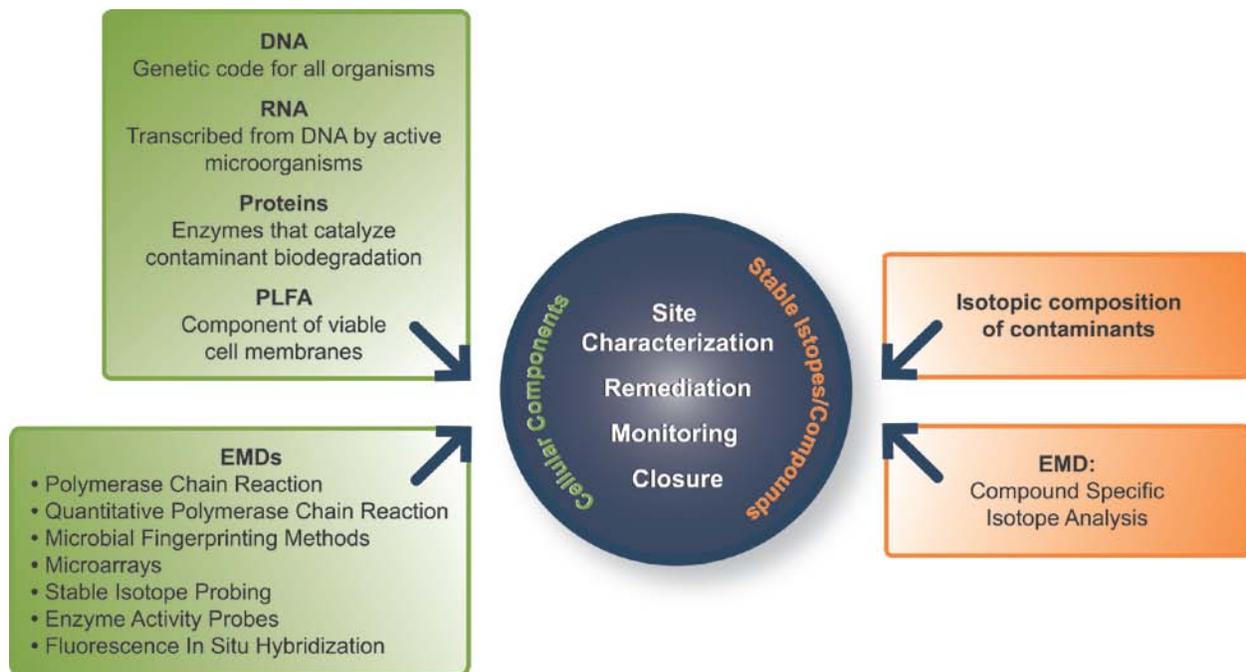


This fact sheet, developed by the ITRC Environmental Molecular Diagnostics (EMD) Team, is one of 10 designed to provide introductory information about and promote awareness of EMDs. The following is an introduction to a selection of EMDs applicable to environmental management, including site characterization, remediation, monitoring, and closure. A glossary is included at the end of this fact sheet.

## What are environmental molecular diagnostics?

“Environmental molecular diagnostics” is a collective term that describes a group of advanced and emerging techniques used to analyze biological and chemical characteristics of soils, sediments, groundwater, and surface water. Many of these tools were originally developed for applications in medicine, defense, and industry. However, over the last decade, great advances have been made in adapting and applying EMDs for site characterization, remediation, monitoring, and closure. EMDs are important and valuable because they can provide key information not available using traditional analytical methods (e.g., groundwater analysis for volatile organic compounds). While they are intended to complement these traditional methods, EMDs can bring a new perspective to all stages in the environmental management decision-making processes. Figure 1 illustrates the connection between the stages of the environmental site management process and the EMDs presented in this and the other fact sheets.



**Figure 1. Environmental molecular diagnostics.**

The EMDs discussed in these fact sheets fall into two distinct categories: compound specific isotope analysis (CSIA), which analyzes the isotopic composition of contaminants, and various methods that analyze biomolecules.

Generally speaking, CSIA measures the amounts of stable isotopes (typically carbon, hydrogen, or chlorine) in contaminants to determine the extent of specific chemical and biochemical reactions impacting the contaminant. As a contaminant degrades through natural or engineered processes, the relative amount of each stable isotope in the contaminant can change. In contrast, the isotopic composition of contaminants is largely unaffected by processes such as dilution that do not result in

degradation of the contaminant. Questions pertaining to a chemical's source, degradation mechanism, and rate of degradation can be answered, supported, and resolved through CSIA.

