

VERIFICATION OF CALCULATED BIODEGRADATION RATES BY STABLE CARBON ISOTOPE AND SOIL SAMPLE ANALYSES

James G. Mueller and Susanne Borchert (SBP Technologies, Pensacola, FL)
Luis A. Cifuentes (Texas A&M University, College Station, TX)
Eduard Alesi and Chris Leins (IEG Technologie GmbH, Kirchheim, Germany)

ABSTRACT: Methods in isotope biogeochemistry were employed to help assess performance of a full-scale *in situ* bioremediation system at Neureut, Germany where soil and groundwater were impacted by petroleum hydrocarbon constituents of interest (COI). Component to the existing monitoring program was the collection of respirometry data (evolved CO₂ and CH₄ from soil gas). In an effort to provide insight into the interpretation of field data, we used stable C isotope analysis ($\delta^{13}\text{C}$) to identify the source of the C in the evolved CO₂. Using a combination of gas chromatography-mass spectrometry (GC/MS) and gas chromatography/combustion isotope ratio mass spectrometry (GC/C/IR/MS), COI-derived CO₂ released in the off-gas of the *in situ* treatment system was measured specifically. The source-specific fingerprint offered by these analytical tools ($\delta^{13}\text{C}$) confirmed that COI carbon was mineralized and helped confirm the significant contribution of biodegradation (*i.e.*, mineralization) to the overall system efficacy.

INTRODUCTION

In situ bioremediation is described herein as the non-invasive stimulation of native microflora to biodegrade target organic molecules through the management of certain environmental parameters. Hence, the aim of *in situ* bioremediation is often to deliver essential co-reagents, such as oxygen and nutrients, to the indigenous microflora to enhance the rate of biodegradation of organic constituents of interest (COI). Towards this end, many *in situ* bioremediation efforts rely on the manipulation (*e.g.*, stripping, aerating, pumping, sparging, *etcetera*) of impacted matrices. As such, many physicochemical processes may, in fact, be responsible for a large proportion of the "biological" remediation observed. While it is often desirable to track specifically the biological component of degradation, corollary data necessary to document "biodegradation" with the removal of organic COI are often difficult to obtain.

Stable isotope ratios of carbon have long been used to study organic carbon biogeochemistry and to elucidate trophic dynamics in terrestrial and aquatic food webs (Boutton, 1991; Fogel & Cifuentes, 1993). Thus, one promising means of tracking the biological component of COI biodegradation (mineralization) is the use of the natural ratio of ¹²C to ¹³C ($\delta^{13}\text{C}$) in the COI. These ratios reflect the natural proportion of the ¹³C carbon (6 protons, 7 neutrons) to ¹²C carbon (6 protons, 6 neutrons) in a given organic compound. In simple terms, the biochemicals from a heterotrophic organism reflect the $\delta^{13}\text{C}$ ratio of their principal growth substrate. Similarly, the CO₂ that is respired from metabolic activity also reflect the $\delta^{13}\text{C}$ ratio of the principal growth substrate (Landmeyer *et al.*, 1996). Autotrophic organisms (plants and photosynthetic microorganisms), on the other hand, are generally responsible for establishing the variation seen in $\delta^{13}\text{C}$ ratios of organic matter since their different carbon assimilation pathways (C3, C4, CAM) are selective (to differing degrees) for ¹²C. If understood, this information can be used as a tool for biomonitoring because variations in $\delta^{13}\text{C}$ can be used to follow the flow of organic

matter through biogeochemical cycles (Fogel & Cifuentes, 1993).

Objective: The purpose of these efforts was to determine the source of C in CO₂ from a Multifunctional Well *in situ* remediation system. These data were then used in conjunction with microbial respiration and soil chemistry data to more accurately calculate the contribution of bioremediation *per se* to the overall removal efficiency of the *in situ* remedial system.

