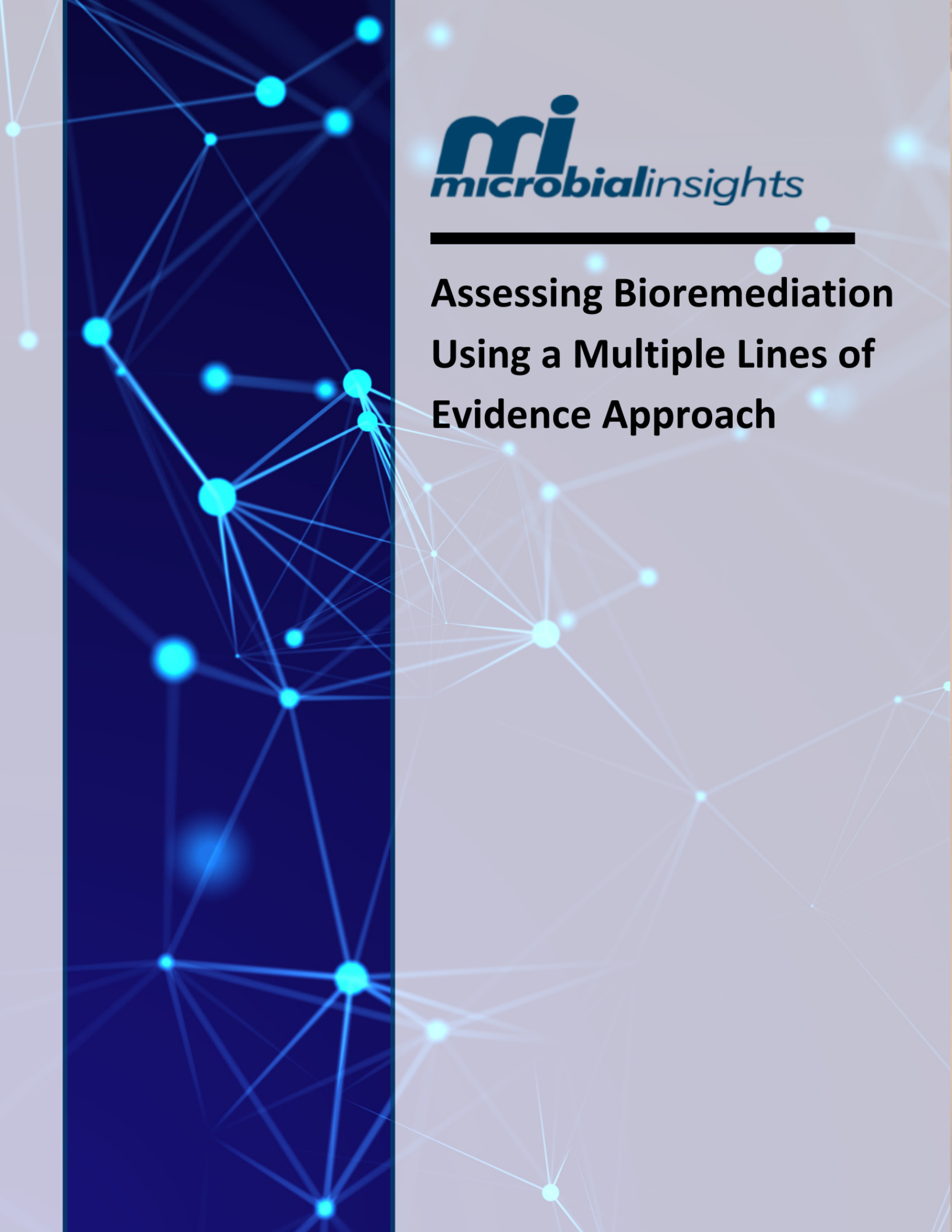

microbialinsights

Assessing Bioremediation Using a Multiple Lines of Evidence Approach

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Table of Contents

1.0 Importance of a Multiple Lines of Evidence Approach 2

2.0 Collecting Microbial Data with Molecular Biological Tools 3

2.1 Measuring contaminant-degrading microorganisms, functional genes, and competitors 3

2.2 Proof of Contaminant Degradation 4

2.3 Microbial Community Composition 4

2.4 Screening Treatment Options 4

3.0 Case Studies 5

3.1 Cost Effective Remedy Selection at a TCE-Impacted Site 5

3.2 Using CENSUS® qPCR to Evaluate MNA at a Petroleum Pipeline Release 7

Assessing Bioremediation Using a Multiple Lines of Evidence Approach

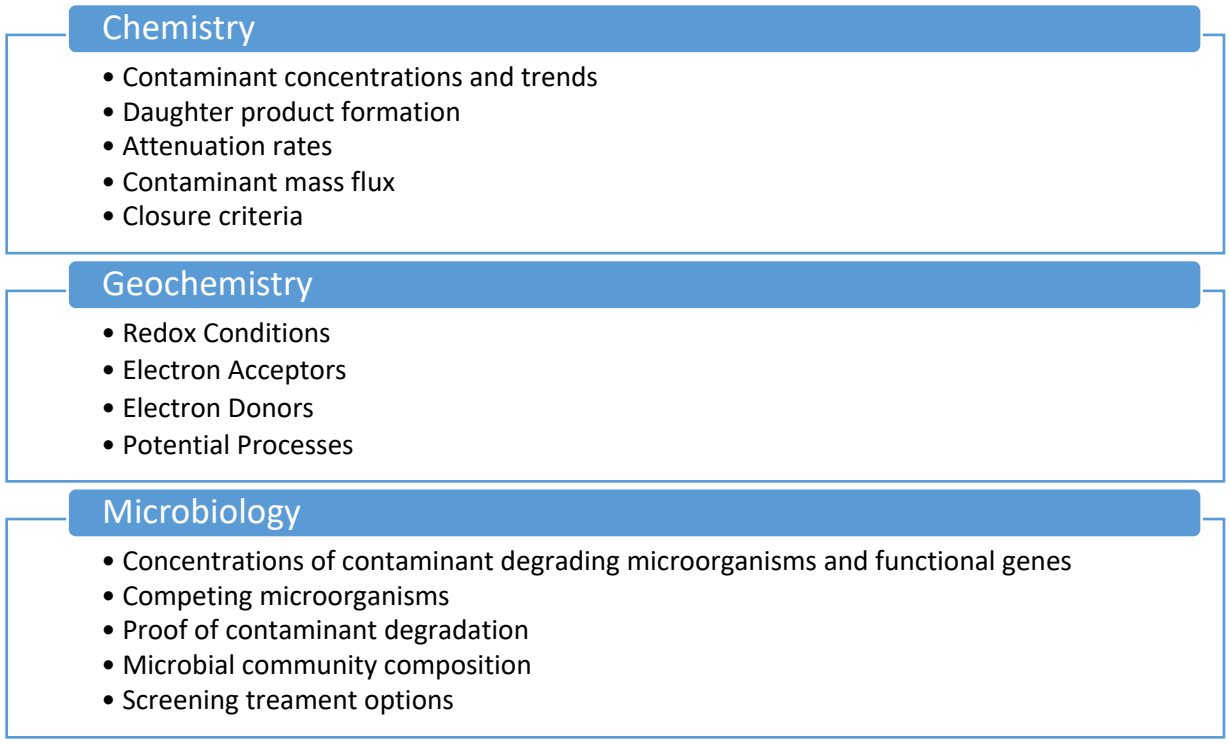
1.0 Importance of a Multiple Lines of Evidence Approach

Microbial Insights highly recommends a multiple lines of evidence approach at remediation sites—synthesizing chemical, geochemical, and microbial data with site history and hydrogeological information to develop and refine the conceptual site model (CSM). Collecting direct microbial data is particularly important at sites where either natural or enhanced bioremediation is part of the corrective action plan. Inferring the potential for biodegradation from chemical and geochemical data alone can underestimate the role of bioremediation in site cleanup and increase uncertainty in CSMs. However, microbial samples are easy to collect in the field and have been proven to provide actionable data that can increase confidence in site management decisions and help gain stakeholder acceptance. A great reference is an article by Madison, et al about, "Increasing *in situ* bioremediation effectiveness through field-scale application of molecular tools" (Madison, 2023).

