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Next Generation Sequencing (NGS)

Characterization of Microbial Communities at a Pipeline Release

PROJECT SUMMARY

- At a pipeline release, CENSUS qPCR results for functional genes BSS and ABC demonstrated high concentrations of anaerobic BTEX degraders in the dissolved plume (See Case Study CENSUS qPCR: Actionable Data for Evaluating MNA).
- However, site managers also wanted to know "Who is there?" and used next generation sequencing (NGS) to characterize the microbial community.
- NGS revealed that microbial communities in the impacted areas were markedly different than background populations and changed over time likely due to fluctuating subsurface conditions.

PROJECT CHALLENGE

Groundwater concentrations of benzene, toluene, ethylbenzene, and xylenes (BTEX) were decreasing. Furthermore, CENSUS qPCR results for benzylsuccinate synthase (BSS) and anaerobic benzene carboxylase (ABC) had indicated high concentrations of anaerobic BTEX degraders within the dissolved plume, providing a supporting line of evidence for MNA. However, site managers were also interested in answering "Who is there?" and assessing the impact of petroleum hydrocarbons and fluctuating subsurface conditions on the overall bacterial community.

SAMPLING AND ANALYSIS

Next generation sequencing (NGS) provides comprehensive identification of microorganisms present in a sample down to the genus and even species level. While not quantitative, the relative proportions of the microorganisms identified can provide insight into potential microbial processes. At complex sites, NGS is often performed for an overall profile of the microbial community while CENSUS qPCR or QuantArray is used to quantify known contaminant degraders and functional genes.

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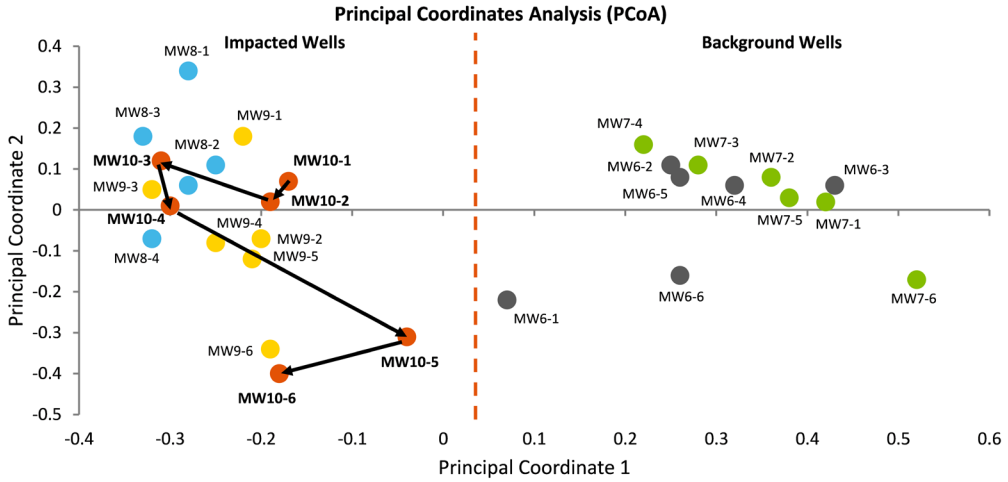
BACKGROUND VS IMPACTED WELLS

Along with tables of top genera and informative descriptions, NGS reports include statistical analyses to aid interpretation. Figure 1 is a Principal Coordinate Analysis (PCoA) of the normalized relative abundances of all genera identified in the samples.



In short, PCoA reveals which samples have similar vs different microbial communities. Samples that are close together or "cluster" on the PCoA plot are more similar. Conversely, samples that are farther apart have notably different microbial communities.

In the current study, the background wells (MW6 and MW7) clustered toward the right on Principal Coordinate 1 whereas the impacted wells (MW8, MW9, and MW10) grouped on the left.

- Thus, the microbial communities in the dissolved plume were substantially different than background populations.

As an example, site managers compared the top genera in background well MW7 to impacted well MW10.

- In MW7, the top genus identified was *Dechloromonas* (~29% of total reads), facultative aerobes which respire oxygen, nitrate or perchlorate. Anaerobes including sulfate reducing *Desulfobulbus* and iron reducing *Geobacter* were identified but at much lower relative abundances (~2%).
- Conversely, *Geobacter* was the top genus in MW10 (~34%) likely due to the anaerobic conditions in the dissolved plume. *Dechloromonas* were much lower (~4%).

The PCoA plot also highlights changes over time for some wells including MW10 (orange circles). Following the black arrows, the first four sampling events (MW10-1 through MW10-4) formed a cluster with other impacted wells. However, time points MW10-5 and MW10-6 were much lower on Principal Component 2 indicating a shift in the microbial community composition.

