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QuantArray®-Petro

Evaluating a Transition to MNA

PROJECT SUMMARY

- After years of successful contaminant mass removal at a former gasoline station BTEX and MTBE concentrations had become asymptotic and site managers were considering transitioning to MNA.
- QuantArray®-Petro and the MI Database demonstrated high concentrations of BTEX and MTBE degraders in monitoring wells exhibiting decreasing concentrations of hydrocarbons and fuel oxygenates supporting a transition to MNA.
- Moreover, Stable Isotope Probing (SIP) studies using Bio-Traps® amended with ^{13}C-benzene or ^{13}C-MTBE conclusively demonstrated *in situ* biodegradation of these compounds was occurring under existing site conditions providing a strong second line of evidence supporting a transition to MNA over most of the site.

PROJECT CHALLENGE

At the study site, system performance and contaminant mass removal had decreased to the point where continued operation would result in high costs with little additional contaminant recovery so stakeholders were evaluating transitioning to monitored natural attenuation (MNA) as a long-term strategy. At most monitoring wells, BTEX and MTBE concentrations were decreasing, but in one area hydrocarbon trends were inconclusive. Site managers needed additional lines of evidence to conclusively evaluate a transition to MNA and potential enhanced bioremediation options.

SAMPLING AND ANALYSIS

Along with chemical and geochemical monitoring, QuantArray-Petro was performed to quantify a broad spectrum of functional genes responsible for biodegradation of BTEX, MTBE and TBA in select monitoring wells across the site including locations with and without decreasing trends. Stable Isotope Probing (SIP) is an innovative molecular biological tool (MBT) that uses a Bio-Trap amended with a ^{13}C "labeled" contaminant (e.g. ^{13}C benzene) to conclusively determine whether biodegradation has occurred. The ^{13}C label serves much like a tracer which can be detected in the end products of biodegradation – microbial biomass and CO^2.

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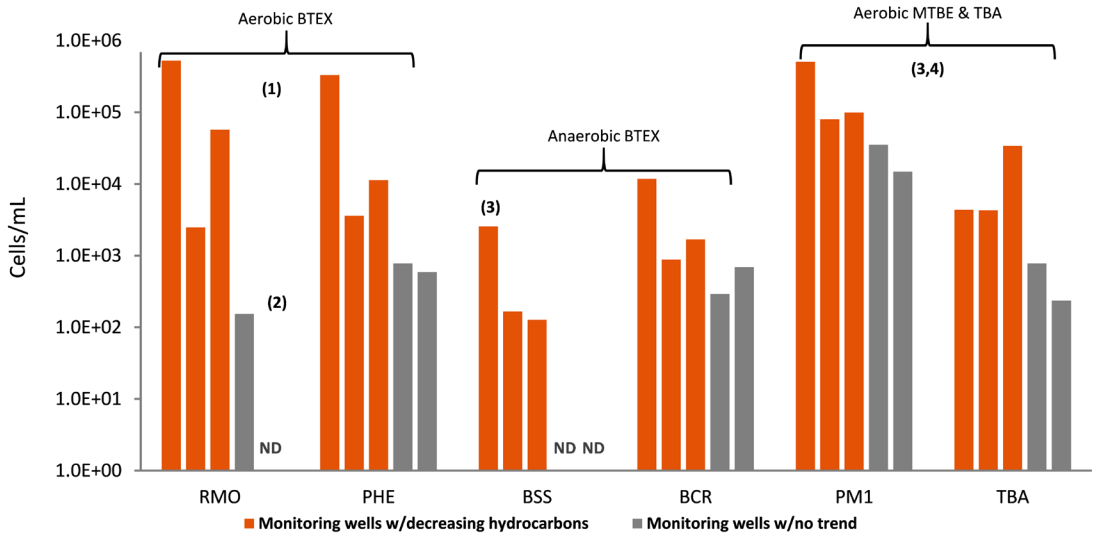
QUANTARRAY-PETRO RESULTS

At the study site, groundwater monitoring demonstrated decreasing trends in BTEX and MTBE concentrations in most monitoring wells. As shown below, concentrations of functional genes involved in BTEX and MTBE biodegradation were substantially greater in these monitoring wells exhibiting decreasing hydrocarbon concentrations (orange bars) compared to the wells in a small area where contaminant concentrations were low but not conclusively decreasing (gray bars).



(1) More specifically, concentrations of toluene/benzene monooxygenases (RMO) and phenol hydroxylase (PHE) genes were on the order of 10^3 to 10^5 cells/mL in wells with decreasing BTEX concentrations (orange bars) suggesting that biodegradation would contribute to attenuation in this area – an inference proven by subsequent SIP studies with ^{13}C-benzene.

