

Microbial Population and Degradation Activities Changes Monitored During Chlorinated Solvent Biovent Demonstration

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Introduction

Characterization of changes in the microbial community is essential in proving the effectiveness of bioremediation. Documentation of changes in the concentration of contaminants in soil and water helps to show the effectiveness of the remediation, but does not definitely determine the fate of the contaminant. Observed reductions in contaminant concentration could be the result of biodegradation but could also be due to transport out of the sampling area, or increased adsorption to sediments. Linking contaminant loss to biodegradation can be accomplished through techniques that document changes in the presence, number, and degradation activity of the contaminant-degrading microorganisms. As part of the Remediation Technologies Development Forum Bioremediation Consortium, we monitored microbial population changes and activities at the co-metabolic biovent demonstration at Dover Air Force Base, Dover, Delaware to help determine the effectiveness of the bioremediation treatment.

Work Description

The targeted site was contaminated with TCE, 1,2-cis DCE and 1,1,1-TCA at levels ranging from non-detect to 15, 35 and 200 mg/kg, respectively. Depth to groundwater varied over time from 1.8 to 3.0 m due to changes in rainfall. A 6-m x 9-m test plot was established that included air/co-substrate injection and soil gas sampling points over a 3-m depth interval below the

surface (Figure 1). Based on laboratory column studies of aerobic cometabolic degradation performed by Zeneca, propane was chosen as the co-substrate. Propane addition resulted in greater stimulation of degradation than either methane or toluene addition. Following a period of

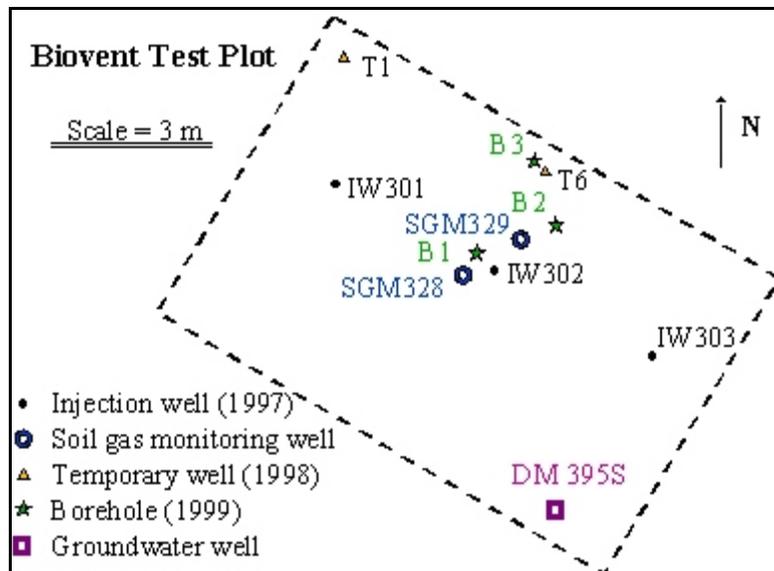


Figure 1 Plan View of the Biovent Test Plot

acclimation of the field site to propane, the air/propane injection system was operated for 16 months.

Sediment and groundwater samples were collected between March 1997 and August 1999 (Figure 1). Samples were collected using techniques developed through the RTDF Bioremediation Consortium and the DOE Subsurface Science¹. While dedicated downhole bladder pumps were used to obtain the groundwater, steam-cleaned split spoon or Geoprobe samplers were used to collect sediments. Sample processing was accomplished with sterile utensils and nitrogen-purged containers. Sediment and groundwater samples were shipped overnight on blue ice for laboratory microbial analysis at the University of Tennessee. Prior to the propane gas injection, sediment samples were analyzed to establish a baseline for microbial populations, diversity, community structure, and degradative capabilities. During the bioventing and prior to completion of the demonstration, sediment and groundwater samples were analyzed to determine shifts in the microbial community, changes in TCE degradation and effectiveness of

the bioremediation.

