EMD Sampling Methods

EMD Team Fact Sheet—November 2011

This fact sheet, developed by the ITRC Environmental Molecular Diagnostics (EMD) Team, is one of 10 designed to provide introductory information about and promote awareness of EMDs. Please review the Introduction to EMDs Fact Sheet along with this one. A glossary is included at the end of this fact sheet.

What EMD sampling methods are used?

Various active and passive microbial sampling methods have been developed to collect microorganisms from an environment (typically groundwater) for analysis using EMDs. **Active** microbial sampling methods are used to collect a grab sample of the microbial community from a particular point in time. **Passive** microbial sampling devices provide a time-integrated sample of the microbial community. Both methods, when combined with EMDs, can be used for assessment of monitored natural attenuation (MNA) and evaluation of enhanced bioremediation alternatives.

How are the data used?

Microbial sampling devices are versatile platforms that can be used in conjunction with a broad spectrum of EMDs, including the following, each of which is described in more detail in other fact sheets:

- quantitative polymerase chain reaction (qPCR and RT-qPCR)
- microbial fingerprinting methods, such as phospholipid fatty acids (PLFA) analysis, denaturing gradient gel electrophoresis (DGGE)
- microarrays
- compound specific isotope analysis (CSIA)
- stable isotope probing (SIP)

Selecting microbial sampling methods and subsequent EMD analyses depends on the site-specific questions that need to be addressed. For example, an appropriate microbial sampling method can be paired with qPCR to quantify known key microorganisms capable of biodegradation of a contaminant of interest to assess MNA.

Active microbial sampling methods are widely used when collecting grab samples for EMD analysis. These sampling methods are similar to traditional soil and groundwater sample collection for volatile organic compound (VOC) analyses (e.g., low-flow groundwater sampling with peristaltic pumps). Since samples are collected from single points in time, the data are representative "snapshots" of the microbial community. Thus, multiple sampling events are typically used to describe how microbial conditions vary over time. The same is also true of sampling for chemical and geochemical parameters. Typically, samples are collected quarterly or annually from selected groundwater monitoring wells as they are for chemical or geochemical analyses. For example, *Dehalococcoides* analyses are quantified as cells per milliliter before, during, and after bioremediation treatment to evaluate system performance.

Passive microbial sampling devices are incubated within the sampled environment for several weeks (typically 30–90 days) and depend on the formation and collection of biofilms that grow on or within a solid matrix. Thus, the passive microbial samplers provide a more time-integrated sample of microorganisms from the sampled environment. Passive microbial sampling devices can be amended with potential remediation amendments (e.g., electron donors, electron acceptors, etc.) and/or microbial cultures of known degraders. These amended passive microbial sampling devices, combined with EMD analysis, have been used to evaluate biostimulation and bioaugmentation as remediation strategies. If the passive microbial sampler contains an adsorptive surface, such as activated carbon, the sampler can be amended with a specially synthesized form of the contaminant (e.g., VOC) containing "heavy" stable carbon (^{13}C) isotope as a label. Since ^{13}C is relatively rare, carbon originating from labeled contaminant can be readily distinguished from carbon (predominantly ^{12}C) from other sources (see the SIP Fact Sheet for additional information). During in-well deployment, the ^{13}C-labeled contaminant is subject to the same

physical, chemical, and microbiological processes as the unlabeled contaminant present at the site. For many contaminants (e.g., benzene, methyl *tert*-butyl ether), biodegradation is a process whereby microorganisms use the contaminant as a carbon and energy source producing new cells (biomass) and carbon dioxide. Thus, if biodegradation is occurring during field deployment, the ^{13}C label from the synthesized contaminant in the passive microbial sampling device will be incorporated into the end products of biodegradation: microbial biomass and dissolved inorganic carbon (HCO_3^- and CO_2). Upon recovery of the passive microbial sampling device and subsequent EMD analysis, incorporation of the ^{13}C label into biomolecules (DNA or PLFA) and dissolved inorganic carbon provides evidence of in situ biodegradation. Figure 1, an example of SIP, illustrates the process. Here the passive microbial sampling device is a Bio-Trap™ in which the solid matrix is Bio-Sep®. This matrix contains powdered activated carbon to which ^{13}C-labeled compounds can be tightly adsorbed prior to incubation in groundwater.

