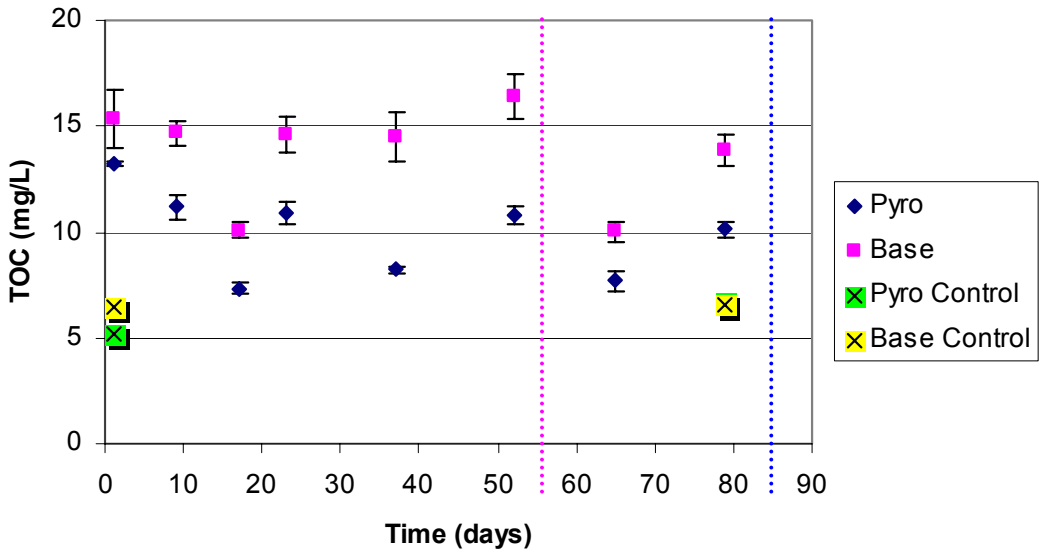

Figure 32. MLS20 8-10 sediment live bioassays aqueous TOC concentrations (mg/L) monitored for a period of 79 days. The initial and final TOC concentrations are shown for the control bioassays. The blue line indicates were CO_2 production ceased in the pyro bioassays, and the pink line indicates this time for the base bioassays.

Conversion of Aqueous TOC to Headspace C-CO_2

The shallow pyro bioassays converted 21 to 40% TOC to C-CO_2, with the last sample showing 25% greater CO_2 production than there was loss of TOC. The base bioassays converted 31 to 65% TOC to C-CO_2. However, days 9 and 23 showed a 159% and 182% conversion to C-CO2. This corresponds with much less TOC loss than was observed on other days, but high CO_2 generation. Most of the bioassays appear to have a burst of CO_2 production at the beginning regardless of whether much TOC is removed from the system or not. This is likely due to incomplete flushing of the headspace after construction of the bioassays. Some CO_2 is most likely being generated, but with little observed TOC degradation, residual CO_2 probably remains.

VFA Analysis

No VFAs were detected in the shallow sediment bioassays throughout the experiment.

Conclusions from MLS20 (8-10 ft) Sediment

The large differences in the time 0 TOC concentrations and the extract concentrations indicate either a loss of TOC in the extract sample or a sampling or analysis error. The pyrophosphate extractions pulled more organic carbon off the sediment than did the final base and pyrophosphate extractions (Table 11). Not only did they extract greater quantities of carbon, but the pyrophosphate extractions also extracted more biodegradable carbon.

The shallow pyro bioassays appear to decrease in TOC over time, losing 18% TOC. Although there is fluctuation in the data, the fluctuations occur after the initial decrease at concentrations lower than the time 0 concentrations, indicating that there was some degradation. This is supported by evidence of higher CO_2 production during the first part of the experiment before decreasing to slightly lower values.

It is unclear whether any degradation occurred in the base bioassays. The difference between the initial concentration and the TOC concentration at the time that CO_2 was no longer detected indicates a loss of 9%; however, given the fluctuation occurring throughout the extent of the experiment, it cannot be concluded that TOC was broken down by the microorganisms. There is an initial burst of CO_2 production in the first sampling, immediately followed by a sharp decline to negligible amounts of CO_2 in the headspace. However, the levels of CO_2 observed in the base live bioassays closely match the amounts observed in the killed controls, indicating that the CO_2 observed may be equivalent to background concentrations. This would support that negligible degradation occurred in these bioassays.

Comparison to MLS20 (8-10 ft) Aerobic Live Bioassays

Unlike the aerobic bioassays, the anaerobic bioassays did display biodegradation in the shallow sediment. The deep aerobic bioassays displayed much greater biodegradation than did the anaerobic bioassays. These results are summarized in Table 8.

MLS20 (22 ft)

Headspace CO_2

The deep pyro bioassays increased in CO_2 production through day 65 reaching a high of 0.046 mg before decreasing again to 0.017 mg CO_2 on day 93 (Figure 33). Generation of CO_2 was observed in the deep pyro bioassays throughout the experiment. Like the shallow base bioassays, the deep base bioassays decreased exponentially from the first sample through the end of the experiment producing 0.215 mg during the first sample and decreasing to .008mg on the last day (Figure 34). Negligible levels of CO_2 were seen after day 52. The deep pyro controls produced levels of CO_2 that fluctuated around levels similar to those produced in the live bioassays through day 52 before decreasing to negligible amounts. The deep base controls generated similar amounts of CO_2 as the live bioassays throughout the experiment. No CO_2 was found in the unsampled deep pyro control, while the deep base control contained only 0.0016 mg CO_2. No methane was detected.

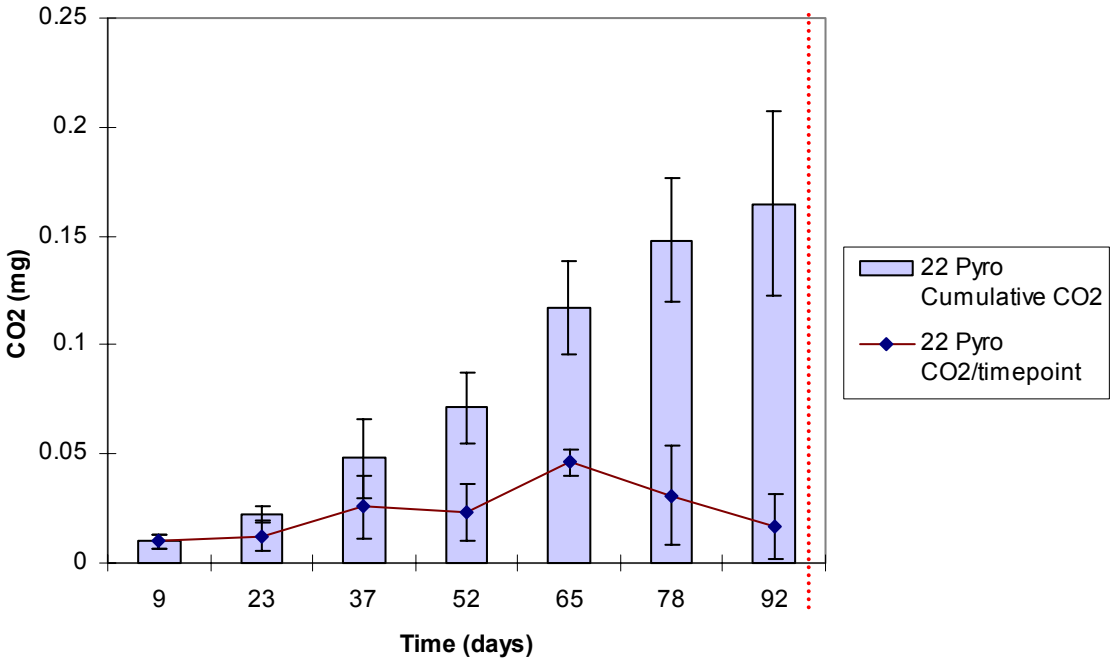

Figure 33. Generation of CO_2 in the headspace of the MLS20 22 pyro bioassays. The red line indicates the point at which CO_2 production was no longer detected.

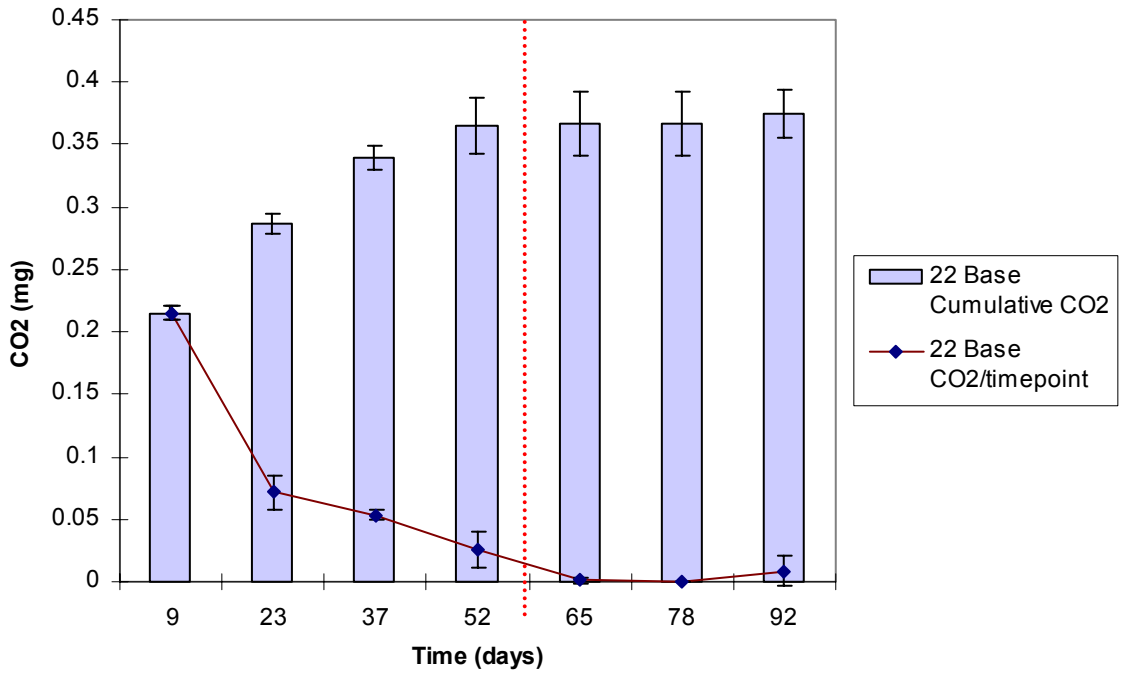

Figure 34. Generation of CO_2 in the headspace of MLS20 22 base bioassays. The red line indicates the point at which CO_2 was no longer being generated.

Anaerobic Live Bioassays TOC

The 22 feet pyro and base bioassays showed 0% and 14% loss of TOC respectively. This corresponds to a loss of 0 mg/L and 2.3 mg/L TOC, with the lowest TOC concentration for pyro bioassays occurring on day 23 for both sets of bioassays. The pyro bioassays show a downward trend in TOC concentration through day 23 before increasing again to values close to the initial concentration (Figure 35). The base bioassay on the other hand does not appear to degrade. No trend of loss was observed and with the exception of the low point on day 23, the concentrations all hovered around 20 mg/L TOC. Neither set of bioassays appear to be decreasing at the end of the experiment.

