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Next Generation MBTs: A Pathway to Precision Bioremediation

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Background/Objectives. Advances in understanding of the microbiology contributing to the degradation and detoxification of groundwater contaminants enabled the implementation of enhanced in situ bioremediation approaches. In particular, the demonstration that organohalide-respiring bacteria can detoxify chlorinated contaminants was a breakthrough discovery. *Dehalococcoides*, *Dehalogenimonas* and *Dehalobacter* are highly specialized bacteria that require chlorinated compounds to fuel their energy metabolism, and some strains possess reductive dehalogenases (RDases) that dechlorinate priority pollutants including chlorinated solvents, chlorinated benzenes, and polychlorinated biphenyls. Several RDases responsible for specific reductive dechlorination steps have been identified and serve as process-specific biomarkers. The enumeration of phylogenetic (i.e., 16S rRNA gene) and process-specific (e.g., RDase gene) biomarkers using quantitative PCR (qPCR) provides valuable information about the presence and abundance of specific organisms capable of contaminant degradation; however, DNA-based approaches merely inform about potential, but not actual degradation activity. Further, the same qPCR approaches are applied to all samples even though the diversity of organisms and RDase genes differs between sites. Thus, the informational content of qPCR data alone is limited and leaves ample room for interpretation, which can lead to very different site management decisions with different outcomes.

Approach/Activities. Based on available information, a suite of qPCR assays targeting reductive dechlorination biomarker genes was designed for the QuantStudio 12K Flex Real-Time PCR System. This qPCR system allows the enumeration of up to 224 target genes and transcripts in a highly parallel format. In addition, global and targeted proteomics approaches were applied to identify and enumerate biomarker proteins. Attempts were made to correlate the biomarker gene-, transcript-, and protein-centric approaches with observed reductive dechlorination rates.

Results/Lessons Learned. The QuantStudio qPCR system provides an ideal platform for processing many samples simultaneously and generating robust qPCR data. Reductive dechlorination biomarker proteins can be quantified in groundwater, and in concert with qPCR, quantitative proteomics (qProt) can provide rate estimates for specific reductive dechlorination steps of interest. Metagenomic workflows can provide information about the specific target genes present at a site of interest, and site-tailored qPCR and qProt approaches can be designed and applied. Thus, the integrated application of metagenomics, qPCR and qProt promises to link biomarker abundances to in situ rates, which will be a major step towards the implementation of precision bioremediation. At many sites, biostimulation, with or without bioaugmentation, is implemented to stimulate the reductive dechlorination process. Contemporary qPCR approaches cannot inform about appropriate electron donor amounts to be added, the rate of electron donor applications, or if enhanced treatment is required at all. While such brute-force bioremediation approaches have been successfully implemented at many sites, it is very likely that more refined treatment (i.e., precision bioremediation) would have resulted in a similar reduction of contaminant concentrations at substantially lower costs and lesser environmental impacts. This presentation will outline how the integrated application qPCR and omics tools generates information, including contaminant degradation rate estimates, that can transform bioremediation from an empirical practice to a science with predictable outcomes.