In contrast, molecular biology-based EMDs are used to determine the biochemical capabilities of microorganisms present in the environment. In many cases, specific microorganisms are responsible for the degradation of specific contaminants. Some molecular biology-based EMDs can be used to detect and quantify these known microorganisms. Other molecular biology-based EMDs can be used determine whether microorganisms are actively degrading specific contaminants and can also identify currently unknown microorganisms involved in these processes. Questions pertaining to biochemical capabilities and activities of microorganisms and changes in microbial population sizes in natural and engineered environments can be answered, supported, and resolved through these types of analyses.

### Why use EMDs?

The environmental management process typically involves site characterization, remediation, monitoring, and closure. A variety of chemical and biological analyses (e.g., analyses for contaminants, geochemical parameters, heterotrophic plate counts) have been developed and are widely accepted as the means to understand environmental and biological conditions at each of these stages. However, characterization and remedial evaluation is best based on converging lines of chemical, geochemical, and microbiological evidence. EMDs provide useful new analytical techniques intended to complement those already in use.

For example, traditional chemical analyses of contaminants by gas chromatography or gas chromatography coupled with mass spectrometry accurately quantify the amount of each contaminant but provide little or no information about the isotopic composition of the contaminants. The ability to measure stable isotopes is important because the relative amounts of each stable isotope in contaminants often change in different and predictable ways in response to biological and physical processes. The isotopic analysis of contaminants using CSIA can therefore answer otherwise irresolvable questions about a contaminant's source, its rate of degradation, and the underlying degradation mechanism. Similar benefits can also be obtained from applying EMDs to biomolecules. Traditionally, cultivation-based methods like heterotrophic plate counts or most-probable-number analyses have been used to estimate numbers of microorganisms and the biodegradation potential of a site (Fredrickson and Balkwill 1998). Unfortunately, the overwhelming majority (>99%) of microorganisms cannot be grown in a laboratory; therefore, cultivation-based methods can dramatically underestimate the size and composition of microbial communities (Amann, Ludwig, and Schleifer 1995 and references therein). The EMDs discussed here can be used to identify and quantify key microorganisms, enzymes, and/or genes involved in specific biodegradation processes without growing microorganisms in the laboratory. These analyses can therefore answer previously unanswerable questions about the potential of sites to support biological remediation processes and the effectiveness of existing remediation approaches.

While traditional techniques are still useful and often appropriate, EMDs can provide an unprecedented level of reliability, efficiency, and precision. Each EMD has advantages and disadvantages that must be considered, and the various EMDs offer more direct, comprehensive, and accurate assessment of contaminant degradation. The EMD results, along with contaminant concentration trends and geochemical information, provide consultants, regulators, and stakeholders with knowledge and information to make informed decisions.

### Example Environmental Remediation Questions EMDs Can Help Answer

- **Site Characterization**
  - Are multiple sources contributing to a groundwater plume?
  - Is biodegradation occurring?
- **Remediation**
  - Is monitored natural attenuation (MNA) feasible?
  - Is biodegradation already occurring? Will it continue in the future?
  - Is complete degradation occurring? Will there be by-products that require remediation?
  - Is biostimulation necessary? Should an electron donor or acceptor be added?
  - Is bioaugmentation necessary? What microorganisms need to be added?
- **Monitoring**
  - Is the chosen remedial technology performing as designed?
  - Did electron donor or acceptor addition promote growth or activity of contaminant-degrading microorganisms?
  - Are bioaugmentation microorganisms surviving in situ?
  - Are contaminant-degrading communities maintained over time under existing site conditions?
  - Will concentrations decrease to remedial objectives?
- **Closure**
  - Is contaminant degradation likely to continue?

The EMDs detailed in the other fact sheets in this series include CSIA, polymerase chain reaction (PCR), quantitative PCR (qPCR), microbial fingerprinting methods, microarrays, stable isotope probing (SIP), enzyme activity probes (EAPs), and fluorescence in situ hybridization (FISH). Lastly, a fact sheet pertaining to EMD sampling methods is also provided. Table 1 summarizes the underlying principle and purpose of each EMD.