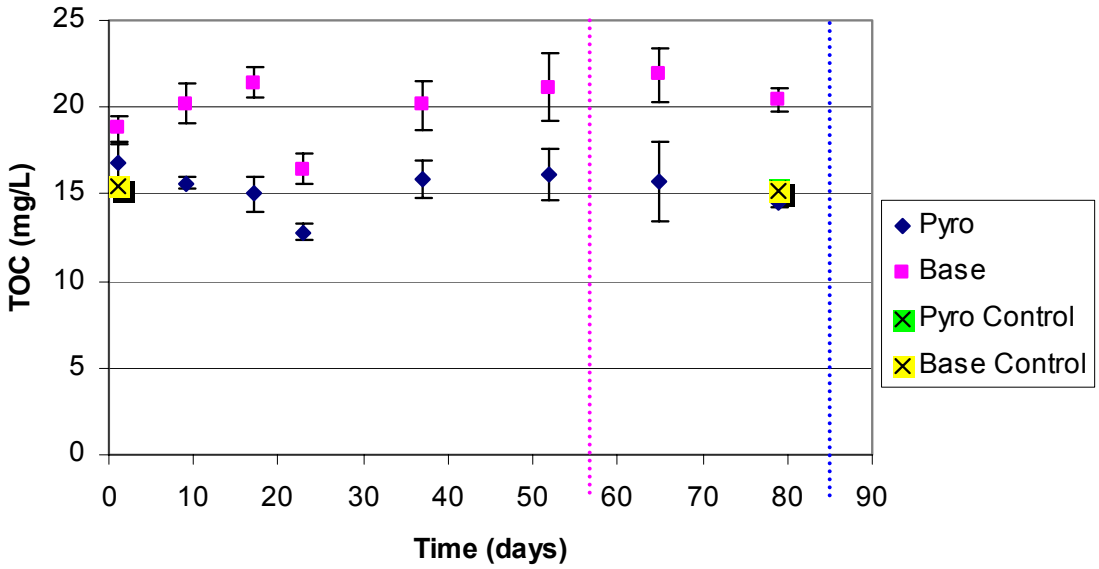

Figure 35. MLS20 22 feet sediment live bioassays aqueous TOC concentrations (mg/L) monitored for a period of 79 days. The initial and final TOC concentrations are shown for the control bioassays. The pyro and base controls are almost identical and therefore it is difficult to see the pyro control points. The blue line indicates the point at which CO_2 production ceased in the pyro bioassays, and the pink line indicates this point for the base bioassays.

Conversion of Aqueous TOC to Headspace C-CO_2

In comparing C-CO_2 production and loss of TOC, the shallow pyro bioassays converted 5 to 89% TOC to C-CO_2. The base bioassays showed a conversion of only 1% TOC to C-CO_2 on day 23. Otherwise, there was more C-CO_2 generation than there was loss of TOC. Day 23 was the only day in the base bioassays that showed any decrease in TOC from the initial concentration.

VFA Analysis

Very low levels of isocaproic acid were detected on day 8 in 2 of the deep pyro bioassays. One of the base bioassays contained very low levels isobutyric acid on day 78. These concentrations are listed in Table 12.

Table 12. ***Summary of VFAs (mg/L) found in MLS 20 bioassays.***

	22 Pyro	22 Base
Acetic Acid		
Proprionic Acid		
Isobutyric Acid		1
Butyric Acid		
Isovaleric Acid		
Caproic Acid		
Isocaproic Acid	2	
Hexanoic Acid		
Heptanoic Acid		
Total as equivalents of Acetic Acid (mg/L)	3	1

Conclusions from 22 Feet Sediment

Like the extractions from the shallow sediment, the pyrophosphate extractions pulled less carbon off of the sediment; however the pyrophosphate extract exhibited greater biodegradation of TOC than did the extract from the combined base and pyrophosphate extractions. The pyro bioassays did experience degradation during the first 23 days, after which TOC concentrations increased again. A CO_2 spike on day 65 occurred but does not correspond to loss of TOC in the bioassays. Otherwise, CO_2 levels remained at approximately 0.01 to 0.02 mg C-CO_2 in the headspace, with no strong ties to aqueous TOC loss.

It does not appear that biodegradation occurred in the base bioassays. The CO_2 in the base controls closely matched that of the live bioassays, suggesting that the CO_2 observed in the base live bioassays is background CO_2, not a result of conversion from TOC. This supports that no carbon was broken down in these bioassays.

VFAs were detected on day 8 in two of the pyro bioassays, and none were seen again in any of the bioassays until isobutyric acid was detected in one of the base bioassays on day 79.

Comparison to MLS20 (22 ft) Aerobic Live Bioassays

The shallow aerobic bioassays experienced no TOC utilization whereas the anaerobic bioassays did experience minimal TOC utilization. However, the anaerobic bioassays contained much more TOC at the start of the experiment than did the aerobic bioassays. The deep aerobic bioassays exhibited greater loss of TOC than did the anaerobic bioassays. Whereas the aerobic base bioassay experienced a loss of 43% TOC, the anaerobic bioassays actually showed an increase in TOC. Looking at those sets of bioassays that did experience a loss of TOC, the base bioassays exhibited greater biodegradability except in the case of the deep anaerobic set.

Conclusions

This research was conducted with three identified objectives:

- determine the extent to which extracted natural organic carbon (NOC) is degraded under anaerobic conditions
- establish whether VFAs are produced from the carbon degradation, therefore providing a link between carbon and hydrogen generation
- determine the extent of hydrogen production as the extracted carbon is degraded

In order to evaluate the anaerobic biodegradability of NOC, extractions were performed on sediments from 3 different sites. The centrate from these extractions was then used to construct bioassays that were monitored for approximately 12 weeks. During these 12 weeks, aqueous TOC concentrations and headspace CO_2 were quantified for evidence of biodegradation.

The samples collected from NSB Kings Bay are characterized by low, medium, and high organic carbon content (KBA, 5/11/02, and Outcrop, respectively), which allowed an analysis of the differences in biodegradation and extraction based on the concentration of the carbon content of the sediment. In both aerobic and anaerobic bioassays, the pyrophosphate extractions removed more biodegradable carbon than the final base and

pyrophosphate extractions. Although this same trend was seen in the aerobic and anaerobic bioassays, large differences existed between the extent of biodegradation in the aerobic and anaerobic bioassays, except in the case of the Outcrop pyro bioassays. Whereas the aerobic bioassays biodegraded approximately 60 to 70% TOC in the pyro bioassays and approximately 40% in the base bioassays (except for the 5/11/02 base), the anaerobic bioassays displayed larger differences in biodegradability between the sediments. In the anaerobic bioassays, the higher carbon content correlated to more biodegradable material. Although the Outcrop base extractions removed more carbon from the sediment than any of the other extractions, none of this material appears to be bioadegradable.

The Outcrop sediment contained higher quantities of condensed humic material than did the other sediments. The milder pyrophosphate extractions are less likely to break this material apart and therefore are unable to access the mineral surfaces for extraction. The harsher base extraction can better disperse the condensed substances and access more surface area from which to solubilize carbon. Although the Outcrop base extraction removed much more carbon from the sediment, the bioassays showed no trends of degradation, possibly indicating that the microorganisms are not as effective at breaking down the humic material in anaerobic conditions.

The sediment samples from NAB Little Creek, MLS10 and MLS20, allowed an examination of differences seen in sediment inside a chlorinated solvent plume and outside of the plume. MLS10 is a relatively low carbon sediment outside of the plume, while MLS20 is also a low carbon sediment but is found inside the chlorinated solvent plume. The base extractions removed more biodegradable material in the shallow sediments, while the pyro extractions removed more biodegradable material in the deep extractions. Greater biodegradation occurred in sediment taken outside the chlorinated solvent plume than occurred inside the plume. This was the same trend observed in the aerobic bioassays.

Although a clear link was not formed between trends observed in the aerobic and anaerobic live bioassays, the NOC in the sediment that was extracted using the procedure developed by Rectanus et. al (2005) was shown to be anaerobically biodegradable. Therefore, the NOC extracted is significant in anaerobic aquifers for driving reducing conditions to create a suitable environment for active reductive dechlorinators.

The second objective was to determine if VFAs were produced in the anaerobic bioassays. Although few VFAs were observed, their presence confirmed anaerobic conditions within the bioassays. They appeared as early as day 8 in both MLS10 and MLS20 and day 15 in Kings Bay. Even if the system was not immediately anaerobic, the presence of VFAs shows that within a short period of time, the bioassays did become anaerobic systems. The VFAs indicate that as more complex organics are broken down, they are converted into VFAs that are subsequently fermented yielding hydrogen, the primary electron donor driving reductive dechlorination. Most of the VFAs observed were just above detection limit, showing that there were likely more present than recorded, but analysis was not able to identify them.

The low TOC loss observed in MLS10 coupled with the high VFA production indicates that complex organics were being degraded, but the environment was not conducive for VFA fermentation. It is likely that not enough carbon was available to drive the system to further reduced conditions that allow this fermentation to occur.

The last objective involved checking for the presence of hydrogen, thus providing further evidence that the NOC quantified using the extraction procedure will drive the system to a reduced state sufficient for VFA fermentation. These bioassays were monitored only for hydrogen concentrations so the trends of hydrogen production were not tied directly to VFA concentrations, CO_2 generation or to loss of TOC. The deep bioassays produced more hydrogen than did the shallow bioassays, and the base bioassays produced much greater quantities of hydrogen than did the pyro bioassays. The bioassays constructed showed that hydrogen production was generated as NOC was broken down. While the pyro bioassays did not generate hydrogen throughout the experiment, the base bioassays

contained high levels even in the last sample taken 101 days after the bioassays were constructed. Thus hydrogen was generated in the bioassays constructed using extracts from aquifer sediment. This hydrogen could be used by dechlorinating organisms as an electron donor in the process of reductive dechlorination leading to the remediation of chloroethene contaminated sites.

Reductive dechlorination requires appropriate redox conditions and an active community of dechlorinating microorganisms. This research suggests that even in an anaerobic environment, the NOC present in aquifer sediment is anaerobically biodegradable and will drive the system to further reduced conditions. Results from bioassay data do not closely suggest any strong trends connecting aerobic and anaerobic biodegradability. However, the NOC in the extracts was shown to be biodegraded anaerobically with conversion to VFAs and further to CO_2.

pH Experiment

Introduction

In developing a method for extracting organic carbon from aquifer and stream-bed sediment, the effects of pH may be important in determining the effectiveness of the extractant. The nature of soil matter changes under different pH's and the response to extracting solutions may differ under these varying conditions. Higher pH's increase soil organic matter solubility de-protonating the functional groups on humic substances in the soil. The loss of protons decreases the attractive forces within the organics, resulting in a more loosely associated structure. This allows for physical expansion and higher solubilization of organics. The deprotonated functional groups now possess a negative charge and cations introduced into the system would satisfy this charge, thus causing even greater repulsion within the structure. This expansion allows the extracting solution better access to the surface of the organics, solubilizing more of the organic matter (McBride 1994; Stevenson 1994).

