

**ASSESSMENT OF IN SITU BIODEGRADATION POTENTIAL OF BENZENE
USING ¹³C-LABELED BENZENE AND BIO-SEP[®] BEADS**

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Conventional indicators of intrinsic bioremediation of BTEX include the distribution of hydrocarbons and metabolites and the correlation of temporal trends with the concentrations and distributions of geochemical parameters (electron acceptors, products of reduction of electron acceptors, DO, redox potential, hydrogen, etc.). These data are typically collected over the entire plume and in suitable control areas over a period of time at significant cost. The actual extent of site characterization required to support a risk-based management strategy varies from state to state. However, the goals remain the same: to deduce the prevalent bioprocesses in the subsurface and to determine whether natural attenuation will prevent exposure of environmental receptors to the hydrocarbon plume. With respect to the prevalent bioprocesses the key word here is "deduce" – that is, these data amount to circumstantial evidence of intrinsic bioremediation.

The biodegradation potential of benzene in a gasoline-contaminated aquifer was determined using *in situ* microcosms containing Bio-Sep beads with ¹³C₆-benzene (8% ¹³C-benzene and 92% ¹²C-benzene). The microcosms were deployed in triplicate for 45 days about one foot below the water table. Retrieved microcosms were extracted with CHCl₃:MeOH and lipids were derivatized with trimethylchlorosilane (TMCS) yielding fatty acid methyl esters (FAMES). Gas chromatography coupled to isotopic ratio monitoring mass spectrometry (GC-IRMS) was used to measure ¹³C incorporation in phospholipid fatty acids (PLFA). Beads were analyzed for residual ¹²C-benzene and ¹³C-benzene by GC-MS.

About 80% of the original benzene (about 1 mg/bead and 400 beads per microcosm) was depleted during incubation. There was a significant increase in the percent ¹³C-labeled benzene in the retrieved microcosms compared to the pre-incubation composition. This difference strongly suggests that the observed depletion of benzene in the bio-traps during incubation was due to biodegradation since it is well known that bacteria show a preference for ¹²C-labeled substrates. Therefore, the pool of benzene left in the beads would be expected to be enriched for ¹³C over time since ¹²C-benzene would be degraded at a faster rate. Several FAMES were found to be enriched for ¹³C with δ ¹³C as high as +6000 ‰ compared to -26 ‰ for natural benzene. This provides absolute proof that benzene was biodegraded by indigenous organisms under *in situ* aquifer conditions.

The potential use of this technology for other hydrocarbons as well as MTBE and TBA will also be discussed.