

# Role of Microorganisms in the Operation of the Savannah River Site

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## Abstract

Microorganisms are invisible to the naked eye, but their size belies their important role in nature and their role in the operation of the Savannah River Site (SRS). This contribution to the 50-Year Celebration of Excellence in Science and Engineering at the Savannah River Site details one of the microbial investigations that have provided greater insight into the versatility and applicability of our smallest allies to solve some of the greatest needs of humanity. The microbiological investigations at the Savannah River Site have opened new avenues for research into the interactions between the biosphere and the geosphere. The studies at SRS have shown the extension of the biosphere deep within the geosphere and that life may only cease to exist when temperature and pressure become inhospitable. These investigations have expanded our horizons about the habitats where microorganisms live and their ability to adapt and alter their selected niches. As we better understand the microbial niches around and under us, the sophisticated microbe continues to amaze its viewers, and in turn provides solutions to some of mankind's most pressing needs.

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Proverbs 3:13 "Happy is the man that findeth wisdom, and the man that getteth understanding  
14 For the merchandise of it is better than the merchandise of silver, and the gain thereof than fine gold."- Solomon

## Introduction

Microbiological investigations at the Savannah River Site have never taken on major mission status. It has been difficult for management to appreciate how invisible life plays a substantial role in the enormous science of big energy generation and nuclear weapon technology, but they have and they do. Microorganisms are important and affect all we do even in the nuclear industry. This is one of their stories as told by one of their friends. The narrative is of how subvisual life has influenced the science of subatomic particle physics. The reactors at SRS await their call. Environmental cleanup ensues, but the microbial world thrives as information flows from data defined by knowledge with a little wisdom of the microbial world around us.

In our family a clear message that has passed through the generations is that if one sees a turtle on a lamp post, one knows that the turtle did not get there by itself. It had lots of help. It is with this same sense of inadequacy and privilege that I have the honor of sharing one of the fascinating achievements at SRS in its 50-year history. Much of the data discussed in this contribution have been documented elsewhere (Fliermans and Balkwill 1989; Hazen et al. 1996; Massmann unpublished results).

When I arrived at SRP in 1974, Dr. Todd Crawford gave instructions to "set up a laboratory for microbial ecology". What a beautiful challenge. Little did he nor anyone else dream where that challenge would lead. Interests in the early days were of what microorganisms were able to do "to you" as opposed to the current emphasis at SRS as to what they can do "for you". Fresh from a post doc and studies on and in the extreme environments in Yellowstone National Park, I had eager excitement of how the microorganisms in natural thermal habitats compared to those in the man-made thermal habitats of the cooling water canals from the Site's nuclear reactors.

In the southeastern United States just a little thermal impact into already warm surface waters provides a perfect temperature habitat for those organisms that are capable of causing disease. With Ray Harvey and Lawrence Tilly as my mentors and J.J. Foreman as my right hand side kick, we began the adventure that continues. The journey serpentine through the intricacies of medical microbiology, consummating, in what has been described by the late Professor Rene Dubos, as a “very elegant marriage to microbial ecology” with the discover of the natural habitat of the bacterium that causes Legionnaires’ Disease. That work has continued for the past 22 years and has been a vanguard in moving the understanding of microbial systems and interactions from data to information to knowledge with a little wisdom.

## **Microbiology of the Deep Subsurface**

As thermal research became less of a DOE mission after the reactors were shut down, our interests began to shift towards understanding the ability and role of microbial systems in remediating contaminants left by the nuclear legacy in the groundwaters and terrestrial subsurface environments of the Site. There was but one problem. Extensive microbiological investigations had been confined to the upper few meters of the earth’s crust. Until the beginning of DOE’s Subsurface Microbiology Program in 1985 at the Savannah River Plant, scientists considered it unlikely that communities of microorganisms could inhabit the deeper sediments of the biosphere. In his seminal textbook Professor Martin Alexander of Cornell University, the foremost soil microbiologist of his day, stated, “THAT LIFE DOES NOT EXIST BELOW THE ROOT ZONE OF PLANTS” (Alexander 1977). Since the subsurface contaminants were relatively deep at SRS, and certainly below the root zone of plants where the conventional wisdom said “all” the microorganisms resided, such “dogma” had a dampening affect on any investigations that used microbial

systems to clean up deeply affected aquifers and nonsaturated soils called the vadose zones.

