United States
Environmental Protection
Agency

Robert S. Kerr Environmental
Research Laboratory
Ada, OK 74820

Research and Development

EPA/600/S2-89/042 Sept. 1989

EPA

Project Summary

In Situ Bioremediation of Spills from Underground Storage Tanks: New Approaches for Site Characterization Project Design, and Evaluation of Performance

John T. Wilson, Lowell E. Leach, Joseph Michalowski, Steve Vendegrift, and Randy Callaway

The full report presents a systematic approach for the design of in situ bioremediation of hydrocarbon contamination in ground water from the determination of the total quantity of hydrocarbons in the aquifer to the utilization of that information in an actual field bioremediation demonstration. The full report explains why the total quantity of hydrocarbons in an aquifer can only be determined by collecting cores. A procedure to acquire cores from a contaminated aquifer is described. The procedures described in the report were field-tested in designing a demonstration of the bioremediation of an aviation gasoline leak. The performance of the demonstration was consistent with the expected performance based on the preliminary site characterization using the described procedures.

This Project Summary was developed by EPA's Robert S. Kerr Environmental Research Laboratory, Ada, OK, and the Environmental Monitoring Systems Laboratory, Las Vegas, NV, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

The full report presents a systematic approach for the design of in situ bioremediation of hydrocarbon contamination in ground water from an initial determination of the total quantity of hydrocarbons in the aquifer to the utilization of that information in an actual field bioremediation demonstration.

Bioremediation of ground water contaminated with hydrocarbons such as gasoline is an on-site treatment technology that is both potentially technically feasible and more cost-effective than "pump and treat" technologies that involve pumping of contaminated ground water to the surface and removal of the contaminant by air-stripping or carbon adsorption. In situ bioremediation usually consists of modifying the environment of an aquifer by adding oxygen and other inorganic nutrients in order to enhance the activity of native microbial populations in degrading contaminants. Bioremediation is especially promising with hydrocarbons which are potentially biodegradable by native subsurface bacteria under the right environmental conditions to harmless byproducts.

Successful bioremediation is dependent upon a number of factors, including the hydrogeology at the site and the

availability of critical nutrients in the aquifer. The primary limiting factor with hydrocarbons is the aviability of oxygen. If sufficient oxygen is not present naturally, then oxygen must be provided by circulating oxygenated water through the contaminated area until degradation is complete.

The primary factor that determines how much oxygen and nutrients must be supplied to a hydrocarbon leak and how long remediation will take is the quantity of the hydrocarbon at the site. Normally, the amount of the leak is not known and available methods to determine the amount of contaminant at the site and its location are not acceptable.

Almost all techniques that have been applied for the analysis of oily contaminants in aquifers emphasize the compounds of regulatory interest, and few are appropriate for both solids and water. All too frequently, the only information available from a leak site is the concentration of selected organic contaminants in water from wells. Such information is inadequate for determining the total quantity of hydrocarbons in the aquifer. Therefore, it is impossible to determine how much oxygen and nutrients must be delivered to the aquifer to support sufficient microbial activity to degrade all of the contaminant to harmless by-products.

The full report explains why the total quantity of hydrocarbons in an aquifer can only be determined by collecting cores. A procedure to acquire cores from a contaminated aquifer is described. Before the procedure was developed, it was very difficult to recover good-quality cores of unconsolidated sandy material from below the water table. The report also describes two procedures to determine how much contamination the cores contain. Results of the two procedures are in good agreement, even though they are based on different principles.

The two techniques were developed and evaluated by scientists at the Robert S. Kerr Environmental Research Laboratory (RSKERL) as part of a large bioremediation research program. An oil-and-grease method was adapted to estimate total hydrocarbons in core samples. A second method was adapted from techniques for the analysis of fuels that determines the total content of hydrocarbons as well as the specific content of individual compounds of interest.