There are benefits to collecting multiple lines of evidence at every stage of a bioremediation project (site assessment, remedy selection, performance monitoring, closure). For example, baseline data can help delineate the contaminant source zone and plume, identify potential limitations in electron acceptors or donors, and determine the presence and abundance of indigenous contaminant-degrading microorganisms. Whether a pilot test or in situ microcosm study is used to assess the feasibility of a biostimulation or bioaugmentation treatment option, a sampling program that includes microbial data can verify that the remedy performed as expected or identify any issues prior to investing in a full-scale application.

After a treatment has been applied, analyzing spatial and/or temporal trends in chemical, geochemical, and microbial data can confirm whether the remedy was effective and help explain unexpected datapoints. For instance, if contaminant concentrations initially stay the same or even increase after treatment, microbial data showing an increase in contaminant degraders can be an important leading indicator to support that biodegradation is occurring. The concentration gradient resulting from enhanced biodegradation can lead to increased contaminants in the dissolved phase, which may complicate analysis of groundwater contaminant trends until more contaminant mass has been degraded.

The following chart provides examples of chemical, geochemical, and microbial lines of evidence to consider when assessing bioremediation. The molecular biological tools recommended for collecting microbial data will be discussed in Section 2.0.



2.0 Collecting Microbial Data with Molecular Biological Tools

2.1 Measuring contaminant-degrading microorganisms, functional genes, and competitors

CENSUS® qPCR and QuantArray® are DNA-based molecular biological tools used to accurately quantify contaminant-degrading microorganisms, relevant functional genes, and competing microorganisms. For sites impacted with chlorinated solvents, the QuantArray®-Chlor suite is recommended as it provides simultaneous quantification of over 20 different gene targets associated with reductive dechlorination, aerobic co-metabolism, and competing biological processes. For petroleum-impacted sites, QuantArray®-Petro is recommended as it quantifies 20+ gene targets associated with aerobic and anaerobic degradation pathways for BTEX, polycyclic aromatic hydrocarbons, and fuel oxygenates. For additional contaminants of concern like 1,4-dioxane and perchlorate, relevant CENSUS® qPCR assays may be available. Please reach out to a Microbial Insights project manager for more information.

There are several different strategies for interpreting CENSUS® qPCR and QuantArray® data. For baseline and monitored natural attenuation (MNA) sampling events, compare results from impacted monitoring wells to background concentrations. Higher concentrations of key microorganisms and functional genes in impacted monitoring wells indicates growth of contaminant degraders within the dissolved plume and the potential for biodegradation. For sites undergoing enhanced bioremediation, compare results from baseline and post-treatment sampling events to evaluate remedy effectiveness.

The Microbial Insights Database is a helpful resource for adding context to microbial results, as it includes CENSUS® qPCR and QuantArray® data from more than 250,000 field samples from sites around the world. The database provides percentile rankings to help site managers understand if their results are low, medium, or high compared to thousands of other environmental samples Microbial Insights has

analyzed. Finally, threshold concentrations have been established for some qPCR assays that can be used as benchmarks. For example, a *Dehalococcoides* (DHC) concentration of 10^4 cells/mL has been established by the Microbial Insights database and in the literature as a threshold associated with generally effective rates of complete reductive dechlorination of chlorinated ethenes.

Bonus Tip

If combined biotic/abiotic or chemical treatment approaches are being considered, Min-Traps® can be used to passively collect mineralogical samples, and the QuantArray®-BGC suite is a recommended line of evidence to better understand the biogeochemical processes occurring in the subsurface.

2.2 Proof of Contaminant Degradation

Stable Isotope Probing (SIP) is an innovative method to track the environmental fate of a ^{13}C-labeled contaminant of concern to unambiguously demonstrate contaminant degradation. The label serves as a tracer which can be detected in the end products of biodegradation: new biomass and carbon dioxide (CO_2) or dissolved inorganic carbon (DIC). A SIP study can be used to assess MNA where incorporation of the ^{13}C label into biomass and DIC conclusively demonstrates that biodegradation of the contaminant is occurring in situ. A SIP study is also beneficial when evaluating enhanced bioremediation where greater levels of ^{13}C incorporation into biomass and DIC relative to a control would demonstrate that the addition of an amendment (electron acceptor, nutrients, etc.) promoted biodegradation.

