Molecular Genomic Approaches for Tracking In Situ Anaerobic Benzene Degradation

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Background/Objectives. Petroleum hydrocarbons are widespread anaerobic groundwater pollutants arising from thousands of accidental releases at industrial facilities, oil refineries, pipelines, and mining operations globally. Groundwater contamination with benzene is of particular concern due to its proven carcinogenicity and low drinking water standards (typically 5 µg/L). Physicochemical and aerobic microbial remediation methods have long been the standard for benzene removal. However, these technologies are not feasible at all sites (i.e., deep anaerobic aquifer systems where treatment options are challenging and expensive to implement). Bioremediation strategies relying on anaerobic degradation processes may therefore be a more effective approach at benzene removal at anoxic sites. While anaerobic benzene remediation does sometimes occur by intrinsic microorganisms, they are often absent or in abundances too low to stimulate degradation (< 2 copies/mL). Clearly there is a need to explore what might be inhibiting the growth of anaerobic benzene degraders at these sites.

Approach/Activities. We sought to use genomic tools and microbial community analyses to help develop hypotheses for what might be promoting or preventing the growth of anaerobic benzene degraders at specific sites. To accomplish this, laboratory-based treatability studies were conducted using site materials from 10 benzene-contaminated sites across Canada, the US, Germany, and China. Microcosms were prepared using groundwater slurries of contaminated site materials and treated with exogenous electron acceptors (biostimulation), cultured benzene degraders (bioaugmentation), or were left untreated (natural attenuation). Bottles were incubated under laboratory conditions for 1 to 2 years, and routinely monitored for analytical and molecular evidence of benzene degradation/microbial activity. The key organisms and functional genes associated with anaerobic benzene degradation were quantitatively tracked using recently developed biomarker assays (qPCR, 16S rRNA gene sequencing).

Results/Lessons Learned. Laboratory treatability tests using biostimulation and bioaugmentation increased the probability of anaerobic benzene degradation by 50% in surveyed site materials, as compared to natural attenuation. These technologies successfully increased the abundance of active benzene degraders (> 10^6 copies/mL) in site materials that already harboured intrinsic but low abundances of these microorganisms (specifically, *Peptococcaceae* spp. and *Deltaproteobacteria* ORM2). This also corresponded with an increase in rates of anaerobic benzene degradation (> 25%). When these taxa could not be detected by our biomarker assays, the queried bioremediation strategies were also found to be unsuccessful. Thus, increasing the abundance of anaerobic benzene degraders is only sometimes possible. Two main classes of growth inhibitors were identified in the experimental site materials; 1) petroleum co-contaminants (BTEX, alkanes) that support the preferential growth of other microorganisms, and 2) anionic salts at concentrations greater than 2,000 mg/L. In the case of the former, laboratory demonstrations suggest that diluting or degrading co-contaminants can eventually allow for conditions suitable for benzene bioremediation. We are now using these data to help plan an in situ anaerobic benzene bioremediation pilot study later this year.