Developing a Molecular Methods-based Tool for Assessing MNA and Biostimulation Potential for Remediation of Released Fuels in the Environment

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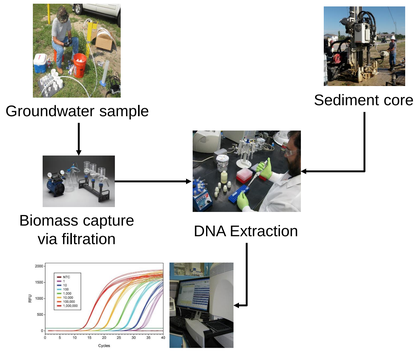
Abstract

Microbial biodegradation of petroleum products by native microflora within the terrestrial subsurface is a sustainable, effective, and cost-efficient mechanism for remediation of fuels released from leaking underground storage tanks. Evaluating biodegradation potential for remedial monitored natural attenuation (MNA) or biostimulation strategies as part of site characterization poses a challenge for the agencies responsible for each site. A low-cost tool for determining whether microorganisms with hydrocarbon biodegradation capacity exist within a contaminated site is being developed utilizing signature genetic biomarkers. Based upon the microbial physiology of petroleum biodegradation, genes for the activation/degradation of specific hydrocarbon compound classes (e.g. aliphatic, monoaromatic, polyaromatic, etc.) under oxic or suboxic conditions are identified and enumerated using molecular biology techniques within any subsurface environmental sample matrix. In addition, these methods can be utilized to screen for which electron acceptor would be the best option to enhance remediation as a biostimulant. This tool will aid remediation project managers in assessing the potential for biodegradation strategies when paired with standard geochemical site characterization data.

Sample Evaluation Methodology



Enumeration of target biodegradation genes by quantitative polymerase chain reaction (qPCR)

Molecular Biology & Environmental Processes

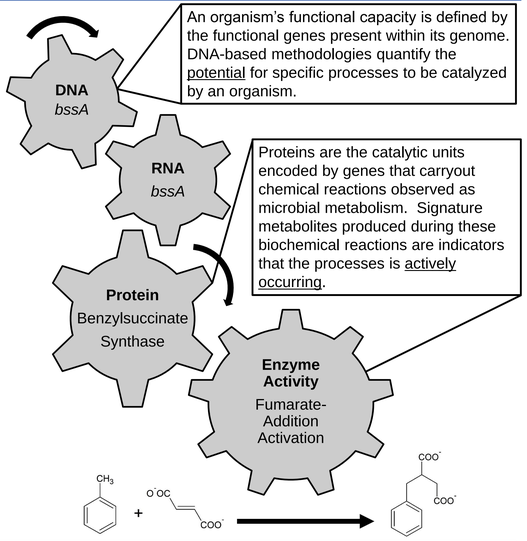


Figure 1. Graphical schematic representing the relationships between biomacromolecules and microbial metabolism observed as environmental reactions.

Fumarate-Addition Pathways of Anoxic Hydrocarbon Biodegradation

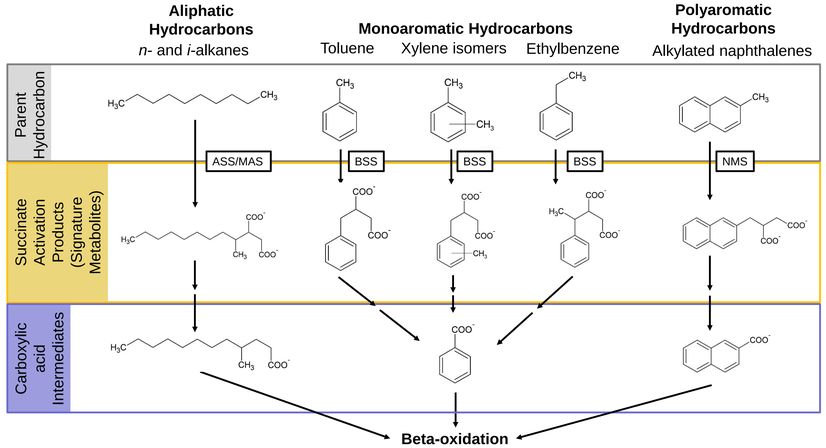


Figure 2. Diagram of anaerobic hydrocarbon biodegradation pathways with initial activation by fumarate-addition reactions. Fumarate-addition enzyme abbreviations (black-outlined boxes): ASS/MAS - alkylsuccinate synthase/methylalkylsuccinate synthase; BSS – benzylsuccinate synthase; NMS – 2-naphthylmethylsuccinate synthase.

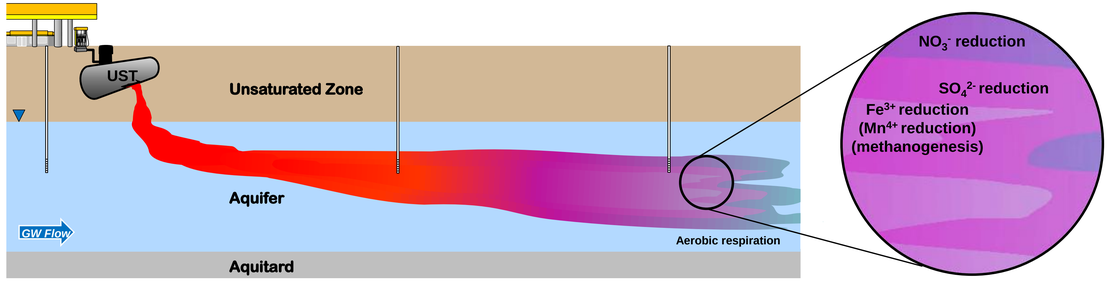


Figure 3. Schematic representation of expected functional gene and signature metabolite presence in various zones within a BTEX plume originating from a leaking underground storage tank (UST). Color gradation within the plume highlights the small spatial scale separating electron-accepting processes at the plume fringe. Varying quantities of *bssA* functional gene and alkylbenzylsuccinate metabolites are anticipated within the biologically active anoxic zones of the plume.

Proposed Tool-Supported Decision Tree

Are MNA or Biostimulation viable options for anoxic petroleum remediation at this site?

1. Are Fumarate-Addition Enzyme (FAE) genes present at this site?

- No → Bioremediation may not be a viable remediation strategy.
- Yes →

2. Is there an enrichment of FAE genes in the contaminated zone relative to the uncontaminated area?

- No → Bench-scale testing should be determined to evaluate potential inhibitory conditions (e.g. toxicity, limitations, etc.).
- Yes →

3. Is the FAE gene abundance >10^x copies/mL within the contaminated zone?

- No → **Biostimulation may be required for successful bioremediation strategy.**
- Yes → **MNA is likely a viable remediation approach.**

Ongoing Research and Application Potential

Research on field-derived samples from BTEX-contaminated groundwater sites is being conducted to evaluate the statistical significance of correlations between functional gene (*bssA*) abundance and the presence of associated signature metabolites (alkylbenzylsuccinates). This low-cost molecular-based method can be incorporated into site characterization, and is aimed at providing remedial strategy decision support to contaminated site managers. In addition, this method can also be employed for monitoring the progress of both MNA and biostimulation remediation efforts throughout the life of site cleanup projects.

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