Toluene-Producing Bacteria from Sediments and Groundwater of the Southeastern US

Raymond J. Poche, Kalina G. Namikas, Morgan M. L'Hoste, and ***William M. Moe***
(moemwil@lsu.edu) (Louisiana State University, Baton Rouge, LA, USA)

Background/Objectives. The subsurface injection of fermentable substrates is frequently employed to establish anaerobic conditions conducive to growth of reductively dechlorinating bacteria (e.g., *Dehalogenimonas* and *Dehalococcoides*) and supply them with electron donors (e.g., H_2). At a site located in southeastern Louisiana, toluene was observed in groundwater following the implementation of a biostimulation approach that involved subsurface injection of molasses. Toluene was not detected in upgradient groundwater or in molasses-amended injection water and other pollutants typically present with toluene resulting from hydrocarbon spills (e.g., benzene, ethylbenzene, and xylenes) were not present. A combination of field monitoring, enrichment cultures, and radio-labeling experiments ultimately demonstrated that microbially-mediated processes led to toluene production at the site. The research described in this presentation was conducted to explore the spatial distribution and diversity of toluene producing bacteria at a regional scale in an effort to assess whether the potential for in situ toluene production was unique to the Superfund site or if toluene-producing bacteria are widespread in the environment.

Approach/Activities. Sediments and surface water collected from 17 locations encompassing multiple watersheds spanning the region from northwest Florida to eastern Louisiana were used to inoculate anoxic enrichment cultures supplied with phenylacetic acid as a potential toluene precursor. Following incubation, toluene concentrations were measured with purge and trap gas chromatography. Cultures that accumulated appreciable toluene (>100 mg/L) were sequentially transferred and maintained over a two-year period. Community DNA extracted from the sediment-derived enrichment cultures was used as template in PCR reactions targeting 16S rRNA genes and genes coding for a key enzyme in the pathway for toluene production. Community DNA extracted from a toluene-producing enrichment culture derived from Superfund site groundwater where toluene accumulation was observed following biostimulation was also included in testing.

Results/Lessons Learned. Toluene is an environmental pollutant most often associated with gasoline and other refined petroleum products; however, it may also be produced by bacteria harboring genes encoding the enzyme phenylacetate decarboxylase (PhdB) which was recently discovered in an uncultured *Acidobacteriota* species. Enrichment cultures inoculated with a mixture of sediment and surface water from 15 of 17 locations in Louisiana, Alabama, and Florida produced toluene that accumulated to concentrations over 100 mg/L when grown in an anoxic medium supplemented with phenylacetic acid. Primers targeting genes encoding the enzyme phenylacetate decarboxylase (PhdB) were amplified by PCR and then sequenced for all of the toluene-producing enrichment cultures. Sequencing of the *phdB* genes and 16S rRNA genes revealed appreciable diversity among the toluene-producing bacteria. Results demonstrate that the metabolic potential that can lead to toluene production is widespread in the environment, at least along inland areas of the northern U.S. Gulf Coast region.