(2) In the other wells (gray bars), RMO and PHE concentrations ranged from below detection limits to 10^2 cells/mL – one to three orders of magnitude lower.

(3) The results for functional genes responsible for anaerobic BTEX biodegradation were similar. Concentrations of benzylsuccinate synthase (BSS) genes were on the order of 10^2 to 10^3 cells/mL in the wells with decreasing hydrocarbons but were below detection limits in the other area.

(4) The PM1 and TBA assays quantify *Methylibium petroleiphilum* PM1, one of the few organisms isolated to date which is capable of utilizing MTBE, and TBA hydroxylase that catalyzes continued oxidation of *tert*-butyl alcohol.

(5) Concentrations of MTBE-utilizing PM1 were substantial throughout the study wells indicating the potential for MTBE biodegradation. However, PM1 concentrations in areas with decreasing hydrocarbon concentrations were on the order of 10^5 compared to 10^4 cells/mL in other monitoring wells. Likewise, concentrations of TBA hydroxylase genes were an order of magnitude greater.

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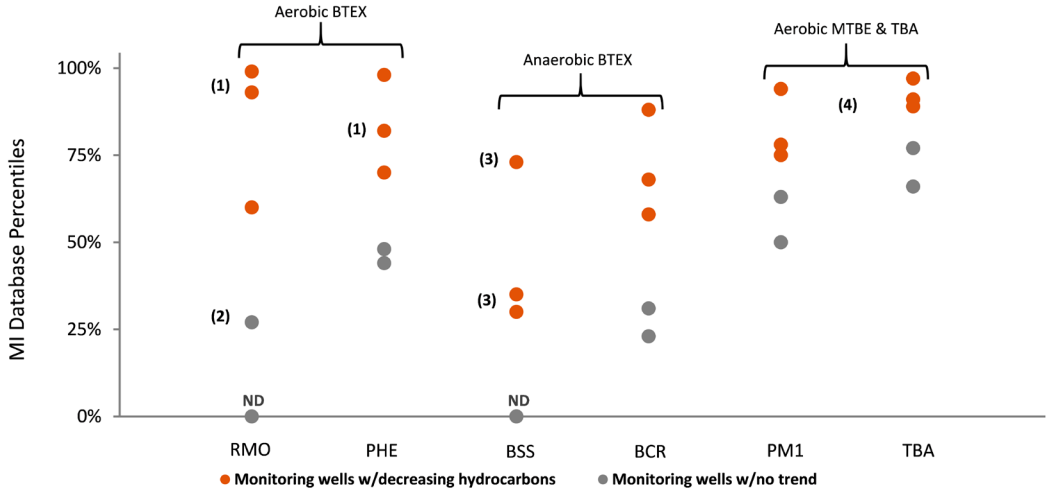
MICROBIAL INSIGHTS DATABASES

The MI qPCR Database puts microbiology in context. Percentile rankings of your CENSUS and QuantArray-Petro results based on thousands of samples from sites from around the world lets you answer a critical question: Are my results low, medium, or high?



(1) In monitoring wells with decreasing hydrocarbon concentrations (orange circles), RMO and PHE gene copies were "high" - not only compared to the other wells at the site (gray circles) - but also compared to sites around the world. PHE and RMO concentrations in most samples at the study site were greater than 70% to 95% of samples where PHE and RMO have been detected.

(2) Conversely, PHE and RMO concentrations in site monitoring wells in the area with lingering hydrocarbons were "low" or "below average" compared to other sites in the MI Database.

(3) BSS genes involved in anaerobic TEX biodegradation were only detected in monitoring wells with decreasing hydrocarbon trends. Consistent with the geochemical conditions however, BSS concentrations were relatively low (~35th percentile) in two of these wells.

(4) Concentrations of MTBE-utilizing strain PM1 and TBA hydroxylase genes were "greater than average" in all study monitoring wells. In the wells with decreasing trends, PM1 concentrations were in the upper quartile (75th – 95th percentiles) nd TBA hydroxylase concentrations were high (>90th percentile).

Overall, QuantArray-Petro and the MI Database demonstrated high concentrations of BTEX and MTBE degraders in monitoring wells exhibiting decreasing hydrocarbon concentrations providing a line of evidence for MNA. In one area of the dissolved plume, concentrations of BTEX degraders were low. While still indicating the potential for aerobic BTEX biodegradation, the low concentrations of key functional genes suggested that biodegradation in this area could be limited under existing conditions.

