Comparison of Whole Genome Sequencing, 16S Amplicons, and qPCR for Assessment and Monitoring of an EPA Superfund Site

Peter Guerra (pguerra@lynker.com)
Rebecca A. Reiss and Oleg Makhnin (New Mexico Tech, Socorro, NM)

Background/Objectives. Tetrachloroethene (PCE) contamination of the sole-source water supply for the city of Española New Mexico and the Santa Clara Pueblo was discovered in 1989. The North Avenue Railroad Plume (NRAP) was the result of leakage from a dry-cleaning facility and was designated an EPA Superfund site in 1999 (National Priority List #NMD986670156). When samples were submitted for initial molecular biological testing in 2006 the state of the art included quantitative Polymerase Chain Reaction (qPCR) to detect the genera of microbes with dehalogenation potential and reductive dehalogenase *(rdh)* genes. Denaturing Gradient Gel Electrophoresis (DGGE) and Phospholipid Fatty Acid Analysis (PLFA) were also used to generate microbial community profiles. *Dehalococcoides*, *Dehalobacter, and Desulfuromonas* were detected as was the gene for trichloroethene reductase (TCE-RDase) qPCR. Combined with the geochemical and hydrological data, these results indicated that enhanced reductive dichlorination via biostimulation was the selected remedy. This involved the addition of bioamendments that included emulsified vegetable oil (EVO), a nutrient mix, and hydrogen gas. Prior to amendment injection, microbial samples from two wells on the Site were stored for future analysis. The objective was to be prepared to apply new techniques as they became available. This made possible the comparison of techniques for assessment, monitoring, and for new discoveries that will lead to the improvement of remediation assessment protocols.

Approach/Activities. The results of WGS for 0 and 5 months were published in 2016. Samples were obtained at 23 and 39 months and all four timepoints were sequenced by the Joint Genome Institute (JGI). These were also subjected to 16S amplicon analysis, in which universal primers were used to create a pool of 16S molecules that were sequenced.

Results/Lessons Learned. While WGS detects more taxa than 16S amplicons, for taxa such as *Dehalococcoides* and *Dehalobacter,* the patterns over the four timepoints are similar for both techniques. For initial assessment, qPCR remains an accurate and affordable method. The more expensive 16S amplicon analysis can replace qPCR for taxa but cannot detect genes involved in degradation. DGGE and PFLA techniques can be replaced by 16S. While WGS is not currently optimal for initial assessment due to the expense, it can detect both important taxa and genes when reads are assembled into scaffolds. WGS facilitates the identification of strains, genes, and genomes without any *a priori* knowledge of community composition. Considering the diversity within *Dehalococcoides* to respire organohalides, genus level detection and quantification can overestimate the potential for complete dehalogenation of PCE to ethene. The TCE-RDase gene first detected by qPCR is present in a scaffold attributable to *Dehalococcoides*. Taxa not yet known to be involved in degradation are present at NRAP that contain RDase genes, evidence that other genera may be capable of respirating organohalide contaminants. WGS will advance treatment options, but only if baseline samples are available to compare the profiles of later timepoints. A recommended approach is to use qPCR and/or 16S for initial site assessment but to collect and store sample material from the baseline that can be subjected to WGS techniques when needed. Another sampling event is planned before the end of 2023 that will mark 16 years since remediation began. This sample will be analyzed using proximity ligation sequencing, a new WGS technique that facilitates the assembly of genomes from complex metagenomes that requires less DNA than current protocols.