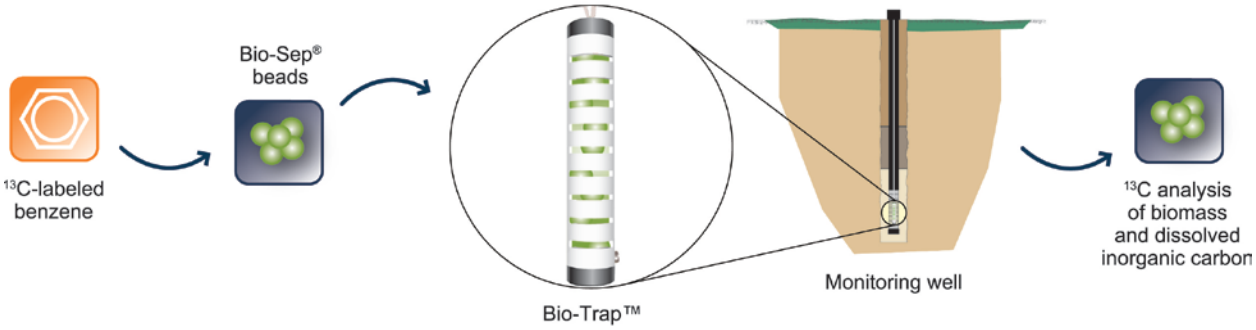

Figure 1. Illustration of stable isotope probing with a Bio-Trap™.
Source: Microbial Insights, Inc., 2010, used with permission.

Both active methods and passive devices are easy to use and are useful tools for microbial sampling and supporting remedial investigation and design efforts.

How does it work?

Descriptions for how both active sampling methods and passive sampling devices work in conjunction with EMDs are presented separately below.

Active Microbial Sampling Methods—For practical reasons, active sampling for EMDs at remediation sites generally focuses on groundwater. The focus on groundwater is justified for the analysis of targets like *Dehalococcoides* that are found in the aqueous phase (e.g., planktonic microbial cells which grow in a suspended state in an aqueous environment as opposed to attached to a surface). Various active microbial sampling approaches are available for collection of biomass from environmental media, ranging from commonly used peristaltic pumps for groundwater sampling to direct-push coring or split-spoon sampling for soils that incorporate aseptic techniques for collecting microbial samples. Until recently, groundwater samples were typically collected and sent to a laboratory for biomass extraction. However, based on field trials conducted as part of the Environmental Security Technology Certification Program (ESTCP) Project ER-0518 and guidance from commercial vendors, field filtration is recommended for collection of biomass from groundwater (Lebrón et al. 2011, Ritalahti et al. 2010). Field filtration increases the likelihood of collecting suspended particles, decreases shipping costs, and significantly reduces the costs associated with laboratory extraction procedures. Whether sending samples to a laboratory for biomass extraction or using the field filtration approach, the active sampling methods enable analysis of virtually all of the biomass (alive, dead, and dormant) within the sample.

Passive Microbial Sampling Devices—When sampling groundwater, passive microbial sampling devices typically consist of a solid matrix as a surrogate for aquifer material within a slotted or otherwise permeable housing. Although a number of solid matrix materials have been used (e.g., sterilized sand, glass or ceramic beads, glass wool, granular activated carbon), Bio-Trap samplers are commonly used

and commercially available passive microbial sampling devices. Bio-Traps contain Bio-Sep beads, a composite of Nomex® and powdered activated carbon (PAC), as the solid matrix. Nomex allows beads to be heat sterilized prior to in-well deployment, while the PAC provides adsorptive properties and a large surface for microbial growth. When sampling groundwater, passive microbial sampling devices are typically deployed in an existing monitoring well for 30–90 days. During in situ deployment, active microorganisms grow on and/or within the solid matrix similar to biofilm formation on native aquifer materials. Once recovered from the well, DNA, RNA, or phospholipids can be readily extracted from the solid matrix for analysis by the EMD methods to characterize the subsurface microbial community. If the solid matrix contains activated carbon, organic aquifer contaminants will adsorb to the matrix during incubation and may also be extracted for VOC/semivolatile organic compound analyses or CSIA.

The solid matrix in passive microbial sampling devices is not a perfect surrogate for the aquifer material; thus, the microbial community colonizing the surface or interior of this solid matrix may not perfectly reflect the community composition of the aquifer.

Advantages of Active Microbial Sampling Methods

- Active microbial sampling methods can be easily integrated into existing site monitoring programs since the sample collection techniques are comparable (e.g., low-flow groundwater sampling from monitoring wells).
- Since actual environmental media (e.g., soil and groundwater) are collected and biomass extraction/filtration methods have become highly efficient, the resulting EMD data are considered to represent in situ conditions at the time of sampling relatively well.
- Field filtration increases the likelihood of collecting suspended particles, decreases shipping costs, and significantly reduces costly laboratory extraction procedures.

Limitations of Active Microbial Sampling Methods

- Active microbial sampling devices give a “snapshot” of the microbial community; therefore, periodic sampling is required to evaluate variations over time.
- Active microbial sampling is targeted at collection of site media samples only and does not allow for in situ assessments (e.g., in-well SIP or treatability studies).
- Filters can clog during sampling, which would limit the sample size and potentially reduce the representativeness of the sample.
- Active sampling methods may use sterilized materials and aseptic techniques, requiring additional training for field personnel.