MATERIALS AND METHODS

Remediation System Installation. An *in situ* “Multifunctional-Well” (derived from the UVB™ Technology) was installed at a site North of Karlsruhe, Germany, at the Neureut Barracks, formerly US Army property. A 354 m² area downgradient of a tank farm was chosen as the location for a pilot remediation study designed to show *in situ* bioremediation could be enhanced through the installation of vertical groundwater circulation well technologies, *e.g.* the UVB and its related GZB™ and SZB™ systems (Stamm, 1995). The Multifunctional Well construction incorporated a unique “phased” design approach that allowed the remediation well to change its function with the changing needs during the course of the remediation. The well’s basic phases entail: 1) free product recovery, 2) bioventing and soil vapor extraction (SVE), 3) SZB operation (soil flushing in unsaturated zone, capillary fringe, and uppermost saturated zone), and 4) UVB operation for vertical groundwater circulation in the saturated zone. All processes are patented by IEG mbH, Reutlingen, Germany.

Performance Monitoring. Multiple soil borings were augured before and after the pilot test as a means of determining the efficiency of the remediation on impacted soil. Averaging initial COI concentrations in the soil and groundwater, and taking into account other site parameters, a total COI concentration of 13,755 kg (1,788 kg as BTEX + 11,967 kg as TPH) was approximated to be in the 354 m² area of active remediation. To monitor performance of the remediation system, influent and effluent (off-gas) were analyzed regularly for the following parameters: volume, temperature, humidity, COI (BTEX) concentrations, CO₂ and CH₄. Respired methane was consistently <0.1% of the CO₂ production measured (<0.2 ppb/day) thus it was not further considered. Soil and groundwater were measured for BTEX and TPH. From the air volume and COI measurements, the mass COI removal rates were calculated. The amount of COI physically extracted from the subsurface (via SVE/bioventing or *in situ* stripping) could be computed for each phase of the remediation. Likewise, using the total CO₂ and CH₄ respired during each stage, subsurface biodegradation rates and consequently the amounts of COI mineralized were also determined.

Isotope Monitoring. The use of the isotopic fractionation phenomenon in bioremediation monitoring requires sensitive instrumentation for the determination of δ¹³C ratios in CO₂, COI, bulk organic matter, and biochemicals. It also requires that the δ¹³C ratio of the COI be sufficiently different from the δ¹³C ratio of background organic material found at the site. Thus, the sampling plan included off-gas monitoring of evolved CO₂, and analysis of background CO₂, free product and indigenous organic matter.

Soil Gas. Petroleum hydrocarbons (BTEX) and natural soil organic material (humics) represented the two potential sources of C in the evolved CO₂. Mineralization of each of these sources was assessed by sampling soil gases near the *in situ* treatment unit, and soil gases from a nearby location not impacted by

BTEX (background). Soil gas samples were collected in Tedlar™ bags according to manufacturer's recommendation and variation of US EPA standard method T03. Soil gases were collected with the objective of minimizing the potential impact of atmospheric CO₂. Gas samples were analyzed as described below.

Soil Humics. The δ¹³C of humics was assessed by collecting replicate samples of soil from background locations not impacted by BTEX. Soil samples were placed in 10% hydrochloric acid to remove carbonates. Acidified soils were retained on a 47 mm GF/F filter and washed with distilled water until neutralized. After drying (60°C, 24 hr), the soil was scrapped off the filter. Organic carbon in these samples was then converted to CO₂ by a modified Dumas combustion (see Salata *et al.* 1996). The CO₂ gas was separated from other gases by cryogenic distillation and transferred to sample bulbs. The precision of analysis was typically <±0.1‰.

Source (Product) Sampling. Petroleum hydrocarbons (COI source) were sampled by collecting free product from an above-ground tank that served as an accumulation point for six product recovery wells prior to start-up of the system. The δ¹³C of the source material was determined as described below.