SIP studies can be performed for any compound that microorganisms use carbon and energy sources. Although remediation sites may have a mixture of contaminants present, when planning a SIP study, it is recommended to choose the contaminant of greatest concern. For gasoline sites, SIP studies are most frequently performed with ^{13}C-benzene, and ^{13}C-MTBE studies are also common. At diesel and manufactured gas plant sites with polycyclic aromatic hydrocarbons, naphthalene is often chosen.

2.3 Microbial Community Composition

Next generation sequencing (NGS) identifies the microorganisms present in an environmental sample down to the genus level, requiring no prior knowledge of microbial community composition. The NGS profile of the microbial community can provide insight into potential microbial processes. While not quantitative, NGS reports include the relative proportions of the microorganisms identified in the sample based on number of classified sequence reads as well as brief descriptions of the top genera identified, which highlight the metabolic capabilities commonly associated with each genus. NGS is frequently used at sites impacted by emerging contaminants where biodegradation pathways are unknown and CENSUS ® qPCR and QuantArray® assays are not available.

2.4 Screening Treatment Options

In Situ Microcosm (ISM) studies are performed to cost effectively evaluate multiple remediation options including MNA, biostimulation, and bioaugmentation. As illustrated in Figure 1, an ISM assembly is a group of individual microcosm units, each representing a different treatment strategy. Each individual ISM unit contains passive samplers that provide the chemical, geochemical, and microbial data to evaluate the effectiveness of each remediation option:

- Passive diffusion bag for volatile organic compounds (VOCs) and dissolved gases
- Geochemistry sampler for anions and volatile fatty acids

- Bio-Trap® microbial sampler
- Amendment suppliers, as appropriate

Following in-well deployment, the ISM units are retrieved, and the passive samplers are shipped to Microbial Insights for analysis to determine the impact of each treatment option on contaminant concentrations, daughter product formation, geochemistry, and concentrations of microorganisms and functional genes of interest.

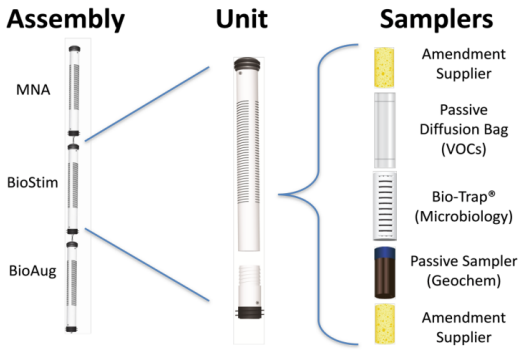


Figure 1. Detail of the samplers and ISM units that comprise an ISM assembly, which is deployed in a monitoring well

At chlorinated solvent sites, an ISM assembly typically has an unamended MNA unit, a BioStim unit amended with an electron donor, and a BioAug unit containing a commercial culture and electron donor. At petroleum-impacted sites, an ISM assembly usually includes an MNA unit and BioStim unit(s) amended with an electron acceptor and/or nutrients.

Action Item

Once a treatment is selected, consult with the product manufacturer/vendor and use vendor design tools to determine the recommended dosing and delivery of the chosen amendment.

3.0 Case Studies

3.1 Cost Effective Remedy Selection at a TCE-Impacted Site

A shallow groundwater aquifer at a former industrial facility was impacted by trichloroethene (TCE). During historical groundwater monitoring, cis-1,2-dichloroethene (cis-DCE) had been detected at notable concentrations. However, vinyl chloride concentrations were low and ethene was typically near or below detection limits, suggesting that cis-DCE may continue to accumulate under existing site conditions. Site managers wanted a cost-effective way to evaluate MNA, biostimulation, and bioaugmentation as potential remediation strategies, so an ISM study was designed to assess these three remedial approaches.

Is complete reductive dechlorination likely?