The extraction method that is being developed aims to find the most efficient process for quantifying potentially biodegradable carbon. Extractants vary in pH and this experiment seeks to examine the effects of pH on the ability of the extractant to remove biodegradable organic carbon from the sediment.

Study Site

Aquifer Sediment

The NSB Kings Bay sediment previously described was used for conducting the experiment examining the effects of pH on the extraction process. The low, medium, and high carbon containing aquifer sediments were used: KBA, 5/11/02, and Outcrop (described in Site Characterization).

Materials and Methods

Extraction

Triplicate extractions were performed for each of the sediments adjusted to two different pHs. Fifteen grams of sediment were combined with 30 mL of nanopure water in a 50 mL centrifuge tube. After vigorously shaking, the pH of the solution was measured. The nanopure water addition resulted in a pH of approximately 5. Another set of solutions were adjusted to pH 11 using sodium hydroxide (NaOH). These were placed on a rotary tumbler for 24 hours, removed and centrifuged for 25 minutes at 2000 rpm. The solution was decanted and the pH was recorded. A 2 mL sample was taken and acidified with 100 uL H_3PO_4 to be later analyzed for TOC concentration.

Bioassay Set-up

Bioassays were constructed in sterilized carbon free (acid washed, baked at 350°C, autoclaved) 40 mL glass vials. Triplicate bioassays were set up with both pH 5 and pH

11 extracts for each of the three sediments. The live bioassays combined 2mL MSM, 2mL soil culture, and 20 mL extract. After combining the pH was recorded and neutralized using H_3PO_4 and NaOH. The headspace was flushed with sterilized nitrogen, and the microcosms were placed on an orbital shaker table in a 20°C constant temperature room. Triplicate controls were constructed with both pH 5 and pH 11 extracts for each of the three sediments. This involved combining 20 mL extract and 4 mL MSM. Again, the pH was brought to near neutral, the controls were autoclaved, and 92 uL NaN_3 was added to the microcosms to minimize the possibility of microbial growth. A 2mL sample was taken for TOC concentration analysis, the headspace flushed with sterilized nitrogen for 10 minutes, and the bioassays were placed on the shaker table with the live bioassays. The matrix used to construct the bioassays is listed in Table 13.

Table 13. ***Kings Bay pH Experiment Microcosm Matrix.***

	Centrate	# of Duplicates	Extract (mL)	MSM (mL)	Culture (mL)	NaN3 (mL)
KBA	pH 5	3	20	2	2	0
	pH 11	3	20	2	2	0
5/11/2002	pH 5	3	20	2	2	0
	pH 11	3	20	2	2	0
Outcrop	pH 5	3	20	2	2	0
	pH 11	3	20	2	2	0
KBA Control	pH 5	3	20	4	0	0.92
	pH 11	3	20	4	0	0.92
5/11/02 Control	pH 5	3	20	4	0	0.92
	pH 11	3	20	4	0	0.92
Outcrop Control	pH 5	3	20	4	0	0.92
	pH 11	3	20	4	0	0.92

Continuous Sampling

Bioassays were removed from the shaker table in order to sample for TOC concentrations and headspace CO_2 concentrations. Sampling took place every 5-7 days for the first 2 weeks and then every 8-10 days for the next two weeks. After this, two more samples were taken spaced approximately 3 weeks apart. Except that sampling was not conducted in a glovebox, the procedure described in materials and methods was used for CO_2 and TOC analysis.

Results and Discussion

Initial TOC

The extractions at both pH's removed increasing amounts of carbon as the indigenous carbon concentrations increased in the sediment. The pH 11 extraction removed more carbon from the sediment than did the pH 5 extraction in all three sediments. Table 14 lists the extraction TOC concentrations and the percent difference of carbon removed between the pH 5 and pH 11 extractions. While 45.9% more carbon was removed by the pH 11 extraction in KBA, 63.6% more carbon was removed by the pH 11 extraction in the 5/11/02 and Outcrop sediments.

Table 14. ***TOC concentrations of extractions performed on Kings Bay sediment using nanopure water adjusted to pH 5 and pH 11.***

	pH5	pH11	% Difference
KBA	3.7	6.8	45.9
5/11/02	11.0	30.3	63.6
Outcrop	26.1	71.8	63.6

There was little difference in the time 0 TOC concentrations and the extract TOC concentrations, except in the case of the pH 11 outcrop. This set of bioassays showed a decrease of 37.6 mg/L TOC in the time 0 samples. However, the rest of the timepoint data was consistent with the time 0 concentrations. It seems unlikely that there was that much decrease in the extract TOC concentrations. There could have been error in the sampling or analysis of the extract sample.

Kings Bay pH Controls

Only ½ of the controls maintained stable TOC concentrations showing no loss of TOC over time. This indicates that the loss of TOC in these live bioassays was due to the biotic activity of the culture that was added. The KBA and 5/11/02 pH 11 and the Outcrop pH 5 control bioassays did show a loss of TOC, indicating that these bioassays likely experienced regrowth of microorganisms at some point during the experiment. However, this cannot be proven and therefore does not allow for conclusive statements regarding the biodegrability of this material. The initial and final TOC concentrations for the control bioassays can be seen in Figure 38, Figure 41, and Figure 44.

KBA Sediment Sample

Headspace CO_2

As seen in Figure 36, steady CO_2 production was sustained through day 32 in the pH 5 bioassays maintaining 0.19 to 0.26 mg CO_2 in the headspace before decreasing below .1 mg CO_2. Generation of CO_2 was sustained until after day 64. The pH 11 bioassays steadily decreased in CO_2 production with each sampling, but maintained 0.21 to 0.79 mg CO_2 through day 32 before decreasing to below 0.07 mg CO_2 (Figure 37). Controls produced substantially less CO_2 than did the live bioassays. The pH 5 control produced approximately 0.06 mg CO_2 through day 32 before decreasing to negligible amounts. The pH 11 control produced negligible amount throughout the experiment with the exception of two timepoints which reached 0.05 mg CO_2. No CO_2 was detected in the pH 5 controls that remained sealed, and only 0.009 mg CO_2 was detected in the pH 11 unsampled controls.

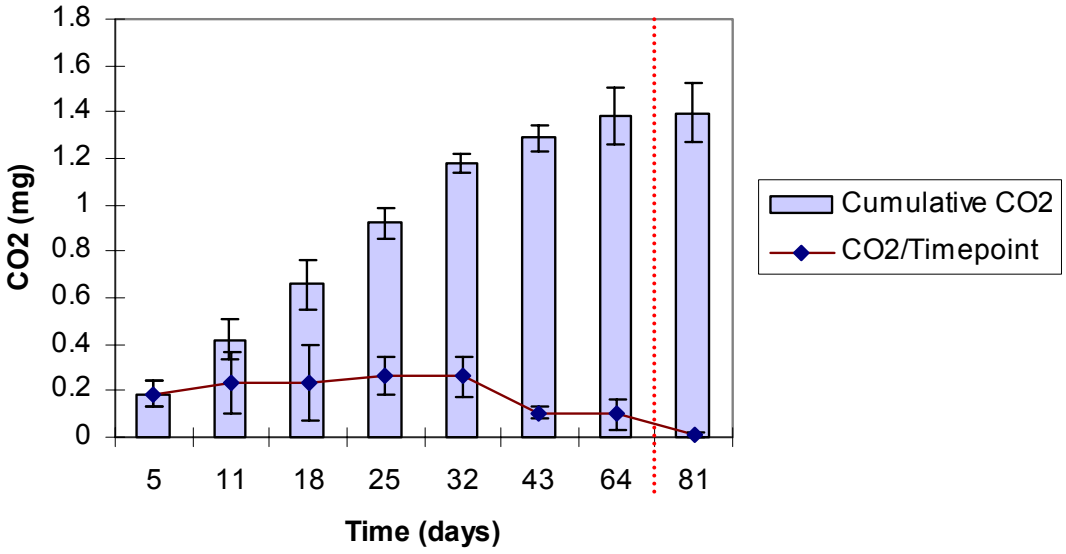

Figure 36. Headspace CO_2 (mg) observed in the KBA pH 5 bioassays over time.

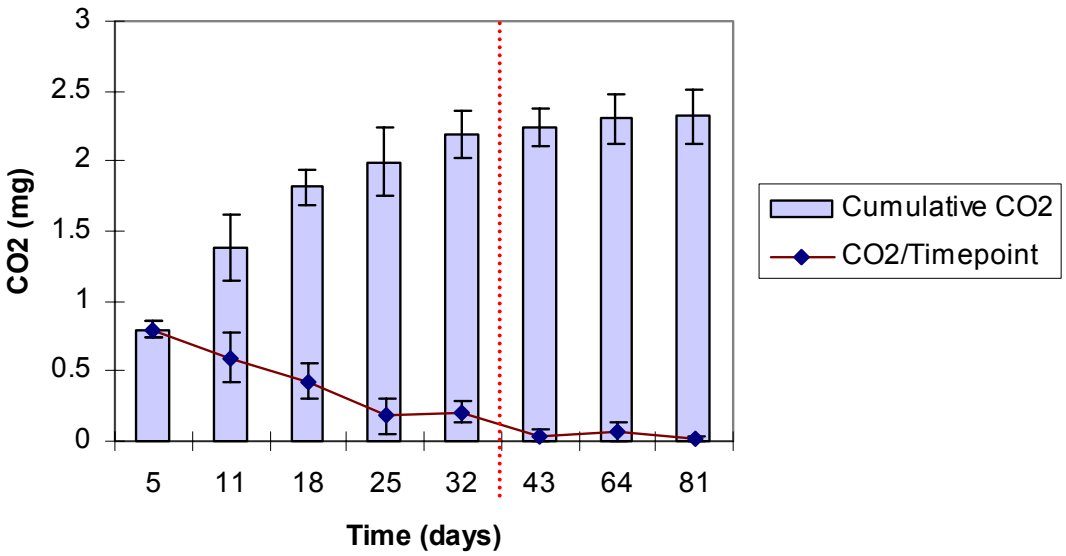

Figure 37. Headspace CO_2 (mg) observed in the KBA pH 11 bioassays over time.

Aerobic Live Bioassays TOC

The pH 5 and pH 11 live bioassays showed losses of 43% and 0%, respectively. This corresponds with a loss of 2.1 and 0.0 mg/L TOC. With some fluctuation, the pH 5 bioassays show a steady decline of TOC during the first 43 days. Although calculated as

no loss, the pH 11 bioassays do show a drop in TOC after day 43. Because CO_2 was no longer being produced at this point, these values were not taken into consideration. In addition, the rise in TOC in the final sample causes the lower values on the previous two timepoints to be questionable. The pH 11 controls showed significant decrease in TOC, therefore not allowing for any conclusive statement to be made about biodegradability of this material. No loss of TOC appears to be occurring at the end of the experiment (Figure 38).