**Table 1. Description of EMDs**

EMD	Principle	Purpose
Compound specific isotope analysis	Analyzes the relative abundance of various stable isotopes (e.g., $^{13}\text{C}/^{12}\text{C}$ , $^2\text{H}/^1\text{H}$ ) of the component elements of contaminants (e.g., trichloroethene [TCE]). Degradation processes can cause measureable shifts in the isotopic ratios.	<ul style="list-style-type: none"> <li>• Determine whether contaminant degradation is occurring.</li> <li>• Investigate the degradation mechanism.</li> <li>• Identify contaminant source.</li> </ul>
Polymerase chain reaction	Amplifies (makes copies of) the genetic material of microorganisms to levels that can be further analyzed using other techniques.	<ul style="list-style-type: none"> <li>• Detect microorganisms or target genes responsible for contaminant biodegradation.</li> <li>• Process genetic material for use in other EMDs.</li> </ul>
Quantitative polymerase chain reaction	Quantifies a target gene based on DNA or RNA.	<ul style="list-style-type: none"> <li>• Quantify the abundance and expression of specific functional genes, microorganisms, or groups of microorganisms responsible for contaminant biodegradation.</li> </ul>
Microbial fingerprinting methods	Differentiates, and in some cases identifies, microorganisms by unique characteristics of universal biomolecules, including phospholipid fatty acids (PLFA) and nucleic acids (DNA and RNA).	<ul style="list-style-type: none"> <li>• Provide a profile of the microbial community.</li> <li>• Identify a subset of the microorganisms present.</li> <li>• Quantify living biomass.</li> </ul>
Microarrays	Detects and estimates the relative abundances of hundreds to tens of thousands of genes simultaneously.	<ul style="list-style-type: none"> <li>• Provide a comprehensive evaluation of the microbial diversity and community composition.</li> </ul>

EMD	Principle	Purpose
Stable isotope probing	Detects the presence of an added synthesized form of the contaminant containing a stable isotope (e.g., $^{13}\text{C}$ ). If contaminant biodegradation is occurring, the isotope will be detected in biomolecules (e.g., phospholipids, DNA) and metabolites (e.g., $\text{CO}_2$ ).	<ul style="list-style-type: none"> <li>Determine whether biodegradation of a specific contaminant is occurring.</li> <li>Identify the microorganisms responsible for this activity.</li> </ul>
Enzyme activity probes	Detect the transformation of surrogate compounds that resemble specific contaminants.	<ul style="list-style-type: none"> <li>Quantify the activity of microorganisms with specific biodegradation capabilities.</li> </ul>
Fluorescence in situ hybridization	Detects the presence of targeted genetic material in an environmental sample.	<ul style="list-style-type: none"> <li>Estimate the number of and/or relative activity of specific microorganisms or groups of microorganisms.</li> </ul>
EMD sampling methods	Active sampling methods (e.g., low-flow groundwater sampling) and passive microbial sampling devices in which subsurface microorganisms colonize a solid matrix.	<ul style="list-style-type: none"> <li>Methods for collection of biomass from environmental media to be used in conjunction with EMDs.</li> </ul>

EMDs differ in the level of information they provide, the type of data they provide, costs, and availability. As of the writing of these fact sheets in 2011, the most often used EMDs are CSIA and qPCR. The level of information provided by EMDs can range from the identity of microorganisms (i.e., genus or species), to the potential activity (i.e., Are the microorganisms present capable of performing a desired task?), to the expressed activity (i.e., Are the microorganisms performing the desired task?). The type of data provided can be qualitative or quantitative. The per sample costs can range \$75–\$5,000, and analysis may be available commercially or through university or other research laboratories. Table 2 summarizes these differences between the EMDs.

**Table 2. Comparison of EMDs**

EMD	Identity/potential activity/expressed activity <sup>a</sup>	Quantitative, qualitative (Qa/QI)	Cost range <sup>b</sup> (\$)	Availability <sup>c</sup>
Compound specific isotope analysis	E	Qa	100–2,500	C/R
Polymerase chain reaction	I/P	QI	225–350	WC
Quantitative polymerase chain reaction	I/P/E <sup>d</sup>	Qa	275–425	WC
Microbial fingerprinting methods	I	Qa/QI	300–570	WC
Microarrays	I/P/E <sup>d</sup>	QI	1,250–5,000	C/R
Stable isotope probing	I/P/E	Qa/QI	1,500 and up	C/R
Enzyme activity probes	E	Qa	250–2,500	C/R
Fluorescence in situ hybridization	I/P/E <sup>d</sup>	Qa/QI	250–5,000	C/R
EMD sampling methods	I/P	Qa/QI	75–200	WC

<sup>a</sup> I = identity of microorganisms (i.e., genus or species), P = potential activity (i.e., genetically capable of completing the activity), E = expressed activity (i.e., actually completing the activity at a given time).

<sup>b</sup> Estimated range of per sample costs. The low end of the cost range represents a very restricted analysis and may not be applicable to every site (e.g., for only one compound/target with very limited quality control documentation).

<sup>c</sup> WC = widely commercially available, C = minimally commercially available, R = available through university or other research laboratory.

<sup>d</sup> RNA rather than DNA is used to assess activity.

## When can EMDs be used?

EMDs can be used at environmental cleanup projects affected by a variety of contaminants. These range from volatile organic compounds, such as chlorinated solvents or petroleum constituents, to inorganic metals. Additional applicable contaminants include polycyclic aromatic hydrocarbons, polychlorinated biphenyls, radionuclides, pesticides, explosives, nitrates, perchlorates, and/or chromates. EMDs can be applied to a broad variety of projects because research has been performed to understand the chemical changes and microbial metabolism associated with degradation (Löffler and Edwards 2006, Lovely 2003, Watanabe 2001, Watanabe and Hamamura 2003). Each of the individual fact sheets includes additional information and references.

