26 Case Study: Full Scale In Situ Bioremediation Demonstration of the Savannah River Site Integrated Demonstration Project[1]

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26–1 INTRODUCTION

Bioremediation has proven to be one of the most cost effective and environmentally sound remediation technologies available at sites where it will work. The Savannah River Site recently completed an Integrated Demonstration on "Clean-up of Soils and Groundwater Contaminated with Chlorinated VOCs." More than 20 laboratories, several companies, and several government agencies were involved in the planning, execution, and evaluation of this demonstration. The demonstration showed how gaseous nutrients (methane, nitrous oxide, and triethyl-phosphate) could be injected into an aquifer via a horizontal well to stimulate indigenous bacteria (methanotrophs) to degrade trichloroethylene and other microbes to reduce tetrachloroethylene to trichloroethylene. Sediment, water, and soil gas samples were taken before, during, and after the demonstration. Indeed, >90 measurements were done on >2000 sediment samples, 173 different analyses of >1000 groundwater samples, and >30 different measurements of >3000 soil gas samples. The 14-month demonstration showed how nucleic acid probes, fluorescent antibodies, and phospholipid fatty acid analyses could be used to directly characterize and monitor bioremediation in the sediment and groundwater. A number of other assays were cross compared with varying degrees of success. The direct functional group assays were extremely effective at showing quickly who was present, how important they were to the remediation, and how happy they were. Evaluations and modeling by several laboratories showed that this aerobic methane stimulation in situ bioremediation process was at least 40% more effective than any physical stripping process (also tested at this site), and five times more effective than any pump and treat process. The process removed 78% of all of the trichloroethylene (TCE) and tetrachloroethylene (PCE) present during the demonstration, with initial concentrations higher than 1000 ppb and final concentrations in the most effected areas reaching less than 2 ppb.

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26–2 INTRODUCTION

26–2.1 Demonstration Objectives

The principal objective was to demonstrate the utility of in situ methanotrophic bioremediation for cleanup of nonarid waste sites contaminated with chlorinated solvents. The ancillary objectives were to (i) establish the optimal conditions for complete biodegradation of chlorinated solvents by in situ nutrient stimulation of microorganisms, (ii) demonstrate the utility of horizontal wells as a nutrient delivery technique for in situ bioremediation, (iii) demonstrate the utility of biomolecular probes (nucleic acids, fluorescent antibodies, and enzymes) and other direct analysis assays for characterization, monitoring, and controlling the biological aspects of an in situ bioremediation, and (iv) to establish, via process optimization studies (bioreactors) compared with in situ data, an explanatory and deterministic environmental model of the in situ methanotrophic bioremediation process. Chlorinated solvents are the most common organic contaminants in groundwater in the USA (Craun, 1986) and the U.S. DOE complex. Due to their small size and chlorination they are extremely recalcitrant, yet they must be treated to <5 ppb in drinking water. At DOE sites chlorinated solvent plumes from disposal areas are as large as 20 sq miles and as deep as 1000 ft and growing. It is thus with great urgency that DOE searches for better, faster, and cheaper technologies to remediate these contaminants (U.S. DOE, 1989).

26–2.2 Brief Background of Technology and Site

For longer and more detailed descriptions of the technology and the site see the following references: Hazen (1991), Looney et al. (1991), Eddy et al. (1991), Eddy Dilek et al. (1993), Wilson and Wilson (1985), Wackett et al. (1989), and Semprini et al. (1988).

Methanotrophs, methane-oxidizing bacteria, oxidize methane via a series of enzymes that are unique to this group. The primary enzyme in this oxidation chain is methane monooxygenase. Methane monooxygenase is an extremely powerful oxidizer, thus giving it the capability of oxidizing a wide variety of normally recalcitrant compounds including TCE. Wackett (Newman & Wackett, 1991; Tsien et al., 1989) and others (Chaudhry & Chapalamadugu, 1991; Wilson & Wilson, 1985; Fogel et al., 1986; Little et al., 1988) have shown that the soluble methane monooxygenase induces formation of TCE-epoxide from TCE. TCE-epoxide is extremely unstable and therefore spontaneously breaks down to simpler compounds like formate, and others. All of the daughter compounds are either unstable or small and easily metabolizable compounds, thus making the final and almost immediate end products of TCE-epoxide formation, CO_2, and chloride salts.

Although development of methanotrophic bioreactors for TCE bioremediation is progressing well, in situ biodegradation of TCE is an emerging technology that has not yet been demonstrated at a practical scale. Tests on a small area of a shallow aquifer at the Moffett Naval Air Station in California (Semprini et al., 1988) have shown that indigenous microorganisms can be stimulated with methane and O_2 to degrade TCE. Their results were very encouraging. Their

experiences in these studies are a large part of the basis for the process design for this in situ demonstration at the Savannah River Site.

