ESTCP Executive Summary

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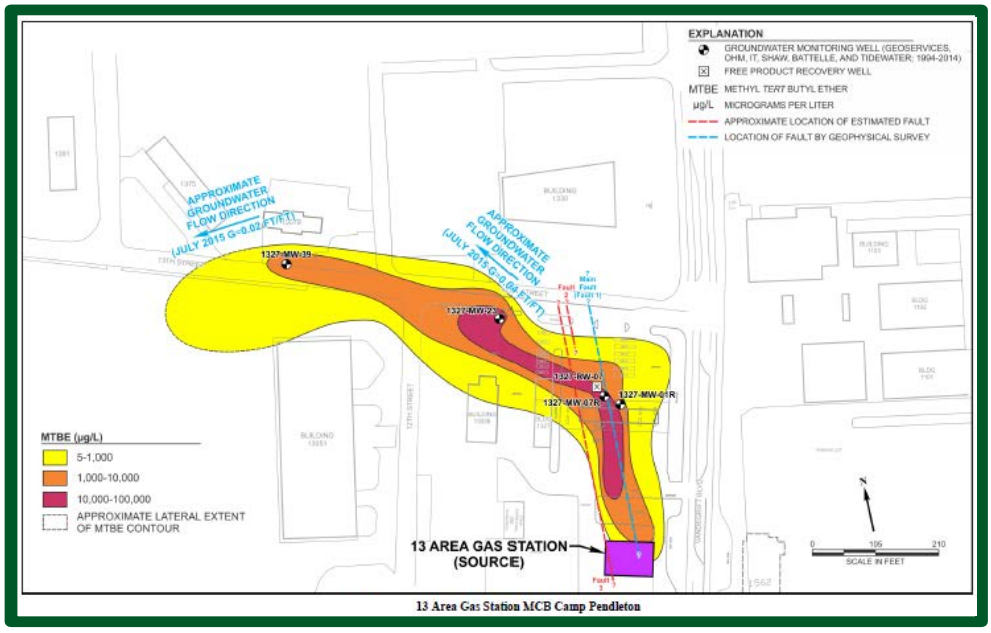

Assessment of Post Remediation Performance of a Biobarrier Oxygen Injection System at an MTBE Contaminated Site

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14. ABSTRACT

This Environmental Security Technology Certification Program (ESTCP)-funded project was performed to evaluate the long-term performance of monitored natural attenuation (MNA) at a site where natural attenuation of methyl tert-butyl ether (MTBE) is being used as a polishing step following in situ bioremediation. To achieve this goal, two primary objectives were identified:

-Evaluate the current microbial activity supporting natural attenuation of MTBE using a combination of conventional contaminant concentration data and geochemistry trend analyses and advanced molecular biological tools (MBTs), including metaproteomics and metagenomics.

-Assess the long-term impact of the biobarrier system on formation permeability.

In addition to evaluating data collected using conventional monitoring techniques, this project applied metagenomics and metaproteomics to improve the understanding of long-term impacts of the remedy on biodegradation at the site. Use of these advanced MBTs for quantification and detection of biomarkers, especially deoxyribonucleic acid (DNA) and peptides (protein fragments) in environmental samples has been rapidly expanding over the last few decades.

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Bioremediation, monitored natural attenuation, MTBE, proteomics, metagenomics, groundwater

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EXECUTIVE SUMMARY

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1.0 INTRODUCTION

This Environmental Security Technology Certification Program (ESTCP)-funded project was performed to evaluate the long-term performance of monitored natural attenuation (MNA) at a site where natural attenuation of methyl tert-butyl ether (MTBE) is being used as a polishing step following in situ bioremediation. In addition to evaluating data collected using conventional monitoring techniques, this project applied metagenomics and metaproteomics to improve the understanding of long-term impacts of the remedy on biodegradation at the site. Use of these advanced MBTs for quantification and detection of biomarkers, especially deoxyribonucleic acid (DNA) and peptides (protein fragments) in environmental samples has been rapidly expanding over the last few decades.

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2.0 OBJECTIVES

For this project, two primary objectives were identified:

- Evaluate the current microbial activity supporting natural attenuation of MTBE using a combination of conventional contaminant concentration data and geochemistry trend analyses and advanced molecular biological tools (MBTs), including metaproteomics and metagenomics.
- Assess the long-term impact of the biobarrier system on formation permeability.

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3.0 TECHNOLOGY DESCRIPTION

Unlike conventional MBTs, such as quantitative polymerase chain reaction (qPCR) or microarrays, metagenomics provides insight into gene sequence information for whole communities. Metagenomic sequencing of environmental samples provides a comprehensive picture of all bacterial and archaeal sequences within a sample, not just those microorganisms targeted with qPCR assays. Providing a larger snapshot of microbial community composition not only allows detection of microorganisms related to the degradation of a specific chemical, but also has a potential to link composition of microbial consortia and geochemical characteristics of the site (Pérez-de-Mora et al. 2014).

