

Pilot-Scale Evaluation of *In Situ* Cometabolic Bioremediation of TCE in Groundwater Using PHOSter[®] Technology

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A study was conducted to evaluate the efficacy of PHOSter[®] technology for treating groundwater contaminated with trichloroethene (TCE) at Edwards Air Force Base, California. The technology consists of injecting a gaseous mixture of air, methane, and nutrients into groundwater with the objective of stimulating the growth of methanotrophs, a naturally occurring microbial group that is capable of catalyzing the aerobic degradation of chlorinated solvents into nontoxic products. Injection operations were performed at one well for a period of three months. Six monitoring wells were utilized for groundwater and wellhead vapor monitoring and for groundwater and microbial sampling. In the five monitoring wells located within 44 feet of the injection well, the following results were observed: dissolved oxygen concentrations increased to a range between 6 and 8 milligrams per liter ($\mu\text{g/L}$); the biomass of target microbial groups increased by one to five orders of magnitude; and TCE concentrations decreased by an average of 92 percent, and to below the California primary maximum contaminant level (MCL; 5 micrograms per liter [$\mu\text{g/L}$]) in the well closest to the injection well. © 2008 Wiley Periodicals, Inc.*

INTRODUCTION

The U.S. Air Force implemented a treatability study at Site 14, located at the leading edge of the Sites 5/14 Contaminant Plume, Operable Unit No. 2, Edwards Air Force Base (AFB), California. The purpose of the study was to evaluate the efficacy of *in situ* cometabolic bioremediation using the PHOSter[®] technology for treating groundwater contaminated with dissolved-phase trichloroethene (TCE). The primary objectives of the study were to: (1) evaluate the capability of the technology to stimulate naturally occurring microbial populations that catalyze the degradation of TCE; (2) evaluate the capability of the technology to reduce the concentration of TCE in groundwater to below the MCL; and (3) determine the radius of influence (ROI) of a typical injection well at the site.

Site Description

The Sites 5/14 Contaminant Plume (Exhibit 1) is a commingled fuel and solvent plume that originates at Site 5 and extends approximately 5,600 feet downgradient to Site 14. The source area consisted of the former Southern Fuel Depot, a cluster of 20 underground storage tanks (USTs) that were installed in the early 1940s and supplied aviation gasoline to the Muroc Army Airfield flightline until 1955. In 1972, three of the USTs were converted to waste petroleum, oil, and lubricants (POL) storage tanks and used until 1984. Based on the contaminants present in the soils beneath the waste POL tanks and within the plume, it is apparent that waste solvents were commingled with the other waste liquids. Solvents were not released as free phase. The USTs were removed between December 1993 and December 1994.

The study was conducted at Site 14, the leading edge of the plume, which historically has only dissolved-phase TCE contamination in the upper 10 feet of the saturated zone. The study period was from July to November 2005. In July 2005, TCE concentrations in the study area ranged from approximately 200 to 600 µg/L. Exhibit 2 shows a layout of the pilot study area including the injection well (Well 14-SW01) and six monitoring wells, five of which are located within 44 feet of the injection well and one (Well 14-MW03) located 154 feet downgradient of the injection well.

The Site 14 groundwater extraction and treatment system (GETS) (Exhibit 1) has been operated at the leading edge of the plume since December 1998 with the objective of preventing further downgradient migration of the plume. The Site 14 GETS and the