Basically, the oil-and-grease method uses infrared spectroscopy to measure the absorbance of carbon-hydrogen chemical bonds. Quantitation is sensitive to the type of hydrocarbon but is relatively insensitive to the particular organic constituents of the fuel. In the fuel carbon technique the hydrocarbons are extracted into methylene chloride, then separated and quantified by gas chromatography. Representative peaks are selected, and the quantity of total hydrocarbons is calculated by comparing the area of the representative peaks in a standard sample of the fuel to the area of the same peaks in the extract. The method works well if the standard is representative of the material being analyzed. If the proper calibrations are done, the concentrations of compounds of regulatory interest, such as the alkylbenzenes, can be determined in the same analytical run. The techniques for core analysis and their performance is discussed in Section III of the full report.

The procedures described in the report were field-tested in designing a demonstration of the bioremediation of an aviation gasoline leak. The performance of the demonstration was consistent with the expected performance based on the preliminary site characterization using the described procedures.

Site Characterization for In Situ Bioremediation of Hydrocarbon Leaks from Underground Storage Tanks

The pattern of contamination from a leak is complex. As the release drains through the unsaturated zone, a portion is left behind trapped by capillary forces. If the released material is volatile, a plume of vapors soon forms in the soil air in the vadose zone. If the release is a light hydrocarbon, it will drain down to the water table, and then spread laterally. Ground water moving through the aquifer comes in contact with the release, and leaches out the more water-soluble components. As a result there are three distinct regions or "plumes" formed at the leak site: a plume of volatile fumes in the soil air, a ground-water plume, and the region primarily in the unsaturated zone that contains the oily-phase material which serves as a source area for both plumes.

In practice the source area is usually the object of remedial activities. There is little point in treating the ground water or vapors if the source area is left to spread more contamination. Therefore, the first step is to remove any leaking tanks, transmission pipes, and the most visibly contaminated fill-material around the tank. Although necessary, such practices usually do not remove all of the sou The material trapped in the earth so beneath the tank will remain and serve as a continuous source of leacl contaminants for many years.

To intelligently remediate such a using in situ bioremediation require detailed understanding of the thr dimensional distribution of the sou area in the subsurface and good infor tion on the quantity of contaminant in source area.

Unless it is known how much conta inant has escaped into the subsurfa and where it is located, there is sensible way to locate injection a extraction wells, or to optimize pump rates and concentrations of any ame ments. Further, there is no way determine how much time a reme action will take, or how much it will cos

Conventional monitoring wells c accurately define the geometry of ground-water plume, but often th cannot distinguish the source area fr the rest of the plume. In fresh spi differential sorption of individual cc ponents of the plume to the aquifer sol can result in chromatographic separat of the components and alter the ratic their concentrations in water from w distant from the source area. However older spills, whose plumes have come sorptive equilibrium with the aquifer, concentration of contaminants dissol in the ground water is similar in source area and in the plume, altho the total amount of contaminant in source area is much greater.

For example, comparisons of grou water analyses vs. core analyses at aviation gasoline spill site in Michig showed that the ground water analys underestimated the amount of toluene the aquifer significantly. Further analy showed that the core contained pet eum hydrocarbons that sorbed most the toluene. If the data from t monitoring well had been used to des a remedy, the effort and exper required to restore the aquifer would ha been underestimated by a factor of six.

Obviously, the distribution of t source area and the extent of conta ination can only be characterized collecting and analyzing cores, becau they sample the entire aquifer, not j the ground water. Very precise inforr tion is needed on the vertical extent contamination, particularly for in situ b restoration. The injected waters are v expensive, and water injected into a cle part of the aquifer is wasted. If injec water moves underneath the conta

inated interval and breaks through in a monitoring well, it can also give the false impression that the region of aquifer between the two wells is clean.

Accurate techniques for analyzing cores to determine the total quantity of petroleum hydrocarbons in the aquifer and the concentration of individual compounds of regulatory concern are necessary not only for estimating the ultimate demand for oxygen, but also for documenting at the end of the remediation that the clean-up is complete.

