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# Environmental Molecular Diagnostics for Green Remediation

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## Extended Abstract

In situ bioremediation is inherently considered "green remediation". The mechanisms of destruction by in situ biotechnologies, however, are often unseen and not well understood. Further, physical effects of amendment application effect concentration data in an identical manner as the desired reactive mechanism. These uncertainties have led to the weight of evidence approach when proving viability: multiple rounds of data collection, bench studies, and pilot studies, etc. Skipping these steps has resulted in many failed in situ applications. Traditional assessment data are often tangential to the desired information; e.g., "Is contaminant being destroyed or just being pushed around and diluted?" and "What is the mechanism of the destruction and can it be monitored directly?"

Environmental molecular diagnostic tools (EMDs), 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. EMDs can be classified into two major categories of analytical techniques: chemical techniques, specifically compound specific isotope analysis (CSIA), and a variety of molecular biological techniques (MBTs).

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. CSIA therefore can be useful for answering several important questions regarding a chemical's source, degradation mechanisms, and rate of degradation.

MBTs evaluate the types, abundance, and biochemical capabilities of microorganisms present in the environment. Often, the microorganisms responsible for the degradation of specific contaminants cannot be detected and quantified by conventional methods and MBTs can overcome these limitations. Several types of MBTs are available - some can be used to detect known microorganisms, others are also useful for quantification, some can be used to determine whether microorganisms are actively degrading specific contaminants, and some can identify currently unknown microorganisms involved in degradation. To date, the most commonly used MBTs are polymerase chain reaction (PCR), quantitative PCR (qPCR) and reverse transcriptase-qPCR (RT-qPCR), and DNA microarrays.

EMDs have application in each phase of environmental site management, including site characterization, remediation, monitoring, and closure activities. EMDs can provide unique information valuable in conjunction with more conventional data. For example, CSIA can assess viability of in situ biotechnologies by providing definitive data on contaminant destruction that are not concentration related. The most commonly used stable isotopes in environmental studies include 13C, 2H, and 37Cl, etc. The development of combined gas chromatography-isotope ratio mass spectrometry (GC-IRMS) in 1990s has led to an explosion in applications using the technique of CSIA in the environmental fields.

This presentation outlines the fundamentals of environmental molecular diagnostic tools and their benefits are highlighted through a series of case studies at chlorinated solvent and petroleum hydrocarbon contaminated sites around the world (United States, Canada, Japan, Italy, South Africa, Argentina, and

Brazil). The CSIA tools located source zones and apportion remediation cost by identifying plumes of different isotope signatures and fractionation trends. Further, a combining use of such advanced site diagnostic tools allowed remediation professionals to evaluate effectiveness of treatment and make better decisions to expedite site closure for monitored natural attenuation (MNA) and minimize costs, consistent with US EPA's initiative for “Green Remediation”.