EMDs can be useful at each stage of environmental management, including site characterization, remediation, monitoring, and closure. Depending on the contaminant, site conditions, and location, different EMDs help answer different management questions. During site characterization, EMDs can determine what microorganisms are present, their relative abundance, what metabolic capabilities and activities are present and occurring, and how contaminants are being degraded. In the case of adjacent contaminant plumes and when combined with other information and analytical results, CSIA can be used to identify sources. During remediation efforts, EMDs are useful in evaluating what approaches are feasible, optimizing remedial strategies, and troubleshooting unsuccessful treatment approaches. EMDs can confirm remediation effectiveness by presenting scientific evidence that biodegradation is occurring, or not. If biodegradation is occurring, some EMDs can be used to estimate the rate of contaminant degradation to determine whether site-specific cleanup goals will be attained within an acceptable time frame. This information may, along with contaminant concentration trends and geochemical information, support closure. It may serve the remedial project well to discuss the use of EMDs with the regulators and the stakeholders, prior to project initiation. This step would provide the opportunity for education and to gain support from involved parties.

### Examples of When EMDs Can Provide New Information

- **Site Characterization**
  - Two neighboring gasoline stations have groundwater releases (plumes) of methyl *tert*-butyl ether (MTBE). In addition to traditional data (e.g., concentration trends, groundwater flow data), CSIA can be used to identify which plume is affecting a downgradient receptor.
- **Remediation**
  - A groundwater plume of chlorinated solvents is being evaluated for MNA. qPCR can be used to quantify whether *Dehalococcoides* microorganisms are present in sufficient number to pursue MNA or active remediation with biostimulation or bioaugmentation will be required.
- **Monitoring**
  - An electron donor was added to stimulate biodegradation of chlorinated solvents (e.g., TCE) in groundwater. qPCR can be used to detect and quantify the genes encoding the enzymes responsible for complete degradation of vinyl chloride to ethene and to monitor the effectiveness of the treatment approach.
- **Closure**
  - A case for MNA of a groundwater gasoline plume is being evaluated for closure. CSIA can be used to estimate the degradation rate of the contaminants and also provide an estimated cleanup time.

## How do EMDs work?

The geochemical and microbiological characteristics of soil, groundwater, or surface water typically change when contaminants are introduced into these environments. As the contaminants degrade either naturally or in response to engineered systems, these geochemical and microbiological characteristics also continue to change, as do the chemical features of the contaminants themselves. The EMDs

discussed in these facts sheets are used to characterize and quantify these changes. For example, CSIA quantifies changes in the natural isotopic composition of contaminants. The molecular biology-based EMDs can quantify changes in the microbial community by determining the numbers of specific microorganisms or specific biochemical reactions at any given time. Specific details pertaining to each of the individual EMDs are found in the applicable fact sheet.

## Who could use EMDs?

Individuals currently using traditional and other analytical techniques as part of environmental management could use EMDs to help create multiple lines of evidence. Typically, this group includes environmental managers (e.g., consultants or regulators) responsible for site characterization and remediation. Samples collected for EMD analysis are sent to a laboratory for analysis. Results are interpreted by the laboratory or the consultant and used to provide a better understanding of environmental conditions. These analyses are documented and presented to regulators and stakeholders via reports and presentations.

There exists an increasing potential for regulators to be called on to review results from EMDs as part of routine reports provided by environmental consultants. One of the primary reasons for developing these fact sheets is to provide regulators with an understanding of EMDs to make more informed decisions. These fact sheets also serve to provide stakeholders with background information, basic technical knowledge, and examples of applicability for EMDs. Community stakeholders may be presented with EMD results as part of public meetings and outreach programs.

## Sampling Protocols

Most sample matrices (soil, sediment, groundwater, in-field filters) can be collected for EMD analyses. Sampling procedures for EMDs are straightforward and, in most cases, can be readily integrated into routine monitoring programs. The following items are typical requirements for microbiological sampling: (a) use of aseptic sample collection techniques and sterile collection containers, (b) shipment of the samples to the laboratory within 24 hours of collection, and (c) maintenance of the samples at an appropriate temperature during handling and transport to the laboratory, usually 4°C. Sample collection techniques and containers may vary depending on the matrix sampled and the laboratory analyzing the samples. Users should work with the analytical laboratory to ensure sampling protocols for collecting, handling, and transporting the samples are in place and understood. In addition, specific active and passive sampling methods have been developed to be used in conjunction with EMDs (see the EMD Sampling Methods Fact Sheet). Although methods may vary according to site-specific conditions, it is important that the sampling protocol for a given site be defined, documented, and maintained for the duration of sampling efforts.