- Chemistry: Are daughter products such as vinyl chloride and ethene detected?
- Geochemistry: Is the groundwater anaerobic? Are competing electron acceptors present in high concentrations?
- Microbiology: Is *Dehalococcoides* present at concentrations greater than the 10^4 cells/mL benchmark?

For this site, the ISM assembly was composed of an unamended MNA unit, a BioStim unit amended with an electron donor, and BioAug unit pre-inoculated with a commercial *Dehalococcoides*-containing culture as well as an electron donor amendment. The ISM assembly was deployed in an existing monitoring well at the site for approximately 60 days.

Microbial Results: Results of CENSUS® qPCR quantification of *Dehalococcoides* (DHC) and vinyl chloride reductase genes (VCR) are shown in Figure 2 below. Low DHC concentrations were detected in the MNA unit, indicating limited potential for complete reductive dechlorination of TCE to ethene under existing site conditions. In the BioStim unit, concentrations of DHC and VCR were over an order of magnitude greater compared to the MNA unit—a substantial increase considering the short ISM deployment relative to the life of a full-scale electron donor injection. For the BioAug unit, the pre- and post-deployment concentrations of DHC were comparable, indicating survival of the bioaugmentation culture in the field.

Overall, DHC concentrations were highest in the pre-inoculated BioAug unit. CENSUS® qPCR results for the MNA and BioStim units indicated that DHC was present at low concentrations and electron donor addition would stimulate growth of this key group of halorespiring bacteria.

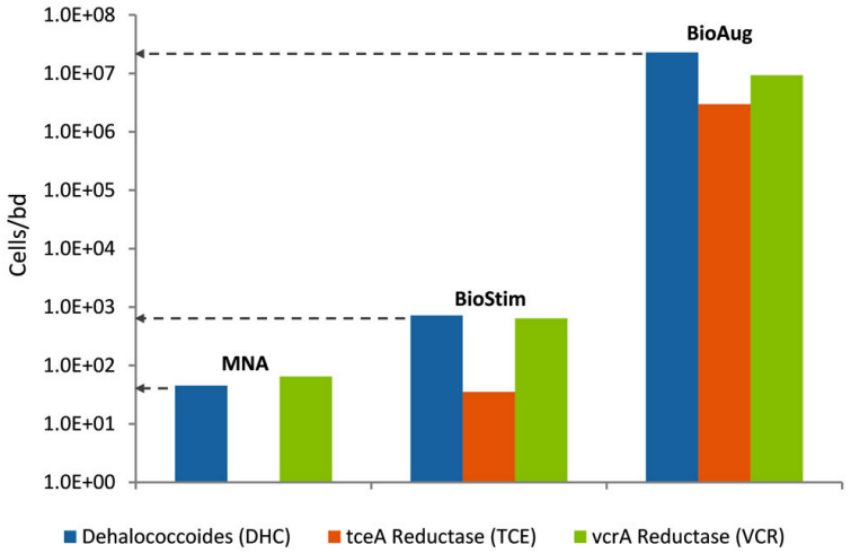


Figure 2. CENSUS® qPCR results from the MNA, BioStim, and BioAug ISM units

Chemical Results: VOCs and dissolved gases were analyzed from passive diffusion samplers in each ISM unit. The mole fractions of TCE and daughter products are show in Figure 3. In the MNA unit, TCE was present in the highest proportion with cis-DCE as the remainder. In the BioStim unit, the mole fraction of TCE had been reduced to <0.1, cis-DCE was predominant, and vinyl chloride and ethene were also detected. This is consistent with the observed growth of DHC in response to the electron donor addition.

In the BioAug unit, TCE was below detection and cis-DCE comprised the highest proportion. Vinyl chloride and ethene were detected in low proportions only slightly greater than those seen in the BioStim unit.

