FACT SHEET

Environmental Molecular Diagnostics (EMDs): Molecular Biology-Based Tools

Introduction

Environmental molecular diagnostics (EMDs) are a group of advanced techniques used to analyze biological and chemical characteristics of environmental samples. EMDs facilitate decision making throughout the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) process (Figure 1) and at other appropriate cleanup sites. This fact sheet focuses on biological tools for use in environmental restoration (ER) applications including: quantitative polymerase chain reaction (qPCR), metagenomic sequencing of the 16S ribosomal ribonucleic acid (16S rRNA) gene, whole genome sequencing (WGS), and proteomics. A companion fact sheet focuses on EMDs using chemical-based tools.

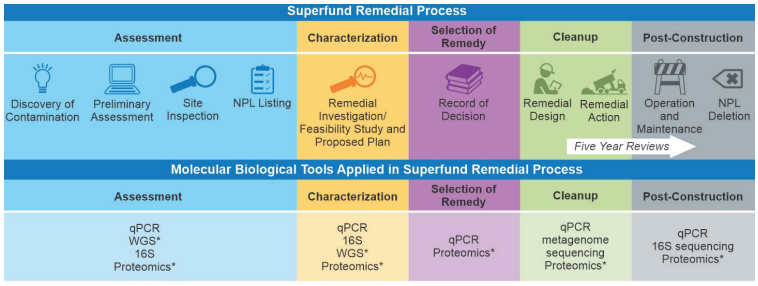

Figure 1. CERCLA Process (Courtesy of Battelle)

Technology Background

EMDs based on biological techniques rely on the analyses of genetic material (deoxyribonucleic acid [DNA], ribonucleic acid [RNA]) or compounds produced by microbes.

qPCR: PCR is a technique used to amplify (makes copies of) specific genes of microorganisms to levels that can be further analyzed. qPCR is a quantitative, targeted technique used to assess concentrations of specific DNA or RNA sequences in a sample. The qPCR method relies upon a fluorescent dye that binds to a certain fragment of DNA or RNA and can be correlated to the number of genes present. This technique requires prior knowledge of the microbes or metabolic pathways of interest. The qPCR method has been applied commercially to detect genes encoding enzymes capable of degrading chlorinated solvents, benzene, toluene, ethylbenzene, and xylene (BTEX), 1,4-dioxane, and other contaminants of concern (COCs). With the use of qPCR microarrays, multiple genes can be screened at once in a given sample.

16S rRNA Sequencing: The gene or nucleotide sequence that codes for 16S rRNA is present in all microbes because it is required in protein synthesis. Evolution has resulted in differences in the genetic sequence of the 16S rRNA gene across microorganisms. Metagenomic sequencing is used to study variations in the genes of microorganisms. Metagenomic sequencing of 16S rRNA genes can be used to identify microbes at the genus level and to provide a snapshot of the microbial community present in a sample.

WGS: The WGS method analyzes all genomic data in a sample providing for species identification and functional gene identification. WGS is an application of next generation sequencing (NGS) where the entire DNA sequence is determined instead of a targeted portion. WGS requires no cultivation and is helpful in environments where microorganisms or gene functions are not well known. This technique is available primarily from academic or specialized laboratories and requires expertise in bioinformatic analyses of sequencing data.

Proteomics: Proteomics provides direct information on microbial metabolism by measuring the composition and quantity of proteins produced. Proteins are extracted, purified, and analyzed using advanced mass spectrometry instrumentation. Specialized computational tools are then applied to identify and quantify proteins and/or peptides in the sample. Once specific proteins are quantified (e.g., reductive dehalogenases involved in degradation of chlorinated ethenes), their concentrations may provide evidence of degradation processes ongoing at a site. This technique is an emerging technology that is mostly available from academic laboratories.

Use of EMDs in the CERCLA Process

How Does It Work?

These biological EMDs are used to characterize and quantify the changes in microbial communities over time. Various EMDs may be selected for use depending on the needs for the site as described below.

How Can It Help?

These EMDs provide multiple lines of evidence to help make decisions throughout the CERCLA process to:

- ☐ Identify if biodegradation is occurring under natural conditions or if intervention is required.
- ☐ Determine microbial activities and presence to track degradation over time and ensure long-term efficiency.
- ☐ Investigate potential causes of degradation stall.
- ☐ Evaluate if re-application of additional electron donor or microorganisms is necessary.
- ☐ Identify correlations to changes in groundwater geochemistry (e.g., shift to methanogenic conditions and the increase in *vcrA* genes).

What CERCLA Questions Does Each Tool Answer?

