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Lipid Analysis to Determine the Effect of a Source Remedial Technology in Microbial Ecology

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ABSTRACT: Microbial community structures and related changes in the subsurface environment were investigated following in situ chemical oxidation (ISCO) treatment at Launch Complex 34, Cape Canaveral Air Station, Florida. The site has dense, nonaqueous-phase liquid (DNAPL) concentrations of TCE over a wide areal extent in relatively sandy soils with a shallow groundwater table. The investigation stemmed from concerns that ISCO remediation could have a variety of effects on the indigenous biological activity including reduced biodegradation rates and a long-term disruption of community structure with respect to the stimulation of TCE degraders. Phospholipid ester-linked fatty acid (PLFA) analyses of the aquifer material were used to assess structural and functional differences between microbial communities. The technique is independent of the bias inherent in classical culturing techniques and provides a more accurate estimation of in situ microbial populations. The data suggested that the ISCO treatment significantly increased the biomass of the test site sediments at 6 and 40 ft, and decreased the diversity in terms of the loss of *Actinomycetes* a the 6-ft depth, and oligotrophs at the 30- and 40-ft depths. The 15-ft samples had the lowest biomass across all sites and dates. The Recovery samples had slightly less biomass than the Oxidation samples, and there was not a great difference in their community structures.

INTRODUCTION

Historical disposal practices of chlorinated solvents have resulted in the widespread contamination of groundwater resources. It has been estimated that, in the United States, public groundwater systems provide 6.3 million people with drinking water that contain reportable levels (>0.005 mg/L) of tetrachloroethene (PCE); 6.8 million people are similarly exposed to trichloroethene (TCE), and 1.7 million people to dichloroethene (DCE) (U.S. EPA, 2002). These groundwater contaminants exist in the subsurface as free products, residual and vapor phases, and in solution. The remediation of these contaminants often require a sequenced train of treatments, the success of which is ultimately dependent on the hydraulic control of local flow regimes. Although each phase requires specialized remediation technologies, the removal of dissolved DNAPLs from groundwater is important in preventing migration to sources of water use until long after contaminants at the source have been removed. The focus of this study was to employ a groundwater treatment involving both biological and chemical processes; ISCO for the removal of the source and bioremediation as the polishing step. Hence, the effect of ISCO on the indigenous microbial activities, especially with respect to the stimulation of TCE degraders, represented a strategy for the use of in situ remediation technologies.

Quantitative analysis of the PLFA composition provides insight into the microbial community structure. Fatty acids are known to differ in chemical composition depending upon microbial type and environmental conditions. These differences allow a quantitative

insight into three important attributes of microbial communities including viable biomass, community structure, and metabolic activity (Lehman et al., 1995). In practice, this chemotaxonomic tool is based on the extraction and separation of lipid classes followed by quantitative analysis using gas chromatography mass spectrometry (GC/MS) (White and Ringelberg, 1997). PLFA analyses for the characterization of microbial ecologies have been reported for a variety of investigations including the bioremediation of hazardous waste sites (Bittkau et al., 2004; Peacock et al., 2004; Anderson et al., 2003; Feris et al., 2003; Musslewhite et al., 2003; Jackson et al., 2003; Brigmon, 2002).

The aim of this study was to assess the immediate effect of ISCO treatment on microbial abundance and community structure. Microbiological vertical profiling of core samples was accomplished using PLFA biomarkers.

METHODS

The data consisted of PLFA profiles from 66 soil samples. These were from 5 sampling sites labeled 6 through 10. The first sampling event occurred approximately one month prior to the treatment initiation. The following two sampling events took place 1 and 6 months after the treatments terminated. The sampling events are referred to here as "Control," "Oxidation," and "Recovery." Sampling depths were consistent over most of the data, and are named for the top of the soil sample analyzed — "6 ft," "15 ft," "30 ft," and "40 ft." Samples from Site 8 depth 15 ft and Site 10 depth 40 ft were provided in duplicate for all sampling dates.

The collection of the core samples for vertical profiling was accomplished using a Cone Penetrometer equipped with a Mostap™ sampler (20-inch long with a 1.5-inch diameter) that contained three sterile sleeves (brass or stainless steel) and one spacer. Core samples were collected at four different depths: approximately 7 ft (capillary fringe), 15 ft (upper sand unit below water table), 30 ft (middle fine-grained unit), and 40 ft (lower sand unit) at each sampling location. The samples were frozen in liquid nitrogen (−150°C) on site and were shipped overnight (−70°C) to the laboratory for PLFA analysis (White et al., 1979; White et al., 1997).

RESULTS AND DISCUSSION

In terms of the variation of viable microbial biomass (as PLFA) with sample site, depth, date, and percent moisture, there was no effect of sample site or percent moisture on microbial biomass. Biomass with depth showed a minimum at the 15-ft sample depth, a maximum at the shallowest depth, and the 30-ft and 40-ft samples were intermediate and indistinguishable. The typical pattern is decreasing biomass with depth. Microbial biomass clearly increased from the Control samples to the Oxidation samples, and then slightly decreased again to the Recovery. The same observation was reported by Bittkau et al. (2004) in that after chemical treatment higher total PLFA concentrations were encountered that may indicate higher numbers of viable cells.