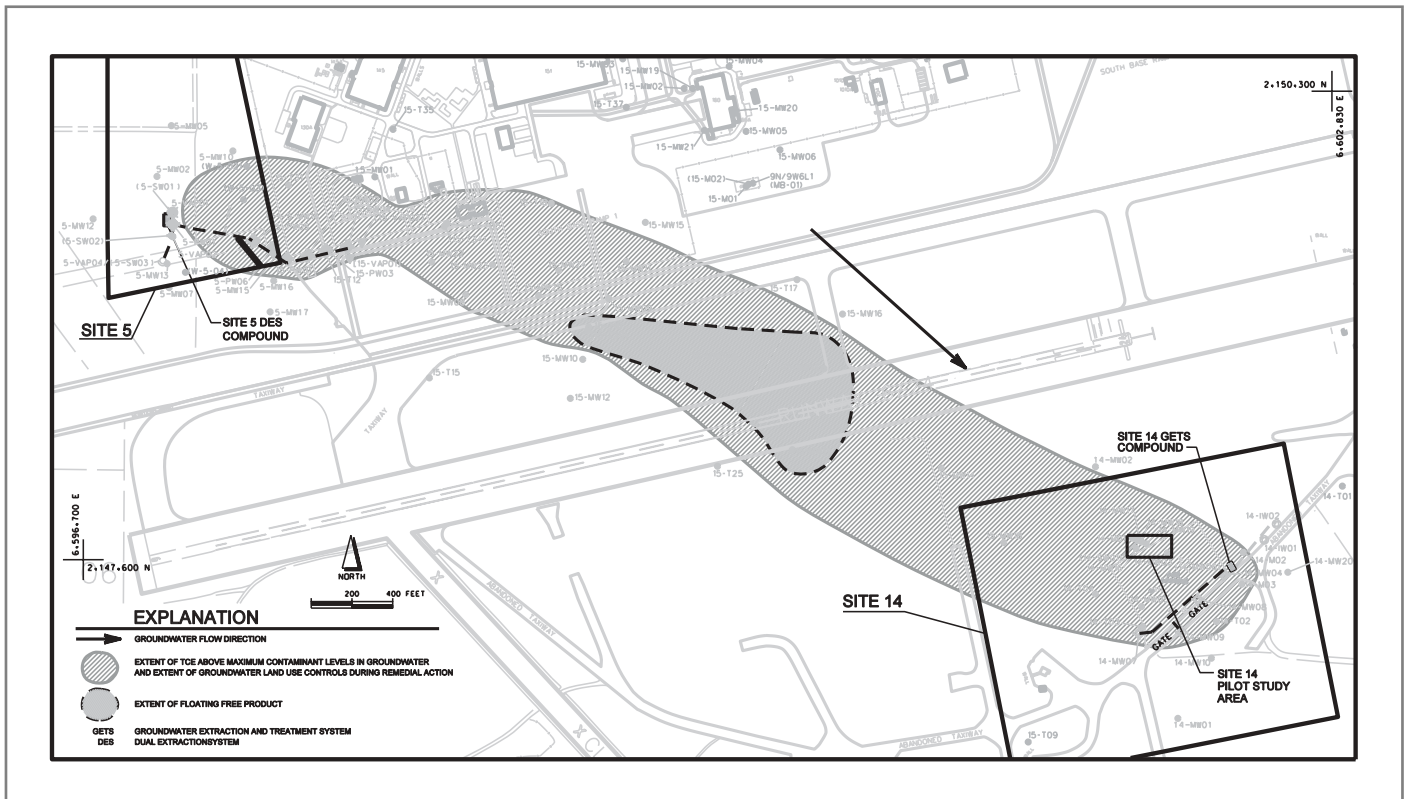


Exhibit 1. Sites 5/14 contaminant plume

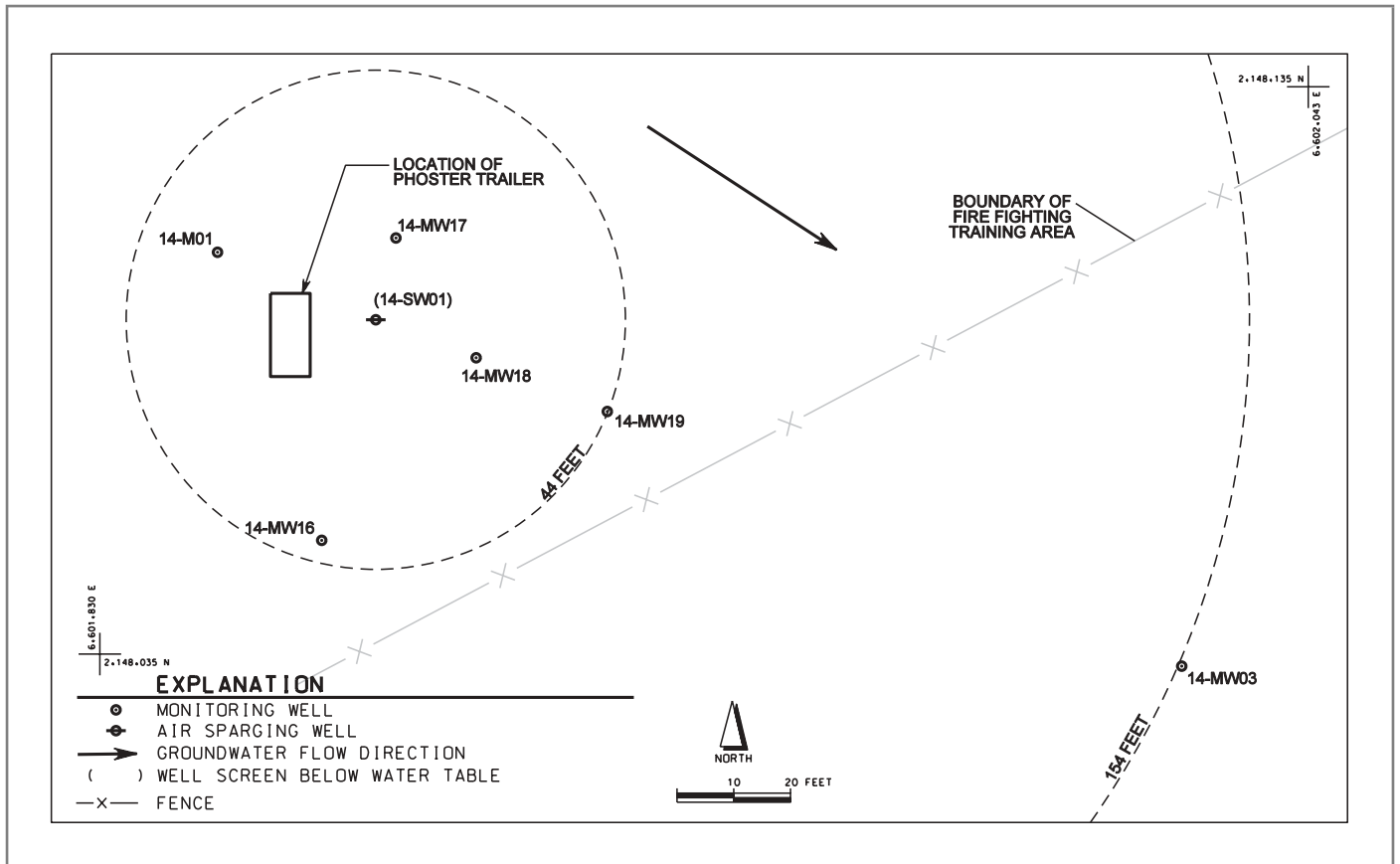


Exhibit 2. Site 14 treatability study layout

closest groundwater extraction well are located approximately 300 feet downgradient of Well 14-MW03, the furthest downgradient well in the pilot study area.

Geology and Hydrogeology

The geology at Site 14 is characterized as alluvial sediments overlying granitic bedrock. The sediments in the upper portion of the saturated zone are predominantly characterized as clayey and silty sands. Depth to weathered bedrock is approximately 300 feet below ground surface (bgs). Groundwater in the study area occurs in unconfined alluvial sediments at a depth of approximately 51 to 55 feet bgs. The average hydraulic gradient is approximately 0.001 feet per foot to the southeast.

PHOSTER® TECHNOLOGY AND SYSTEM DESCRIPTION

The PHOSTer® technology was developed by the Westinghouse Savannah River Company and the U.S. Department of Energy (DOE) at the Savannah River Site in Aiken, South Carolina. PHOSTer® is patented by the DOE. Two important features of the patent are the choice of a phosphorus source (triethyl phosphate [TEP]) and the means for contacting it with a carrier gas to produce gaseous-phase phosphorus (U.S. Patent and Trademark Office, 1998).

Gaseous-Phase Additives

The PHOSter® technology consists of injecting a gaseous mixture of air (oxygen [O₂] source), nitrous oxide (N₂O; nitrogen source), TEP (phosphorus source), and methane (CH₄; carbon source) into contaminated groundwater. The injection of air maintains an aerobic environment, which is necessary for the growth of the target microbial group. Nutrients (nitrogen and phosphorus) are necessary for bacteria to build cell mass and reproduce. The addition of CH₄ specifically targets the growth of *methanotrophs* that possess the capability to degrade chlorinated solvents through a cometabolic process (Hazen, 1993).

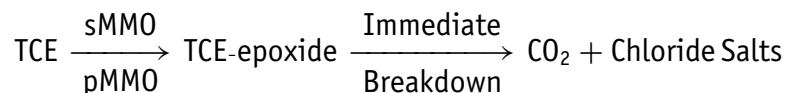
The addition of CH₄ specifically targets the growth of *methanotrophs* that possess the capability to degrade chlorinated solvents through a cometabolic process.

Methanotrophs and Cometabolism

Methanotrophs are a unique and ubiquitous, predominantly aerobic, bacteria group, which metabolize CH₄ as their sole source of energy. In order to metabolize CH₄, methanotrophs generate an enzyme, methane monooxygenase (MMO), that exists in two distinct forms, soluble and particulate (sMMO and pMMO, respectively) (Dalton, 2005). In addition to CH₄, MMO degrades a wide range of carbon substrates. This degradation is referred to as cometabolic, being the result of the incidental activity of MMO toward compounds that do not serve as an energy source for the bacteria. Methanotrophs are divided into two distinct physiological groups: Type I and Type II, which have cellular membranes that are composed of predominantly 16-carbon and 18-carbon fatty acids, respectively. Both Type I and Type II methanotrophs form clusters within the larger *proteobacteria* group. The sMMO enzyme is believed to be found only in a few species of Type II methanotrophs (Brigmon, 2001) and has been shown to degrade TCE at a rate at least an order of magnitude faster than the pMMO enzyme (Sullivan et al., 1998). Therefore, the desired effect of the technology is to stimulate and enhance methanotrophs capable of producing sMMO.

