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Next Generation Sequencing and qPCR as Complementary Approaches for Evaluating MNA at a Petroleum Hydrocarbon-Impacted Site

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Background/Objectives. Advances in DNA sequencing and bioinformatics have allowed unprecedented exploration of microbial communities in environments ranging from the human gut to ocean thermal vents. In environmental restoration, sequencing most often refers to amplicon sequencing of regions of the 16S rRNA gene to give a profile of the overall microbial community composition and answer the question “Who is there?”. Comparing 16S sequence profiles between samples provides insight into impacts on the microbial community resulting from groundwater contaminants and site management activities. However, specific biological functions such as the anaerobic biodegradation of BTEX cannot be conclusively inferred from microbial community profiles. In the current study, next generation sequencing (NGS) was used to monitor changes in microbial community composition while qPCR assays were employed to quantify functional genes involved in anaerobic biodegradation of BTEX.

Approach/Activities. The study site resulted from a petroleum pipeline release and is currently undergoing monitored natural attenuation (MNA). Total BTEX concentrations in impacted wells are on the order of 4 to 7 mg/L. Groundwater samples were periodically obtained from background wells and monitoring wells within the dissolved plume for a period of two years. Along with traditional groundwater monitoring, samples were submitted for NGS of 16S rRNA genes and qPCR analyses. Principal components analysis (PCA) was performed with NGS results to elucidate differences in microbial community composition and any changes over time. To evaluate potential for anaerobic BTEX biodegradation, qPCR analyses included quantification of benzylsuccinate synthase (*bssA*), anaerobic benzene carboxylase (*abcA*) and functional genes (*gmet*0231*, gmet*0232) linked to anaerobic benzene biodegradation initiated by hydroxylation in *Geobacter metallireducens*.

Results/Lessons Learned. PCA of the NGS results revealed marked differences in the microbial communities in the impacted areas relative to the background locations. In the background samples, *Dechloromonas* and *Methylotenera* spp. were among the most frequently detected genera detected while iron reducing bacteria such as *Geobacter* spp. and *Albidoferax ferrireducens* (formerly *Rhodoferax ferrireducens*) were dominant samples in impacted monitoring wells. Microbial community composition in the background areas was relatively stable over time. In impacted areas, however, a clear shift was noted over time. While *Geobacter* spp. were still frequently detected, the relative abundance of *Albidoferax* spp. was notably greater during the more recent sampling events which may be linked to subtle changes in subsurface conditions. In terms of assessing the potential for BTEX biodegradation, concentrations of *bssA* genes were below detection limits in background samples but were routinely detected at concentrations on the order of 10^3 to 10^4 gene copies/mL within the dissolved plume. Benzene carboxylase genes (*abcA*) were also detected at high concentrations confirming the potential for benzene biodegradation under existing conditions but was not detected at all locations. Overall however, the combination of NGS and qPCR highlighted enrichment of iron reducing bacteria within the dissolved plume, revealed shifts in the community over time but also demonstrated continued potential for anaerobic BTEX biodegradation.