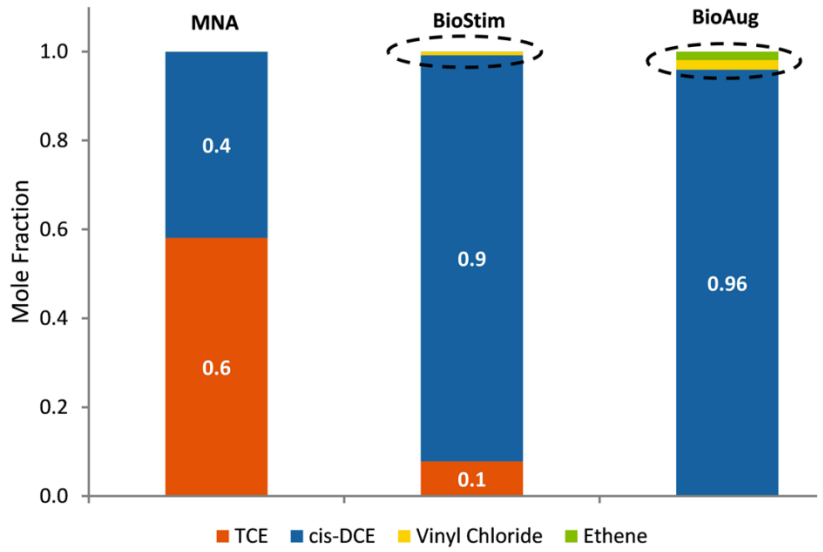


Figure 3. Contaminant mole fractions from the MNA, BioStim, and BioAug ISM units

Decision

Based on the multiple lines of evidence collected in the ISM units, site managers decided that bioaugmentation was not necessary and selected biostimulation as the treatment strategy. The increased DHC concentration in the BioStim unit along with increased production of cis-DCE, vinyl chloride, and ethene daughter products indicated that electron donor addition would stimulate reductive dechlorination without requiring a bioaugmentation culture.

3.2 Using CENSUS® qPCR to Evaluate MNA at a Petroleum Pipeline Release

A petroleum pipeline release in a remote area had gone undiscovered for several years. Groundwater was impacted with benzene, toluene, ethylbenzene, and xylenes (BTEX). Groundwater monitoring results since discovery suggested that BTEX concentrations were decreasing. However, contaminant concentrations and geochemical conditions exhibited considerable variability. Site managers needed additional lines of evidence to evaluate if MNA would be an appropriate management strategy.

CENSUS® qPCR quantification of benzylsuccinate synthase (BSS) and anaerobic benzene carboxylase (ABC) genes was incorporated into routine monitoring of plume and background wells to evaluate the BTEX biodegradation potential and the feasibility of continuing MNA. BSS initiates anaerobic biodegradation of toluene, ethylbenzene, and xylenes. Although additional pathways are likely, the only characterized pathway for anaerobic benzene biodegradation is initiated by ABC.

During on-going groundwater monitoring, Bio-Traps were submitted for CENSUS® qPCR analysis from two background wells (MW-6 and MW-7) and three BTEX-impacted wells (MW-8, MW-9, and MW-10).

Comparing the CENSUS® qPCR results for background wells to impacted wells across six sampling events indicated growth of anaerobic BTEX degraders within the dissolved plume (Figures 4 and 5).

