Applications of Anaerobic Petroleum Hydrocarbon Bioremediation

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Benzene, Toluene, Ethylbenzene and Xylene (BTEX) are widespread groundwater pollutants. Groundwater contamination with benzene is of particular concern due to its persistence in anoxic environments and confirmed carcinogenicity. Intrinsic anaerobic processes impact the fate of BTEX as well as other hydrocarbons at petroleum contaminated sites. Ongoing applied research has shown that anaerobic bioremediation processes represent viable options for plume control and site cleanup for BTEX. Benzene, the most toxic of these compounds, is also the most challenging for bioremediation, because the requisite microorganisms are relatively slow growing and reaction mechanisms are not well understood.

Recent advancements in molecular genomics have allowed us to identify the microorganisms responsible for anaerobic benzene, toluene and xylene (BTX) transformation and SiREM has commercialized an anerobic BTX culture (DGG™ Plus) for field application. The microbial composition of DGG™ Plus is relatively complex (i.e., not a pure culture) due to its origin as enrichments from diverse natural microbial communities. DGG™ Plus is a blend of three separately grown cultures that are comprised of mixture of prokaryotic Bacteria and Archaea. Anaerobic hydrocarbon-degrading consortia rely on a synergistic web of activities of different groups of microorganisms to ultimately achieve the complete degradation of BTX to innocuous products.

Based on the microbial community composition and metagenome of the cultures (Devine, 2013; Luo et al., 2016; Toth et al., 2021), the consortium has the predicted (functional) capability of participating in the following biogeochemical pathways:

- Fermentative BTX degradation (Deltaproteobacteria ORM2, *Desulfosporosinus*, and *Peptococcaceae*)
- Fermentation of a range of organic compounds
- Fermentation of dead microbial biomass (***Ca.*** Nealsonbacteria, and others)
- Facultative sulfate reduction (Deltaproteobacteria ORM2, *Peptococcaceae*, *Desulfovibrio*, *Desulfobacca*, and others)
- Metals reduction (*Geobacter*)
- Facultative aerobic metabolism of organic compounds (minor organisms including *Pseudomonas*, and others)
- Methanogenesis (*Methanosaeta*, *Methanoregula*, *Methanomethylovorans*, and other methanogenic archaea)

Results from laboratory treatability studies demonstrated bioaugmentation promoted enhanced benzene biodegradation rates and provided information to aid in field pilot-test design. One field pilot-test performed in November 2019 at a site in Saskatchewan included three injection points, two of which received up to 10 liters of the culture. A third injection point received killed culture, which will serve as a control to rule out if dead cells, or media components, can promote benzene degradation. It is anticipated that benzene degradation rates will be accelerated ***in situ*** through bioaugmentation as observed in corresponding treatability studies. Two additional field applications (one in October 2019 and one in April 2020) with DGG-B™ and one field injection (Summer 2020) with DGG™ Plus are also being monitored.

These first-to-field projects using these novel bioaugmentation cultures, are providing a better understanding of dosing requirements, timeframes for obtaining results and ranges of conditions over which the cultures are effective. As with chlorinated solvents, bioaugmentation for BTEX compounds has the potential to decrease remediation time frames and increase the range of sites to which bioremediation is applicable providing a much-needed, cost-effective alternative for BTEX remediation in groundwater.

Jeff Roberts

Jeff is the Operations Manager at SiREM with extensive technical experience in the laboratory assessment and field implementation of soil, sediment and groundwater remediation technologies at sites containing contaminants including chlorinated solvents, petroleum hydrocarbons and other recalcitrant compounds. Over the past nineteen years he has conducted and managed hundreds of bench-scale batch and column treatability studies and also has technical experience in the growth, scale up and field implementation of several anaerobic microbial cultures for bioremediation remedies. Jeff has several years of passive sampling experience and was a lead member in the development and commercialization of the SP3™ and Speeper™ sampler.