Aerobic Degradation of TCE

Unlike the anaerobic degradation of TCE, the aerobic process does not produce any toxic daughter products (e.g., *cis*-1,2-dichloroethene [DCE] and vinyl chloride) and results in nontoxic end products. Studies have shown that the sMMO enzyme induces the transformation of TCE into TCE-epoxide, which is a highly unstable compound. The TCE-epoxide is almost immediately mineralized into carbon dioxide (CO₂) and chloride salts (Hazen, 1993). The process is described in the following equation:



PHOSter® System

Exhibit 3 presents a layout of the trailer-mounted system used during the study. The system includes a rotary screw type air compressor, refrigerated air dryer, air receiver tank, TEP tank, N₂O and CH₄ gas cylinders, interconnecting piping, pressure gauges and

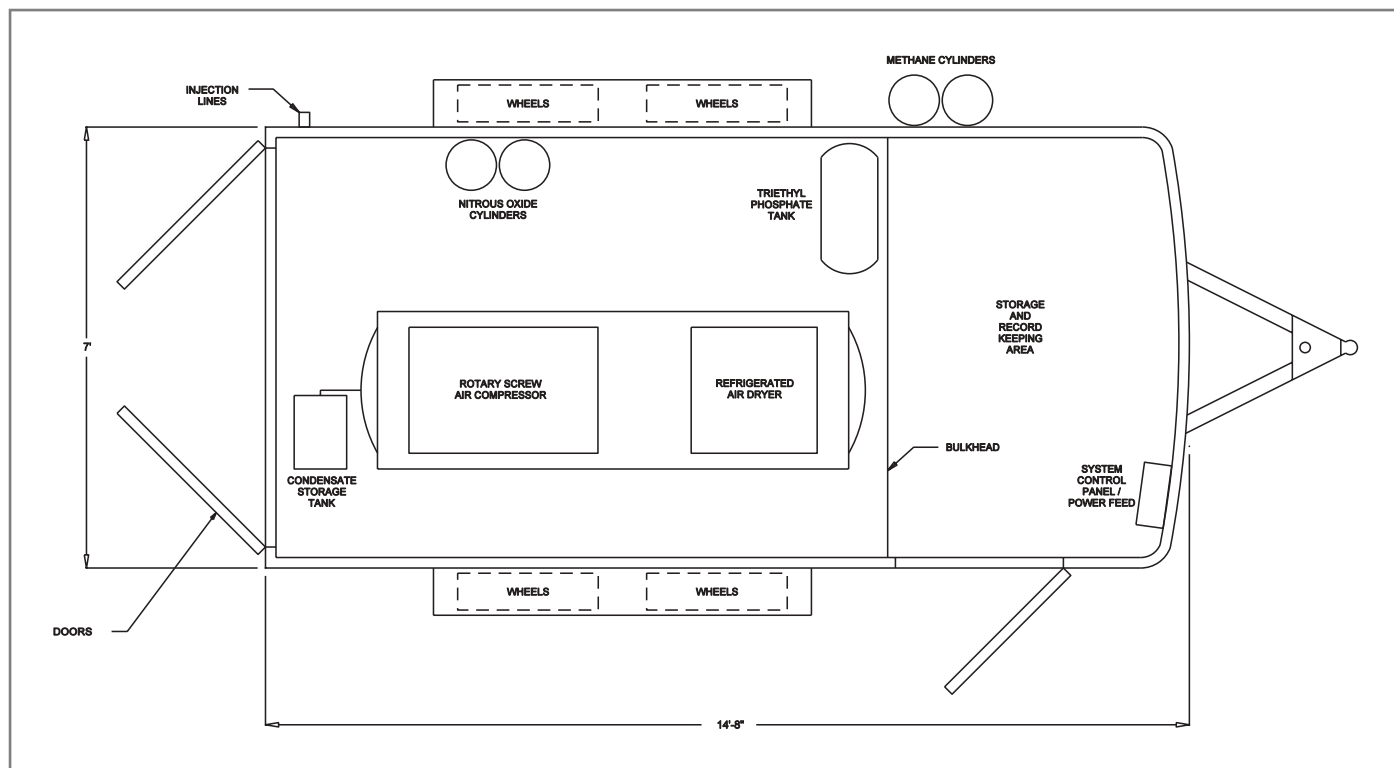


Exhibit 3. Trailer-mounted PHOSter[®] system

regulators, and flow meters. The system's discharge was connected directly to the injection wellhead.

PHOSTER[®] SYSTEM OPERATIONS

Injection operations were performed at one injection well for a period of three months between August 2 and November 9, 2005. The system operated for 2,396 hours, or approximately 99.9 percent of the available operating time. The system was offline for 0.1 percent of the time due to regular maintenance operations that were performed on the air compressor and air dryer. The system was monitored on a weekly basis and primarily consisted of adjusting gas flow rates and replacing N_2O and CH_4 gas cylinders.

The total injection flow rate was relatively stable throughout the study with a mean of 2.9 standard cubic feet per minute (scfm) and a standard deviation of 0.2 scfm. The air flow rate through the TEP tank was also relatively stable with a mean of 27 percent of the total air flow and a standard deviation of 4 percent. The injection of CH_4 was controlled by a timer to 12 hours per day. The flow rates of N_2O and CH_4 fluctuated more than the air flow mainly due to the gas cylinders being emptied. The mean concentrations by volume of N_2O and CH_4 were 0.8 percent and 1.4 percent, respectively. The standard deviations for the N_2O and CH_4 gas flow rates were 0.5 percent and 1.1 percent, respectively.

WELLFIELD MONITORING

Wellfield monitoring was performed at six monitoring wells prior to starting injection (i.e., baseline, on July 27, 2005), daily during the first week, weekly during the first month, and monthly thereafter. Groundwater monitoring consisted of measuring the depth to groundwater, dissolved oxygen (DO) concentration, and oxidation-reduction potential (ORP) *in situ* using downwell instruments. Wellhead vapor monitoring consisted of measuring the induced pressure using magnehelic gauges, the concentration of volatile organic compounds (VOCs) using a photo ionization detector, and the concentrations of CH₄, O₂, and CO₂ using a landfill gas analyzer.

Groundwater Monitoring Results

Groundwater Levels

Immediately following start-up of injection (August 2, 2005), the groundwater elevations increased significantly, with the largest increase of approximately 7 feet at Well 14-MW17, the well closest to the injection well. Exhibit 4 shows the change in water levels from baseline (i.e., mounding) in monitoring wells during the study. Following the initial effect and optimization of the total injection flow rate, water levels returned to near-static levels in all wells, with the exception of Wells 14-M01 and 14-MW17. At these two wells, the groundwater levels remained consistently higher than static levels, suggesting that the injected air was continually lifting water from deeper in the aquifer to the water surface.