Procedure for Acquiring Core Samples

Problems with Unconsolidated Sediments

Traditionally, unconsolidated soils or sediments are sampled through a hollow-stem auger with a split-spoon core barrel or a conventional thin-walled sample tube. The hollow-stem auger acts as a temporary casing to keep the borehole open until a sample can be acquired. A borehole is drilled down to the depth to be sampled. Then the core barrel is inserted through the annular opening in the auger and driven or pushed while rotating the auger into the earth to collect the sample. These tools work extremely well in both unsaturated and saturated cohesive materials. Unfortunately, they work poorly in noncohesive aquifer materials, such as unconsolidated sands.

There are two technical challenges to sampling noncohesive material below the water table. The first challenge is to keep aquifer material out of the annular area of the hollow stem auger. During augering, the annular area of the hollow-stem auger is plugged with a solid drill head that pushes the sand out onto the auger flights. To sample, the drill head is removed and replaced with a core barrel. When the drill head is pulled out of the auger in consolidated sands, pressure on the aquifer sediment is reduced, and water and fluidized sand rush into the annular area of the auger. This inconvenient phenomenon is commonly referred to as "heaving." The core barrel must push through (and sample) this heaved material inside the auger before it reaches the undisturbed sediment underneath. When the core is recovered, it is usually impossible to determine how much of the core is the fluidized material and how much is an authentic sample of the aquifer. Occasionally the amount of sediment in the auger is so great that the core barrel cannot be pushed, and no sample can be acquired.

The second challenge is to keep the sample in the core barrel while it is being retrieved to the surface. When the sampling tool is pulled out of the aquifer, the pressure holding the sample in the tool is reduced. Noncohesive sediment will often fluidize and dribble out of conventional core barrels.

Special Piston Sampling

Conventional practice to keep sediments out of the hollow-stem of an auger is to fill the hollow annular column with drilling mud. As the borehole is advanced, the weight of the mud stabilizes the hydraulic pressure of the aquifer. The use of drilling mud is not acceptable in geochemical assessments because fluids or chemicals introduced into the borehole can drain into the aquifer and alter the geochemistry of the pore water or contaminate the sample with foreign microorganisms. Such compromised samples cannot be used to assess prospects for bioremediation, and there is a strong possibility of microbial alteration of the sample during shipment or storage.

The staff of RSKERL have developed and tested new tools and protocols that consistently provide samples of the quality needed to characterize spills from underground storage tanks (Leach et al., 1988). The tools and protocols are modifications of techniques pioneered by others, principally researchers at the Institute for Ground Water Research, University of Waterloo, Ontario, Canada (Zapico et al., 1987).

Zapico et al. (1987) recently described a sampling device that effectively retains unconsolidated sands inside a cannister fitted inside a core barrel. A sliding piston inside the cannister maintains an air-tight seal on the core. Vacuum and friction keep the core in place. This device was modified to meet the special requirements of the RSKERL protocol.

During field evaluation at Traverse City, Michigan, the piston core barrel worked very well, but only when a core retainer basket was used. The piston core sampler without a core retainer basket often lost half or more of the sample before it could be recovered. A conventional core barrel with a core retainer basket recovered no sample at all. The combination of the two consistently recovered more than 95% of the cored interval (12 boreholes, more than 50 cores).

After the piston core barrel is brought to the surface, the end of the sampler is quickly covered with a plastic bag and tightly sealed to minimize aeration of the exposed core. The sampler is then quickly disassembled by removing the drive cap and manually pulling the piston free from the top of the sample tube. Then one end of the core barrel is connected to a hydraulic ram mounted on the rig, and the core is extruded. The cores are collected in wide-mouth canning jars. If possible, each jar is entirely filled with sample. The seal on the lid of the canning jar effectively excludes oxygen and prevents loss of volatiles.

