## Assessing the Genetic Potential for Natural Source Zone Depletion at a Petroleum-Contaminated Site

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Background/Objectives. As researchers continue to identify the impact that natural source zone depletion (NSZD) has on site cleanup timeframes, there is a growing need for methods and tools that provide microbial-based metrics to aid in conceptual site modelling. Through the identification and quantification of microorganisms and functional genes related to NSZD at hydrocarbon release sites, project managers are better able to: 1) identify the potential for NSZD, 2) improve site-specific models, and 3) provide direct microbial data to regulatory bodies as proof of NSZD potential. QuantArray<sup>®</sup>-NSZD is an advanced gPCR method that guantifies a broad spectrum of microorganisms and functional genes associated with NSZD processes in a single analysis for a comprehensive and cost-effective evaluation of bioremediation potential. Similarly, QuantArray<sup>®</sup>-Petro quantifies a suite of functional genes involved in aerobic and anaerobic biodegradation of BTEX, PAHs, and other petroleum hydrocarbons. Both QuantArray<sup>®</sup> analyses include over 20 assays performed on a single sample. By incorporating these molecular biological tools into baseline sampling and routine monitoring the subsurface microbiome will no longer be an unknown. In this study, QuantArray®-NSZD was employed to identify the presence of key gene targets related to NSZD at a petroleum hydrocarbon-impacted site.

**Approach/Activities.** QuantArray<sup>®</sup> is a hybrid technology that combines the highly parallel detection of DNA microarrays with the accurate and precise quantification of qPCR into a single platform. The approach employs nanoliter fluidics for low volume, solution phase qPCR allowing simultaneous quantification of different gene targets and therefore more comprehensive sample assessment. QuantArray<sup>®</sup>-NSZD is used to quantify 21 different taxonomic and functional gene targets related to NSZD. Examples of target microbial groups and functional genes include acetogens, fermenters, methanogens, acetoclastic methanogens, biosurfactant production-based genes, and metal reducing bacteria. Other methods like multiplex qPCR have been described that achieve some level of parallel quantification, but unlike multiplex qPCR, QuantArray<sup>®</sup> employs discrete through-holes for individual qPCR reactions so reaction kinetics are not compromised. A petroleum hydrocarbon-impacted site suspected to be undergoing NSZD was analyzed via chemical and geochemical techniques as well as QuantArray<sup>®</sup>-NSZD. The goals of this study were to determine if NSZD was occurring and to identify correlations between chemical parameters and the measured microbial gene targets.

**Results/Lessons Learned.** As demonstrated within this presentation, the bulk of NSZD processes rely on microbial activity. Although the final products ( $CO_2$  and  $CH_4$ ) of NSZD can be measured using traditional analyses, only the microbial data can provide insights into the actual functions taking place. At the study site, QuantArray<sup>®</sup>-NSZD data were used to determine that key NSZD gene targets were present and that methane production could be correlated to a specific target. Often, three lines of evidence are recommended for a clearer understanding of the activity occurring in the subsurface of a contaminated site: chemical, geochemical, and microbiological. The results from this study will not only highlight the necessity for multiple lines of evidence, but will illustrate that microbiology is the only line of evidence that can truly connect the other two lines of evidence during processes like NSZD.