If SRP were going to use microbial systems to affect the clean-up of hazardous and toxic wastes in the deep subsurface, then substantial microbial populations present in the subsurface would be required. Using state-of-the-art microbiological technologies, the Subsurface Program initiated at the Savannah River Plant focused on detecting microorganisms at great depths, establishing fundamental scientific information, including their ecology, and exploring their potential use in cleaning up contaminated deep terrestrial sediments and groundwater environments from energy and defense production activities (DOE 1988). Results from this program destroyed the traditional scientific concept of an abiological terrestrial deep subsurface. These investigations demonstrated that the terrestrial deep subsurface is a habitat of great biological diversity, and that activity does not decrease significantly with increasing habitat depth.

The enormous diversity of the microbiological communities in deep terrestrial sediments is most striking. Even at depths greater than 1000 feet, the number of microorganisms are greater than 10,000,000 bacteria per each gram of sediments. The organisms varied widely in their appearance and the way they transform or degrade a variety of organic and inorganic compounds. Regardless of the depth sampled, microorganisms were able to perform their traditional vital roles of recycling carbon, nitrogen, sulfur, manganese, iron, and phosphorus. Although the organisms were not of the same physiological types, each geological niche contained a basic cast of microbial players capable of these nutrient transformations. Such versatility was surprising, and contrary to conventional thinking about soil microbiology, because the deep subsurface was presumably a nutrient-limited environment where the driving force of life—photosynthesis and its products—are not abundant or absent.

The Microbiology of the Deep Subsurface Program has opened new avenues for research into the interactions between the biosphere and the geosphere. The biosphere now appears to extend a substantial distance into the geosphere and only ceases where temperature and pressure become incompatible with life. Recognition of the terrestrial subsurface as a microbiologically active environment applies to a variety of industrial and governmental concerns. It has influenced the planning for fossil fuel discovery, recovery, and storage; deep hazardous waste repositories; and groundwater remediation, storage, and retrieval. This recognition has provided new opportunities to produce and enhance biomedical and biological products and expanded thinking about extraterrestrial life. Knowledge about deep subsurface microbiology is likely to increase understanding of the transport and fate of groundwater contaminants, and it may offer new opportunities for *in situ* bioremediation strategies of deep groundwater and unsaturated vadose zone sediments. Microbial populations in these sediments are more active than had been expected from the scientific literature, and thus are likely to play a significant role in groundwater chemistry and geological processes. Additionally, these investigations have expanded horizons about the habitat of microorganisms and their ability to adapt to the parameters of the habitat and the ability of the microbial populations to alter their habitats. As we better understand the microbial niches around and under us, the sophisticated microorganisms we discover may help solve contamination problems, as well as provide useful products for humanity.

### ***In Situ* Bioremediation Demonstration of the Savannah River Integrated Demonstration Project**

One of those applications of the Microbiology of the Deep Subsurface has been conceived and completed at SRS. The U.S. Department of Energy (DOE), Office of Technology Development, sponsored a full-scale environmental

restoration technology demonstration at the Savannah River Site. The Integrated Demonstration Project, which began in 1989 in the M Area, enjoys national and international recognition for contributions to fundamental and applied research on innovative technologies for characterizing and cleaning soils and groundwater. The Integrated Demonstration has been described as the best bioremediation demonstration ever done. The primary emphasis of the subsurface remediation activities occurred in M Area at SRS, where the subsurface was contaminated by chlorinated solvents from the metal manufacturing facilities. This groundwater contamination resulted from surface spills and discharges from a variety of locations. The chlorinated solvents and degreasers are not unique to the Department of Energy facilities and are the most common soil and groundwater contaminants other than petroleum products. The two main solvents used in M Area were tetrachloroethylene (PCE) and trichloroethylene (TCE), man-made chemicals that do not occur naturally in the environment.