Field Glove Box Sampling

If the cores are to be used for treatability studies to evaluate the prospects for bioremediation, they must be protected from contamination by foreign microorganisms. If naturally occurring microbial processes are to be evaluated, they must also be protected from the atmosphere because many anaerobic microorganisms are killed by oxygen.

To protect from foreign microorganisms, a core is collected by extruding a small portion of the core, breaking off a small section to reveal an uncontaminated face, then installing a sterile paring device onto the end of the sample tube. This tool peels away the outer contaminated wall of the core as the material is extruded.

To protect the sample from the atmosphere, the sample is extruded inside a nitrogen-filled glove box. The core barrel is introduced into the glove box through an iris port that makes a tight seal around the barrel.

The glove box is prepared for sample collection by filling it with the desired number of sterile canning jars and sterile paring devices, sealing the box, and then purging it with nitrogen gas. To prevent oxygen contamination when the jars are opened to receive the core in the field glove box, the jars are filled with nitrogen before they are brought to the field. They are passed into a laboratory anaerobic glove box, opened, then sealed air-tight. A slight positive pressure of nitrogen is maintained in the box during extrusion and collection of the cores.

Procedures to Determine the Concentration of Contaminants

The two techniques were developed and evaluated by scientists at the

RSKERL as part of a large bioremediation research program. An oil-and-grease method was adapted to estimate total hydrocarbons in core samples. A second method was adapted from techniques for the analysis of fuels that determines the total content of hydrocarbons as well as the specific content of individual compounds of interest.

Basically, the oil-and-grease method uses infrared spectroscopy to measure the absorbance of carbon-hydrogen chemical bonds. Quantitation is sensitive to the type of hydrocarbon but is relatively insensitive to the particular organic constituents of the fuel. In the fuel carbon technique the hydrocarbons are extracted into methylene chloride, then separated and quantified by gas chromatography. Representative peaks are selected, and the quantity of total hydrocarbons is calculated by comparing the area of the representative peaks in a standard sample of the fuel to the area of the same peaks in the extract. The method works well if the standard is representative of the material being analyzed. If the proper calibrations are done, the concentrations of compounds of regulatory interest, such as the alkylbenzenes, can be determined in the same analytical run

Comparison of the Methods

The fuel carbon method and the oil and grease method compare favorably, even though they are based on entirely different principles (Powell et al., 1988). The fuel carbon analysis is preferred at RSKERL because it also provides information on the concentration of alkylbenzenes in waste oils.

Field Demonstration of Sampling and Analytical Procedures in Designing a Bioremediation

In 1969, a spill of aviation gasoline from an underground storage tank at the U.S. Coast Guard Air Station at Traverse City, Michigan, contaminated a shallow, sandy, water-table aquifer. Ground water moving through the spill produced a large plume that eventually moved off the base and ruined a large number of domestic water wells in a residential area. The spill contained at least 25,000 gallons of aviation gasoline, which drained to the water table 16 feet below land surface, then spread laterally in the capillary fringe to contaminate a section of aquifer about 80 yards in diameter.

Design of the Experiment

In 1988 the U.S. Coast Guard and the U.S. EPA installed a pilot-scale study of bioremediation in the area of the original spill. The alkylbenzenes are the object of the regulatory concern, and the bioremediation will be finished when their concentration is brought below 5 ug/liter, as specified in a consent decree between the Michigan Department of Natural Resources and the U.S. Coast Guard.

Cores were acquired from the source area to determine the vertical and horizontal extent of contamination, the concentration of total hydrocarbons in the contaminated interval, and concentrations of individual alkylbenzenes. The aviation gasoline was composed primarily of branched-chain alkanes. The material spilled at Traverse City was 38% 2,2,4-trimethylpentane; 15% 2,2,5-trimethylhexane, 14% 2,3-dimethylpentane; 13% 2,4-dimethylhexane; 7% 2,3-dimethylhexane; and 5% 2,4-dimethylpentane. Only 10% of the original spill was alkylbenzenes.