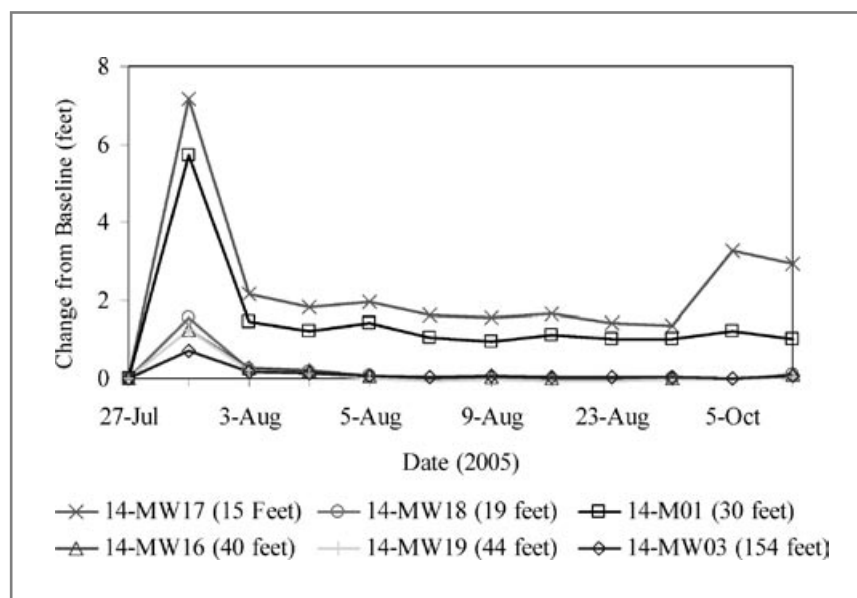


Exhibit 4. Changes in groundwater levels from baseline versus time

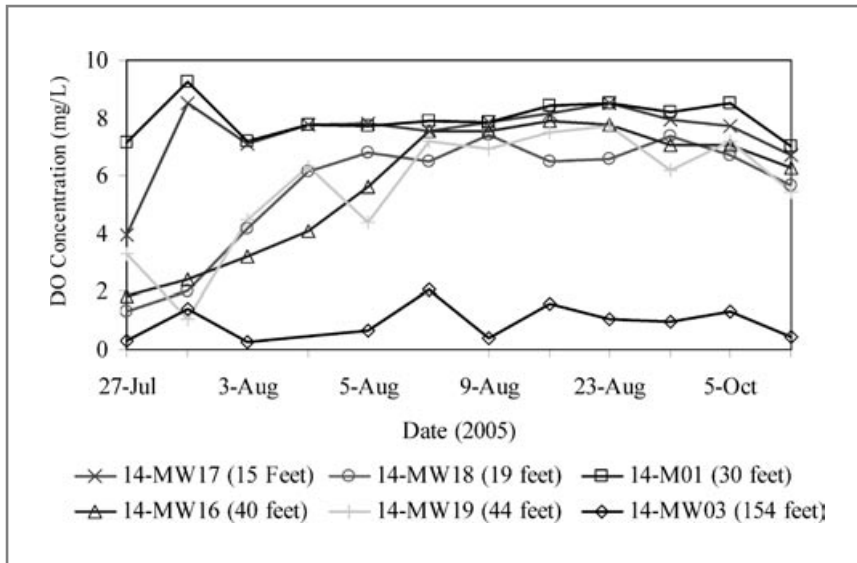


Exhibit 5. Dissolved oxygen concentrations versus time

Dissolved Oxygen Concentrations

Exhibit 5 shows the DO concentrations measured in the monitoring wells during the study. Baseline DO concentrations varied greatly between monitoring wells, ranging from 0.3 to 7.1 mg/L. Following start-up of injection operations, DO concentrations increased in all monitoring wells, with the exception of Well 14-MW03, which is located furthest from the injection well. The ROI for air injection appears to be greater than 44 feet (Well 14-MW19), but less than 154 feet (Well 14-MW03). The DO concentrations within the ROI reflect an aerobic environment adequate for the growth of the target microbial group.

Oxidation-Reduction Potential

Exhibit 6 shows the ORP measured in the monitoring wells during the study. Baseline ORP also varied greatly between monitoring wells and indicated generally aerobic conditions (greater than 50 millivolts [mV]) in all monitoring wells. During the study, all wells, with the exception of Well 14-MW03, exhibited a nearly identical pattern of change in ORP, all converging to essentially the same value at the end of the study (approximately 400 mV). Changes in ORP appear to support an ROI for air injection of greater than 44 feet but less than 154 feet.

Wellhead Gas Monitoring Results

Induced Pressure

Induced pressure was consistently registered in all monitoring wells during the injection operations, with the highest pressures recorded in Wells 14-M01 and 14-MW17 (Exhibit 7). Although induced pressure is indicative of the influence the injection has on the subsurface (saturated and unsaturated zones), it is not necessarily indicative of the ROI

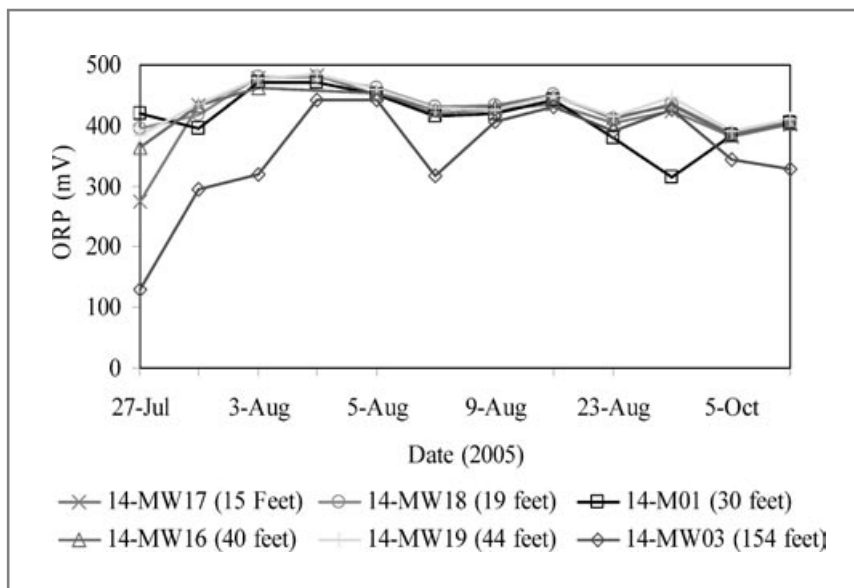


Exhibit 6. Oxidation-reduction potential versus time

for air injection or introduction of additives into the saturated zone. The induced pressure measured at a monitoring well could be due to air that reached the unsaturated zone at the vicinity of the injection well and only then flowed to the wellhead through unsaturated sediments.

Wellhead Vapor Concentrations

During baseline vapor monitoring, VOCs were not detected at the monitoring wells. During injection operations, VOCs were detected sporadically at concentrations below 2

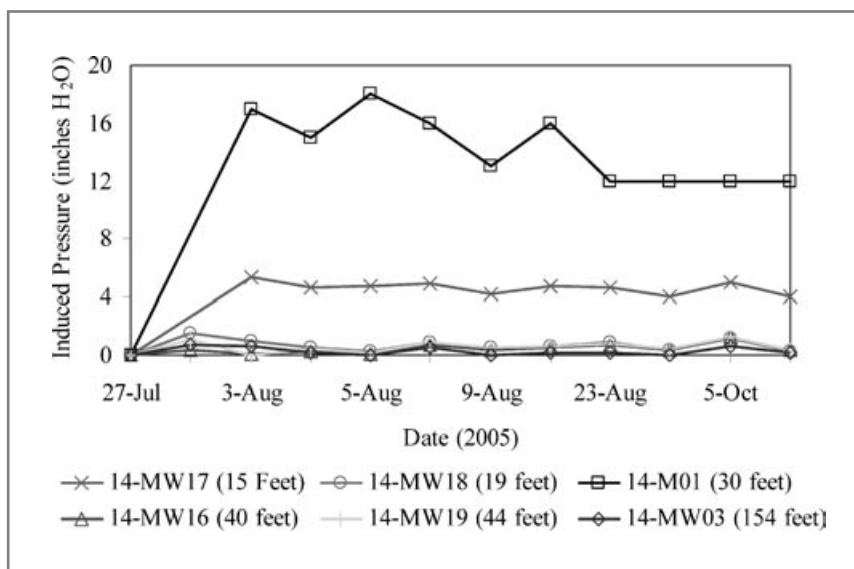


Exhibit 7. Induced pressure versus time

parts per million by volume (ppmv) at Wells 14-M01 and 14-MW17, the two wells where the highest induced pressures were recorded. These data suggest that few, if any, contaminants were being stripped from the groundwater as a result of the injection.

Vapor-phase CH₄ was not detected at the monitoring wells during baseline monitoring. During injection operations, CH₄ was detected at all monitoring wells, with a maximum concentration of 5.8 percent, indicating that not all the injected CH₄ was being dissolved into the groundwater.

During baseline monitoring, CO₂ was not detected at the monitoring wells, while O₂ was detected at concentrations between 20.2 and 20.4 percent, which is slightly below the atmospheric level of 20.9 percent. During injection operations, CO₂ was detected at all the monitoring wells, with a maximum concentration of 0.9 percent. The O₂ concentrations were less than baseline, with minimum concentrations ranging between 16.2 and 18.6 percent. These data indicate that microbial activity was occurring as a result of the injection.