The Integrated Demonstration Program at SRS focuses on cleaning up soils and groundwater contaminated with volatile organic compounds (VOCs). To optimize resources, the project simultaneously evaluated and tested a large number of drilling, monitoring, characterization, and remediation technologies developed by SRS, other DOE sites, national laboratories, industries, and universities. During a single fiscal year (1992) over 44 different technologies were tested and evaluated. The principal remediation technology that this paper discusses is *in situ* bioremediation in conjunction with *in situ* air stripping. *In situ* air stripping was first demonstrated at Savannah River Site using parallel horizontal wells. These wells were placed in the subsurface, one below the water table and another above the water table. The initial *in situ* air stripping demonstration was successful in that it provided excellent characterization and monitoring data, which served as the background for the *in situ* biostimulation. Several collaborators had demonstrated in the laboratory the ability of a

certain kind of bacteria to completely degrade or mineralize chlorinated solvents. These bacteria feed on methane and are naturally found in soils and aquifer material. The Integrated Demonstration Program injected methane mixed with air into the contaminated aquifer through horizontal wells and extracted from the vadose zone via parallel horizontal wells. This configuration has the advantage of simultaneously stimulating methanogenic activity in both the groundwater and vadose zone, and inhibiting spread of the organic plume.

Subsurface soils and groundwater adjacent to an abandoned process sewer line in M Area were found to contain elevated levels of the degreaser and cleaning fluid solvent, TCE (trichloroethylene). It has been estimated that roughly 3.5 million pounds of solvents were discharged to the subsurface in M Area. This area of subsurface and groundwater contamination was the focus of the Integrated Demonstration Program. TCE and PCE were first detected in the groundwater of M Area in 1981 where concentrations of PCE and TCE exceeded the drinking water level of 0.005 mg/L. This contaminant plume extended over an area greater than 1200 acres with a circumference of roughly 5 miles. The M-Area settling basin received solvents from about 1958 to 1979. Between 1958 and 1976, these solvents came from the 321-M facility. After 1976, solvents from Building 313-M were also discharged to the M-Area basin. Of the 2.1 million pounds discharged to the M-Area basin (1.9 million from Building 321-M and 220,000 from Building 313-M), it has been estimated that 84% was PCE, 15% was TCE, and 1% was TCA. Although the disposal of solvents to the M-Area basin was stopped in 1979, the basin continued to receive process effluents until 1985. The basin was certified as closed under RCRA in 1991.

PCE and TCE can be biodegraded or destroyed by naturally occurring microorganisms found in many soils and aquifer materials, including those at SRS (Fliermans et al. 1988). The primary biodegradation pathway for PCE and TCE

involves bacteria that prefer to live in environments with low oxygen concentrations. These bacteria, called facultative anaerobic, prompt the sequential removal of chlorine atoms from the solvent molecules. PCE, with four chlorine atoms, is reduced to TCE and then to a compound with two chlorine atoms (DCE) and DCE is further reduced to vinyl chloride (VC), which has only one chlorine atom per molecule. Once the last chlorine atom is removed from the vinyl chloride, ethane is formed, which is a compound easily mineralized to carbon dioxide and water by a diverse group of bacteria.

These solvents were discharged into the vadose zone beneath M Area. The term "vadose zone" is used to describe the area between the ground surface and the water table. The water table identifies the area where the soil pores are completely filled with water. Above the water table (in the vadose zone) the pores contain both water and soil gases. The vadose zone is important to environmental restoration because essentially all groundwater contamination is derived from liquids initially introduced into the vadose zone.