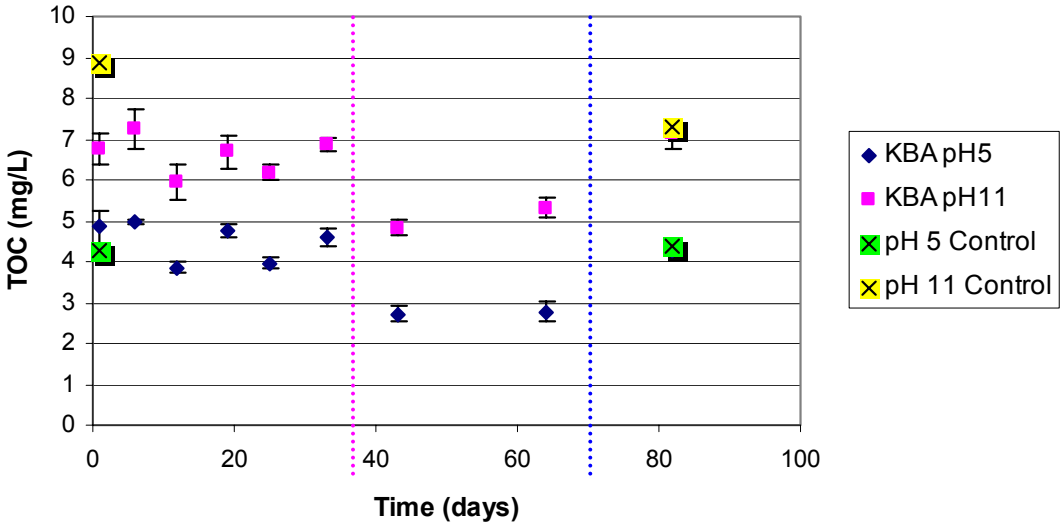

Figure 38. KBA pH 5 and pH 11 bioassays TOC concentrations monitored for a period of 82 days. The initial and final TOC concentrations are shown for the control bioassay.

Conversion of Aqueous TOC to Headspace C-CO_2

With each time point, a higher fraction of the TOC loss was seen as C-CO_2. The pH 5 bioassays converted 10.0% TOC to C-CO_2 on day 12 and increased all the way to an 89.8% conversion during the last sampling. The pH 11 bioassays showed a 24% conversion on day 12 and a 94% conversion during on day 64. The pH 11 bioassays resulted in a higher conversion of TOC to C-CO_2 throughout the experiment.

5/11/02 Sediment Sample

Headspace CO_2

CO_2 generation was sustained through day 32 in both sets of bioassays before decreasing to below .1 mg CO_2 in the headspace (Figure 39 and Figure 40). Both sets ceased CO_2 production after day 64. The pH 5 bioassays maintained 0.13 to 0.17 mg CO_2, and the pH 11 bioassays contained 0.30 to 0.48 mg CO_2 in the headspace through day 32. Controls produced substantially less CO_2 than did the live bioassays. The pH 5 control produced approximately 0.02 to 0.07 mg CO_2 through day 32 before decreasing to negligible amounts. The pH 11 control produced negligible amount throughout the experiment. In the controls that remained sealed throughout the experiment, only 0.001 mg CO_2 was detected in the pH 5 bioassays and only 0.004 mg CO_2 was detected in the pH 11 bioassays.

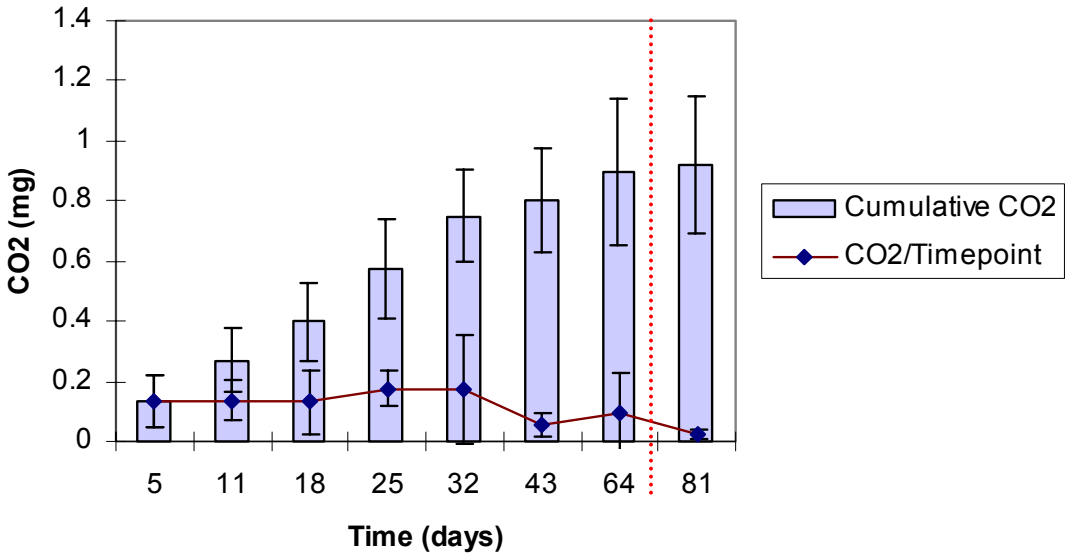

Figure 39. Headspace CO_2 (mg) observed in the 5/11/02 pH 5 bioassays over time.

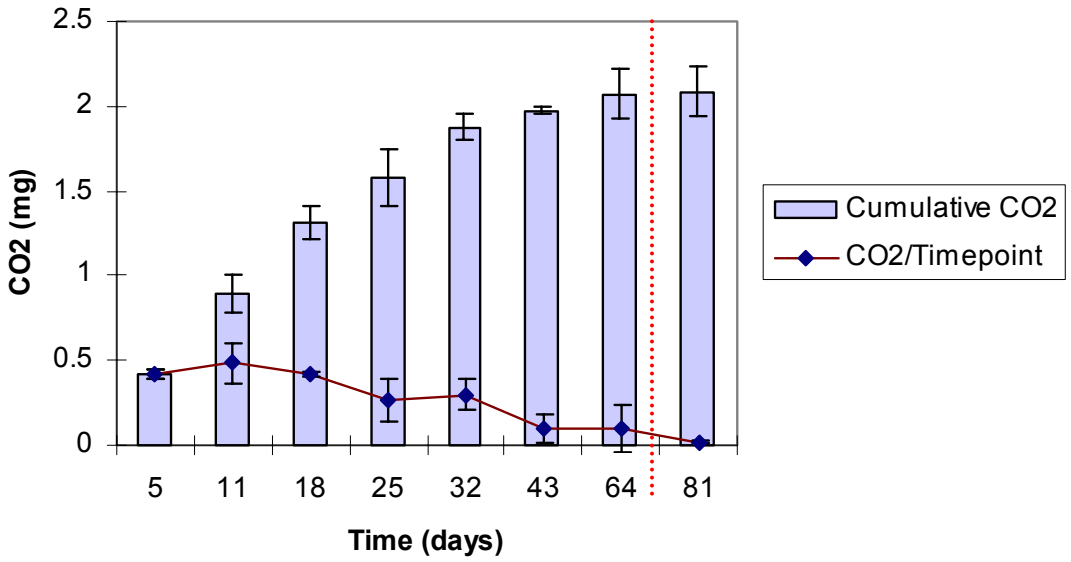

Figure 40. Headspace CO_2 (mg) observed in the 5/11/02 pH 11 bioassays over time.

Aerobic Live Bioassays TOC

Losses of 44% and 32% were observed in the pH 5 and pH 11 bioassays, respectively. This corresponds with a loss of 3.0 and 5.4 mg/L TOC. Utilization of TOC is not apparent in the pH 5 bioassays until after day 33, whereas the pH 11 bioassays show a steady decrease soon after inoculation (Figure 41). No loss of TOC appears to be occurring at the end of the experiment. Although the pH 5 controls remain steady from beginning to end, the pH 11 controls decrease almost as much as the live bioassays. This is likely due to contamination or regrowth in the controls, however this doesn't allow conclusive statements in regards to the biodegradability of this material.

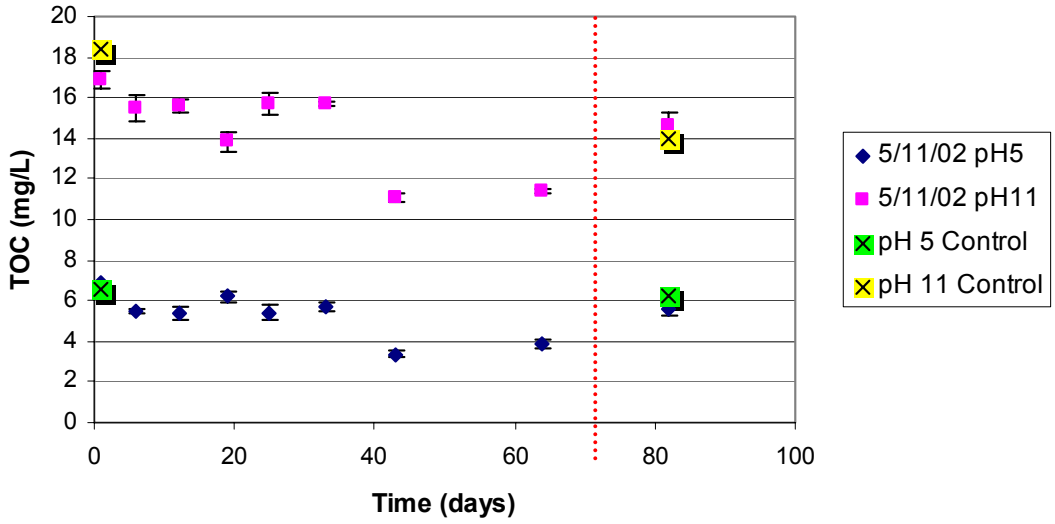

Figure 41. 5/11/02 pH 5 and pH 11 bioassays TOC concentrations monitored for a period of 82 days. The initial and final TOC concentrations are shown for the control bioassay.

Conversion of Aqueous TOC to Headspace C-CO_2

The 5/11/02 pH 5 bioassays showed an average conversion of 9.5 % TOC to C-CO_2, with a peak conversion of 25.0 % on the last sampling day. The pH 11 bioassays experienced an average conversion of 12.0 % TOC to C-CO_2, with a peak conversion of 19.0 %. In both sets of bioassays, the percent conversion tended to increase over time. The two series of bioassays experienced similar % conversions throughout the experiment.

Outcrop Sediment

Headspace CO_2

CO_2 generation was sustained through day 32 in both series of bioassays (Figure 42 and Figure 43) After this point, levels began to decrease. The pH 5 bioassays were no longer generating CO_2 after day 64, and the pH 11 bioassays were no longer generating CO_2 after day 43. The pH 5 bioassays maintained 0.20 to 0.27 mg CO_2, and the pH 11 bioassays contained 0.48 to 0.66 mg CO_2 in the headspace through day 32. The pH 5 controls produced similar levels of CO_2 through day 11 before decreasing to levels near 0 mg CO_2. The pH 11 controls generated similar levels of CO_2 through day 18 before

decreasing substantially. In the controls that remained sealed throughout the experiment, 0.008 mg CO_2 was observed in the pH 5 bioassays and only 0.06 mg CO_2 was observed in the pH 11 bioassays.