GROUNDWATER SAMPLING RESULTS

Groundwater samples were collected prior to system start-up and monthly thereafter for a total of four sampling events (baseline, 30-day, 60-day, and 90-day [final]). The groundwater samples were collected using dedicated sampling pumps and the low-flow/minimal-drawdown sampling method. The samples were analyzed for VOCs, CH₄, total Kjeldahl nitrogen (TKN), total phosphorus, and total organic carbon (TOC).

Volatile Organic Compounds

Three VOCs were detected in groundwater samples during the study. Acetone was detected in two monitoring wells (Wells 14-MW16 and 14-MW18) at estimated concentrations below the reporting limit of 10 µg/L. *Cis*-1,2-DCE was also detected in two monitoring wells (Wells 14-MW03 and 14-MW19) at estimated concentrations below the reporting limit of 4 µg/L. TCE was detected in all the monitoring wells.

Exhibit 8 presents the TCE concentrations in the monitoring wells during the study. TCE concentrations consistently decreased between sampling events in all monitoring wells except for Well 14-MW03, with a total decrease between 86.6 percent (Well 14-MW16) and 99.8 percent (Well 14-MW17). In addition, the concentration of TCE in Well 14-MW17, the well closest to the injection well, was reduced to 0.42 µg/L (below the MCL). In Well 14-MW03 (the well furthest from the injection well), TCE concentrations decreased by 28 percent between the baseline and final sampling events. However, there was neither a consistency nor a pattern in the TCE reduction at this well throughout the study. Therefore, there is no conclusive evidence to suggest that the apparent TCE reduction at this well was caused by the injection and not by natural variations in sample collection techniques and/or acceptable tolerance of laboratory instrumentation. The ROI for TCE reduction appears to be greater than 44 feet (Well 14-MW19), but less than 154 feet (Well 14-MW03); the same ROI as for air injection.

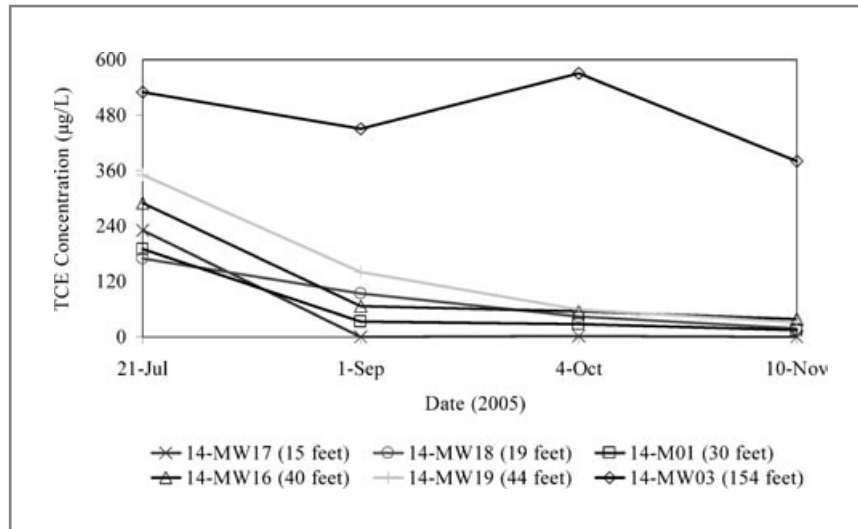


Exhibit 8. TCE concentrations versus time

Methane

Methane was not detected in baseline groundwater samples, but, with the exception of Well 14-MW03, was detected in all monitoring wells (i.e., within the air injection ROI) in all subsequent sampling events (Exhibit 9). The initial increase in CH₄ in wells within the air injection ROI is indicative of the system's efficacy in introducing dissolved-phase CH₄ into the aquifer. In subsequent sampling events, concentrations of CH₄ decreased in all wells, except for Well 14-M01. This decrease may be attributed to increasing CH₄ demand due to the growth of methanotrophs. The elevated concentrations at Wells 14-M01 and 14-MW17 correspond to the higher induced pressures (see Exhibit 7) and consistent mounding (see Exhibit 4) measured at these wells during the study.

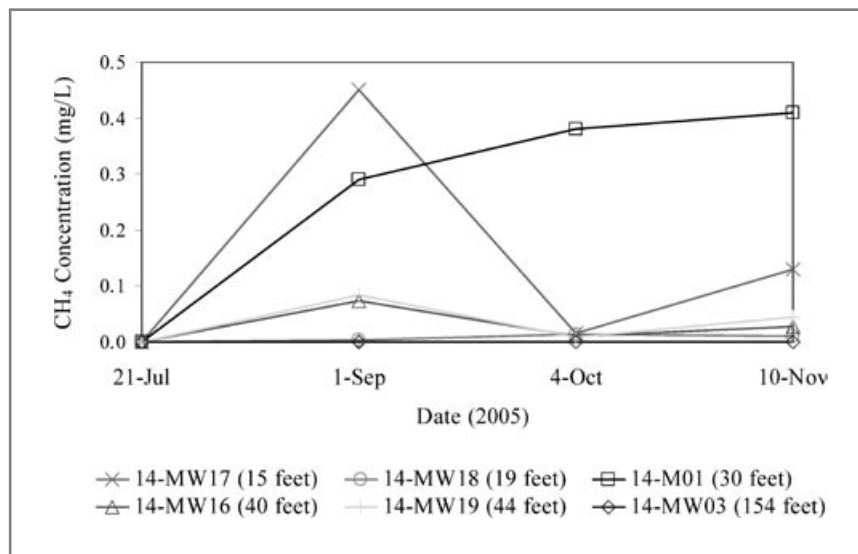


Exhibit 9. CH₄ concentrations versus time

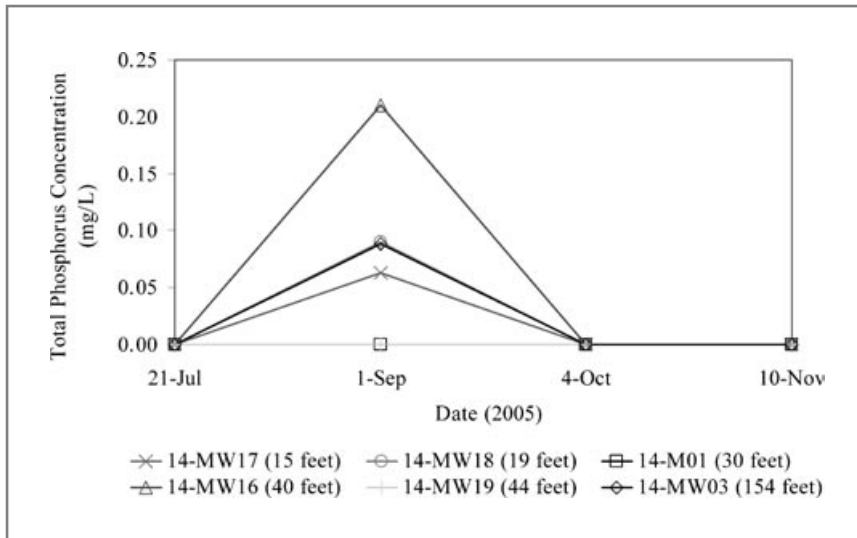


Exhibit 10. Total phosphorus concentrations versus time

Nutrients

Total phosphorus was not expected to be detected frequently or at elevated concentrations due to microbial demand and intake. TEP, the source of phosphorus, is a plasticizer and if supplied in excess will react with and damage plastic well materials (e.g., polyvinyl chloride [PVC]). Total phosphorus was not detected in baseline samples but was detected in four wells in the 30-day samples (Exhibit 10). No total phosphorus was detected in subsequent sampling events, which may be attributed to increasing intake due to the increases in microbial population densities.

