



## Comparison of commercially available $^{13}\text{C}$ methods for demonstration of enhanced in- situ biodegradation of plume hydrocarbons following oxygen injection

Mike Spence (1), Gerard Spinnler (2), Cristin Bruce (2), and Dieter Stupp (3)

(1) Shell Global Solutions (UK), Chester, UK (mike.spence@shell.com), (2) Shell Global Solutions (US), Houston, US, (3) Dr. Stupp Consulting GmbH, Kohn, Germany

The effect of pulsed oxygen injection remediation (OPIS) on in-situ biodegradation rates in groundwater was evaluated using two commercially available in-situ microcosm technologies: Biotraps (Microbial Insights, US) and Bactraps (Isodetect, Germany). The traps, supplied pre-loaded with  $^{13}\text{C}$ -hydrocarbon substrates, were incubated for a period of 2 months in boreholes located in anaerobic groundwater, and aerobic groundwater oxygenated to  $>30$  mg/L dissolved oxygen. The traps were later removed and analysed to determine the  $^{13}\text{C}$ -content of dissolved inorganic carbon (DIC) and phospholipid fatty acid (PLFA) in the microbial biomass. Biodegradation was quantified through the  $^{13}\text{C}$ -enrichment of PLFA in the biomass.

PLFA recovered from Bactraps incubated in aerobic groundwater shows clear  $^{13}\text{C}$ -enrichment ( $\delta^{13}\text{C} = +11,491\text{‰}$ ), but there is no significant  $^{13}\text{C}$ -enrichment in PLFA from traps incubated in anaerobic groundwater ( $\delta^{13}\text{C} = -24$  to  $-31\text{‰}$ ). This is consistent with enhanced biodegradation only at elevated oxygen concentrations.

Data from the Biotraps shows  $^{13}\text{C}$ -enrichment of PLFA in both aerobic ( $\delta^{13}\text{C} = +17,703\text{‰}$ ) and anaerobic groundwater ( $\delta^{13}\text{C} = +2249\text{‰}$ ), although the degree of enrichment is greater where oxygen is present. This is consistent with biodegradation under both aerobic and anaerobic conditions, but with higher rates at elevated oxygen concentrations.

The reason for increased incorporation of  $^{13}\text{C}$  into PLFA recovered from the anaerobic Biotraps is not yet clear. Possible reasons include differences in the  $^{13}\text{C}$ -substrates used (Bactraps were loaded with 100%  $^{13}\text{C}$ -labelled substrates, Biotraps with 10%  $^{13}\text{C}$ -labelled substrates), or differences in the sorbent materials used in the construction of the devices. Subtle variations in the chemistry of the anaerobic groundwater could also have contributed to the difference, since the Bactraps and Biotraps were incubated in different boreholes.

The  $^{13}\text{C}$  content of DIC was quantified differently by the two laboratories, with Isodetect analyzing a groundwater sample recovered from the borehole after the incubation period and Microbial Insights isolating CO<sub>2</sub> recovered from the Biotrap. Isotopic analysis of DIC was found to be a less sensitive indicator of biodegradation activity, with only one of the substrates tested yielding significant  $^{13}\text{C}$  enrichment of associated DIC under aerobic conditions. Exchange of DIC between the devices and the groundwater prevented the  $\delta^{13}\text{C}$ -DIC data being used to calculate the % of  $^{13}\text{C}$ -substrate degraded.

In summary, results from both the Bactrap and Biotrap investigations clearly show that pulsed oxygen injection increased in-situ biodegradation rates for all the compounds tested relative to untreated groundwater. This finding is in agreement with the results of aerobic microcosm studies, carried out using samples of aquifer sediment and groundwater from the same site