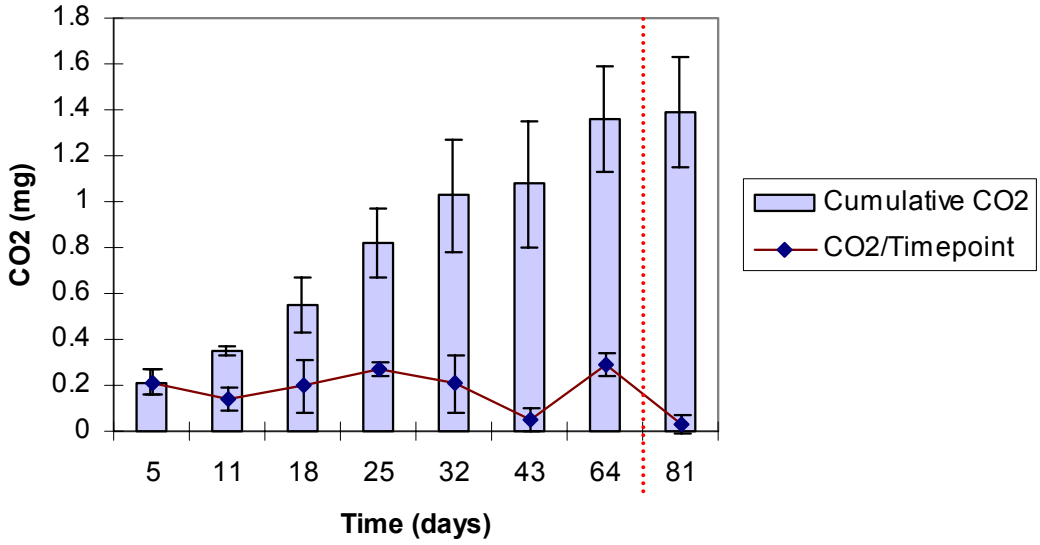

Figure 42. Headspace CO_2 (mg) observed in the Outcrop pH 5 bioassays over time.

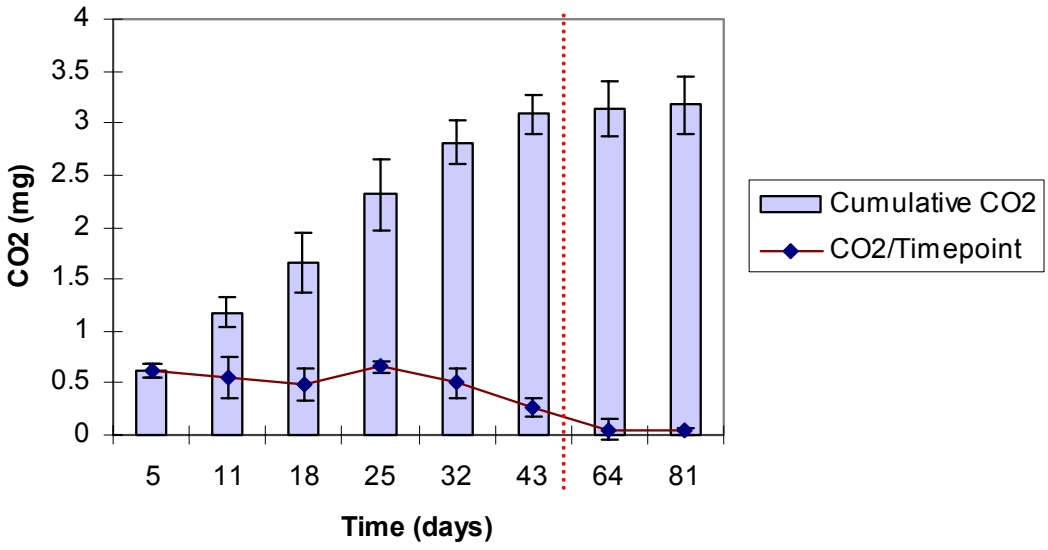

Figure 43. Headspace CO_2 (mg) observed in the Outcrop pH 11bioassays over time.

Aerobic Live Bioassays TOC

Losses of 51% and 6% were observed in the pH 5 and pH 11 bioassays, respectively. This corresponds with a loss of 10.0 and 2.1 mg/L TOC. While the pH 5 bioassays experienced a steady decline from the beginning, the pH 11 bioassays show no steady trend of biodegradation (Figure 44). It is unclear whether any degradation occurred in the pH 11 bioassays. Controls remained steady.

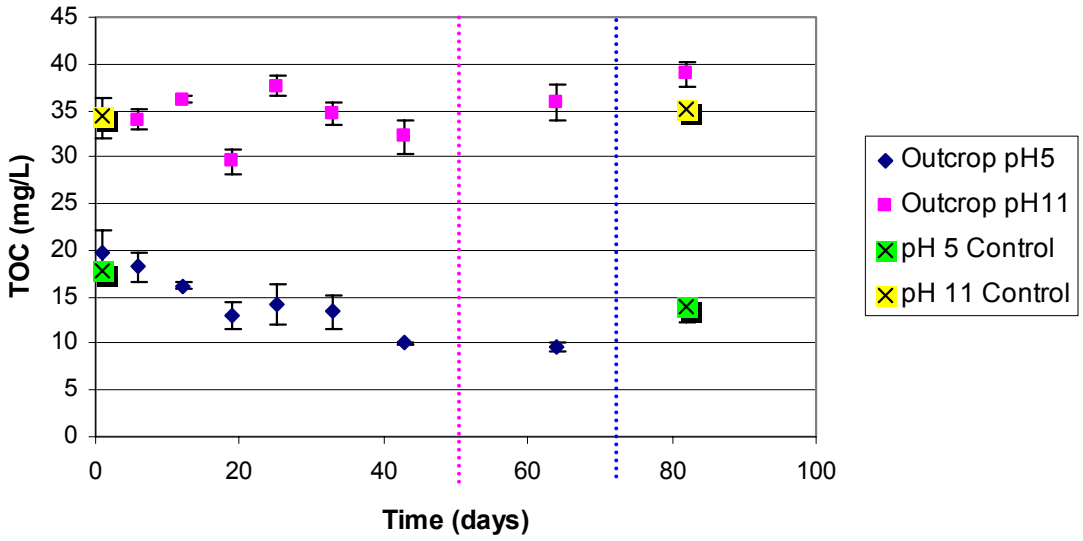

Figure 44. Outcrop pH 5 and pH 11 bioassays TOC concentrations monitored for a period of 82 days. The initial and final TOC concentrations are shown for the control bioassay.

Conversion of Aqueous TOC to Headspace C-CO_2

The Outcrop bioassays showed much lower levels of TOC to C-CO_2 conversion than did the other two sets of bioassays. The pH 5 bioassays remained around 1% to 3% conversion throughout the experiment, peaking at 5.9% conversion on the last sampling day. The pH 11 bioassays experienced 2% to 17% conversion of TOC to C-CO_2.

Conclusions

Although the pH 11 extraction removed more organic carbon from the sediment, the pH 5 extract experienced a greater percent loss of TOC in all sets of bioassays. The 5/11/02 bioassays were the only set in which this did not also correspond to a higher loss of TOC concentration. With increasing indigenous carbon, the pH 5 extract bioassays experienced higher % biodegradation. In those bioassays that did experience biodegradation, TOC was utilized for approximately 6 weeks before reaching steady concentrations.

Because the higher pH disperses tightly coiled humic substances and allows better access to more surface area for the extractant, it was expected that the pH 11 extractant would removed more organic matter from the soil than did the pH 5 extractant. However, this did not correspond to the same level of biodegradability as that experienced by the material removed by the lower pH extractant. The two different pH levels appear to remove different pools of organic carbon. If the higher pH were able to access the same pool as the lower pH in addition to the carbon bound by condensed material, then a higher loss of TOC would be expected. It is unknown if the higher pH changes the nature of the organic carbon and thus affects the biodegradability during the extraction process. Based on the results of this experiment, it is not recommended that a higher pH extractant is solely used as a measure of the biodegradable fraction of organic carbon in sediment.

The results of the pH experiment tie to the anaerobic experiment in that similar trends were observed. The pyro extractions are a lower pH and therefore correspond to the pH 5 extraction, while the base extraction corresponds to the pH 11 extraction. In both experiments, the higher pH removed more organic carbon, but the lower pH extracted material was shown to have greater biodegradability. Although pH plays a role in the ability of an extractant to remove organic carbon from the sediment, other factors play a role in the process. This is seen by the fact that the method developed by Rectanus *et al.* (2005) removed an average of 10 times more organic carbon from the sediment than did the nanopure water.

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Appendix A: Positive Control Data

Table A-1. TOC concentrations (mg/L) for the positive controls.

	4-Apr	8-Apr	12-Apr	19-Apr	25-Apr	4-May	19-May	1-Jun	21-Jun
Positive1	144.30	118.63	108.13	119.60	101.63	104.01	120.09	132.88	140.61
Positive 2	125.13	153.74	138.83	147.47	129.46	131.74	165.83	173.16	175.70
Positive 3	138.44	153.26	136.17	144.48	132.70	132.02	166.64	180.30	178.55

Table A-2. Headspace CO_2 concentrations (mg/L) for the positive controls.

	6-Apr	12-Apr	19-Apr	25-Apr	4-May	19-May	1-Jun
Positive 1	17.79	17.82	25.08	22.95	24.98	25.29	19.69
Positive 2	2.46	7.97	10.15	8.60	11.85	3.29	4.60
Positive 3	2.71	7.35	9.97	7.55	10.41	3.32	7.13

Table A-3. VFA concentrations (mg/L) in the positive controls.

