USING ENVIRONMENTAL MOLECULAR DIAGNOSTICS (EMDS) TO DEFINE TRANSITIONS IN REMEDIAL STRATEGIES; CHEMICAL OXIDATION TO BIO-STIMULATION VIA AN *IN SITU* BIOREACTOR (ISBR)

Eric J Raes*
Engineering and Land Planning Associates, Inc.
54 Old Highway 22, Suite 300
Clinton, NJ 08809
Voice: 908-238-0544
Fax: 908-238-9572
eric@elp-inc.com

Kerry L. Sublette
Katherine Key
The University of Tulsa
Tulsa, OK

Dora Ogles
Greg Davis
Brett Baldwin
Microbial Insights, Inc.
Rockford, TN

Stable isotope probing was used to quantify relative biodegradation and contaminant mass destruction rates, before, during, and after two chemical oxidation events. Biofilms characteristic of aquifer conditions can be rapidly and efficiently collected using in situ microcosms or “Bio-Traps” containing Bio-Sep® beads. The Bio-Sep beads were “baited” with a 13C compound by vapor phase adsorption onto the powdered activated carbon component of the beads. The adsorbed organics are bioavailable to bacteria that form biofilms in the beads during incubation in a contaminated aquifer. Since the beads are clean of biomarkers and sterile when deployed, incorporation of 13C in phospholipids provides proof of in situ biodegradation of a target compound by indigenous microorganisms under actual aquifer conditions. The Bio-Sep beads were analyzed for the abundance of known petroleum degrading bacteria via quantitative Polymerase Chain Reaction, and 13C incorporation into the residual carbon dioxide in the beads was analyzed using GC-IRMS to determine whether the compound had been mineralized.

Using these Environmental Molecular Diagnostics (EMDs), the fortuitous occurrence of significant bio-stimulation was observed once the harsh chemicals had dissipated, as indicated by the microbial analysis of a Bio-Trap installed in the remediation area 30 to 60 days after the chemical oxidation events. However, the bio-stimulation was not sustainable as the partially oxidized materials and dissolved oxygen flux were not maintained.

To promote sustainable bio-stimulation, a novel, in-well “bio-reactor” was installed in the plume. The *in situ* Bioreactor (ISBR) design builds on existing Bio-Sep bead technology. Bio-Sep beads provide a substrate that can be rapidly colonized by the active members of the microbial community and serve to concentrate indigenous degraders. Oxygen and nutrients are also delivered to the bioreactor to maintain conditions favorable for growth and reproduction. The contaminated groundwater is treated as it is circulated through the bed of beads. Groundwater moving through the system also transports degraders released from Bio-Sep beads away from the bioreactor, increasing biodegradation of the residual petroleum hydrocarbons in the aquifer.

These data were used to define transition points from post-chemical oxidation treatment to a bio-stimulation remedy strategy for two fuel oil release sites.

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