Researchers continue to evaluate the implications for collecting soil or sediment versus groundwater samples for EMD analysis (Amos et al. 2009). However, sampling for EMDs currently focuses on groundwater due to the practical limitations of collecting routine soil or sediment samples. This focus on groundwater is justified for the analysis of microorganisms that are found in the aqueous phase (i.e., planktonic nonattached microorganisms vs. microorganisms that are predominantly attached to the aquifer material [e.g., soils, sediments]). Field filtration is also recommended for collecting biomass from groundwater because field filtration increases the likelihood of collecting suspended particles, it decreases shipping costs, and it significantly reduces the costs associated with laboratory extraction procedures. A guidance protocol providing a step-by-step approach for groundwater sampling using field filtration methods was developed as part of the Environmental Security Technology Certification Program (ESTCP) Project ER-0518 and is available in Lebrón et al. (2011) and Ritalahti et al. (2010).

## How are EMD data reported?

Data collected from each of the EMDs presented in these fact sheets are represented differently. Generally speaking, EMD results can be qualitative or quantitative. Qualitative results include information

regarding the type of microorganisms detected and the presence of targeted genes (e.g., PCR) or the interaction between microbial communities (e.g., FISH). Quantitative results typically include the number of detected cells, gene copies, or other measured parameter per gram of soil or milliliter of water (e.g., qPCR). This is comparable to concentration data provided by traditional chemical analyses. More specifically, CSIA data are reported as the isotopic ratio relative to that in an international standard isotopic reference material.

## Advantages

- Analysis is typically performed directly on the environmental sample. There is usually no need for microbial cultivation, making EMDs more precise and, in most cases, less time-consuming than traditional microbiological methods.
- CSIA provides accurate and reliable information for many environmental management decisions. In addition, CSIA may be used for environmental forensic investigations (e.g., source and age of contamination).
- Molecular biology-based EMDs can evaluate a broad spectrum of microorganisms and biodegradation processes.
- EMDs are sensitive; some can detect as few as 100 cells or genes in a sample or very low chemical concentrations.
- Analytical results are available in a short turnaround time, typically within a couple of days for most EMDs.
- Molecular biology-based EMDs define microbial characteristics such as community, species, and/or activity which can be used to monitor or improve remedial processes.
- EMDs can be performed on a variety of environmental sample types, such as surface water, groundwater, soil, and sediment.
- EMD data can impart a better understanding of contaminant plume heterogeneity, especially for MNA or in situ remedy selection and monitoring.
- EMDs have been shown to be valuable at each stage of environmental management, including site characterization, remediation, monitoring, and closure.

## Limitations

- While some are still maturing, a number of EMDs are well established. Yet, even as some EMDs have become readily available and applied in the field, widespread understanding and acceptance by consultants, regulators, and stakeholders have not yet been achieved.
- Many molecular biology-based EMDs are limited to currently known biodegradation pathways and gene sequences. However, with ongoing research, EMDs will likely be adapted to emerging contaminants and newly identified biodegradation pathways.
- It is possible for elevated concentrations of minerals, metals, or organic matter to interfere with laboratory analysis.
- Technical expertise is required to evaluate EMD applicability, select the appropriate EMD, and interpret the results.
- Standard EMD protocols for sample collection, storage, analysis, and quality assurance (QA)/quality control (QC) have not yet been established across all laboratories.
- Currently, EMD analyses may be more expensive than traditional site investigation analyses (see Table 2). However, EMDs do not need to be applied with the same frequency; rather, they are typically performed on selected samples, over space and time.

## Quality Assurance/Quality Control

To date, most EMDs do not have standardized protocols accepted by the U.S. Environmental Protection Agency (EPA) or other government agencies. However, standards are in development. For example, CSIA and PCR have EPA guidance documents (EPA 2008, 2004, respectively), and standardized methods for qPCR are under development through the Strategic Environmental Research and

Development Program (SERDP) Project ER-1561. SERDP Project ER-1561, titled “Standardized Procedures for Use of Nucleic Acid-Based Tools,” investigated through a literature review the challenges associated with standardizing qPCR methods (Lebrón et al. 2008). Preliminary results from SERP Project ER-1561 suggest that even in the absence of a standard methodology, a systematic sampling and analysis program can yield accurate and reproducible results. In addition, each laboratory works under its own standard operating procedures (SOPs) and good laboratory practices, which can be provided to the user (e.g., consultant, state regulator) on request.