8-Apr	Acetic	Proprionic	Isobutyric	Butyric	Isovaleric	n-Valeric	Isocaproic	Hexanoic	Heptanoic
Positive 1	34.63	5.91	22.97	0.00	19.36	0.00	17.91	2.14	1.70
Positive 2	198.68	28.78	19.69	2.37	47.71	0.00	45.21	0.00	2.14
Positive 3	173.27	25.93	7.95	0.00	23.42	0.00	15.22	0.00	1.79

12-Apr	Acetic	Proprionic	Isobutyric	Butyric	Isovaleric	n-Valeric	Isocaproic	Hexanoic	Heptanoic
Positive 1	78.03	8.18	28.79	0.00	18.07	0.00	22.38	3.30	1.50
Positive 2	213.65	28.12	48.41	12.01	54.94	1.63	52.23	3.32	2.34
Positive 3	251.73	31.07	52.59	47.18	56.33	4.97	58.93	4.89	2.30

20-Apr	Acetic	Proprionic	Isobutyric	Butyric	Isovaleric	n-Valeric	Isocaproic	Hexanoic	Heptanoic
Positive 1	137.14	9.97	28.48	7.77	19.42	0.00	22.78	3.35	1.47
Positive 2	246.47	28.81	47.37	27.46	54.00	3.15	53.40	4.95	2.48
Positive 3	240.02	27.80	46.05	45.03	50.02	6.79	52.82	6.68	2.20

25-Apr	Acetic	Proprionic	Isobutyric	Butyric	Isovaleric	n-Valeric	Isocaproic	Hexanoic	Heptanoic
Positive 1	109.53	9.29	25.37	10.91	17.98	20.84	0.00	2.84	0.00
Positive 2	240.84	26.29	43.65	33.43	49.42	3.91	48.73	5.27	2.29
Positive 3	237.70	27.27	46.07	45.62	50.39	7.13	52.24	7.18	2.16

4-May	Acetic	Proprionic	Isobutyric	Butyric	Isovaleric	n-Valeric	Isocaproic	Hexanoic	Heptanoic
Positive 1	165.19	10.29	29.20	0.00	9.95	0.00	0.00	0.00	0.00
Positive 2	34.18	0.00	0.00	0.00	26.10	0.00	0.00	0.00	0.00
Positive 3	235.15	24.33	42.19	41.51	45.39	6.28	45.55	0.00	0.00

19-May	Acetic	Proprionic	Isobutyric	Butyric	Isovaleric	n-Valeric	Isocaproic	Hexanoic	Heptanoic
Positive 1	153.38	14.81	35.93	13.00	26.24	0.00	24.26	0.00	0.00
Positive 2	299.83	26.53	44.81	38.49	46.97	3.92	44.15	0.00	0.00
Positive 3	335.11	33.07	61.61	56.21	58.92	7.97	57.92	1.24	0.48

1-Jun	Acetic	Proprionic	Isobutyric	Butyric	Isovaleric	n-Valeric	Isocaproic	Hexanoic	Heptanoic
Positive 1	334.05	32.77	89.43	32.19	64.92	1.31	58.66	0.00	0.00
Positive 2	458.75	46.45	73.76	67.47	70.97	5.95	61.00	0.56	0.00
Positive 3	378.94	39.66	64.35	63.41	66.70	8.59	62.67	1.13	0.00

21-Jun	Acetic	Proprionic	Isobutyric	Butyric	Isovaleric	n-Valeric	Isocaproic	Hexanoic	Heptanoic
Positive 1	162.90	17.40	43.09	14.41	34.05	0.53	28.59	0.00	0.00
Positive 2	396.76	34.99	58.80	49.79	59.44	4.75	56.00	0.49	0.00
Positive 3	364.92	35.73	62.28	61.11	66.47	8.01	62.23	0.86	0.00

Appendix B: Hydrogen Data

PPB

Table B-1. Hydrogen concentrations (ppb) in the hydrogen bottles.

	Pyro 8-10 Feet	Pyro 20.5-22 Feet	Base 8-10 Feet	Base 20.5-22 Feet
6-Apr	174.47	192.73	8770.43	20469.33
14-Apr	199.53	542.23	19013.33	16278.33
19-Apr	0.00	375.40	23773.33	35945.33
4-May	47.63	83.63	42007.67	64328.67
18-May	0.00	0.00	25376.00	61462.67
1-Jun			47333.33	52838.28
6-Jul			39560.00	85574.00

Conversion from ppb to nM: ppb/1000 * 0.82

Table B-2. Hydrogen concentrations (nM) in the hydrogen bottles.

	Pyro 8-10 Feet	Pyro 20.5-22 Feet	Base 8-10 Feet	Base 20.5-22 Feet
6-Apr	0.14	0.16	7.19	16.78
14-Apr	0.16	0.44	15.59	13.35
19-Apr	0.00	0.31	19.49	29.48
4-May	0.04	0.07	34.45	52.75
18-May	0.00	0.00	20.81	50.40
1-Jun			38.81	43.33
6-Jul			32.44	70.17

Appendix C: Kings Bay Data

Table C-1. TOC concentrations (mg/L) in the King's Bay bioassays.

Vial	Time0	Time1	Time2	Time3	Time4	Time5	Time6	Time7	Time8	Time9	FINAL
KBA Pyro 1	55.02	56.13	64.09	60.43	52.13	62.79	57.94	46.86	50.62	37.80	
KBA Pyro 2	57.98	62.41	39.05	61.87	55.32	58.63	51.28	43.80	51.61	36.20	
KBA Pyro 3	58.73	35.53	52.62	42.98	52.21	53.61	49.39	42.36	46.06	33.67	
KBA Base 1	83.50		80.11	92.09	82.77	78.99	68.51	68.30	67.46	68.14	
KBA Base 2	83.47		78.16	83.82	75.54	77.82	69.24	67.68	64.22	66.23	
KBA Base 3	80.31		83.70	74.81	37.60	76.24	77.32	65.58	65.58	67.13	
KBA Pyro Control 1	46.47	35.66	45.24	52.96	24.61	45.72	49.44	31.93	38.04	30.23	
KBA Pyro Control 2	48.80										38.15
KBA Pyro Control 3	46.20										30.51
KBA Base Control 1	70.88		72.08	74.91	39.43	65.39	55.52	44.96	52.35	49.48	
KBA Base Control 2	72.95										59.96
KBA Base Control 3	66.43										59.32
5/11/02 Pyro 1	119.70	155.58	74.48	141.78	71.31	140.98	69.45	110.40	129.51	107.80	
5/11/02 Pyro 2	122.38	126.11	133.91	146.20	70.13	136.14	66.50	111.81	129.08	109.33	
5/11/02 Pyro 3	116.24	127.07	137.66	114.76	92.62	136.02	127.00	114.02	124.40	110.12	
5/11/02 Base 1	61.89		73.43	77.74	47.08	67.70	35.16	53.99	59.73	56.60	
5/11/02 Base 2	65.19		72.69	73.75	43.98	64.89	68.32	55.83	62.36	57.86	
5/11/02 Base 3	61.37		64.74	72.48	44.89	70.37	72.72	55.79	61.10	60.11	
5/11/02 Pyro Control 1	117.32	128.29	132.61	137.96	80.02	143.88	142.83	116.37	127.25	108.13	
5/11/02 Pyro Control 2	135.69										124.59
5/11/02 Pyro Control 3	118.22										121.22
5/11/02 Base Control 1	67.05		63.71	68.87	44.72	66.89	59.66	58.13	59.27	60.99	
5/11/02 Base Control 2	67.30										61.36
5/11/02 Base Control 3	65.45										60.04
Outcrop Pyro 1	217.49	213.30	236.04	218.88	98.14	114.99	137.66	274.44	119.28	103.02	
Outcrop Pyro 2	122.52	177.95	239.85	131.72	92.71	118.06	123.92	271.33	113.62	107.79	
Outcrop Pyro 3	121.43	219.13	230.41	130.95	86.27	117.31	132.65	267.08	113.49	118.78	
Outcrop Base 1	383.47		0.00	449.17	414.58	397.60	401.34	926.31	438.86	429.10	
Outcrop Base 2	411.20		1006.16	437.86	373.06	420.13	446.41	657.53	436.33	434.79	
Outcrop Base 3	432.88		652.39	424.12	390.13	382.29	418.19	572.08	439.50	431.16	
Outcrop Pyro Control 1	113.40	205.08	219.93	120.86	121.76	117.79	129.68	211.71	107.13	96.50	
Outcrop Pyro Control 2	101.05										109.78
Outcrop Pyro Control 3	141.83										110.58
Outcrop Base Control 1	391.46		524.19	464.49	395.93	456.80	518.49	490.64	458.47	483.01	
Outcrop Base Control 2	386.37										481.04
Outcrop Base Control 3	396.48										498.32

Table C-2. King's Bay Extraction Data

	Day 1	Day 2	Day 3	Day 4	Day 5
KBA 1	88.708	50.874	42.639	86.321	43.800
KBA 2	80.644	45.576	43.103	94.918	42.242
KBA 3	78.766	45.361	45.765	91.026	45.406
KBA 4	76.185	47.587	49.450	98.749	42.371
KBA 5	72.811	47.952	59.601	124.944	44.963
KBA 6	71.228	47.669	78.056	118.524	38.217
5/11/02 1	461.467	193.685	147.737	173.765	49.480
5/11/02 2	441.874	224.820	145.406	212.821	54.184
5/11/02 3	381.837	228.849	141.565	164.127	38.161
Outcrop 1	457.197	330.447	235.462	1288.524	204.469
Outcrop 2	395.549	307.205	202.570	1164.992	204.475
Outcrop 3	372.607	346.185	220.165	2219.020	285.105
Control	7.835	8.727	4.357	3.432	0.998

Table C-3. TOC concentrations (mg/L) of combined extract TOC samples.

	TOC (mg/L)
KBA DAYS 1-3	55.770
KBA DAYS 4-5	72.187
5/11/02 DAYS 1-3	156.913
5/11/02 DAYS 4-5	67.238
OUTCROP DAYS 1-3	261.303
OUTCROP DAYS 4-5	419.354

Table C-4. Headspace CO_2 (mg) in the KBA bioassays.

KBA	21-Feb	25-Feb	1-Mar	6-Mar	20-Mar	3-Apr	10-Apr	16-May
Pyro 1	0.019	0.015	0.021	0.045	0.012	0.007	0.000	0.000
Pyro 2	0.015	0.024	0.014	0.012	0.014	0.016	0.002	0.004
Pyro 3	0.020	0.033	0.025	0.024	0.008	0.002	0.000	0.001
Base 1	0.170	0.157	0.147	0.083	0.132	0.051	0.040	0.027
Base 2	0.103	0.088	0.074	0.070	0.032	0.012	0.019	0.009
Base 3	0.225	0.196	0.138	0.094	0.091	0.070	0.000	0.003

Table C-5. Headspace CO_2 (mg) in the 5/11/02 bioassays.

5/11/02	21-Feb	25-Feb	1-Mar	6-Mar	20-Mar	3-Apr	10-Apr	16-May
Pyro 1	0.002	0.006	0.010	0.009	0.002	0.000	0.010	0.000
Pyro 2	0.008	0.014	0.012	0.011	0.004	0.000	0.009	0.000
Pyro 3	0.009	0.013	0.012	0.011	0.004	0.005	0.003	0.005
Base 1	0.152	0.130	0.077	0.039	0.029	0.037	0.005	0.000
Base 2	0.124	0.115	0.079	0.042	0.068	0.044	0.002	0.000
Base 3	0.148	0.196	0.090	0.045	0.022	0.040	0.011	0.017

Table C-6. Headspace CO_2 (mg) in the Outcrop bioassays.

Outcrop	21-Feb	25-Feb	1-Mar	6-Mar	20-Mar	3-Apr	10-Apr	16-May
Pyro 1	0.014	0.021	0.020	0.016	0.011	0.012	0.006	0.003
Pyro 2	0.011	0.016	0.017	0.014	0.010	0.002	0.001	0.004
Pyro 3	0.014	0.022	0.025	0.020	0.002	0.000	0.001	0.000
Base 1	0.227	0.162	0.192	0.137	0.158	0.179	0.112	0.033
Base 2	0.182	0.258	0.213	0.125	0.202	0.137	0.081	0.014
Base 3	0.188	0.196	0.086	0.060	0.021	0.091	0.070	0.014

Table C-7. Headspace CO_2 (mg) in the KBA control bioassays.