Table 1 provides examples of information generated by the application of these biological EMDs within the CERCLA framework.

qPCR	16S rRNA Sequencing	WGS	Proteomics
Site Characterization			
• Are COC-degrading microorganisms present in sufficient quantity? • Is biodegradation naturally occurring?	• Are COC-degrading microorganisms present? • Can biodegradation of COCs occur under existing site conditions?	• What microbial communities are present? • Are microorganisms capable of degrading COCs? • Which biodegradation pathways are these microorganisms able to facilitate?	• What proteins are present and are the concentrations high enough to allow for efficient COC degradation? • What biodegradation processes may be occurring?
Remediation			
• Are target microbes/genes present in sufficient quantity to support robust remediation? • Is bioaugmentation and/or biostimulation necessary?	• Did amendment(s) alter the composition of the microbial community to favor the desired biodegradation reactions? • Can microbial presence/abundance explain potential degradation stall? • Is bioaugmentation and/or biostimulation necessary?	• Did amendment(s) alter the composition of the microbial community or genes present? • Can microbial presence/abundance explain potential degradation stall? • Is bioaugmentation and/or biostimulation necessary?	• Are target proteins present in sufficient quantity to support robust remediation? • Can protein levels explain potential degradation stall? • Is bioaugmentation and/or biostimulation necessary?
Long-Term Monitoring			
• Are target microbes/genes present in sufficient quantity to support robust remediation over time?	• Are COC-degrading microorganisms present over time?	• How does the composition of the microbial community change over time?	• Are proteins present in sufficient quantities to support robust remediation or natural attenuation over time?

Table 1. CERCLA Questions

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CASE STUDY 1

qPCR and 16S Sequencing at Naval Air Station (NAS) Jacksonville

Project Objective: The qPCR and 16S sequencing methods were employed to monitor the performance of enhanced in situ bioremediation over a two-year timeframe. The results were used to determine the presence of functional genes and changes in the microbial composition in groundwater as a result of both bioaugmentation and biostimulation.

Site Background: The NAS Jacksonville site in Florida occupies 134 acres and had a former dry cleaning facility within the property as well as two buildings that were sources of chlorinated volatile organic compound (cVOC) contamination. Over 50 direct push technology (DPT) injection locations were used to apply 145,000 gallons of 0.7% emulsified vegetable oil (EVO). The EVO was injected along with 100 liters of KB-1 and KB-1 plus for both biostimulation and bioaugmentation. Chloroethenes and methane were tracked over two years and qPCR and 16S sequencing were performed on samples from upgradient wells (control) compared to the downgradient pilot test wells (Geosyntec, 2016).

Results: The chemical analyses revealed that tetrachloroethene (PCE), trichloroethene (TCE), and cis-dichloroethene (cDCE) in groundwater were degraded over the course of two years (Figure 2). In tandem, the concentration of the *vcrA* gene, as assessed by qPCR, increased by three orders of magnitude over time (Figure 2). The composition of the microbial community was altered with the addition of the carbon source and microbial cultures. *Dehalococcoides* and *Geobacter* increased in relative abundance from 0.01% and 0.2% to 5% and 24%, respectively (Table 2 and Figure 3). These results indicate that the bioaugmentation procedure was effective.

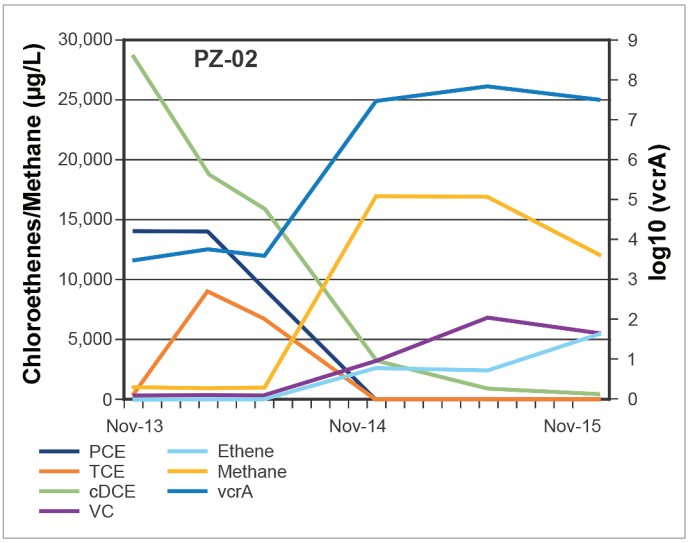

Figure 2. cVOC and qPCR Data Results from NAS Jacksonville Site (Courtesy of RITS, 2020)

