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Standard Guide for Application of Molecular Biological Tools to Assess Biological Processes at Contaminated Sites[1]

This standard is issued under the fixed designation E3354; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This guide provides a framework for the application of molecular biological tools (MBTs) to assess and characterize *in-situ* biological processes to improve contaminated soil and groundwater management. While the focus of this guide is on *in-situ* biological processes, some concepts of how to apply MBTs can also be applied to *ex-situ* bioremediation approaches (for example, biopiles, bioreactors) to support design, operation, and troubleshooting. The intent of this guide is to develop a consistent way in which MBTs are applied at contaminated sites, not to develop expertise. Technical experts need to be engaged whenscoping, planning, executing, and interpreting data for MBTs. Lastly, there is a brief description of isotopic techniques within section 5.2; however, the scope and focus of this guide is the use of nucleic acid-based MBTs to assess biological processes at contaminated sites.

1.2 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.3 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D6771 Practice for Low-Flow Purging and Sampling Used for Groundwater Monitoring

2.2 *U.S. EPA References:*[3]

U.S. EPA. 2018 Sampling, Laboratory and Data Considerations for Microbial Data Collected 32 the Field. EPA/600/R-164. 74 pg

U.S. EPA. 2008 A Guide for Assessing Biodegradation and Source Identification of Organic Ground Water Contaminants Using Compound Specific Isotope Analysis (CSIA). Ada, Oklahoma: Office of Research and Development, U.S. (EPA 600/R-08/148)

U.S. EPA. 1999 Use of monitored natural attenuation at superfund, RCRA corrective action, and underground storage tank sites. United States Environmental Protection Agency, Washington

U.S. EPA 1996 Ground Water Issue: Low-Flow (Minimal Drawdown) Ground-Water Sampling Procedures EPA/540/S-95/504

3. Terminology

3.1 This section includes definitions of key processes that are specific to molecular biological tools and use of abbreviations and acronyms. Definitions are adapted from other sources noted in the referenced documents. A full list of bioremediation-relevant microorganisms is beyond the scope of this guide.

3.2 *Definitions:*

3.2.1 *16S ribosomal ribonucleic acid (rRNA), n*—the RNA component of the 30S unit of the prokaryotic ribosome. The 16S rRNA is produced by 16S rRNA genes (sometimes referred to as 16S rDNA) which are gene sequences used to study bacterial phylogeny and taxonomy.

3.2.1.1 *Discussion*—The 16S rRNA gene has historically been the most common housekeeping genetic marker used by molecular biologists and microbiologists to identify and classify microorganisms (for example, genus and species).

3.2.2 *attenuation, n*—contaminant reduction over space and time due to physical (advection and dilution), chemical (volatilization, adsorption, abiotic transformation), and/or biological processes (biodegradation, biotransformation).

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[3] Available from United States Environmental Protection Agency (EPA), William Jefferson Clinton Bldg., 1200 Pennsylvania Ave., NW, Washington, DC 20460, http://www.epa.gov.

3.2.3 *bioinformatics, n*—a subdiscipline of biology and computer science that focuses on the acquisition, storage, analysis and study of biological data, frequently applied to DNA, RNA, and protein sequences.

3.2.4 *biomarker, n*—a unique characteristic of a biomolecule, such as a specific DNA sequence, that can be measured and used as an indicator of a target microorganism or a specific biological process.

3.2.5 *bioaugmentation, n*—the introduction of microorganisms into the environment for the purpose of enhancing a beneficial biological activity.

3.2.6 *bioremediation, n*—cleanup of sites contaminated with hazardous, toxic, or radioactive substances or wastes, or any combination thereof using biological systems.

3.2.7 *biostimulation, n*—the introduction of electron donor, electron acceptor, and/or nutrients to the subsurface to promote biodegradation by an existing, native microbial community. Manipulation of pH or temperature may also be a form of biostimulation to enhance biodegradation.

3.2.8 *compound specific isotope analysis (CSIA), n*— analytical method that measures the ratio between the stable isotopes (for example, $^{13}C/^{12}C$, $^{2}H/^{1}H$, or $^{37}Cl/\ ^{35}Cl$) of a contaminant. As contaminants are degraded (for example, metabolism of aromatics, reductive dechlorination of chlorinated solvents), contaminants become enriched with the heavier isotopes (for example, ^{13}C) and the associated shift in isotopic ratios can be determined as evidence of bond breaking reactions.