Laboratory CO₂ Analysis. The isotopic ratio of CO₂ was determined by gas chromatography-isotope ratio mass spectrometry (GC-IRMS; see Routh & Cifuentes, 1995). Prior to introduction into the IRMS, the CO₂ was separated cryogenically from other gases (*e.g.*, N₂, Ar, CO, CH₄) with a Carboxen 1006 Plot column (30 m, 0.53 mm I. d., Supelco, Bellefonte, PA) as described by Salata *et al.* (1996). Precision for samples taken in the field in duplicates averaged ±0.3‰.

COI Analysis. Free product and gas samples were analyzed for δ¹³C of the individual petroleum constituents via gas chromatograph-isotope ratio mass spectrometer (GC-IRMS) with a Finnigan MAT 252 GC-IRMS (Routh & Cifuentes, 1995). All C isotope ratios were reported in the standard δ¹³C notation: δ¹³C (‰) = [(R_{sample}/R_{std}) - 1] x 1000 where R_{sample} and R_{std} are the ¹³C/¹²C isotope ratios of the sample and the conventional PeeDee Belemnite standard, respectively, that, by definition, has a δ¹³C of 0.0.

RESULTS

Multifunctional Well Operations. From July 22, 1994 through April 3, 1995, the system operated in Phase 2 with bioventing and SVE removing a total of 4,800 kg of COI (BTEX and TPH) from the unsaturated zone (data not shown). About 25% of this amount was extracted to the surface and captured on granular active charcoal; the remaining 3,600 kg were calculated to have mineralized during biodegradation based on the continuous CO₂ and CH₄ concentration measurements of the system off-gas (calculations not shown). The remediation system was altered to a soil flushing application (Phase 3) and functioned as such from April 4 to August 4, 1995. During this period, another 2,900 kg of COI were estimated to have been biodegraded based on continuous monitoring of CO₂ and CH₄ concentrations in the off-gas. From August 30 to December 19, 1995, the system operated in the in-well aeration and vertical groundwater circulation phase (*i.e.*, UVB technology). During this time, another 3,900 kg of COI from the saturated zone were further mineralized (calculated from respiration data).

In summary, a total of 11,600 kg, or 84% of the total COI at time-zero, had been removed from the subsurface after 17 months of pilot testing. To confirm these reductions, four soil borings were augured during May 1996 and soil samples from various depths were analyzed in similar locations as the borings before

remediation began. Results showed total residual COI of 1,014 kg: this was less than the predicted difference 2,155 kg (total of 13,755 kg at start-up less 11,600 kg removed) existed in the area of study. Of the total 11,600 kg of COI removed, approximately 1,200 kg were physically extracted hence the remaining 10,400 were presumably mineralized (*i.e.*, 90% of the total COI removed were biodegraded/mineralized). In an attempt to confirm the relatively high contribution of bioremediation to the overall removal efficiency, we employed isotope biogeochemistry as described below.

Isotope Monitoring. At the Neureut site, the $\delta^{13}\text{C}$ of indigenous soil organic matter was -24.7 ± 0.6 (n=4), and that of the $\delta^{13}\text{C}$ of the free product was -26.4 ± 0.1 (n=2). This suggested that the indigenous soil organic material was enriched in the heavier isotope, but not too different from the free product. Nevertheless, respired CO_2 , whether of free product or soil humus origin (about -27 to -24 ‰), would be isotopically distinct from atmospheric CO_2 (typically between -8 to -9 ‰; see Boutton, 1991). In turn, soil CO_2 gas, which is mostly a mixture of respired and atmospheric CO_2 , should be about 4‰ more positive than prevailing organic sources, owing to mixing of respired and atmospheric CO_2 and to diffusion isotope effects (Boutton, 1991).

The isotope data for soil CO_2 at both the background and UVB System during November 1995 followed this predicted result (Figure 1). By March, 1996, however, conditions had changed considerably. Now, soil CO_2 at the background site was comparatively ^{13}C -enriched, particularly at the shallow site, indicating more mixing with atmospheric CO_2 . In contrast, the $\delta^{13}\text{C}$ of soil CO_2 in the UVB System was now almost equal to that of soil organic matter and petroleum COI. The absence of the isotopic discrimination usually observed in soil gas CO_2 with respect to organic sources at the UVB site can be explained by a large flux of CO_2 (induced by soil vapor extraction/bioventing of the vadose zone) associated with increased degradation of the soil organic matter and/or COI. Under these circumstances, the 4‰ enrichment usually associated with diffusion and mixing with atmospheric CO_2 in uncontaminated soils was not observed.