The gasoline was confined to a narrow interval between 15 and 17 feet below the land surface. This interval corresponds closely with the seasonal high and low water table at the site.

This information was used to identify the most contaminated flow path through the spill. A series of miniature monitoring wells was installed along and below the most contaminated flow path.

A set of infiltration wells was installed to perfuse the contaminated area with mineral nutrients, and oxygen or hydrogen peroxide.

Injection began the first week of March, 1988. The system was first acclimated to oxygen, then switched to hydrogen peroxide. The concentration of hydrogen peroxide was increased slowly, to allow time for microbial acclimation to concentrations of hydrogen peroxide that are generally toxic to most heterotrophic bacteria.

Estimate of Oxygen Demand Required for Remediation

The concentration of total petroleum hydrocarbons in the most contaminated interval near the infiltration wells was near 300 mg/kg. The highest measured concentration of total hydrocarbons near a monitoring well 31 feet down gradient from the injection wells is 8,400 mg/kg (core 50AE4 in Figures 10 and 11 in the full report). The highest measured concentration 60 feet down gradient is 6,500 mg/kg (core 50I14 in Figures 19 and in the full report). The average of co 50AE4 and 50I14 (7,500 mg/kg) w taken as the best estimate of t concentration of total petroleum hyd carbons in the most contaminated inter between the monitoring wells at 31 a 50 feet. The interval between the inject wells and the monitoring wells could be cored because access was block by a sanitary sewer line. The most c servative estimate would consider t entire interval between the injection we and the monitoring well at 31 feet to contaminated at 7,500 mg/kg. The m liberal estimate would consider the int val to be contaminated at 300 mg/kg. arbitrary intermediate estimate wo average 7,500 and 300 mg/kg. The o gen demand along the most conta inated interval was calculated for all thr estimates.

To calculate the theoretical oxyg demand of the hydrocarbons in a se ment of a flow path, the hydrocarb content (mg hydrocarbon/kg aquifer) w multiplied by the bulk density of t sediment (2.0 kg/liter) and divided by t porosity of the aquifer (0.4 liter po space/liter total volume) to determine t quantity of hydrocarbon exposed to ea liter of pore water in the segment. T quantity of hydrocarbon was multipli by its oxygen demand to estimate t quantity of oxygen that must be deliver to each liter of pore water in the segme

The interval from the injection wells the monitoring well 31 feet down gradi was considered one segment. The d mand in the flow path to the monitori well 50 feet down gradient was estimat as the weighted average of the dema in the segment from the injection wells 31 feet, and in the segment from 31 to feet.

Performance of the Demonstration

The interval between the injecti wells and the monitoring wells was co sidered remediated when detectable o gen broke through and alkylbenzen disappeared. The interval to the mo toring well at 31 feet was remediat after 220 days (Julian Date 281), and t interval to the monitoring well at 50 fe was remediated after 270 days (Juli Date 331).

The seepage velocity (as determin by the tracer tests) was multiplied by t concentration of oxygen or hydrog peroxide in the injection wells to det mine the instantaneous flux of oxygen

hydrogen peroxide along the flow path. The cumulative flux at the time of remediation was considered the actual oxygen demand for remediation.

The aquifer was purged of alkylbenzenes very quickly. Aviation gasoline is composed primarily of branched-chain alkanes. Only 10% of the original spill was alkylbenzenes. The quantity of oxygen and hydrogen peroxide required to remove alkylbenzenes from the wells agreed closely with the projected oxygen demand of the alkylbenzenes alone.

This may, to some extent, be fortuitous. Some of the alkylbenzenes must have been washed from the source area by simple physical weathering, resulting from their relatively high water solubility. Some of the alkylbenzenes may have been removed by anaerobic biological processes before the front of oxygen swept through. Water from anaerobic regions of the demonstration contained significant concentrations of volatile fatty acids and was visibly turbid with microorganisms. In any case, the flow paths to the monitoring wells at 31 and 50 feet from the injection wells were remediated when a small fraction of the oxygen demand of the spill had been supplied.