The Savannah River Site is a 320 square mile facility owned by the U.S. Department of Energy and operated under contract DE-AC09-89R180035 by the Westinghouse Savannah River Company. The site is near Aiken, SC (Fig. 26–1) and has been operated as a nuclear production facility for USDOE since 1950. The 300 M operations area of Savannah River Site were used to fabricate fuel and target elements that were later irradiated in Savannah River Site reactors (Fig. 26–1). During these operations the elements are degreased at several stages in the process. These degreasing operations generated large amounts of metal-degreasing solvent wastes. From 1952 to 1982, M Area used an estimated 13 million pounds of chlorinated degreasing solvents (Marine & Bledsoe, 1984). Evaporation alone accounted for 50 to 95% loss, while the remainder went to the M Area process sewer system. Marine and Bledsoe (1984) estimate that as much as 2 million pounds may have been released to the sewer that leads to the M Area Settling Basin; another 1.5 million pounds went directly to the A-14 outfall at Tims Branch. The discharges to the M Area Settling Basin consisted primarily of trichloroethylene (TCE: 317 000 lb.), tetrachloroethylene (PCE: 1 800 000 lb.), and 1,1,1-trichloroethane (TCA: 19 000 lb.; Marine & Bledsoe, 1984). The solvents discharged into the settling basin spread through the vadose zone and entered the groundwater below the basin. The leaking process sewer line used to

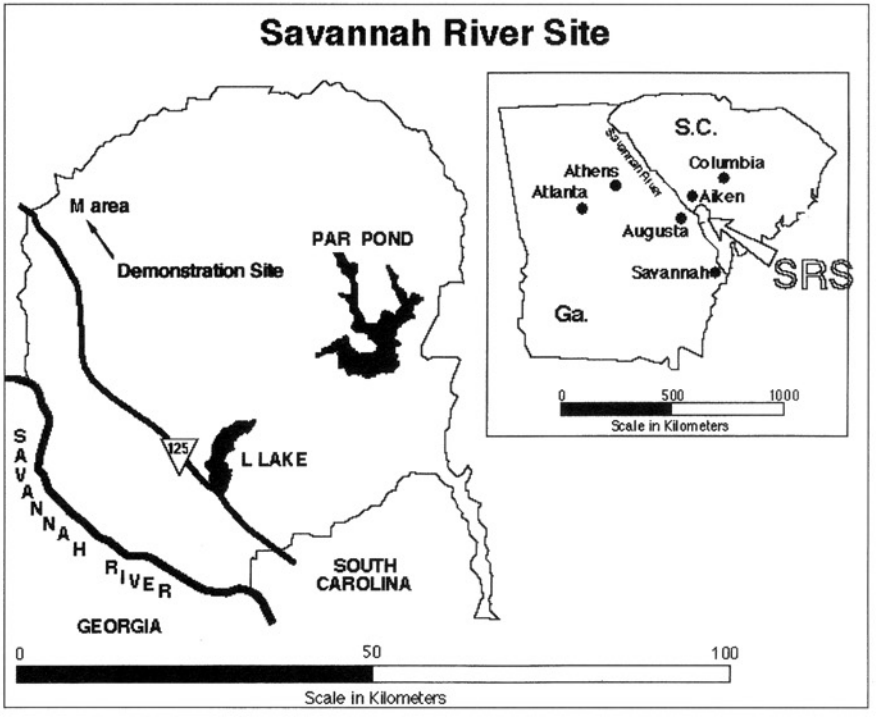

Fig. 26–1. Map of Savannah River Site showing demonstration site location.

convey these wastes to the basin also released large quantities of the solvents into the surrounding vadose zone sediments. A conventional groundwater extraction and treatment system (air stripper) has been in operation since 1984. For detailed descriptions of discharges from the area see Marine and Bledsoe (1984), Christensen and Brendell (1982), and Pickett (1985).

The residual solvents in the vadose zone associated with the abandoned process sewer line and the settling basin continue to leach into the groundwater covering more than 1 square mile. Since the plume caused by the leaking process sewer line was linear, horizontal wells were selected as the injection and extraction system that would best remediate the site. The horizontal wells were installed in 1988 (Kaback et al., 1989) and the area has been extensively characterized in terms of its hydrology, geology, and ecology. For a complete characterization of the site see Eddy et al. (1991). From July 1990 to December 1990 the site was used to demonstrate in situ air stripping via the horizontal wells; for a complete description of the test see Looney et al. (1991).