KBA	21-Feb	25-Feb	1-Mar	6-Mar	20-Mar	3-Apr	10-Apr	16-May	17-May
Pyro Control 1	0.007	0.008	0.005	0.006	0.003	0.008	0.002	0.020	
Pyro Control 2									0.000
Pyro Control 3									0.002
Base Control 1	0.140	0.321	0.138	0.114	0.114	0.132	0.072	0.000	
Base Control 2									0.000
Base Control 3									0.002

Table C-8. Headspace CO_2 (mg) in the 5/11/02 control bioassays.

5/11/02	21-Feb	25-Feb	1-Mar	6-Mar	20-Mar	3-Apr	10-Apr	16-May	17-May
Pyro Control 1	0.008	0.010	0.009	0.007	0.000	0.000	0.000	0.000	
Pyro Control 2									0.006
Pyro Control 3									0.007
Base Control 1	0.103	0.106	0.048	0.021	0.008	0.052	0.013	0.000	
Base Control 2									0.020
Base Control 3									0.000

Table C-9. Headspace CO_2 (mg) in the Outcrop control bioassays.

Outcrop	21-Feb	25-Feb	1-Mar	6-Mar	20-Mar	3-Apr	10-Apr	16-May	17-May
Pyro Control 1	0.021	0.021	0.013	0.014	0.005	0.000	0.000	0.019	
Pyro Control 2									0.000
Pyro Control 3									0.003
Base Control 1	0.275	0.474	0.281	0.185	0.198	0.292	0.191	0.029	
Base Control 2									0.006
Base Control 3									0.035

Table C-10. VFAs detected in Kings Bay bioassays.

3/2/2006		Vial	VFA	Conc (mg/L)
	KBA pyro	A2	Proprionic	**21.541**
	KBA base	A4	Acetic	**9.360**
	KBA base	A5	Acetic	**10.865**
	KBA base control	A10	Acetic	**5.817**
	Outcrop base control	A34	Acetic	**22.465**
3/8/2006				
	KBA pyro	A2	Heptanoic	**29.493**
	KBA base	A4	Heptanoic	**20.748**
3/21/2006				
	KBA base	A4	Acetic	**2.006**
	KBA base	A5	Acetic	**1.972**
5/16/2006				
	Outcrop pyro control	A31	Acetic	**0.192**

Appendix D: MLS 10 Data

Table D-1. TOC concentrations (mg/L) for the MLS10 bioassays.

4/4/2006		Vial	VFA	Conc (mg/L)	VFA	Conc (mg/L)
	8-10 pyro	A101	Acetic	**12.057**		
	20.5-22 base	A117	Acetic	**10.690**		
	20.5-22 base	A118	Acetic	**32.344**		
	20.5-22 pyro ctrl	A119	Acetic	**20.451**		
5/10/2006						
	8-10 pyro	A101	Acetic	**2.307**	Isobutyric	**1.376**
	8-10 pyro	A103	Acetic	**2.273**		
	8-10 base	A104	Isobutyric	**0.529**		
	20.5 pyro ctrl	A119	Acetic	**0.878**		
5/23/2006						
	8-10 pyro	A101	Acetic	**present**		
	8-10 pyro	A102	Acetic	**0.539**		
	8-10 pyro	A103	Butyric	**0.991**		
	8-10 base	A104	Isocaproic	**0.772**		
	8-10 base	A106	Isocaproic	**0.406**		
	8-10 pyro control	A107	Isocaproic	**0.542**		
	8-10 base control	A110	Isovaleric	**0.199**		
	20.5-22 base	A116	Acetic	**0.610**		

Table D-2. TOC concentrations (mg/L) from the MLS10 extractions.

	Day1	Day2	Day3	Day4	Day5
8-10 #1	21.767	7.264	5.148	11.409	5.082
8-10 #2	18.190	6.657	4.187	11.374	3.920
8-10 #3	19.175	7.374	4.572	11.449	3.010
8-10 #4	16.214	7.388	4.014	10.617	3.229
8-10 #5	17.248	6.978	4.026	12.496	3.173
22# 1	20.042	13.728	11.972	46.684	14.780
22 #2	20.192	12.382	12.659	53.900	11.977
22 #3	19.642	11.216	11.317	43.683	11.726
22 #4	17.032	13.666	11.334	37.765	15.352
22 #5	17.578	12.808	11.084	39.785	10.916
Control 1	2.763	2.702	2.689	3.645	2.466
Control 2	4.181	3.273	3.013	3.359	2.814
Control 3	2.540	3.106	3.174	3.768	2.920

Table D-3. Headspace CO_2 (mg) in the MLS10 8-10 feet bioassays.

	4-Apr	12-Apr	25-Apr	9-May	23-May	7-Jun
8-10 Pyro 1	0.030	0.039	0.043	0.018	0.023	0.007
8-10 Pyro 2	0.027	0.034	0.043	0.042	0.028	0.016
8-10 Pyro 3	0.030	0.039	0.043	0.038	0.039	0.033
8-10 Base 1	0.098	0.116	0.099	0.058	0.057	0.110
8-10 Base 2	0.042	0.053	0.061	0.065	0.069	0.033
8-10 Base 3	0.052		0.113	0.083	0.039	0.062

Table D-4. Headspace CO_2 (mg) in the MLS10 20.5-22 feet bioassays.

	4-Apr	12-Apr	25-Apr	9-May	23-May	7-Jun
20.5-22 Pyro 1	0.039	0.055	0.041	0.016	0.018	0.000
20.5-22 Pyro 2	0.036	0.054	0.048	0.032	0.009	0.000
20.5-22 Pyro 3	0.043	0.048	0.056	0.056	0.021	0.045
20.5-22 Base 1	0.114	0.144	0.125	0.069	0.030	0.000
20.5-22 Base 2	0.093	0.128	0.136	0.106	spilled	
20.5-22 Base 3	0.072	0.100	0.096	0.087	0.044	0.000

Table D-5. Headspace CO_2 (mg) in the MLS10 8-10 feet control bioassays.

	4-Apr	12-Apr	25-Apr	9-May	23-May	7-Jun	21-Jun
8-10 Pyro Control 1	0.000	0.018	0.001	0.000	0.000	0.000	
8-10 Pyro Control 2							0.019
8-10 Pyro Control 3							0.014
8-10 Base Control 1	0.014	0.040	0.026	0.037	0.013	0.000	
8-10 Base Control 2							0.009
8-10 Base Control 3							0.072

Table D-6. Headspace CO_2 (mg) in the MLS10 20.5-22 feet control bioassays.

	4-Apr	12-Apr	25-Apr	9-May	23-May	7-Jun	21-Jun
20.5-22 Pyro Control 1	0.000	0.005	0.000	0.000	0.000	0.000	
20.5-22 Pyro Control 2							0.011
20.5-22 Pyro Control 3							0.022
20.5-22 Base Control 1	0.040	0.049	0.060	0.056	0.043	0.029	
20.5-22 Base Control 2							0.051
20.5-22 Base Control 3							0.047

Table D-7. VFAs detected in the MLS10 bioassays.

4/4/2006		Vial	VFA	Conc (mg/L)	VFA	Conc (mg/L)
	8-10 pyro	A101	Acetic	**12.057**		
	20.5-22 base	A117	Acetic	**10.690**		
	20.5-22 base	A118	Acetic	**32.344**		
	20.5-22 pyro ctrl	A119	Acetic	**20.451**		
5/10/2006						
	8-10 pyro	A101	Acetic	**2.307**	Isobutyric	**1.376**
	8-10 pyro	A103	Acetic	**2.273**		
	8-10 base	A104	Isobutyric	**0.529**		
	20.5 pyro ctrl	A119	Acetic	**0.878**		
5/23/2006						
	8-10 pyro	A101	Acetic	**present**		
	8-10 pyro	A102	Acetic	**0.539**		
	8-10 pyro	A103	Butyric	**0.991**		
	8-10 base	A104	Isocaproic	**0.772**		
	8-10 base	A106	Isocaproic	**0.406**		
	8-10 pyro control	A107	Isocaproic	**0.542**		
	8-10 base control	A110	Isovaleric	**0.199**		
	20.5-22 base	A116	Acetic	**0.610**		

Appendix E: MLS 20 Data

Table E-1. TOC concentrations (mg/L) in the MLS20 bioassays.

Vial	Time0	Time1	Time2	Time3	Time4	Time5	Time6	Time7
8-10 Pyro 1		11.799	7.652	11.465	8.220	10.876	8.158	10.365
8-10 Pyro 2	13.321	11.180	7.247	10.585	8.380	10.386	7.273	9.880
8-10 Pyro 3	13.135	10.644	7.101	10.566	8.039	11.149	7.599	16.399
8-10 Base 1	13.943	15.372	10.505	15.233	13.537	17.600	10.480	10.154
8-10 Base 2	16.674	14.444	9.922	14.045	14.197	15.445	10.089	14.413
8-10 Base 3	15.298	14.238	9.827	12.628	15.837	16.182	9.452	13.330
8-10 Pyro Control 1	5.029	5.787	6.918	11.463	9.456	8.225	7.969	8.310
8-10 Pyro Control 2	5.566							6.663
8-10 Pyro Control 3	4.973							6.753
8-10 Base Control 1	6.719	6.432	6.955	12.144	10.891	7.898	8.452	10.979
8-10 Base Control 2	6.319							7.339
8-10 Base Control 3	6.368							5.722
22 Pyro 1	16.618	15.316	15.568	14.295	14.759	17.750	13.619	14.331
22 Pyro 2	17.910	16.063	15.646	13.636	16.919	15.017	18.136	14.883
22 Pyro 3	15.891	15.594	13.805	13.724	15.744	15.476	15.403	14.310
22 Base 1	19.060	21.313	22.159	19.167	21.710	23.350	23.582	20.792
22 Base 2	19.342	19.127	20.385	18.296	18.962	19.807	21.586	19.632
22 Base 3	17.969	20.246	21.731	17.584	19.802	20.271	20.534	20.885
22 Pyro Control 1	14.964	16.134	15.598	22.386	17.440	15.643	14.397	14.297
22 Pyro Control 2	15.521							15.343
22 Pyro Control 3	15.837							15.288
22 Base Control 1	15.156	14.589	15.309	20.109	18.069	15.749	13.646	15.231
22 Base Control 2	14.976							12.149
22 Base Control 3	16.409							12.775

Table E-2. TOC concentrations (mg/L) from the MLS20 extractions.

	Day1	Day2	Day3	Day4	Day5
8-10 #1	6.802	4.968	5.390	1.969	1.599
8-10 #2	6.314	5.245	10.195	2.789	2.009
8-10 #3	7.075	5.632	4.197	2.914	1.597
20.5-22 #1	22.629	10.322	6.978	10.591	7.726
20.5-22 #2	21.516	10.661	7.141	8.382	6.710
20.5-22 #3	23.487	10.283	6.525	9.922	7.264
Control 1	1.030	0.682	1.266	1.408	0.529
Control 2	0.966	2.645	1.068	1.140	3.681
Control 3	2.713	1.211	1.404	1.096	3.402

Table E-3. Headspace CO_2 in the MLS20 8-10 feet bioassays.