When microbial biomass was plotted by site, depth, and date, it was observed that there was a great deal of variation in the microbial biomass measured, from 1.5 pmol/g to 9.2 nmol/g, plus one sample with no fatty acids detected. The increase in biomass from Control to Oxidation samples was evident, but more, the pattern of the biomass down each well was replicated across the sampling times. For example, the biomass at sampling Site 6 at the Control time decreases in the order 6, 40, 30, 15 ft. This same pattern is

seen at the Oxidation and Recovery sampling times. The consistency of the sampling sites allows pooling them for statistical analysis. Tukey's honestly significant difference was chosen for significance because it is a very rigorous test, while still maintaining an overall p value of 0.05 (Table 1). The Oxidation treatment at 6 ft was significantly higher in biomass than all other samples. All of the 15-ft samples were at the bottom of the table, with the other sampling depths decreasing in biomass in the order Oxidation, Recovery, Control.

TABLE 1. Tukey's Honestly Significant Difference analysis of the ln(X + 1) transformed biomass data. Data was pooled over sample sites. Biomass decreases down the table. The vertical bars enclose samples not significantly different.

Oxidation 6 ft
Oxidation 40 ft
Oxidation 30 ft
Recovery 6 ft
Recovery 30 ft
Recovery 40 ft
Control 6 ft
Control 30 ft
Control 40 ft
Recovery 15 ft
Oxidation 15 ft
Control 15 ft

Functional group analysis, breaking the PLFA into biosynthetically related groups such as saturates and iso-/anteiso-branched fatty acids, did not successfully distinguish treatments or sampling depths (data not shown). In order to apply factor analysis to these samples, the low-biomass 15-ft samples were removed from the data, since too few fatty acids were detected in these to give information on the microbial community structure. The data was also restricted to the 22 most abundant fatty acids. The mole percent of each remaining fatty acid was renormalized, and the data submitted to factor analysis by the communalities = multiple R^2 method, the factor rotation method was varimax normalized (Statistica ver. 4.2, StatSoft 1993).

The first factor extracted had the fatty acids 20:0, 22:0, and 24:0 significantly loaded in the negative direction (Figure 1). They were highest in the Control samples from 30- and 40-ft. Long-chain unbranched saturates are characteristic of some soil oligotrophs.

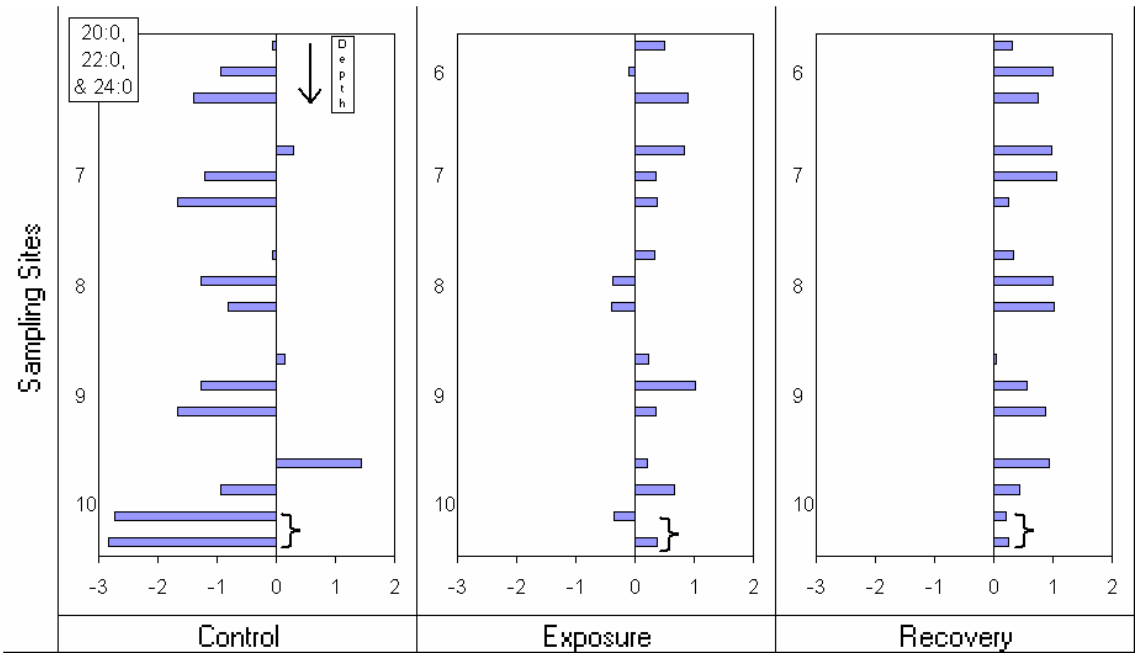

FIGURE 1. Factor 1 by site, depth, and date.

