An Unintended Consequence of Biostimulation for Treatment of Chlorinated Solvents: Biologically-Mediated Toluene Production

William M. Moe (moemwil@lsu.edu), Samuel J. Reynolds, and M. Aaron Griffin (Louisiana State University, Baton Rouge, LA, USA)
J. Bryan McReynolds (Dayspring Group, LLC, Baton Rouge, LA, USA)

Background/Objectives. Groundwater at a former waste disposal site located near Baton Rouge, Louisiana (USA) contains a mixture of chlorinated alkanes and alkenes including 1,2-dichloroethane, 1,2-dichloropropane, 1,1,2-trichloroethane, and vinyl chloride. To prevent the further migration of the halogenated organic contaminants in groundwater, a series of wells was installed near the leading edge of the contaminant plume to allow subsurface injection of electron donors that could stimulate in situ anaerobic reductive dechlorination. The subsurface injection of a fermentable substrate (agricultural feed grade cane molasses) and buffer (sodium bicarbonate) was successful in establishing conditions conducive to growth of reductively dechlorinating bacteria, and the chlorinated alkane and alkene concentrations decreased over time. While the molasses injection strategy was successful in mitigating chlorinated solvent contamination, groundwater concentrations of the aromatic hydrocarbon toluene transiently increased over time following molasses injections. Here, we report on studies conducted to investigate the potential of indigenous microbial communities to synthesize toluene.

Approach/Activities. Field monitoring was conducted to measure groundwater concentrations of contaminants and geochemical parameters before and after multiple molasses injections into multiple groundwater wells over a four-year period. Additional sampling was conducted upgradient from the molasses injection zone to investigate background contaminant concentrations. Laboratory enrichment cultures were established using groundwater collected from two groundwater wells during different years. The enrichment cultures and uninoculated abiotic controls were prepared using strictly anaerobic protocols and aseptic techniques. Cultures were incubated at ambient laboratory temperature (21 ± 2 °C) in the dark without mixing. The enrichment cultures were supplied with a variety of different compounds to assess their ability to produce toluene when supplied with different precursors at different starting concentrations. A subset of experiments was conducted using ^{13}C labeled compounds to confirm whether toluene was synthesized from these precursors.

Results/Lessons Learned. Although below detection (<1 μg/L) prior to molasses injection, toluene concentrations in groundwater at the field site were found to increase to levels that sometimes far exceeded the US drinking water maximum contaminant level (MCL) of 1 mg/L after molasses was injected into the subsurface with the intent of providing electron donors for reductive dechlorination. Laboratory testing of enrichment cultures derived from the site demonstrated that indigenous microorganisms can biologically produce toluene. Precursors demonstrated to be converted to toluene included phenylacetic acid, phenylalanine, phenyllactate, and phenylpyruvate. When grown in defined medium with phenylacetic acid at concentrations ≤350 mg/L, the molar ratio between toluene accumulated and phenylacetic acid supplied exceeded 0.9:1. Experiments conducted using ^{13}C labeled compounds confirmed that toluene was synthesized from these precursors by two independently developed enrichment cultures. Results suggest that monitoring of aromatic hydrocarbons is warranted during enhanced bioremediation activities where electron donors are introduced to stimulate anaerobic biotransformation of chlorinated solvents. Potential sources of phenyl-containing precursors that can be converted to toluene by microbial populations during biostimulation activities will be discussed during the conference.