Contribution of Water Washing

A significant fraction of the alkylbenzenes may simply be washed out of the demonstration area by the flow of water, instead of being destroyed by biodegradation. The significance of this physical weathering can be evaluated by comparing the retardation factor of each alkylbenzene in the most contaminated interval to the number of pore volumes of water that have been delivered to a particular point.

After comparing the number of pore volumes of water delivered along the most contaminated interval to the predicted retardation ratios of individual alkylbenzenes in the field demonstration, it is evident that benzene could easily have been removed by water washing, and that a fraction of the toluene may have been removed, but hardly any removal of the xylenes, ethylbenzene, or trimethylbenzene can be expected.

Confirmation of Remediation

The spill was cored in August 1987 to provide information to design the demonstration, then cored again in March 1988, just before the demonstration began, to define the initial conditions. The proportion of alkylbenzenes in the spill declined modestly over the time interval. This was probably due to anaerobic microbial degradation as discussed earlier.

Shortly after the breakthrough of oxygen in monitoring well BD 31-2, the area near the monitoring well was cored and analyzed for alkylbenzenes and total fuel hydrocarbons. The aliphatic hydrocarbons remained at their initial concentration, but the alkylbenzenes were below the analytical detection limit. It is not surprising that the non-aromatic fraction of the spill remained in the aquifer. A very minor fraction of their oxygen demand had been supplied when the aquifer was cleansed of alkylbenzenes.

When the region near BD31-2 was cored in March of 1 989, almost all the petroleum hydrocarbons had been removed, including the branched-chain alkanes.

A core taken from a region in the demonstration area where oxygen was depleted showed an interesting pattern. Toluene is depleted at one location even though significant quantities of benzene and ethylbenzene remain. It is difficult to rationalize the selective removal of toluene through some purely physical mechanism.

References

Leach, Lowell E., F. P. Beck, J. T. Wilson, and D. H. Kampbell. 1988. Aseptic Subsurface Sampling Techniques for Hollow-Stem Auger Drilling. Proceedings of the Second National Outdoor Action Conference on Aquifer Restoration, Ground Water Monitoring and Geophysical Methods, Vol. 1, pp. 31-51.

Powell, R. M., R. W. Callaway, J. T. Michalowski, S. A. Vandegrift, M. V. White, D. H. Kampbell, B. E. Bledsoe, and J. T. Wilson. 1988. Comparison of Methods to Determine Oxygen Demand for Bioremediation of a Fuel Contaminated Aquifer. Intern. J. Environ. Anal. Chem., Vol. 34, pp. 253-263.

Zapico, Michael M., S. Vales, and J. A. Cherry. 1987. A Wireline Piston Core Barrel for Sampling Cohesionless Sand and Gravel Below the Water Table. Ground Water Monitoring Review, Vol. 7, No. 3, pp. 74-82.

The EPA authors, ***John T. Wilson*** *(also the EPA Project Officer, see below) and* ***Lowell E. Leach*** *are with the Robert S. Kerr Environmental Research Laboratory, Ada, OK 74820; Joseph Michalowski, Steve Vendegrift, and Randy Callaway are with N.S.I. Technology Services, Inc., Ada, OK 74820.*

The complete report, entitled "In Situ Bioremediation of Spills from Underground Storage Tanks: New Approaches for Site Characterization Project Design, and Evaluation of Performance," (Order No. PB 89-219 976/AS; Cost: $15.95, subject to change) will be available only from:

National Technical Information Service
5285 Port Royal Road
Springfield, VA 22161
Telephone: 703-487-4650

The EPA Project Officer can be contacted at:

Robert S. Kerr Environmental Research Laboratory
U.S. Environmental Protection Agency
Ada, OK 74820

United States
Environmental Protection
Agency

Center for Environmental Research
Information
Cincinnati OH 45268

Official Business
Penalty for Private Use $300

EPA/600/S2-89/042