Solvent contaminants occur in one of four different phases in the subsurface. A portion of the solvents will be attached to the soil particles; a portion will be dissolved in soil water; a portion will be in the vapor phase within the soil air; and a portion may be present as a separate liquid (non-aqueous) phase. Some of these phases can be removed more easily than others. While it is somewhat difficult to directly remove soil water or organic liquids from the vadose zone, it is relatively easy to remove soil vapor. In most situations, the only practical ways to remove these types of contaminants from most soils are the following:

- Physically remove the soils
- Induce chemical reactions that make the contaminants less toxic or less mobile
- Enhance biological reactions that degrade the contaminants or modify the contaminants to form a less toxic or less mobile phase
- Cause the contaminants to evaporate

The first approach, digging up the soils, is practical for relatively shallow contamination, although the excavated soils must generally be treated and replaced. Chemical modifications are difficult and are usually applicable only when soil concentrations are relatively high. The effectiveness of the biological reactions depends upon site-specific conditions but is often effective. The fourth approach, which is often termed soil vapor extraction (SVE), is usually effective for volatile organics compounds and, when combined with biological remediation, is one of the most common method for addressing subsurface contamination by volatile organic compounds such as TCE and PCE. Bioremediation can potentially enhance the performance of *in situ* air stripping as well as offer stand-alone remediation of contaminated sites. *In situ* air stripping is the mechanism where volatile organics are removed from the soil matrix by enhancing their transport from a liquid to a gaseous phase through increased air flow.

One of the ways a subsurface contaminant plume can be accessed is through drilling wells or boreholes. Contaminant plumes along a discharge pipeline, such as occurred in M Area, are generally elongated and elliptical in shape. Straight vertical wells that intercept the contaminant plume have a limited zone of influence, while horizontal wells, installed parallel to the plume, are much more capable of accessing the entire distribution of the contaminant plume. Dual horizontal wells parallel to each other were positioned in the subsurface of M Area in such a way as to be both above and below the contaminant plume thus manipulating the subsurface by enhancing the movement of gasses throughout the plume. The *In Situ* Bioremediation Demonstration was the first program to use horizontal wells for bioremediation. This technology was effectively demonstrated to recover groundwater contaminants for bioreactor conversions from deep or inaccessible areas (e.g., under buildings) and to enhance the distribution of nutrient or microbial additions in an *in situ* bioremediation.

The horizontal wells are the base of the SRS Integrated Demonstration and are advantageous over conventional vertical wells for bioremediation nutrient delivery techniques. The increased surface area of the horizontal wells delivers more nutrients, recovers gas and water easier, and minimizes clogging in geological formation being remediated. The principal nutrient supplied via the horizontal wells in this test was methane in air, at a low concentration of less than 4%. The reason methane was added was because the microorganisms that degrade the chlorinated hydrocarbons are methanotrophic bacteria. That means that as part of their diet they get energy from eating methane as well as the carbon in carbon dioxide and methane. This is done through a group of enzymes called methane monooxygenases. These enzymes are somewhat sloppy in that they have a difficult time telling the difference between methane and TCE or PCE. Thus the strategy was to stimulate these particular bacteria *in situ* by giving them methane, but then forcing them to change their diet to effectively eat the PCE/TCE, and thus strip off the chlorines.

The lower horizontal well was an efficient delivery system for gases throughout the contaminated region. A vacuum was applied to the upper well in the vadose zone to encourage air/methane to move through the upper saturated zone and lower vadose zone, inhibiting spread of the contaminant plume. Air/methane mixtures have been demonstrated to stimulate selected members of the indigenous microbial communities capable of degrading TCE. Extensive characterization of monitoring wells and periodic sediment borings were used to measure the response of the soil and water following injection of air/methane. In addition, offgas from the upper horizontal well was assayed for methane, total volatile organic carbon, TCE, PCE, and potential breakdown of TCE/PCE (e.g., DCE, VC, and carbon dioxide).

