QuantArray®
MOLECULAR BIOLOGICAL TOOL

Simultaneously quantify functional genes responsible for aerobic and anaerobic biodegradation of petroleum hydrocarbons in a single analysis

Case Study—QuantArray & Evaluating MNA

The study site is a former manufactured gas plant with dissolved benzene and naphthalene concentrations exceeding risk-based closure levels. Historical groundwater monitoring generally indicated stable or decreasing contaminant concentrations. Monitored natural attenuation (MNA) can be an effective remediation strategy at sites impacted by petroleum hydrocarbons, but is sometimes viewed as a "do nothing" solution in which decreases in contaminant concentrations result from physical processes (e.g. dilution) rather than biodegradation. QuantArray- Petro was performed to quantify functional genes responsible for aerobic and anaerobic biodegradation of contaminants of concern to aid in evaluating MNA as a site management strategy.

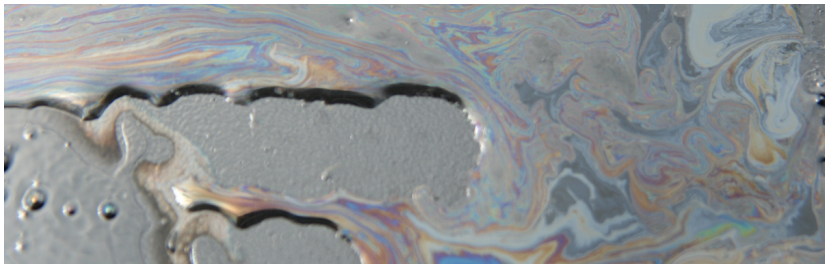
Questions

- Are benzene degrading bacteria present at substantial concentrations under existing site conditions?
- How about naphthalene degrading bacteria?
- Is MNA feasible?

QuantArray®—Petro

Petroleum products are complex mixtures of literally hundreds of aliphatic, aromatic, cyclic and heterocyclic compounds. Moreover, even with a single class of contaminants such as benzene, toluene, ethylbenzene, and xylenes (BTEX), biodegradation can proceed by a multitude of pathways under both aerobic and anaerobic conditions.

QuantArray-Petro has been designed to cost-effectively address both of these issues by providing the simultaneous quantification of the specific functional genes responsible for aerobic and anaerobic biodegradation of BTEX, PAHs and a variety of short- and long-chain alkanes.



QuantArray—Petro Answers (See back for details)

- *Are benzene degrading bacteria present at substantial concentrations under existing site conditions?*

Yes. Although anaerobic benzene carboxylase (ABC) was not detected, genes responsible for initiating multiple pathways for the aerobic biodegradation of benzene were detected in impacted wells but not in the background well suggesting growth of aerobic benzene utilizing bacteria within the dissolved plume.

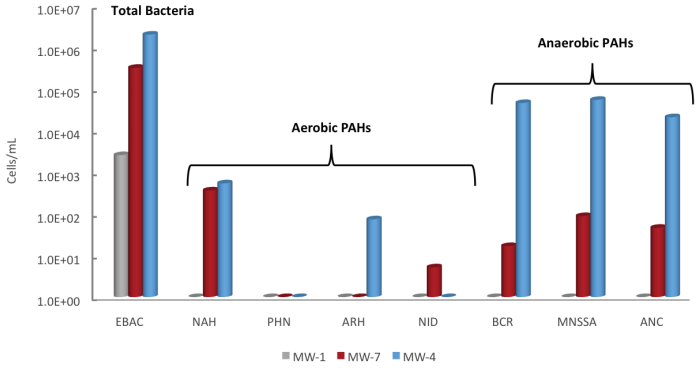
- *How about naphthalene and PAH degraders?*

Yes. Concentrations of genes responsible for initiating aerobic and anaerobic biodegradation of naphthalene and methylnaphthalene were greater in impacted wells than in background samples (which were below detection limits). In fact, naphthalene carboxylase (ANC) and methylnaphthalene succinate synthase (MNSSA) genes were detected at high concentrations in MW-4, indicating the presence of a substantial bacterial population capable of utilizing naphthalene and methylnaphthalene under the prevailing anaerobic conditions.