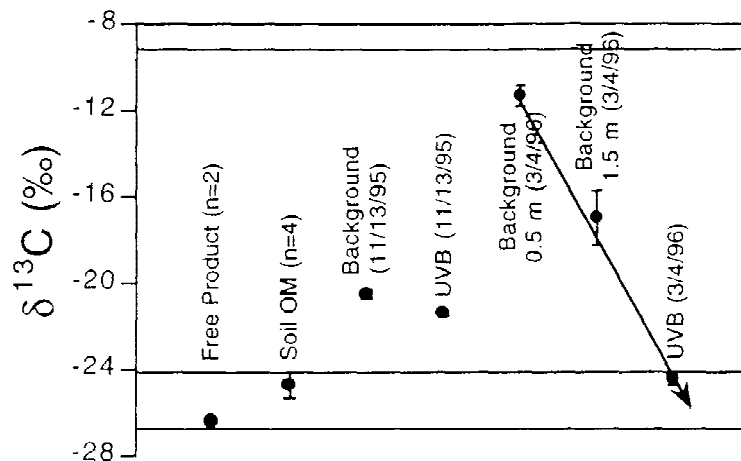


FIGURE 1. Stable carbon isotope ratio ($\delta^{13}\text{C}$) of soil organic matter, free product and soil gas CO_2 from background and UVB sampling sites. Data were taken in November 1995 and March 1996. The stippled areas indicate the $\delta^{13}\text{C}$ of atmospheric CO_2 and of organic sources for microbial respiration. Note that in March 1996 isotopic ratios were ^{13}C -depleted at the UVB site compared with background samples at two depths (refer to arrow).

DISCUSSION

Petroleum-derived materials are comprised of many different chemical components. The $\delta^{13}\text{C}$ values of many of these have been measured in several petroleum products of various origins and maturities. Many of the petroleum products have been found to have distinct suites of carbon isotope ratios associated with them. In other words, a specific source has a distinct "fingerprint" of $\delta^{13}\text{C}$ values. In aerobic environments (such as that in the vicinity affected by the UVB *in situ* treatment system), microbial oxidation of organic matter produces CO_2 with an isotopic ratio similar to that of the organic matter being degraded (Landmeyer *et al.* 1996). In hypoxic environments, C could be reflected in CH_4 . Unfortunately, the isotopic discrimination between soil organic matter and petroleum hydrocarbons at the Neureut site was not sufficient to establish unambiguously which source was responsible for observed increases in microbial mineralization activity (*i.e.*, which carbon molecules were mineralized). Nevertheless, coupled with other data that demonstrated loss of COI, the isotopic data suggest that biological processes were indeed enhanced, and observed COI removals were not simply attributable to myriad abiotic processes associated with UVB operation (*i.e.*, stripping, dilution).

CONCLUSIONS

Over a 17 month period of pilot operation, the multifunctional remediation well removed 11,600 kg of petroleum hydrocarbon which represented a removal efficiency of 84%. A total of 1,200 kg (*ca.* 10%) were removed abiotically (*e.g.*, extracted, stripped) and the remaining 10,400 kg (*ca.* 90%) were attributed to biodegradation (*i.e.*, mineralization to CO_2 or CH_4). Stable C isotope analyses of evolved CO_2 showed that the respired C was isotopically similar to COI source, and that very significant differences were observed in the isotope ratio of CO_2 respired from the background location versus the treatment well. These data helped confirm the relatively high contribution of bioremediation to the overall removal efficiency of the *in situ* system - specifically, enhanced aerobic mineralization of petroleum hydrocarbons (measured as BTEX and TPH).

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