Initially, 1% methane with air was injected continuously into the lower well. However to

ensure process optimization (i.e., to further stimulate the indigenous microorganisms to enhance their biodegradation rates and efficiencies), the injection protocol was altered for subsequent campaigns. At 3-month intervals during the 14-month demonstration, the data were examined by an expert panel, and the final test campaign was developed for use:

- Air injection alone for the upper well at 249 scfm
- Air alone injection was added at 200 scfm in the lower well
- Injection with 1% methane/air in the lower well
- Injection with 4% methane/air in the lower well
- Pulsing 4% methane/air in lower well
- Pulsing 4% methane and continuous injection of nitrous oxide at 0.07% in air and triethyl phosphate at 0.007% in air into the lower well

During the test period, the flow and vacuum conditions of the extraction system have remained constant with a flow of 240 scfm and 7.6 inches of mercury, respectively. VOCs in the offgas were composed entirely of TCE and PCE, while the overall VOC concentrations started 10 times higher and declined rapidly over the next 5 days, and stabilized during the 14 months of the demonstration. Comparison of VOCs in pre-and post-test borings support this observation since sediment concentrations decreased by more than 30%. Interim borings at four holes done at the end of the 1% methane injection also reveal a further 50% decline of VOCs in the sediment. Indeed, few of these samples had detectable levels remaining.

Air injection (200 scfm) seemed to have little effect on the extraction efficiency. Methane injection at 1% and 4% had little effect on extraction efficiency of offgas quality, though overall there was a small decline in VOC concentration over time for both operating campaigns. In addition, the ratio of TCE/PCE significantly and consistently declined over time. This observation is consistent with knowledge that methanotrophs will degrade

TCE, and to a lesser extent PCE, and that PCE is degraded at a slower rate by syntrophic anaerobes.

To attempt to optimize the methane-eating bacteria to favor TCE/PCE use, methane was pulsed-injected into the system. Pulsing methane significantly decreased VOC concentration in the extraction well. When the methane was injected again for 5 days after air-alone injection, the VOC concentration increased, but declined again as soon as this pulse was stopped. These observations coincide with the understanding of competitive inhibition (i.e., when high biomass was achieved then the methane is withdrawn, and more contaminants were degraded, since there were more enzyme-active sites available). In addition, it appeared that the long interval pulsing decreased methanotroph density during the first 6 weeks of the pulsing campaign. During the subsequent 6 weeks, the short-interval pulsing increased methanotroph densities. Carbon dioxide concentrations from the extraction well suggest an upward trend beginning 2 to 3 weeks post-air injection start-up. This may indicate increased microbial respiration in the subsurface caused by the air injection.

There was also a striking positive correlation between VOC concentration in vadose zone soil gas and CO<sub>2</sub> concentrations. After VOCs disappeared, the CO<sub>2</sub> concentration subsequently declined. When new VOCs moved into the area, the CO<sub>2</sub> concentrations subsequently increased until after the VOCs have declined again. Since pulsing began, vadose zone concentrations declined significantly and then increased in some wells. Since nitrogen and phosphorus (N&P) injection began, the concentration of VOC in all vadose zone wells declined dramatically, more than 90%. This again supports the theory of competitive inhibition and nutrient limitations discussed above. More than 108,206,345 scf of air were injected during this test. As expected, even though more than 1,392,774 scf of methane were injected into the subsurface during 53 weeks, only trace quantities of methane were detected in the extraction

wells or any of the vadose zone piezometers during the 1% methane injection campaign (i.e., most, if not all, the methane injected was consumed by the TCE-degrading microflora). Simultaneous injection of helium as a conservative tracer has shown that more than 50% of the injected methane is being consumed.

Groundwater monitoring has shown that methanotrophic bacteria increased at the rate of one order of magnitude every 2 weeks since 1% methane injection began. However, increases substantially slowed and began declining slightly. This change coincides with reduction in nitrates in the water off these wells. Several other measures of microbial activity and abundance have shown a similar response to nitrates. After the 4% methane injection started, (8/5/92) methanotroph densities continued to increase. The wells showing the greatest decrease in TCE/PCE concentrations have experienced as much as a five order-of-magnitude increase in methanotrophs. These same wells have also shown increased concentrations of chloride in the water, an aerobic biodegradation end product for TCE. Stimulation of biodegradation activity by the indigenous microflora appears to have been great during the initial phase of the 1% methane injection. After 2 months of the 4% methane/air campaign, it appeared that the methanotrophic population was further stimulated, but the nitrogen-fixing bacteria were inhibited, causing severe nitrogen limitations. However, wells farther away from the injection point showed significant densities of methanotrophs and for the first time the concentrations of TCE/PCE either remained the same or declined slightly. The 4% methane injection appears to have inhibited nitrogen-transforming bacteria; therefore, we began the pulsing campaign, which initially consisted of air injection alone for 5 to 14 days, followed by injecting 1% methane for 4 to 5 days. It was believed this would reduce competitive inhibition of the methane and TCE for the same enzyme and reduce the inhibition of nitrogen fixers shown to be stimulated by air injection alone.

