Microbially Mediated *p*-Cresol and Toluene Production from Biomass Decay: An Unintended Consequence of Biostimulation for Treatment of Chlorinated Solvents

Samuel J. Reynolds (Sjreynolds@ramboll.com) (Ramboll, Denver, CO, USA)
Alexis B. Gathings (CP&Y, Inc., Houston, TX, USA)
William M. Moe (Louisiana State University, 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 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) 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 hydrocarbons *p*-cresol (4-methylphenol) and toluene, contaminants not present in upgradient monitoring wells, transiently increased over time following molasses injections. Here, we report on a combination of studies conducted to investigate the source of *p*-cresol and toluene.

Approach/Activities. Field monitoring was conducted to measure groundwater contaminant concentrations and geochemical parameters before and after multiple molasses injections into multiple groundwater wells. Laboratory enrichment cultures supplied with 4-hydroxyphenylacetic acid were also established using groundwater collected from multiple groundwater wells. The enrichment cultures and uninoculated abiotic controls were prepared using strictly anaerobic protocols and aseptic techniques. Additional experiments were conducted supplying groundwater microbial communities with molasses-grown biomass or proteinaceous compounds with varying aromatic amino acid content to investigate whether protein hydrolysis associated with anaerobic decay of biomass produced following the addition of a readily fermentable substrate or other sources can provide the precursors that are ultimately transformed to *p*-cresol and toluene in environments undergoing enhanced bioremediation.

Results/Lessons Learned. A combination of field monitoring data and laboratory studies demonstrate that subsurface microbial communities can produce *p*-cresol (4-methylphenol) in addition to toluene as an unintended consequence of enhanced anaerobic bioremediation strategies targeting chlorinated solvents. Following the subsurface injection of agricultural feed grade cane molasses, *p*-cresol transiently accumulated in groundwater, reaching concentrations as high as 63 mg/L approximately 4 months after substrate injection. Enrichment cultures established with site groundwater consistently produced *p*-cresol when provided with 4-hydroxyphenylacetic acid. The results from additional experiments with molasses-grown biomass and two casein-derived products with varying aromatic amino acid composition provide compelling evidence that anaerobic transformation of proteins from biomass produced following the addition of a readily fermentable substrate into groundwater can supply a sufficiently large reservoir of aromatic amino acids to account for *p*-cresol and toluene concentrations observed at the field scale. The implication of these aromatic compounds in site remediation and risk assessment will be presented at the conference.