TKN was also not expected to be detected frequently or at elevated concentrations due to microbial demand and intake. TKN was detected in baseline samples at the two

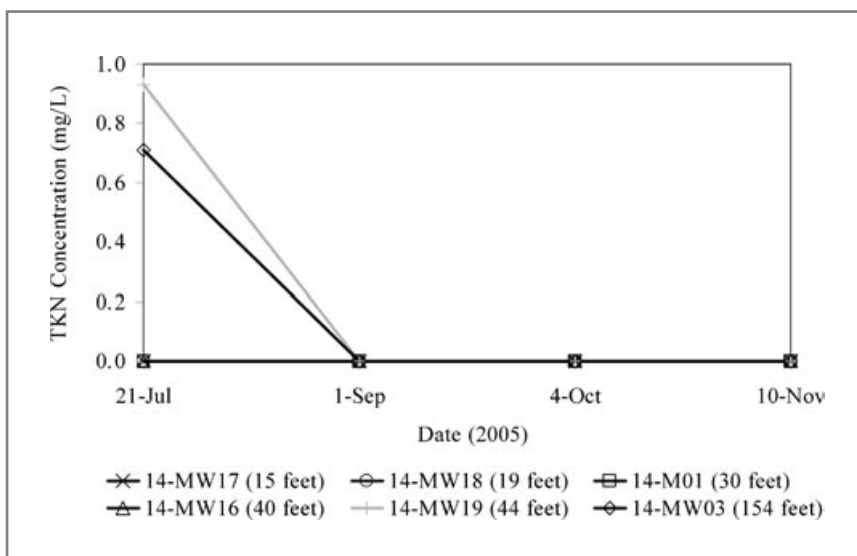


Exhibit 11. TKN concentrations versus time

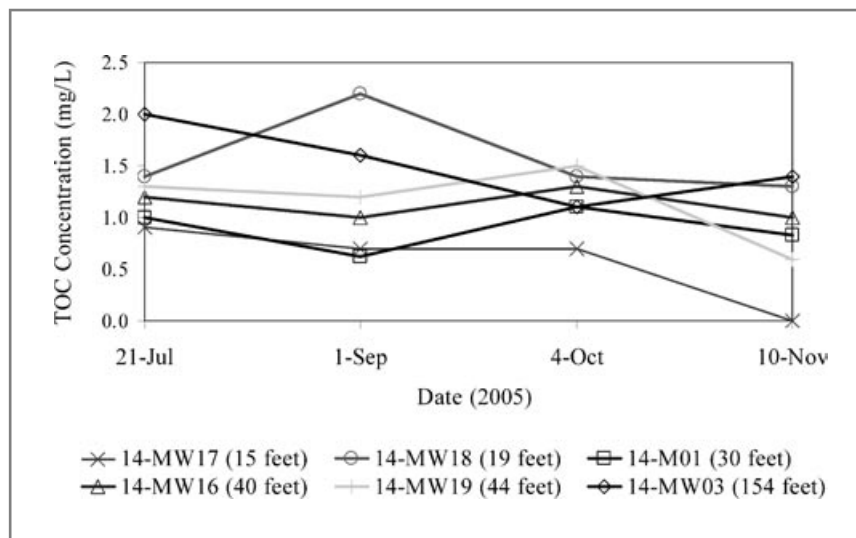


Exhibit 12. TOC concentrations versus time

wells with the highest TCE concentrations (Wells 14-MW03 and 14-MW19) but was not detected in subsequent sampling events (Exhibit 11).

Total Organic Carbon

Total organic carbon is indicative of the amount of organic matter available for microbial degradation. Generally, a decrease in TOC concentrations over time is an indication of microbial activity. TOC was detected in groundwater samples in all monitoring wells in all sampling events (Exhibit 12). With the exception of Well 14-MW17, the TOC concentrations varied in the monitoring wells without exhibiting any consistent trends. In Well 14-MW17, TOC concentrations consistently decreased during the study, corresponding to the largest reduction in TCE concentrations that were observed during the study (see Exhibit 8).

MICROBIAL SAMPLING

Microbial samples were collected using Microbial Insights' (Rockford, Tennessee) Bio-Traps®, which are small canisters containing Bio-Sep® beads upon which representative microbial communities colonize. The Bio-Traps® were installed in the monitoring wells for periods of approximately four weeks before they were retrieved for laboratory analyses of phospholipid fatty acids (PLFAs) and deoxyribonucleic acid (DNA). Bio-Traps® were retrieved prior to system start-up and monthly thereafter for a total of four sampling events (baseline, 30-day, 60-day, and 90-day [final]).

PLFA Analysis and Results

Phospholipid fatty acids are essential components of all cell membranes; therefore, the sum of PLFAs includes all important members of most microbial communities. Broad phylogenetic groups of microbes have different fatty acid profiles, making it possible to

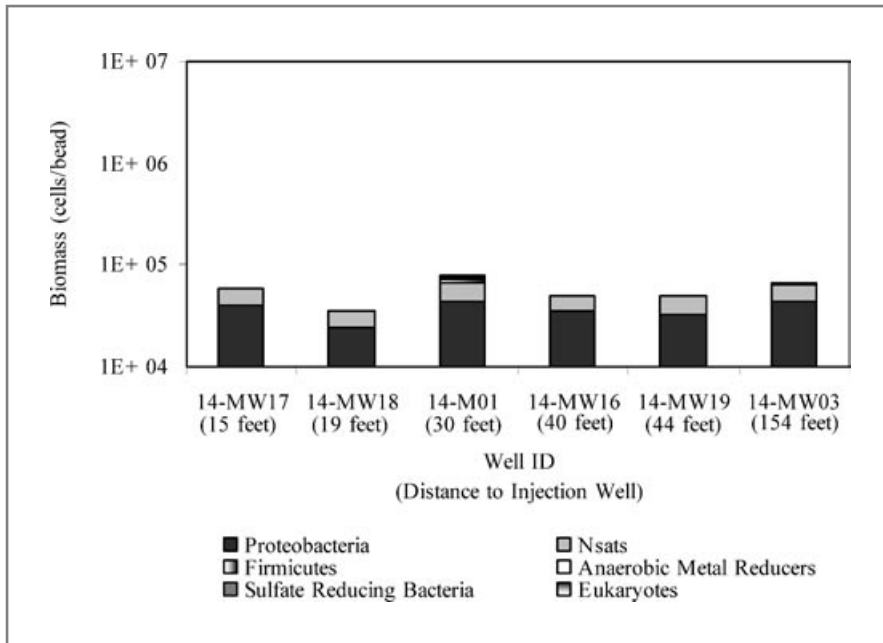


Exhibit 13. Baseline total PLFA biomass

distinguish among them. PLFAs break down rapidly upon cell death; therefore, the PLFA biomass does not include dead cells. As a result, PLFA analysis is the most reliable and accurate method available for the determination of viable microbial biomass (Microbial Insights, 2006).