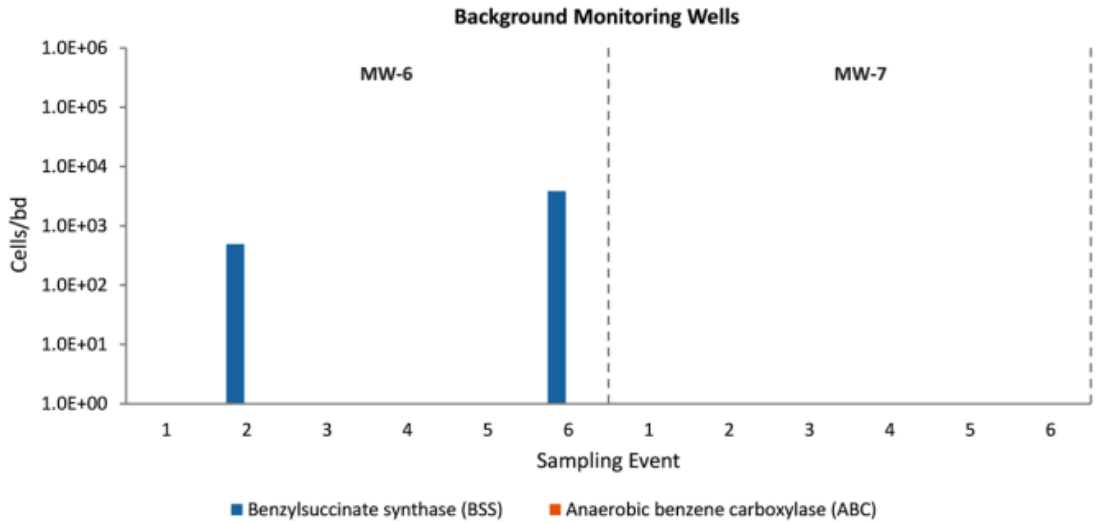


Figure 4. CENSUS® qPCR results for background monitoring wells over six sampling events

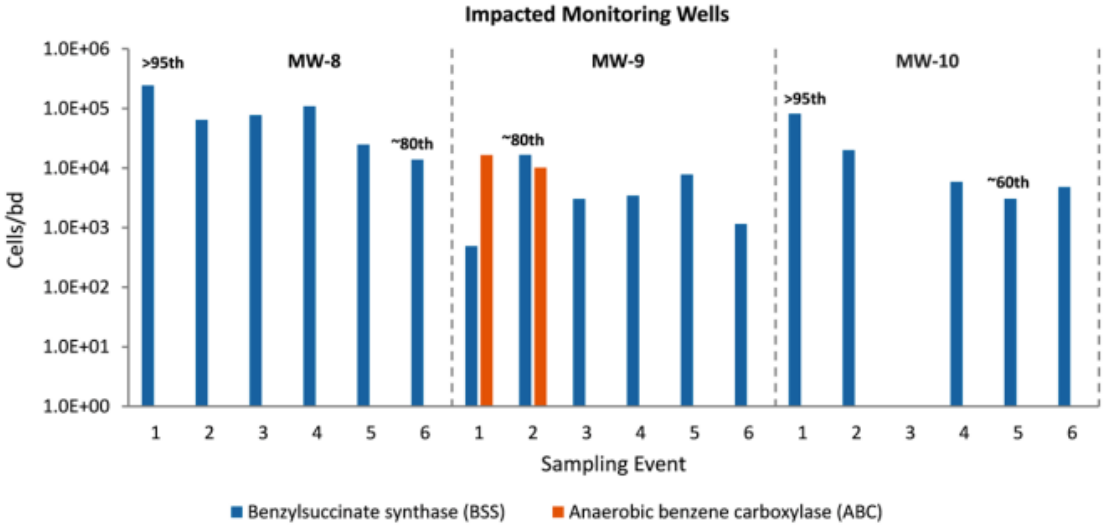


Figure 5. CENSUS qPCR results for impacted monitoring wells over six sampling events

- BSS genes for anaerobic toluene biodegradation were only detected in one of the background wells (MW-6) and only during two of the six sampling events. Conversely, BSS genes were detected in all BTEX-impacted wells (MW-8, MW-9, and MW-10) during all but one sampling event.
- Moreover, concentrations of BSS genes in impacted wells were high. Using the Microbial Insights Database, BSS concentrations in MW-8 were greater than observed in 80% to 95% of samples submitted from sites worldwide. At MW-9 and MW-10, BSS gene concentrations were "above average," ranging between the 60th and 95th percentiles.
- Finally, ABC genes for anaerobic benzene biodegradation were occasionally detected in impacted monitoring well MW-9 at high concentrations.

Overall, CENSUS® qPCR results indicated growth of anaerobic BTEX degraders within the dissolved plume and provided a supporting line of evidence for biodegradation under existing site conditions

Decision

MNA was continued based on the converging lines of chemical, geochemical, and microbiological evidence. Concentrations of targeted functional genes involved in anaerobic BTEX biodegradation ranged from above average to very high using the Microbial Insights Database and were orders of magnitude greater in the dissolved plume compared to background locations.

4.0 References

Madison AS, Sorsby SJ, Wang Y, and Key TA. Increasing *in situ* bioremeditation effectiveness through field-scale application of molecular biological tools. Front. Microbiology 09, Feb 2023.