3.2.9 *constituent of concern (COC), n*—an environmental constituent that is to be assessed, remediated, and or monitored in groundwater, soil, or soil gas at contaminated sites; sometimes referred to as a chemical of concern.

3.2.10 *conceptual site model (CSM), n*—a written and/or graphical representation of a site which includes potential sources and receptors of contaminants as well as the collection of physical, chemical, and biological processes governing contaminant fate and transport. These models are often iterative, revised as more information is learned, and used to aid decision-making throughout the site project lifecycle.

3.2.11 *deoxyribonucleic acid (DNA), n*—a biological macromolecule that carries genetic information. DNA consists of two long chains of nucleotides twisted into a double helix.

3.2.12 *electron acceptor, n*—a chemical compound that accepts electrons transferred to it from another compound. It is an oxidizing agent that is reduced through the process of accepting electrons.

3.2.13 *electron donor, n*—a chemical compound that donates electrons to another compound. It is a reducing agent that is oxidized through the process of losing electrons.

3.2.14 *enhanced attenuation (EA), n*—a remedial strategy that promotes biodegradation via addition of amendments (biostimulation) or cultured microorganisms (bioaugmentation) that stimulate biogeochemical reactions that result in the reduction of COCs *in-situ*.

3.2.15 *functional gene, n*—a segment of DNA that encodes an enzyme or other protein that performs a known biochemical reaction typically of use or interest beyond its role in the general metabolism of the cell (housekeeping gene).

3.2.15.1 *Discussion*—For example, the anaerobic Benzene Carboxylase (*abcA*) gene encodes an enzyme responsible for a known initial activation step in the anaerobic biodegradation pathway of benzene and is therefore a functional gene of interest to bioremediation practitioners.

3.2.16 *genomics, n*—a branch of molecular biology focusing on the structure, function, evolution, and mapping of genomes.

3.2.16.1 *Discussion*—While genetics may focus on the study of individual genes, genomics will consider the entire genetic information of an organism.

3.2.17 *genome, n*—the entire complement of genetic material for an organism.

3.2.18 *metagenomics, or meta-omics, n*—the study of genetic material derived directly from multiple host organisms or environmental samples containing multiple genomes, such as a mixed microbial community.

3.2.19 *molecular biological tools (MBTs), n*—a suite of molecular genetic analyses that can be used to characterize and evaluate microorganisms and their related activity.

3.2.19.1 *Discussion*—MBTs may also be referred to as or included in Environmental Molecular Diagnostics (EMDs).

3.2.20 *metabolism, n*—the myriad of chemical reactions in organisms that support life, including the conversion of the energy in substrates to energy available to perform cellular processes; the conversion of food to building blocks for proteins, lipids, nucleic acids, and some carbohydrates; and the elimination of metabolic wastes.

3.2.21 *metabolite, n*—a low molecular weight intracellular or extracellular molecule which is an intermediate or end product of biological processes required for organism growth, maintenance, and normal function.

3.2.22 *metabolic byproduct, n*—biochemical compounds that are generated by a microorganism during metabolic activity.

3.2.23 *metabolomics, n*—the analysis of intracellular and extracellular intermediates and end products generated during biological processes required for growth, maintenance, and normal function of an organism.

3.2.24 *microcosm study, n*—or "laboratory treatability studies" are a laboratory study in which samples of environmental media (for example, soil, sediment, and/or water) are tested in a contained environment, such as a bottle, to evaluate changes in concentration (or mass) of COCs over time.

3.2.24.1 *Discussion*— Microcosm studies are designed to mimic field conditions (to the extent possible) and may be used to evaluate remedial amendments, or determine natural degradation rates.

3.2.25 *monitored natural attenuation (MNA), n*—remedial approach that relies on demonstration through multiple lines of evidence, that naturally occurring physical (advection and dilution), chemical (volatilization, adsorption, abiotic transformation), and/or biological processes (biodegradation,