	22-Mar	5-Apr	19-Apr	4-May	17-May	30-May	13-Jun
8-10 Pyro 1	0.053	0.033	0.019	0.009	0.000	0.021	0.017
8-10 Pyro 2	0.047	0.015	0.025	0.016	0.030	0.028	0.002
8-10 Pyro 3	0.042	0.035	0.047	0.012	0.033	0.024	0.020
8-10 Base 1	0.108	0.042	0.016	0.004	0.000	0.000	0.000
8-10 Base 2	0.115	0.036	0.012	0.009	0.014	0.000	0.001
8-10 Base 3	0.146	0.044	0.013	0.012	0.000	0.014	0.011

Table E-4. Headspace CO_2 in the MLS20 22 feet bioassays.

	22-Mar	5-Apr	19-Apr	4-May	17-May	30-May	13-Jun
22 Pyro 1	0.013	0.005	0.010	0.033	0.040	0.054	0.027
22 Pyro 2	0.010	0.014	0.038	0.028	0.052	0.031	0.023
22 Pyro 3	0.007	0.018	0.029	0.008	0.046	0.008	0.000
22 Base 1	0.209	0.087	0.053	0.041	0.004	0.000	0.000
22 Base 2	0.217	0.067	0.057	0.015	0.000	0.000	0.003
22 Base 3	0.219	0.061	0.049	0.019	0.000	0.000	0.022

Table E-5. Headspace CO_2 in the MLS20 8-10 feet control bioassays.

	22-Mar	5-Apr	19-Apr	4-May	17-May	30-May	13-Jun
8-10 Pyro Control 1	0.076	0.039	0.028	0.010	0.000	0.000	0.000
8-10 Pyro Control 2							0.013
8-10 Pyro Control 3							0.000
8-10 Base Control 1	0.241	0.016	0.014	0.000	0.000	0.000	0.005
8-10 Base Control 2							0.005
8-10 Base Control 3							0.012

Table E-6. Headspace CO_2 in the MLS20 22 feet control bioassays.

	22-Mar	5-Apr	19-Apr	4-May	17-May	30-May	13-Jun
22 Pyro Control 1	0.026	0.001	0.059	0.013	0.000	0.000	0.000
22 Pyro Control 2							0.000
22 Pyro Control 3							0.000
22 Base Control 1	0.213	0.070	0.046	0.018	0.000	0.000	0.000
22 Base Control 2							0.002
22 Base Control 3							0.002

Table E-7. VFAs detected in the MLS20 bioassays.

3/22/2006	DAY 8	Vials	VFA	Conc (mg/L)
	22 pyro	A214	Isocaproic	**0.826**
	22 pyro	A215	Isocaproic	**0.839**
5/31/2006	**DAY 79**			
	22 base	A214	Isobutyric	0.681

Appendix F: pH Experiment Data

Table F-1. TOC concentrations (mg/L) for pH experiment.

Vial	Time0	Time1	Time2	Time3	Time4	Time5	Time6	Time7	Time8	FINAL
KBA pH 5 1	5.3196769	4.9899404	3.96701	4.8168487	3.9905578	4.7960801	2.5063326	2.5765701	4.200225	
KBA pH 5 2	4.7138426	4.9133093	3.8880445	4.9100452	3.7975252	4.3795773	2.8651721	2.7310873	4.2022864	
KBA pH 5 3	4.5041335	4.9998654	3.7051528	4.553299	4.0612986	4.561237	2.8062012	3.0354824	4.3731967	
KBA pH 11 1	7.0743202	7.7969857	6.3341536	7.1483664	6.3766996	7.0377449	4.9633381	5.2992075	7.393198	
KBA pH 11 2	6.8569691	7.0274847	6.0543377	6.5408461	6.0250238	6.8568056	4.9294245	5.5593394	7.2479985	
KBA pH 11 3	6.3495997	6.9372577	5.4585604	6.3793657	6.1115718	6.7333141	4.6039954	5.0662234	6.7371679	
KBA pH 5 control 1	4.0905709	4.8323455	3.6257037	4.5500852	3.9868767	4.118045	2.7198717	2.9985106	4.0360328	
KBA pH 5 control 2	4.1922876									4.1924329
KBA pH 5 control 3	4.5089071									4.5707261
KBA pH 11 control 1	8.6452795	8.4501709	6.0317015	7.5500771	6.3535692	7.2686676	5.9304033	6.3795064	7.2745533	
KBA pH 11 control 2	8.2864911									5.9157952
KBA pH 11 control 3	9.7165545									7.4655122
5/11/02 pH 5 1	6.918474	5.6044059	5.0884596	6.167012	5.7710103	5.897844	3.5030072	4.0136885	5.656005	
5/11/02 pH 5 2	6.7530671	5.4022228	5.7167452	6.5292746	5.0665202	5.4687621	3.4866653	3.8872592	5.7207033	
5/11/02 pH 5 3	6.8322489	5.4247373	5.3148945	5.9873094	5.4081782	5.6420212	3.1500861	3.6608984	5.2690795	
5/11/02 pH 11 1	17.185762	15.867324	15.852233	14.04824	15.221627	15.683853	11.300367	11.4951	15.172352	
5/11/02 pH 11 2	16.359876	14.774238	15.700381	14.225289	16.261362	15.66628	11.105795	11.500517	13.920617	
5/11/02 pH 11 3	17.086243	15.893681	15.288523	13.319029	15.707635	15.849552	10.910023	11.295728	14.783214	
5/11/02 pH 5 control 1	6.1599218	5.6077971	5.8105355	6.1259866	5.9764892	5.8270225	3.9715135	4.0186121	5.3307937	
5/11/02 pH 5 control 2	6.2150629									5.6764094
5/11/02 pH 5 control 3	7.2178638									6.7459325
5/11/02 pH 11 control 1	17.716535	17.252041	16.774116	13.663569	15.606839	16.886688	11.575432	11.24355	13.979191	
5/11/02 pH 11 control 2	18.700787									12.516216
5/11/02 pH 11 control 3	18.681102									12.737075
Outcrop pH 5 1	16.826936	17.732618	15.823763	11.337231	11.894944	11.762268	9.933788	9.2576688	14.137247	
Outcrop pH 5 2	21.545276	19.861765	16.40314	13.802825	16.217553	15.347149	10.233398	9.5524628	14.067991	
Outcrop pH 5 3	20.620079	16.98414	16.386647	13.950137	14.739718	13.043767	9.8126606	10.057223	12.046209	
Outcrop pH 11 1	31.817087	35.234375	35.851913	28.92369	38.755746	34.478863	34.223841	37.415478	39.934328	
Outcrop pH 11 2	35.708661	33.833615	36.47373	31.072947	36.738776	33.640584	31.227499	36.423329	39.392256	
Outcrop pH 11 3	35.07874	33.026587	36.279948	28.704204	37.257954	35.956565	31.005221	33.655357	37.32128	
Outcrop pH 5 control 1	18.523622	17.902106	15.623772	12.470421	15.745055	16.413462	9.8049801	9.8586764	13.91141	
Outcrop pH 5 control 2	16.574803									12.793629
Outcrop pH 5 control 3	18.503937									13.525992
Outcrop pH 11 control 1	34.301181	35.890827	37.26421	33.043566	35.454568	36.707974	36.507884	37.130515	35.116953	
Outcrop pH 11 control 2	34.822835									37.55939
Outcrop pH 11 control 3	34.261811									36.903879

Table F-2. Headspace C-CO_2 in the KBA pH bioassays.

	KBA pH 5	St Dev	KBA pH 11	St Dev
Tme 1	0.0507415	0.0153239	0.21644022	0.0160026
Time 2	0.0643556	0.0361481	0.16097502	0.048353
Time 3	0.0638017	0.0432445	0.11720258	0.0365163
Time 4	0.0724205	0.0214601	0.04861536	0.033413
Time 5	0.071128	0.024239	0.05614779	0.0213853
Time 6	0.0287569	0.0070729	0.01124101	0.0108513
Time 7	0.0268292	0.017443	0.01872119	0.0165245
Time 8	0.0028611	0.0021752	0.00512872	0.0051287

Table F-3. Headspace C-CO_2 in the 5/11/02 pH bioassays.

	5/11/02 pH 5	St Dev	5/11/02 pH 11	St Dev
Tme 1	0.0361188	0.0229243	0.1131737	0.0079176
Time 2	0.0374846	0.019034	0.1317246	0.0321353
Time 3	0.0355108	0.0139189	0.1135048	0.0039083
Time 4	0.047396	0.0158958	0.0713111	0.0343913
Time 5	0.0473308	0.0488694	0.0817886	0.0253163
Time 6	0.0150215	0.0097777	0.0265395	0.0234964
Time 7	0.0256766	0.0370762	0.0275334	0.0391175
Time 8	0.0065783	0.0051963	0.0019317	0.0019317

Table F-4. Headspace C-CO_2 in the Outcrop pH bioassays.

	Outcrop pH 5	St Dev	Outcrop pH 11	St Dev
Tme 1	0.0586157	0.0161035	0.1694403	0.0198079
Time 2	0.037977	0.0129684	0.1504913	0.0547455
Time 3	0.0534068	0.0139189	0.1307645	0.0416933
Time 4	0.0735849	0.0086419	0.1803183	0.0150512
Time 5	0.0566816	0.0338216	0.1357498	0.0368097
Time 6	0.0132533	0.0126846	0.0745292	0.0235966
Time 7	0.0784031	0.0138514	0.0146381	0.025354
Time 8	0.0078632	0.0099238	0.008173	0.008173

Appendix G: TOC Drift Calculation Procedure

TOC DRIFT CALCULATION

A 10ppm outside check standard was run at the beginning of the analysis, at various points throughout the analysis, and again at the end of the run. For any two check standards and the samples run in between them, the following method was used for calculating the drift.

- A ratio of 10ppm to the TOC value determined for the 10ppm standard was calculated: 10/determined standard concentration (mg/L)
- All concentrations run after this 10ppm check (and before another 10ppm check) were multiplied by this ratio
- A slope between two 10ppm checks was determined
- The % slope was calculated: slope/initial 10ppm check concentration used for determining the slope
- A % slope/sample was calculated: % slope/# of samples run between the two 10ppm checks that determined the slope
- A value was calculated for each sample that was run by multiplying - % slope/sample by the # of samples run since the check and when that sample was analyzed

The drift calculation was then applied to each sample concentration already adjusted by the ratio previously determined. The drift was applied: sample TOC concentration + sample TOC concentration*(% slope/sample * # of samples run since the 10ppm check)

Vita

Catherine Kelly was born April 3, 1980 and is the daughter of Jim and Linda Kelly. After graduating from Marion Senior High School in Marion, Virginia, she attended the University of Virginia where she completed her undergraduate degree in Biology and Environmental Science in May 2002. She began graduate work at Virginia Tech in August 2004 in the Environmental and Water Resources Engineering program. Catherine conducted research under the guidance of Mark Widdowson and defended her thesis in September of 2006 for the completion of the degree of Master of Science in Environmental Science and Engineering.