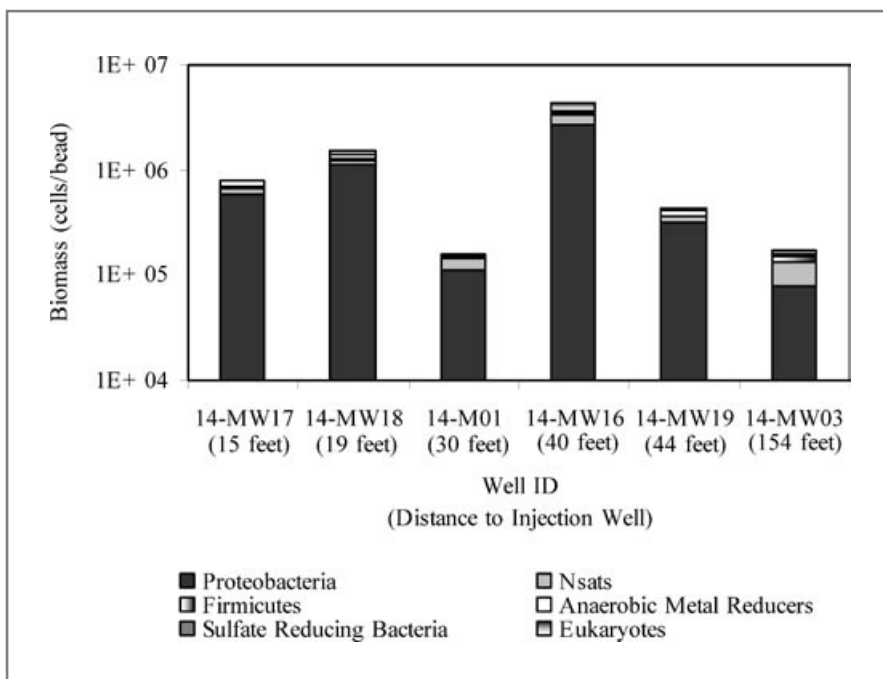


Exhibit 14. 30-day total PLFA biomass

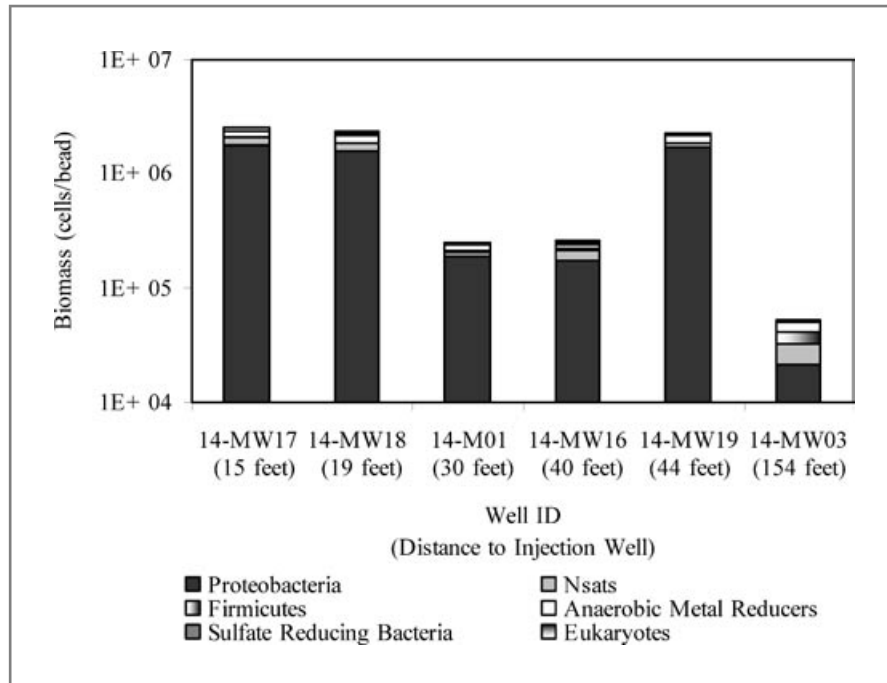


Exhibit 15. 60-day total PLFA biomass

Exhibits 13, 14, 15, and 16 present the total PLFA biomass in monitoring wells and the proportion of the various microbial communities in the baseline, 30-day, 60-day, and final (90-day) Bio-Traps®. In all monitoring wells, total baseline PLFA biomass was 10⁴ cells/bead, which is considered low to medium biomass levels. In the 30-day Bio-Traps®, the total PLFA biomass increased by one to two orders of magnitude in all the monitoring

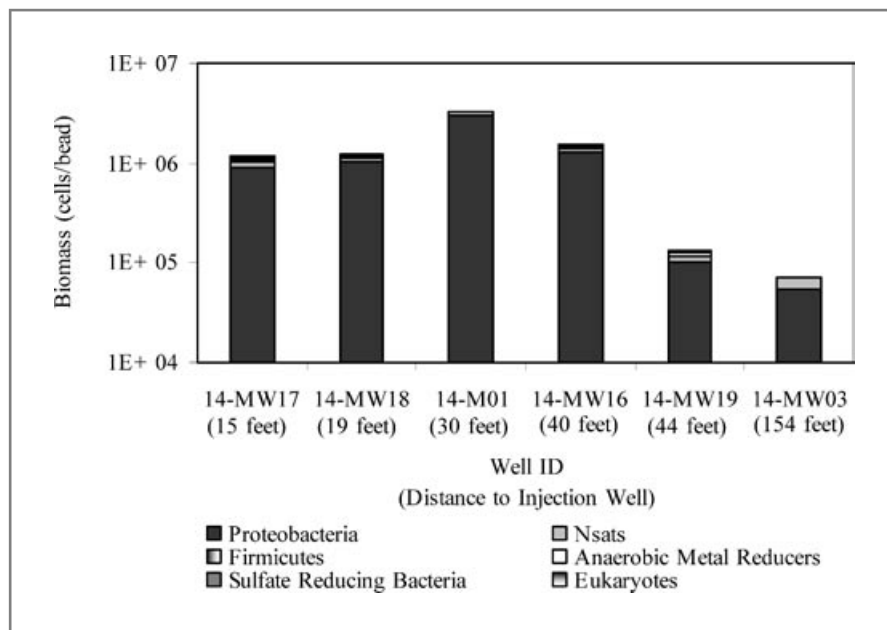


Exhibit 16. 90-day (final) total PLFA biomass

wells with the exception of Well 14-MW03. The total PLFA biomass remained relatively unchanged in all wells in the subsequent 60-day and 90-day Bio-Traps®.

Proteobacteria, the microbial group to which most hydrocarbon-degrading bacteria (including methanotrophs) belong, was the predominant microbial group in all monitoring wells throughout the study. The mean proportion of proteobacteria to the total microbial population was approximately 66 percent in the baseline, 30-day, and 60-day Bio-Traps®. In the 90-day Bio-Traps®, the mean proportion of proteobacteria to the microbial population increased to 81 percent.

The PLFA sampling results indicate that the ROI for stimulating the microbial population appears to be greater than 44 feet (Well 14-MW19), but less than 154 feet (Well 14-MW03), the same as for air injection and TCE reduction.

DNA Analysis and Results

Nucleic acid analysis allows for specific and sensitive detection of microorganisms from a variety of environments. Information can be obtained about the kinds of organisms present (phylogenetic assessment), and about their specific capabilities (functional assessment). Cell counts extrapolated from DNA analysis include dead cells and inactive cells and, therefore, overstate the viable biomass (Microbial Insights, 2006).

The target microbial group of the biostimulation was methanotrophs, particularly Type II methanotrophs that have the functional genes capable of expressing the sMMO enzyme. Exhibit 17 presents the baseline and final (90-day) total methanotroph biomass in the monitoring wells. The baseline total methanotroph biomass was at medium to high levels, ranging from 10⁶ to 10⁷ cells/bead. The final total methanotroph biomass was approximately one order of magnitude higher than the baseline, with the exception of Well 14-MW03, where a slight decline was observed.

