# Long-Term Anaerobic Bioremediation of Petroleum Contaminants by Iron- and Sulfate-Reducing Bacteria following Combined Cement-Persulfate Treatment

***Daniel P. Cassidy*** (daniel.cassidy@wmich.edu)
(Western Michigan University, Kalamazoo, MI, USA)
Vipul J. Srivastava (vipul.srivastava123@gmail.com)
(EcoLogic & Sustainable Strategies, Inc.)

**Background/Objectives.** Laboratory studies performed on 10 different hydrocarbon-contaminated soils investigated a novel remedy combining in situ chemical oxidation (ISCO), in situ stabilization/solidification (ISS), and anaerobic bioremediation in a single application. Portland cement and other ISS amendments were used to activate persulfate to achieve chemical oxidation of hydrocarbon contaminants. The primary purpose of these studies was to investigate the ability of native Iron-reducing bacteria (IRB) and sulfate-reducing bacteria (SRB) to survive exposure to the highly oxidizing conditions and high pH associated with alkaline activation of persulfate with cement and other ISS amendments, followed by growth and degradation of residual hydrocarbon contaminants.

**Approach/Activities.** The 10 test soils were from either manufactured gas plant (MGP) sites or petroleum refinery/storage facilities. The contaminants included polycyclic aromatic hydrocarbons (PAHs) (including naphthalene, the most soluble PAH), total petroleum hydrocarbons (TPH), and benzene, toluene, ethylbenzene, and xylenes (BTEX). Subunits A and B of the dissimilatory sulfite reductase gene (*dsrA and dsrB*) were used to monitor the abundance of native SRB. Because microorganisms can be abundant in soil without being active, concentrations of products of contaminant degradation by SBR were also monitored. Sulfide, generated by use of sulfate as an electron acceptor by SRB, was measured in aqueous samples. The production of 2-naphthoic acid, a specific indicator of naphthalene degradation by IRB and SRB, was also quantified and monitored over time.

**Results/Lessons Learned.** The results showed that the abundance of the native IRB and SRB decreased dramatically during the first week of treatment, due to the oxidizing conditions and high pH resulting from the ISCO/ISS treatment. However, the abundance of IRB and SRB recovered completely within 10 to 30 weeks in all 10 soils, exceeding background values. Sulfide concentrations increased temporarily, but were then reduced by precipitation with iron and other metals. Concentrations of 2-naphthoic acid increased steadily during the first 12 to 24 weeks after ISCO/ISS treatment, demonstrating that IRB and SRB degraded naphthalene. Concentrations of PAHs and BTEX were monitored over a 2-year to 3-year period, and clearly showed significant degradation by of these hydrocarbons by IRB and SRB. Systems treated with Portland cement-activated persulfate, which received significant doses of Iron accompanying the added Portland cement showed much faster recovery and growth of both IRB and SRB, compared with control systems in which the persulfate was activated with NaOH. The results show that activating persulfate with common ISS amendments in a single application synergistically combined ISCO, ISS, and anaerobic bioremediation, and that native anaerobic bacteria can provide long-term biological polishing of residual contaminants after this combined cement-activated persulfate treatment of soils.