Microbial characterization and laboratory studies included enumeration of specific groups of bacteria, and activity studies. For sediment and groundwater samples, enrichments for propane oxidizers, methanotrophs, heterotrophic aerobes, iron reducers, methanogens, sulfate reducers, and heterotrophic anaerobes of microorganisms were performed as dilution series and incubated at 25°C for up to one month². Contaminant degradation potential was estimated in laboratory microcosms by measuring ¹⁴C-daughter products from the degradation of ¹⁴C-TCE using gas chromatography-gas proportional counting. The microcosms consisted of 10 ml groundwater or 2 ml sediment, 0.5 µCi ¹⁴C-TCE, and 2 ml mineral salts solution in 10 ml serum bottles or test tubes with teflon/silicone septa and aluminum seals³. Variations of the microcosm experiment included nutrient amendments and anaerobic conditions. The microcosms were sampled over time.

Results

Microbial analysis showed changes in the microbial community composition over the duration of the demonstration (Figure 2). Propane-degrading populations were not detected prior to propane

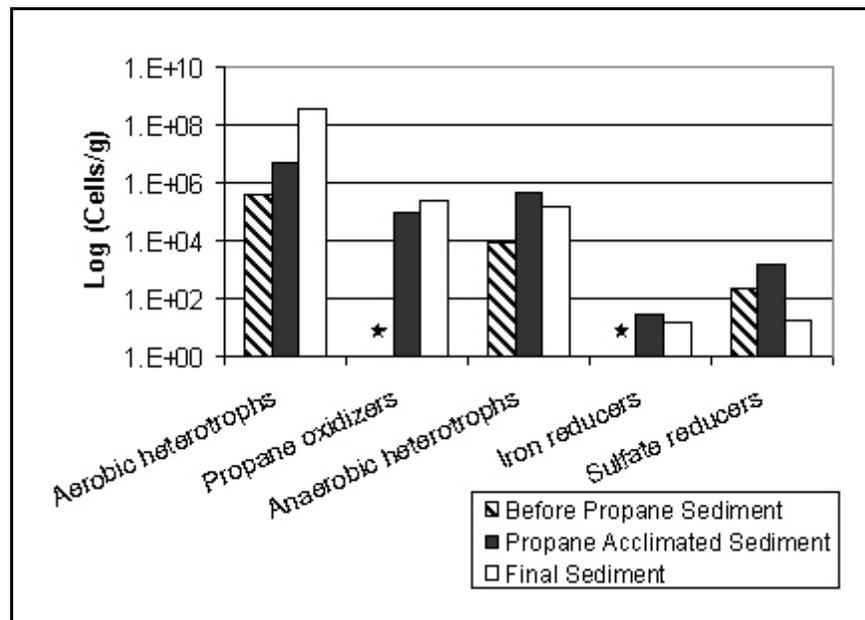


Figure 2. Changes in the Functional Physiology of the Microbial Community during the Biovent Demonstration

injection, but increased dramatically to greater than 10,000 cells/g following propane acclimation. In addition, aerobic heterotrophs increased greater than 100 fold. Although anaerobic populations (heterotrophs, iron and sulfate reducers) were less abundant than the aerobic populations, these anaerobic populations increased after propane acclimation. Apparently as a result of the continued air/propane injection, the sulfate reducers declined in abundance. TCE degradation potentials as measured in the laboratory microcosm analyses changed during the field test. Initially TCE degradation was below detection in unamended microcosms and was minimal in microcosms amended with propane and nutrients. The potential for TCE degradation increased during the biovent demonstration and was greater near the injection well (Figure 3). TCE degradation potential was also greater at shallower depths (Figure 3). Measured TCE degradation potential was greatest in aerobic and nutrient amendment microcosms, suggesting potential for nutrient limitation

in the field. The TCE degradation rates in the laboratory microcosms increased as much as 10 fold during the propane air injection. The percentage of degradative products recovered was 2-3 fold greater in the final sediment samples.