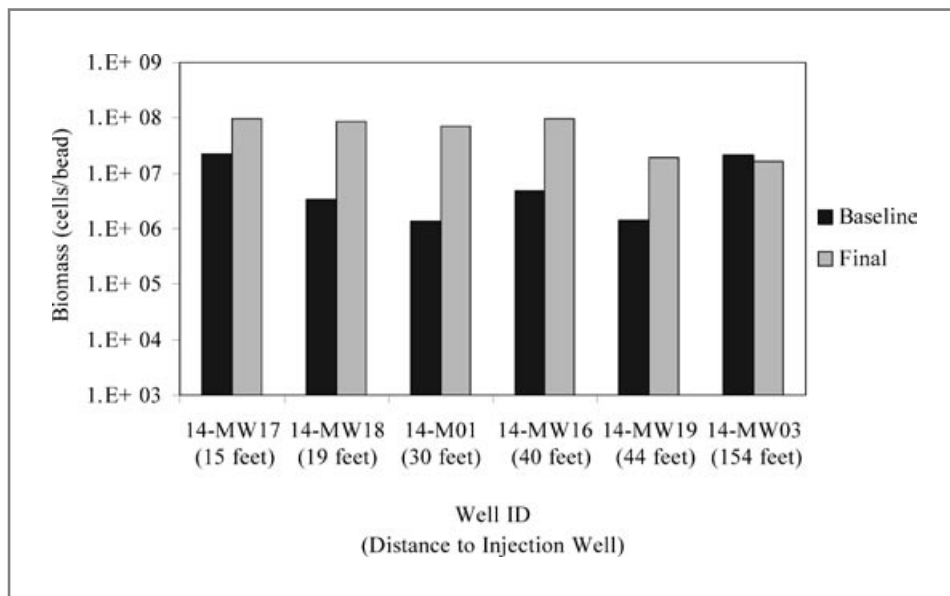


Exhibit 17. Total methanotroph biomass

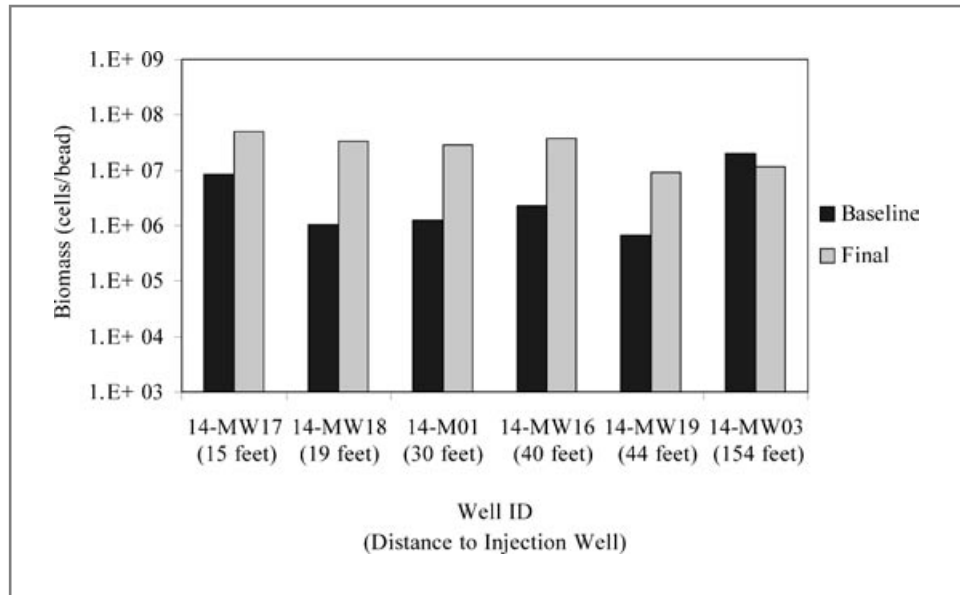


Exhibit 18. Type II methanotroph biomass

Exhibit 18 presents the baseline and final Type II methanotrophs biomass. The baseline Type II biomass levels ranged from 10^6 to 10^7 cells/bead. The final Type II biomass levels were approximately one order of magnitude higher than baseline, with the exception of Well 14-MW03, where a slight decline was observed. The mean proportion of Type II methanotrophs biomass to the total methanotroph biomass was 61 percent in the baseline Bio-Traps®. This decreased to 45 percent in the final Bio-Traps®.

Exhibit 19 presents the baseline and final biomass of methanotrophs with the functional genes for expressing sMMO. Baseline results indicated low levels of

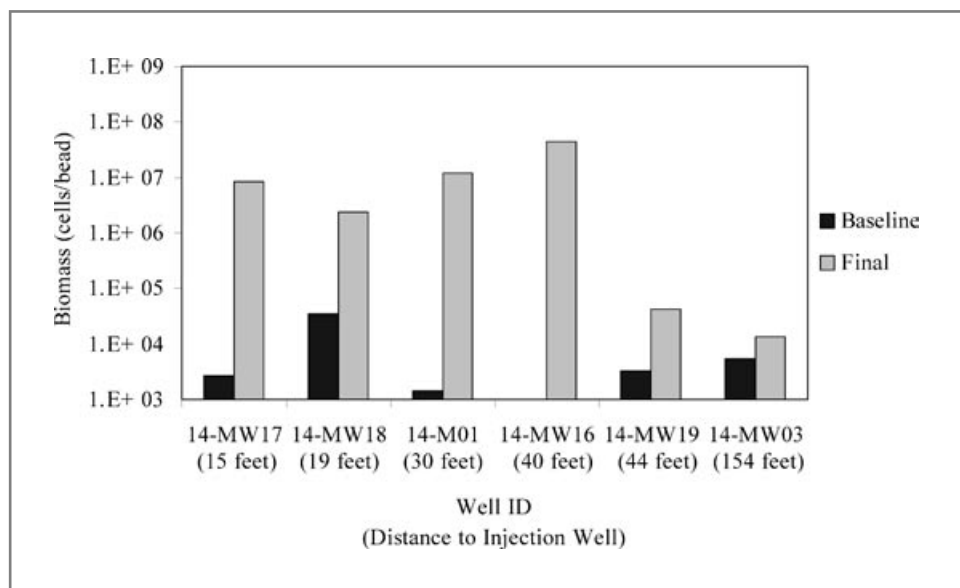


Exhibit 19. sMMO-producing biomass

sMMO-producing biomass ranging from 10^2 to 10^4 cells/bead. Final results indicated low to high levels ranging from 10^4 to 10^7 cells/bead, which was an increase of one to five orders of magnitude over baseline levels. The mean baseline proportion of sMMO producing biomass to the total methanotroph biomass was 0.09 percent. This increased to 17.3 percent in the final Bio-Traps[®].

The ROI for stimulating the target microbial group (sMMO-producing biomass) appears to be greater than 44 feet (Well 14-MW19) but less than 154 feet (Well 14-MW03), the same as for air injection and TCE reduction, and in concurrence with microbial biomass results for PLFAs.

CONCLUSIONS

Results of the treatability study at Site 14 demonstrated that the PHOSter[®] technology (1) stimulated the target microbial groups, including proteobacteria, total and Type II methanotrophs, and sMMO-producing bacteria; (2) reduced the concentration of dissolved TCE in groundwater, with the concentration in the well closest to the injection well (Well 14-MW17) decreasing to below the MCL; and (3) achieved an ROI greater than 44 feet but less than 154 feet. Based on the results of this study, the PHOSter[®] technology was recommended for treatment of TCE-contaminated groundwater at other sites at Edwards Air Force Base.

ACKNOWLEDGMENTS

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