Therefore, factor 1 can be interpreted as a population of oligotrophs at the 30- and 40-ft depths that was lost in the Oxidation treatment, and had not returned by the time of the Recovery sampling. The Tukey's analysis is given in Table 2, which shows the 30- and 40-ft Control samples significantly different from all others.

TABLE 2. Tukey's Honestly Significant Difference analysis of factor 1. The 15-ft depth not included. Data was pooled over sample sites. Factor 1 decreases down the table. The vertical bars enclose samples not significantly different.

Recovery 30 ft
Recovery 40 ft
Recovery 6 ft
Oxidation 6 ft
Control 6 ft
Oxidation 30 ft
Oxidation 40 ft
Control 30 ft
Control 40 ft

The second factor extracted (Figure 2) was significantly loaded by 10Me18, i17:0, and a17:0 in the negative direction. Table 3 gives the Tukey's HSD analysis, which shows that the Control samples from 6 ft were different from all others on factor 2. The fatty acids 10Me18, i17:0, and a17:0 are characteristic of the *Actinomycetes*, so this may be interpreted as a loss of the shallow-depth *Actinomycetes* with Oxidation treatment. Of the 5 sampling sites, Site 8 at 6 ft did show a re-increase of *Actinomycete* markers at the Recovery sampling.

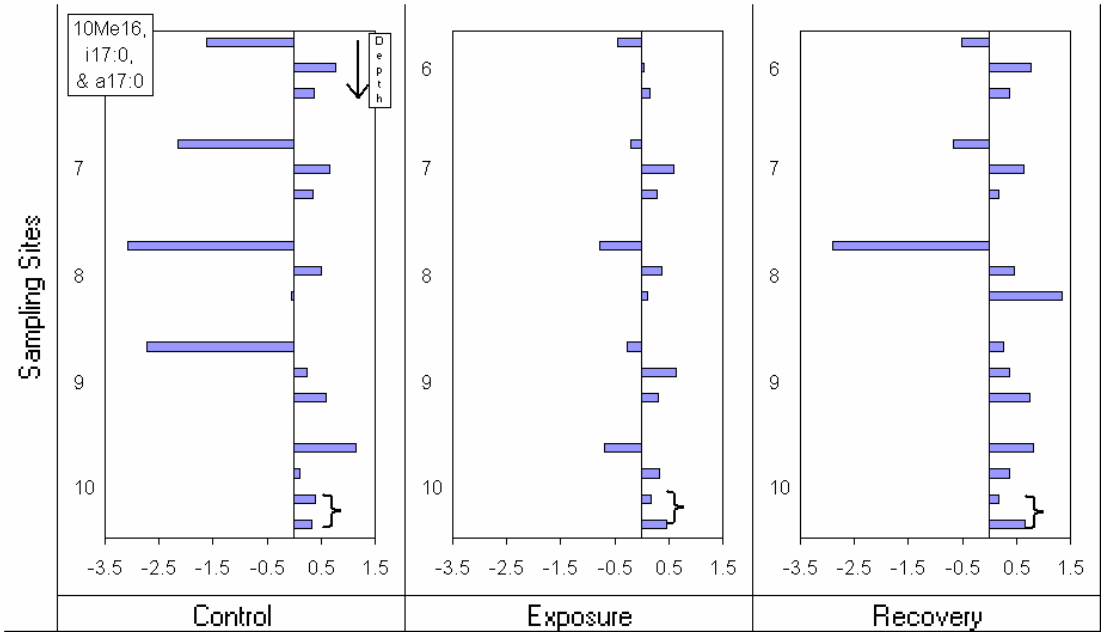

FIGURE 2. Factor 2 by site, depth, and date.

TABLE 3. Tukey's Honestly Significant Difference analysis of factor 2. The 15-ft depth not included. Data was pooled over sample sites. Factor 2 decreases down the table. The vertical bars enclose samples not significantly different.

Recovery 40 ft
Recovery 30 ft
Control 30 ft
Oxidation 30 ft
Control 40 ft
Oxidation 40 ft
Oxidation 6 ft
Recovery 6 ft
Control 6 ft

SUMMARY

The data suggest that the communities from TCE-contaminated groundwater contained high concentration of gram-negative bacteria as indicated by the biomarker 16:1w7c and were in the stationary growth phase as indicated by the abundance of cyclopropyl fatty acids cy17:0 and cy19:0. *Actinobacteria*, the parent group of *Actinomycetes*, and/or metal-reducing bacteria decreased with depth, and showed a modest response to chemical treatment. The branched unsaturates are a minor group of PLFA that are also associated with metal-reducing bacteria. The Recovery samples had slightly less biomass than the Oxidation samples, and there was not a great difference in their community structures.

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