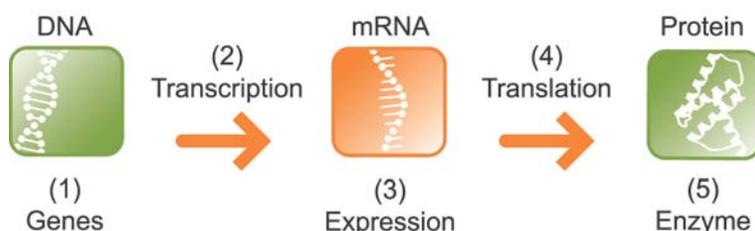
Currently, users can best ensure data quality by detailing the laboratory requirements in a site-specific QA project plan (QAPP). This plan should include identification of the EMDs being used, the field sampling procedures, the SOPs of the laboratory performing the analysis, and laboratory internal QA/QC information. Development of a QAPP facilitates communication between the user and the laboratory, as well as with regulators and stakeholders. With the QA/QC procedures documented, data collected over time can be reviewed against the same criteria.

For users of EMDs and interpreters of results, some points to review may include the following:

- Was the proper environmental medium sampled (e.g., groundwater or sediments)?
- Are the sampling procedures described?
- Were adequate samples taken, preserved, shipped, received, etc.?
- Was there enough environmental sample collected to give an accurate, repeatable result?
- Was the proper biomolecule targeted for analysis (e.g., DNA or RNA)?
- Were all internal laboratory QA/QC criteria met?
- Were sample results outside QA/QC criteria?
- Were oddities found in the sample results (e.g., detections in a blank sample)?
- Did the sample have to be diluted prior to analysis?

## Underlying Principles of EMDs

The ability of microorganisms to degrade contaminants requires that they have the appropriate genetic information (genes) that confers a particular biochemical activity to the microbial cell. EMDs can detect this “information” in several ways. Some EMDs detect and quantify the presence of genes themselves, while other EMDs detect the products of these genes when they are expressed and lead to biochemical activity. Figure 2 summarizes the flow of information in a microbial cell.



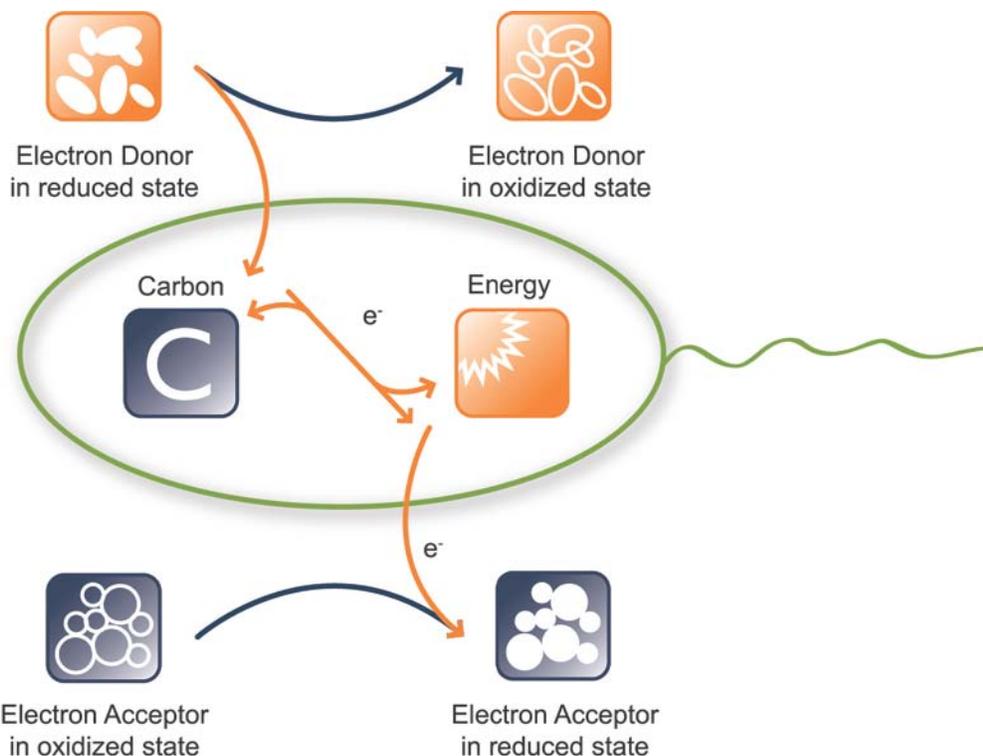
**Figure 2. Flow of information in a microbial cell during gene expression and enzyme production.**

Figure 2 depicts the following steps in the gene expression and enzyme production process:

1. **Genes**—A gene is a segment of DNA within a bacterial cell that contains specialized instructions that direct the cell to make a specific protein. Most proteins (enzymes) act as catalysts that the cell needs in order to metabolize (break down) a contaminant. A typical environmental sample can contain many thousands of different microorganisms. However, only a small fraction may possess the genes necessary to break down a specific contaminant. EMDs, including PCR, qPCR, microarrays, and FISH, are used to detect and/or quantify genes that indicate the presence of specific contaminant-degrading microorganisms (e.g., *Dehalococcoides*) or genes for specific enzymes that biodegrade specific contaminants (e.g., vinyl chloride reductase).