Pulsing caused significant increase in nitrogen-transforming bacteria, a decrease in TCE in well water as well as the vadose zone, and a decrease in methanotroph densities. On December 11, 1993, the short-pulse interval campaign of 8 hr of 4% methane every other day was begun. The final campaign (January 25, 1993) included pulsed injection of methane and continuous injection of nitrous oxide at 0.07% in air and triethyl phosphate at 0.007% in air. The decision was based on enrichment and mineralization studies. It was felt that this last injection would overcome both N&P limitations and allow higher biomass and higher degradation rates of TCE achieved by the methane-stimulated subsurface bacteria. Since the N&P injections, the densities of the methanotrophic bacteria in the water have increased while the TCE concentrations in the vadose zone and water has declined.

The vapor extraction systems at M Area have been effective in terms of the amount of contaminant that has been recovered from the subsurface and the new methodology to allow such transformations. Operating costs indicate that bioremediation used in the *In Situ* Bioremediation Demonstration is far more cost effective (less than 50%) of the cost to remove a pound of contaminants via the groundwater extraction system. However, the total amount of contaminant that has been removed by the vapor extraction system is still a small fraction of the amount that was likely discharged to the subsurface. While the total amount removed via vapor extraction system (roughly 250,000 pounds) is less than 10% of the estimated amount that was discharged to the subsurface, the *In Situ* Bioremediation technology significantly enhanced the tools available to remediate deep subsurface sediments.

These investigations have demonstrated that the gaseous nutrient injections stimulated indigenous soil bacteria to degrade TCE and PCE without risk of forming potentially harmful daughter products or biofouling the remediation wells. One begins these investiga-

tions with a hypothesis that requires the collection of data and advances the collection of data about what microorganisms are present in a particular habitat (i.e., methanotrophs). The use of such data allows one to obtain meaningful information as to what these organism can do under real world conditions to address a particular problem. The next step in moving from data to the acquisition of wisdom is how to use the information to obtain knowledge. Once the information on the effectiveness of methanotrophs had been established, this information was linked with data and information from other disciplines (geology and engineering) to advance information into the application of that information towards knowledge. The application of that knowledge needs to be done with insight that provides a little wisdom in solving the problem of subsurface bioremediation. This project demonstrated not only the feasibility but also the effectiveness of *in situ* bioremediation of groundwater and sediments contaminated with chlorinated solvents. Indigenous microorganisms were stimulated to degrade TCE, PCE and their daughter products *in situ* by adding selective nutrients to the contaminated zone. *In situ* biodegradation is a highly attractive technology for remediation because contaminants are destroyed, not simply moved to another location or immobilized. Such technology decreases costs, risks, and time while increasing efficiency and public and regulatory acceptability. Bioremediation has been found to be among the least costly technologies in applicable situations.

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## Biography

Carl B. Fliermans is a microbial ecologist with a doctorate in limnology and microbial ecology from Indiana University. Following postdoctoral work as a National Institutes of Health Fellow at University of Minnesota, Mr. Fliermans joined Savannah River Site in 1974 and established a microbial laboratory designed to address and solve environmental problems from both a basic research and applied perspectives.

Techniques developed during Mr. Fliermans' tenure at SRS has allowed his laboratory to discover the ecological niche of Legionnaires' Disease Bacterium and provide ecological research instrumental in the identification and control of the bacterium.