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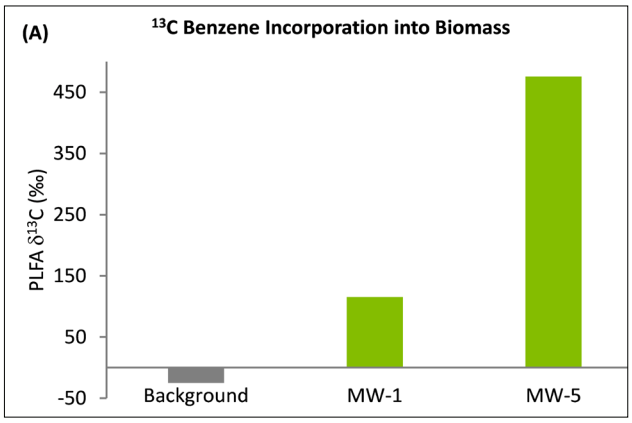
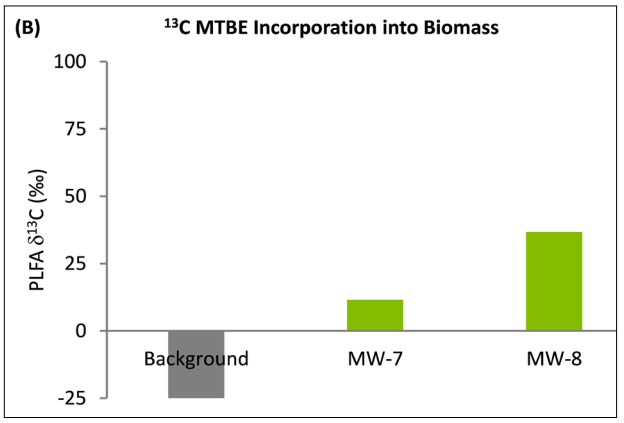
STABLE ISOTOPE PROBING (SIP)

While the nomenclature may be unfamiliar, interpretation of SIP results is straightforward. Biodegradation of benzene and other petroleum hydrocarbons is a process whereby some microorganisms use the contaminant as a carbon and energy source.

- When used as carbon source, contaminant carbon is incorporated into building biomolecules like phospholipid fatty acids (PLFA), a major component of bacterial cell membranes, during growth of new cells (biomass).
- When used as an energy source, contaminant carbon is oxidized to CO2 (mineralization) as part of cellular metabolism.

Therefore, the detection of ^{13}C enriched PLFA and/or ^{13}C enriched CO^2 (dissolved inorganic carbon) during a SIP study conclusively demonstrates *in situ* biodegradation of the contaminant of concern under actual aquifer conditions.





SIP results conclusively demonstrated that ^{13}C-benzene and ^{13}C-MTBE biodegradation occurred during the deployment period at the selected monitoring wells.

- Background: The $\delta^{13}C$ values for most natural substrates/carbon sources are between -20‰ and -30‰. Thus, background PLFA $\delta^{13}C$ values are around -25‰ under natural conditions (gray bars).
- Benzene and MTBE: For both benzene (Figure A) and MTBE (Figure B), the detection of ^{13}C enriched PLFA (green bars) compared to a background level conclusively demonstrated ^{13}C incorporation into microbial biomass and therefore proved *in situ* biodegradation of these contaminants had occurred under existing site conditions.

Decision: MNA vs. targeted enhanced bioremediation. QuantArray-Petro revealed high concentrations of BTEX and MTBE degraders and SIP conclusively demonstrated in situ biodegradation of benzene and MTBE in areas exhibiting decreasing trends in contaminant concentrations providing multiple lines of evidence for MNA. In one area though, low concentrations of BTEX degraders and historical monitoring results suggested that enhanced bioremediation may be warranted.

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KEY BENEFITS

- Saved money: QuantArray-Petro and SIP results supported a transition from an active remediation system to MNA for most of the site. The study also highlighted a small area for enhanced bioremediation
- Comprehensive: QuantArray-Petro accurately quantified a broad spectrum of functional genes involved in biodegradation of BTEX, MTBE, TBA and other petroleum hydrocarbons in a single analysis.
- Context: Percentile rankings from the MI Database revealed wells where concentrations of functional genes responsible for BTEX biodegradation were high compared to other sites around the world.
- Easy: Suspend SIP Bio-Traps in impacted monitoring wells for 30-60 days and then ship to Microbial Insights for analysis, results, and a straight forward report.
- Conclusive: SIP results conclusively demonstrated that in situ biodegradation of benzene and MTBE occurred under existing site conditions.
- Confidence: Increased stakeholder confidence in the site management strategy.

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