- *Is MNA feasible?*

Yes. For this site, multiple lines of evidence suggested that MNA was a feasible remedy. To complement traditional groundwater monitoring which indicated a stable to decreasing plume, QuantArray analysis revealed growth of BTEX- and PAH-utilizing bacteria within the dissolved plume relative to background microbial populations.

BTEX Biodegradation Potential



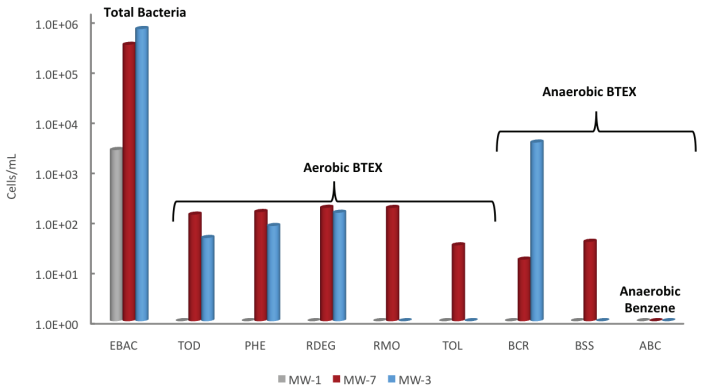
Background Microbial Populations (Monitoring Well MW-1):

- Aerobic BTEX degraders are often considered ubiquitous and are frequently detected in background samples. Therefore, interpretation of QuantArray results at petroleum hydrocarbon impacted sites should emphasize comparisons between samples obtained from a background well (MW-1) vs impacted wells (MW-7 and MW-3). Simply put, higher concentrations of targeted functional genes in impacted wells compared to background samples demonstrates growth and enrichment of BTEX degraders within the dissolved plume, providing a stronger line of evidence for biodegradation under existing site conditions.
- In the current study however, functional genes involved in aerobic and anaerobic biodegradation of BTEX were not detected in the background well, which is somewhat unusual. In this case, total bacteria (EBAC) concentrations at MW-1 were low, suggesting that overall microbial growth was limited under background conditions likely due to lack of growth-supporting substrates.

BTEX Biodegradation:

- In impacted wells MW-7 and MW-3, toluene/benzene dioxygenase (TOD), toluene/benzene monooxygenases (RMO and RDEG), and phenol hydroxylase (PHE) were detected at higher concentrations than in the background groundwater sample, indicating growth of aerobic BTEX degraders within the dissolved plume.
- Benzylsuccinate synthase (BSS) genes were detected at MW-7 indicating the potential for anaerobic biodegradation of TEX. Anaerobic benzene carboxylase (ABC) genes were not detected.
- Although the concentrations of TOD and other target genes were modest, the detection of multiple genes involved in different pathways for BTEX biodegradation (functional redundancy) is also encouraging and suggests the present of a robust BTEX degrading population in the impacted areas.

Naphthalene and PAH Biodegradation Potential



Background Microbial Populations (Monitoring Well MW-1):

- Functional genes involved in aerobic and anaerobic biodegradation of naphthalene and other PAHs were not detected in the background well.

Naphthalene and PAH Biodegradation:

- Naphthalene dioxygenase genes (NAH), not detected in the background groundwater sample, were detected on the order of 10^2 cells/mL in impacted wells MW-7 and MW-3, indicating enrichment and growth of aerobic naphthalene utilizing bacteria within the dissolved plume.
- Furthermore, anaerobic naphthalene carboxylase (ANC) and methylnaphthalene succinate synthase (MNSSA) genes were detected at both impacted wells, indicating the potential for anaerobic biodegradation of naphthalene and 2-methylnaphthalene.
- Concentrations of ANC and MNSAA were particularly high at monitoring well MW-4 located in the source area.

Footnote: In situ biodegradation of benzene and naphthalene was further confirmed with a stable isotope probing (SIP) study.

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