Metaproteomics provides the most direct measure of microbial activity through detection of proteins of interest, providing information on molecular processes used by microorganisms. It can identify proteins encoded by genes in the metagenome and give a snapshot of community metabolic activities at the moment of sampling. The characterization of a proteome can be accomplished by interpreting mass-spectrometry-based peptide sequencing using data derived from 16S ribosomal ribonucleic acid (rRNA) gene sequencing. In environmental metaproteomics, a predicted protein database constructed from metagenomic information of the exact same sample is required to assign peptide sequence information to proteins from which the peptides were derived.

Metagenomic and metaproteomics are cutting-edge environmental microbiological tools that are rapidly developing. With the availability of metagenomic sequences and the increasing number of complete individual bacterial and archaeal genome sequences, it is now possible to apply postgenomic techniques (particularly proteomics) to complex microbial communities. Combined, these powerful tools provide a capability to reveal the presence of specific proteins within the microbial community to provide direct evidence of specific pathways involved in the degradation of contaminants.

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4.0 PERFORMANCE ASSESSMENT

The site selected for this demonstration is the 22 Area Marine Corps Exchange (MCX) Gas Station site, located at Marine Corps Base (MCB) Camp Pendleton, San Diego, California. The treatment system, consisting of a set of two biosparging biobarriers, was installed in 2004. Each barrier was comprised of a number of sparging wells used to inject oxygen into the aquifer. During operation of these biobarriers (from 2004 through 2010), MTBE concentrations in groundwater declined significantly such that only dilute levels of MTBE (i.e., 5 micrograms per liter [µg/L] to 40 µg/L) remained. In 2010 and 2012, regulatory agencies agreed to discontinue operation of the mid-plume and leading-edge biobarrier, respectively. However, since low-levels of MTBE still existed at the site that exceeded the State of California's secondary maximum contaminant level (MCL) for MTBE (5 µg/L), the site was transitioned to MNA after shutdown of the biobarriers. The technical approach for the evaluation at 22 MCX Area Gas Station was designed to test two specific hypotheses including:

1. Current microbial activity supports degradation of the remaining MTBE dissolved in groundwater, indicating that MNA is occurring.
2. Formation permeability within the ROI of the biobarriers decreased over time due to biofouling as a direct result of injecting oxygen into the aquifer.

Historical data combined with analytical results from two rounds of sampling performed during this demonstration were used to test the first hypothesis. The data collected consisted of results from conventional chemical (i.e., contaminant of concern [COC]) concentrations and geochemical analyses (i.e., groundwater quality, anions/cations, etc.) and with metagenomics and metaproteomics. The second hypothesis was tested by performing several slug tests to assess the long-term impact of the biobarrier system on formation permeability and comparing the results to historical data measured before the biobarrier system was in operation.

To provide for a more comprehensive study, a decision was made to perform supplemental sampling and analysis at 13 Area Gas Station site, located approximately 2.5 miles from the 22 Area MCX Gas Station site. This second site, at which a soil vapor extraction and biosparging system is currently in operation to treat high concentrations of MTBE, was used as a positive control to allow for a comparison of measured metagenomic and metaproteomic results to assess where natural attenuation presumably is occurring to treat low residual concentrations.

To test the first hypothesis described above, a tiered approach was used to evaluate MNA at the 22 Area MCX Gas Station that relied on multiple, converging lines of evidence. This evaluation included evaluating contaminant concentrations (primary line of evidence) and geochemical trends (secondary line of evidence), as well as demonstrating and validating metagenomic and metaproteomic methods to determine MTBE degradation microbiology and activity at the site (tertiary line of evidence for MNA). The second hypothesis was tested by performing slug tests at several wells located at the 22 Area MCX Gas Station and comparing the results to similar data collected before the biobarriers were installed and in operation.

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5.0 COST ASSESSMENT

Metageonomics and metaproteomics are two innovative techniques that have the potential to provide robust lines of evidence that degradation of COCs at a site continue to occur after an active remedy has been applied. At present, these techniques serve to augment conventional data, but may not be able to replace and/or reduce the frequency of application of conventional techniques at this time. The cost for these analyses for this demonstration were $350 and $1,800 per sample for the metagenomics and metaproteomic analyses, respectively, based on analysis of a batch of 7 samples. Cost for metagenomic analysis is not anticipated to decrease as the quantity of samples increases; however, the cost for the proteomic analysis will decrease as the number of samples increases. For instance, had 50 samples been analyzed during this demonstration, the resulting cost per sample would have been $750. It is expected as some of the implementation issues are overcome, including lack of widespread regulatory acceptance, lack of commercial laboratories that perform these types of analyses, techniques are refined, and confidence in the data improves, it is expected that cost will decrease substantially.

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6.0 IMPLEMENTATION ISSUES

This project focused on using advanced MBTs that were used to facilitate assessment of MNA. The advanced MBTs and conventional groundwater analyses (contaminant concentration and geochemistry) used are commonly employed for these types of assessments and implementation issues are well understood. The primary end users of advanced MBTs are expected to be DoD site managers and their contractors, consultants and engineers. The general concerns of these end users are likely to include the following: (1) regulatory acceptance; (2) insufficient confidence in results and access to specialized laboratories; and (3) technology cost compared to other more conventional monitoring options. These implementation issues are addressed in the following sections.

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