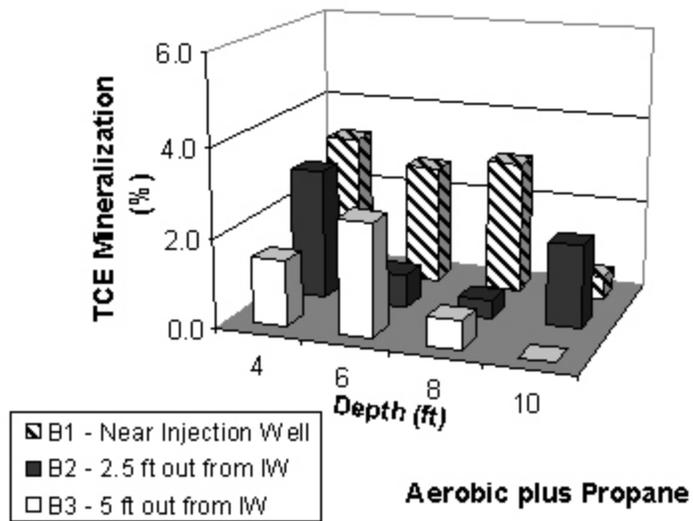


Figure 3. TCE Mineralization Across Test Plot

Chemical monitoring data supported the laboratory microbial results. Large drops in soil gas VOC levels was seen over time indicated significant VOC removal occurred (data not shown). Testing prior to completion of the demonstration showed that propane was consumed rapidly by the microbial populations and that the rate of propane utilization increased during the course of the field test.

Discussion and Conclusions

The monitoring data indicate that the addition of propane resulted in stimulation of specific microbial populations and thus a change in the overall microbial community structure to bacteria capable of using propane. This increase in the population of bacteria capable of using the injected co-substrate is necessary for effective bioremediation and mimics population trends seen in bioventing with methane to remediate TCE. At the Savannah River Site, methane was injected and the population of methanotrophs increased³. In both demonstrations, microbial populations that could utilize the co-substrate increases significantly and measured degradation activity increased significantly. Furthermore, both demonstration showed increased heterotrophic populations, suggesting microbial utilization of cellular metabolic products and recycling of nutrients. In addition, propane/air injection increased TCE degradation. TCE degradation studies were comparable between to DAFB and SRS biovent demonstrations³. At both sites, the injection of co-substrate increased TCE degradation. Monitoring the microbial community characterization and TCE degradation studies were essential in providing microbial evidence to substantiate the chemical monitoring data for determining the effectiveness of this co-metabolic biovent bioremediation. The data from this study indicates that propane can be

used as the co-substrate for biovent remediation of TCE. There may be advantages in using propane rather than methane related to the rates of degradation achieved and the range of compounds that are degraded ^{4,5}.

The data from both this demonstration and the SRS demonstration indicate a potential for nutrient limitation during bioremediation. Nutrient limitations in Eastern coastal plain sediments, such as those found at DAFB and SRS, has been previously documented ⁶. During the SRS co-metabolic bioventing demonstration, the gaseous nutrient addition (with nitrous oxide and triethylphosphate) was tested and proved successful in further stimulating degradation in the field ^{3,7}. At SRS increased degradation of TCE was seen in response to the 1% methane injection, whereas significant degradation of both TCE and PCE occurred in response to the subsequent injection of nutrients. Results demonstrated success in stimulating subsurface methanotrophic populations to achieve higher degradative populations and contaminant degradation rates. Summaries and modeling of the demonstration ^{8,9} indicated the significant impact of the nutrient addition on increasing the rate of TCE degradation and decreasing the treatment time. Based on the microcosm results presented here and on the published literature, we speculate that had additional nutrients been provided in the co-metabolic biovent demonstration at Dover AFB, TCE degradation would have been further enhanced.

Acknowledgments

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