2. **Transcription**—Enzyme production is a two-step process where the gene is first copied (transcribed) into a short-lived, intermediate molecule called messenger RNA (mRNA).
3. **Expression**—A typical cell contains thousands of individual genes. Many of these genes program (encode) production of enzymes responsible for a broad spectrum of different functions. Naturally, a cell does not need all of those enzymes to be produced all of the time. In the interest of efficiency, enzyme production is regulated where transcription of some genes into mRNA is “turned off” and “turned on” only when needed.
4. **Translation**—In the second step of enzyme production, mRNA is translated into the corresponding protein (enzyme).
5. **Enzyme**—The enzyme is the biomolecule that catalyzes the biodegradation of the contaminant. One EMD, EAPs, directly estimates the activity of specific enzymes.

Microorganisms require two types of compounds to gain energy: an electron donor and an electron acceptor (Figure 3). The electron donor is oxidized, and the electrons are transferred to the electron acceptor, producing energy for the cell. In addition to energy, a cell needs carbon to build biomolecules (e.g., DNA, RNA, proteins) and reproduce. For many microorganisms, the electron donor is also the carbon source.



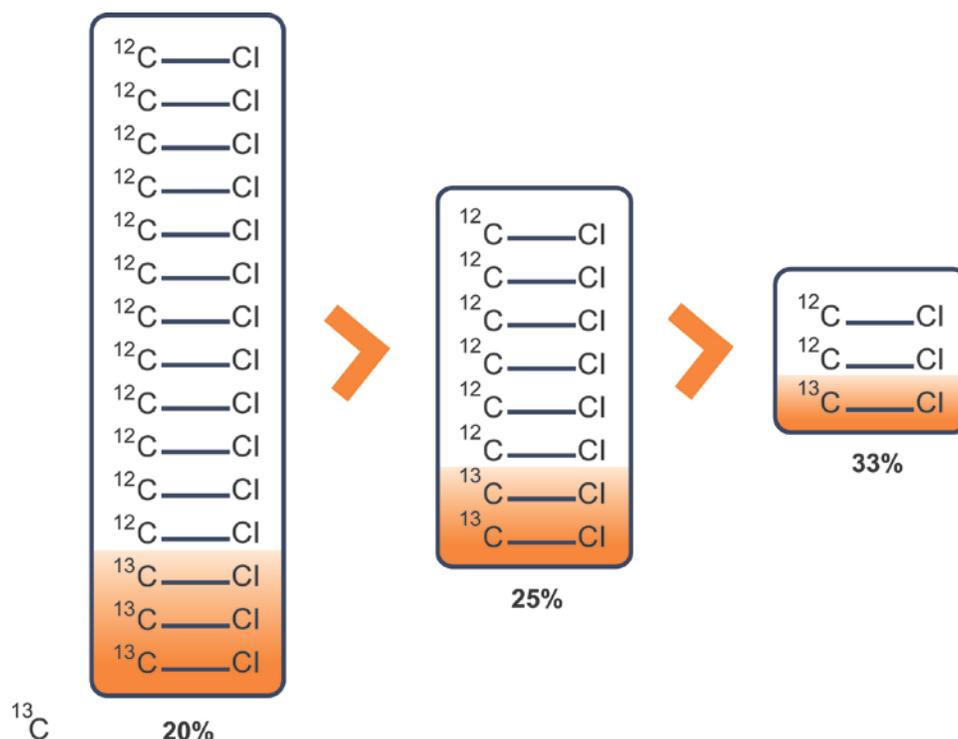
**Figure 3. Microbial metabolism.**

Many common environmental contaminants serve as an electron donor or an electron acceptor. For example, biodegradation of chlorinated ethenes (e.g., TCE) depends on microorganisms that can use them as an electron acceptor. For many other common groundwater contaminants (e.g., benzene, MTBE), biodegradation is a process whereby microorganisms use the contaminant as the electron donor and/or carbon source to produce new cells (biomass).