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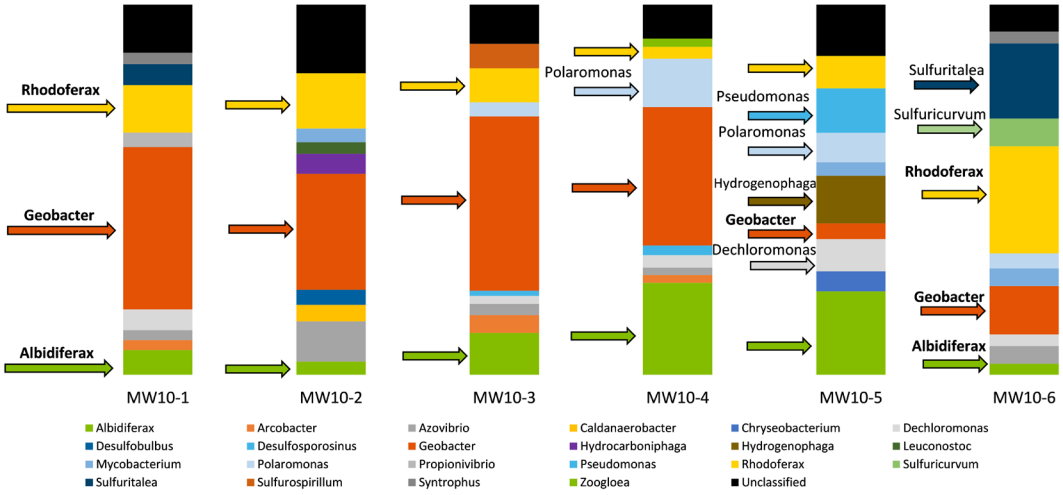
MICROBIAL COMMUNITY CHANGES OVER TIME

The details of these changes in microbial populations over time are evident in Figure 2, the stacked bar portion of the hierarchical cluster dendrogram (HCD) performed as part of an NGS report.



- For the first three sampling events, the microbial community was diverse, but relatively stable overall. *Geobacter* (orange) was the top genus identified in the each of samples ranging from approximately 20% to 40% of total reads. *Rhodoferax* (yellow) varied somewhat but were consistently ~ 10% of total reads.
- At the fourth sampling event (MW10-4), some changes to the microbial community composition were beginning to appear. *Geobacter* remained the top genus identified (~35% of total reads), but the relative proportion of iron reducing *Alibidiferax* (green) had increased substantially to over 21% of total reads. *Rhodoferax* (yellow) had decreased while *Polaromonas* increased.
- By the fifth sampling event (MW10-5), marked changes were evident potentially due to fluctuating subsurface conditions. Most notably, the relative proportion of iron reducing *Geobacter* spp. decreased form over 30% to only ~3% of total reads. Meanwhile aerobic and facultative microorganisms (*Pseudomonas*, *Hydrogenophaga*, *Dechloromonas*, and *Polaromonas*) were detected in notable proportions.
- During the last sampling event (MW10-6), significant changes were also clearly visible. *Geobacter* had increased, but *Rhodoferax* was the top genus identified. *Sulfuritalea* and *Sulfuricurvum*, facultatively anaerobic sulfur oxidizers, were also detected.

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MICROBIAL COMMUNITY CHANGES OVER TIME (CONT.)

Conclusions: NGS revealed that microbial communities in the impacted areas were markedly different than background populations. Moreover, the microbial community composition at the impacted wells changed over time likely due to the observed fluctuations in subsurface conditions. *Geobacter* spp. were typically detected at the highest relative abundances in monitoring wells within the dissolved plume. While at least one species of *Geobacter* is capable of anaerobic BTEX biodegradation, most cannot. Therefore, the presence of *Geobacter* even in high relative proportions does not indicate the potential for BTEX biodegradation. To evaluate the potential for anaerobic BTEX biodegradation, CENSUS qPCR was performed to quantify benzylsuccinate synthase (BSS) and anaerobic benzene carboxylase (ABC) genes. The decision to continue MNA at the site was based on decreasing BTEX concentrations and CENSUS qPCR results demonstrating high concentrations of anaerobic BTEX degraders in the dissolved plume (See Case Study CENSUS qPCR: Actionable Data for Evaluating MNA).

KEY BENEFITS

- **Revealing:** Comprehensive identification of the microorganisms present in each sample provided insight into likely microbial processes such as iron reduction.
- **Statistics:** PCoA and HCD included in NGS reports clearly highlighted that the microbial community composition of impacted wells was considerably different than background populations. Moreover, microbial community composition in impacted wells was dynamic – changing with subsurface conditions.
- **Compatible:** Multiple lines of evidence provide a more complete picture. NGS provided an overall profile of the microbial community while CENSUS qPCR quantified known functional genes in BTEX biodegradation.