Benzene Degradation under Anaerobic Conditions: Using Treatability Studies and Molecular Tools to Provide Insights

Sandra Dworatzek (sdworatzek@siremlab.com) and Jennifer Webb (SiREM, Guelph, Ontario, Canada)
Kris Bradshaw (Federated Co-operatives Limited, Saskatoon, Saskatchewan, Canada)
Fei Luo, Nancy Bawa, Shen Guo, and Elizabeth A. Edwards (University of Toronto, Ontario, Canada)

Background/Objectives. Widespread use of petroleum products has resulted in benzene, toluene, ethylbenzene, and xylenes (BTEX) contamination at numerous sites. Where anaerobic conditions prevail, natural attenuation of BTEX has been observed, however benzene tends to persist due to its recalcitrance to degradation. A methanogenic benzene enrichment culture (DGG), developed at the University of Toronto, transforms benzene into methane, and the key organism, a *Deltaproteobacteria* sp., has been identified.
Current research efforts are designed to determine 1) whether bioaugmentation with the DGG culture is an effective remedy for benzene contaminated sites; 2) if the presence of benzene degrading biomarkers can be correlated to *in situ* biodegradation activity and 3) to scale up the culture for field plot testing application.

Approach/Activities. Numerous anaerobic treatability studies have been conducted under conditions using site materials impacted with petroleum hydrocarbons. Degradation of BTEX was monitored with and without DGG bioaugmentation under various electron acceptor conditions. Samples from the original groundwater materials, as well as microcosm samples were taken, to quantify the potential benzene degraders via qPCR biomarkers. The timeframe for each treatability study is 8-12 months.

Results/Lessons Learned. To date, in three of these studies, bioaugmentation with the DGG culture has sped up benzene degradation under methanogenic or sulfate-reducing conditions, while in another experiment, no benzene degradation was observed despite bioaugmentation. In the experiment that did not display benzene degradation, the total petroleum hydrocarbons (TPH) decreased from 30 to 7 mg/L over the first 200 days, suggesting that degradation of petroleum hydrocarbon is ongoing, however the presence of other organic carbon may inhibit benzene degradation.
The putative benzene carboxylase gene, known to be related to a gram positive *Peptococcaceae* sp., was also observed in two groundwater samples, and in microcosms with geological materials from these sites have started degrading benzene intrinsically, though at much slower rates compared to the bioaugmented microcosms.
Results from ongoing treatability studies will be presented to provide insights into the capacity of the DGG bioaugmentation culture performance at a range of petroleum hydrocarbon contaminated sites, as well as into the correlation between the presence of benzene degradation molecular biomarkers and in situ biodegradation.