For contaminants used as carbon and energy sources, SIP is an EMD that can be used to determine whether biodegradation is occurring. Briefly, an environmental sample is exposed to a specially synthesized form of the contaminant containing a “heavy” isotope such as carbon-13 (<sup>13</sup>C) as a “label.” After a predetermined time, a sample is recovered for isotopic analysis of the contained biomass and dissolved inorganic carbon. If biodegradation has occurred, some of the <sup>13</sup>C from the contaminant will

have been completely mineralized to CO<sub>2</sub>, and some will be incorporated into the biomolecules of the contaminant-degrading microorganisms. In other words, if contaminant biodegradation occurred, elevated levels of the stable isotope “label” will be detected in the products of metabolism: biomolecules and CO<sub>2</sub>. See the EMD SIP Fact Sheet for additional information.

CSIA is based on quantification of the isotopic ratio (e.g., <sup>13</sup>C/<sup>12</sup>C, <sup>2</sup>H/<sup>1</sup>H) of the component elements of the environmental contaminant. Biodegradation and abiotic degradation of a contaminant involve breaking the chemical bonds between the component elements (e.g., C-Cl bond) of the contaminant compound. In general, less energy is required to break a bond between a light isotope and another element (e.g., <sup>12</sup>C-Cl) than is required to break a bond between the heavy isotope and the same atom (e.g., <sup>13</sup>C-Cl). As a result, the ratio of heavy isotopes to light isotopes increases as the contaminant is degraded. Figure 4 illustrates the enrichment of <sup>13</sup>C during degradation. See the CSIA Fact Sheet for additional information.



**Figure 4. Illustration of <sup>13</sup>C enrichment during degradation.**

Source: Microseeps, Inc., 2010, used with permission.

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## Glossary

- activity**—Refers to when a microorganism performs a specific function (e.g., sulfate reduction, metabolism of benzene).
- 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).
- biomolecules**—Classes of compounds produced by or inherent to living cells, including phospholipids, nucleic acids (e.g., DNA, RNA), and proteins.
- bioremediation**—The treatment of environmental contamination through the use of techniques that rely on biodegradation.
- biostimulation**—A remedial technique which provides the electron donor, electron acceptor, and/or nutrients to an existing subsurface microbial community to promote degradation.
- compound specific isotope analysis**—Analyzes the relative abundance of various stable isotopes (e.g.,  $^{13}\text{C}$ : $^{12}\text{C}$ ,  $^2\text{H}$ : $^1\text{H}$ ). Degradation processes can cause shifts in the relative abundance of stable isotopes of the contaminant; changes in isotopic ratios can be measured.
- 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 perchloroethene and TCE to ethene. Some *Dehalococcoides* species are also capable of reductive dechlorination of chlorinated benzenes, chlorinated phenols, and polychlorinated biphenyls.
- 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).
- environmental forensics**—The process of distinguishing contaminants from different sources.
- enzyme**—Any of numerous proteins or conjugated proteins produced by living organisms and facilitating biochemical reactions (based on EPA 2004).
- enzyme activity probes**—Transformation of surrogate compounds (probes) resembling contaminants produces a fluorescent (or other distinct) signal in cells, which is then detected by microscopy.
- fluorescence in situ hybridization**—Detects the presence of targeted genetic material in an environmental sample and estimates the number of specific microorganisms or groups of microorganisms.
- gene**—A segment of DNA containing the code for a protein, transfer RNA, or ribosomal RNA molecule (based on Madigan et al. 2010).
- genus**—A category of organism classification (taxonomy). A particular genus is a group of related species. For example, *Pseudomonas* is a genus of bacteria.
- heterotrophic plate count**—A test used to estimate the total number of bacteria capable of growing on organic compounds in an environmental sample.
- microarrays**—Detects and estimates the relative abundances of hundreds to thousands of genes simultaneously.
- microbial community**—The microorganisms present in a particular sample.
- microbial diversity**—Microbial diversity can have many definitions but in this context generally refers to the number of different microbial species and their relative abundance in an environmental sample (Nannipieri et al. 2003).
- microbial fingerprinting methods**—A category of related techniques that differentiate microorganisms or groups of microorganisms based on unique characteristics of a universal component or section of a biomolecule.
- phospholipid**—A type of biomolecule that is a primary structural component of the membranes of almost all cells.
- polymerase chain reaction**—Makes copies of a specific DNA sequence within a target gene of microorganisms that can be further analyzed.

**protein**—Large organic compounds made of amino acids arranged in a linear chain and joined together by peptide bonds (U.S. Navy 2009).

**quantitative polymerase chain reaction**—A laboratory analytical technique for quantification of a target gene based on DNA or RNA.

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

**species**—The lowest taxonomic rank, and the most basic unit or category of biological classification (Biology Online n.d.).

**stable isotope**—A form of an element that does not undergo radioactive decay at a measureable rate.

**stable isotope probing**—A synthesized form of the contaminant containing a stable isotope (e.g.,  $^{13}\text{C}$ ) is added. If biodegradation is occurring, the isotope will be detected in biomolecules (phospholipids, DNA).

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