Advantages of Passive Microbial Sampling Devices

- Passive microbial sampling devices are relatively easy to deploy and recover.
- Passive sample collection over an extended period of time may be more representative of actual subsurface conditions compared to single, “snapshot” grab-sample collection of a microbial community.
- EMD results based on passive microbial sampling devices can reflect temporal changes in aquifer microbial community composition that cannot always be discerned from analysis of groundwater samples.
- Passive microbial sampling devices can be amended with potential remediation amendments (e.g., electron donors or electron acceptors) or microbial cultures to evaluate treatment alternatives.
- Passive microbial sampling devices that contain activated carbon have been used for SIP studies to provide evidence of in situ biodegradation potential of a contaminant by indigenous microorganisms under actual aquifer conditions.
- Passive microbial sampling devices that contain activated carbon can concentrate contaminants for CSIA.

- Certain passive sampling media, such as Bio-Sep, collect only organisms that are actively reproducing under local aquifer conditions.

Limitations of Passive Microbial Sampling Devices

- Passive microbial sampling devices typically require 30–90 days of incubation in the sampled environment and require two mobilizations to the site to install and then retrieve the sampling devices.
- The solid matrix of most passive microbial sampling devices is a surrogate; thus, differences may exist between organisms colonizing the sampling device and native aquifer material.
- Regulatory approval may be required to deploy amended sampling devices, depending on the amendment and the applicable regulations.
- Data cannot be normalized to a unit volume of groundwater.

Sampling Protocols

Active microbial sampling involves biomass extraction/filtration from environmental media samples. Based on field trials conducted as part of ESTCP Project ER-0518 and guidance from commercial vendors, field filtration is recommended for collecting biomass from groundwater. A field filtration approach involves low-flow groundwater purging and sampling from monitoring wells, using the same methods that are generally recommended when sampling for VOCs. Representative groundwater is passed through a filter (e.g., Sterivex™), which isolates biomass from the sample. The filter is then shipped overnight on ice to a laboratory for analysis. A guidance protocol is available under ESTCP Project ER-0518 (Lebrón et al. 2011; Petrovskis, Amber, and Walker, in press), which provides a step-by-step approach to groundwater sampling using field filtration methods.

Passive microbial sampling devices are typically deployed in purged groundwater monitoring wells located within and upgradient of the dissolved contaminant plume to compare results of analyses between impacted and background conditions. Comparing the impacted area to a background control clearly illuminates the effect of a contaminant on the groundwater community. A typical in-well incubation period is 30–90 days. Following in well deployment, samplers are recovered and shipped overnight on ice for analysis. If recovered passive microbial sampling devices have been frozen, it is important that they not thaw in route to the laboratory for analysis.

Users of all types of microbial sampling devices should work with the analytical laboratory to ensure that sampling protocols for collecting, handling, and transporting the samples are in place and understood.

Quality Assurance/Quality Control

Commercial filters for active sampling and passive microbial sampling devices are assembled under sterile conditions and shipped in sterile bags. Following deployment both types of samplers should be shipped cold by overnight delivery to their respective locations for analysis. Currently, users can best ensure data quality by detailing the laboratory requirements in a site-specific quality assurance project plan (QAPP). This plan should include identification of the sampling devices and procedures being used; the field locations and procedures, including preservation requirements; the EMDs being used; the standard operating procedures of the laboratory performing the analyses; and any internal quality assurance/quality control information available (such as results for positive and negative controls).

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Glossary

bioaugmentation—The introduction of cultured microorganisms into the subsurface environment for the purpose of enhancing bioremediation of organic contaminants (EPA 2011).

biodegradation—A process by which microorganisms transform or alter (through metabolic or enzymatic action) the structure of chemicals introduced into the environment (EPA 2011).

biostimulation—A remedial technique which provides the electron donor, electron acceptor, and/or nutrients to an existing subsurface microbial community to promote degradation.

Dehalococcoides—A specific group (genus) of bacteria. *Dehalococcoides* species are the only microorganisms that have been isolated to date which are capable of complete reductive dechlorination of PCE and TCE to ethene. Some *Dehalococcoides* species are also capable of reductive dechlorination of chlorinated benzenes, chlorinated phenols, and polychlorinated biphenyls (PCBs).

DNA (deoxyribonucleic acid)—A nucleic acid that carries the genetic information of an organism. DNA is capable of self-replication and is used as a template for the synthesis of RNA. DNA consists of two long chains of nucleotides twisted into a double helix (EPA 2004).

electron acceptor—A chemical compound that accepts electrons transferred to it from another compound (based on EPA 2011).

electron donor—A chemical compound that donates electrons to another compound (based on EPA 2011).

phospholipid—A type of biomolecule that is a primary structural component of the membranes of almost all cells.

PLFA—Phospholipid fatty acids derived from the two hydrocarbon tails of phospholipids.

RNA (ribonucleic acid)—Single-stranded nucleic acid that is transcribed from DNA and thus contains the complementary genetic information.

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