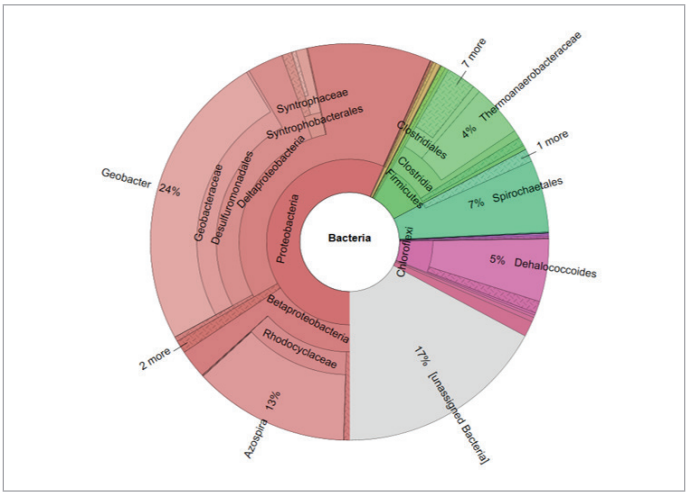

Figure 3. Relevant 16S rRNA Sequence Data Results for Pilot Test Well from NAS Jacksonville Site (Courtesy of RITS, 2020)

	% of Bacteria	
	Upgradient Well MW-40S	**Pilot Test Well PZ-02**
Dehalococcoides	0.01%	5%
Geobacter	0.2%	24%
Methylobacter	9%	9%

Table 2. Relevant 16S rRNA Sequence Data Results from NAS Jacksonville Site (Geosyntec, 2016)

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CASE STUDY 2

Metagenomic Sequencing and Proteomics at Marine Corps Base (MCB) Camp Pendleton

Project Objective: Metagenomic sequencing and proteomics were employed to monitor microbial composition and metabolic activity at two methyl tert-butyl ether (MTBE)-contaminated sites at MCB Camp Pendleton in California. The purpose was to determine if MTBE-degrading microorganisms were present at each site and if evidence could be found of ongoing MTBE biodegradation in groundwater.

Site Background: MCB Camp Pendleton 13 Area Gas Station served as a "positive control" with high MTBE concentrations at >40,000 µg/L and an ongoing air sparging system to stimulate aerobic biodegradation processes. The MCB Camp Pendleton 22 Area Gas Station was the main study site with low MTBE concentrations at <10 µg/L and served as the site of a former biobarrier system. The biobarrier operations and oxygen injection ceased in 2012. Samples for EMD analyses were collected at key locations throughout each MTBE plume at both sites (see Figure 4).

Results: The 16S sequencing revealed that MTBE direct mineralizing microorganisms and co-metabolic degraders were present (Figure 4). For the 13 Area Gas Station site with ongoing air sparging, proteomic data revealed active metabolism associated with MTBE degradation and known MTBE degraders. The metaproteomic data showed that 7 out of 10 known proteins involved in the direct mineralization of MTBE were present in the groundwater samples taken from the 13 Area Gas Station. However, only proteins for cometabolic MTBE degradation were identified at the 22 Area Gas Station site where oxygen injection had ceased (ESTCP, 2017).

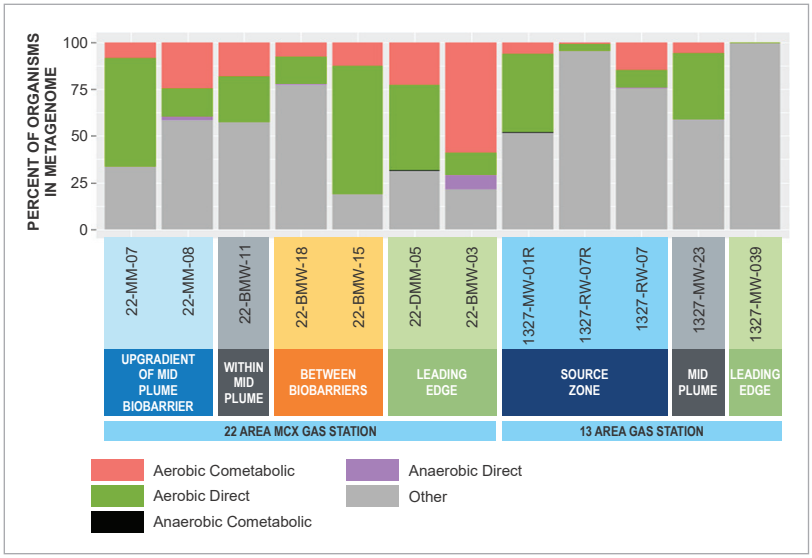

Figure 4. Relative Abundance of Microorganisms with MTBE-Degrading Metabolisms in MCB Camp Pendleton Samples for 22 Area and 13 Area Sites (Courtesy of Battelle)

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