26–2.3 Test Plan, Field Operations, Operating Campaigns

26–2.3.1 Test Plan

The test plan for the field demonstration was compiled after several meetings of the Bioremediation Technical Support Group (BTSG; Expert Panel). The BTSG started meeting in May of 1990. A draft test plan was prepared from the meeting of 9 Nov. 1990. During the 1 Oct 1991 meeting the final draft of the test plan was approved with a prioritized list of analyses. The test plan document was issued as a draft 18 Sept. 1991; final revision no. 3 was issued on 23 Apr. 1992 (WSRC-RD-91-23, see Hazen, 1991). For details of methods and procedures, permits, licensees, patents, QA/QC, funding, and operational management the test plan should be consulted. The operating campaigns are given in Table 26–1.

26–2.3.2 Field Operations

The Gas Research Institute supplied all methane for the project via their contractors: Radian Corporation, Eaves Gas Company, and South Carolina Electric and Gas Company. Since no natural gas was available at the site, this included fabrication of two compressed natural gas tanker trailers and a compres-

Table 26–1. Operating campaigns during the demonstration.

Injection operations	Start date	End date
1. No air injection (air extraction only)	02/26/92	03/18/92
2. Air injection	03/18/92	04/20/92
3. 1% methane–air	04/20/92	08/05/92
4. 4% methane–air	08/05/92	10/23/92
5. Pulsed methane–air	10/23/92	01/25/93
Long intervals (5–14 d air/5 d 1% methane)	10/23/92	12/20/92
Short intervals (36 h air/8 h 4% methane)	12/20/92	01/25/93
6. Pulsed 4% methane (short intervals), continuous 0.07% nitrous oxide and 0.07% triethyl phosphate	01/25/93	04/30/93

sor station. Each trailer had a capacity of 12 500 standard cubic feet (SCF) and thus could supply the gas needs for more than 48 h even at the highest injection rates used (4% in air). The subcontract for field operations, which included leasing of equipment, equipment maintenance and operation, and off-gas and soil gas analyses, was awarded via the federal competitive bid process to ECOVA on November 1991 (see Appendix C of the Test Plan for the detailed scope of work). ECOVA procured the system and began mobilization 26 Jan. 1992. Mobilization was complete, the equipment operational 2 wk later. Operation of the equipment began at 10:15 a.m. on 26 Feb. 1992 after receiving final approval for air emissions from the South Carolina Department of Health and Environmental Control on 24 Feb. 1992 (Fig. 26–2). The demonstration was completed 7:15 p.m. 30 Apr. 1993. The project began a phased startup to provide optimal conditions for measuring the effects of air extraction, air injection, and air-methane, and multiple nutrient injection on the subsurface environment. Air was extracted constantly from the upper, vadose zone, horizontal well, AMH-2 at 240 SCFM (range 239–246, average 245 SCFM). The extracted air was treated via an electrically heated catalytic oxidation system (halo hydrocarbon degradation catalyst, HDC, from Allied Signal, Colonial Heights, VA). Rigorous sampling of both the influent gas to the catalytic oxidation system and the effluent gas revealed that the off-gas treatment system destroyed >94% of the total VOCs. Average daily emissions were <1.9 lbs total VOCs. (Note: After modification of the CatOx system 25 Jan. 1993, by adding more catalyst the operating temperature could be lowered. This temperature reduction resulted in a 41% decrease in energy consumption. At this reduced rate of energy demand the additional cost of the catalyst was recovered in <3 mo. More than 108 206 345 scf of air and 1 392 774 scf of methane were injected during the demonstration.

Air injection was begun 18 Mar. 1992, at 200 SCFM (range 195–230, average 204 SCFM) via the aquifer horizontal well AMH-1. The system operated 9206 h of 10 303 h possible, removing 12 096 lbs VOC, destroying >11 370 lbs VOC, and emitting <726 lbs VOC. The system was down for maintenance, testing, repairs, and experiments for 1097 hours. Of this down time 73 h was due to a control board failure, a single occurrence in the first 7 d and 80 h due to a lightening strike that damaged several electrical components of the system. The system was shutdown for 58 h beginning 31 July 1992 for a special series of experiments and again on 19 Jan. 1993 for 100 h. The system was down 72 h in September for electrical system repairs due to failing circuit boards. The system was down for 89 h due to regulator freezing and minor CatOx repairs. The system also was down 72 h the week of 19 Jan. 1993 for modifications to the CatOx. All remaining down time was due to required maintenance of the system (e.g., changing oil on compressors) and 250 h required because of site scheduled power outages for M area.

26–2.3.3 Pre-Test

The sediment post-test characterization for the in situ air stripping demonstration was also used to provide the most recent pre-test characterization data for the geology, hydrology, and biology data for the in situ bioremediation demonstration. Sampling for the in situ air stripping post-test–in situ bioremediation pre-test characterization began in March 1991 and was completed in June 1991.

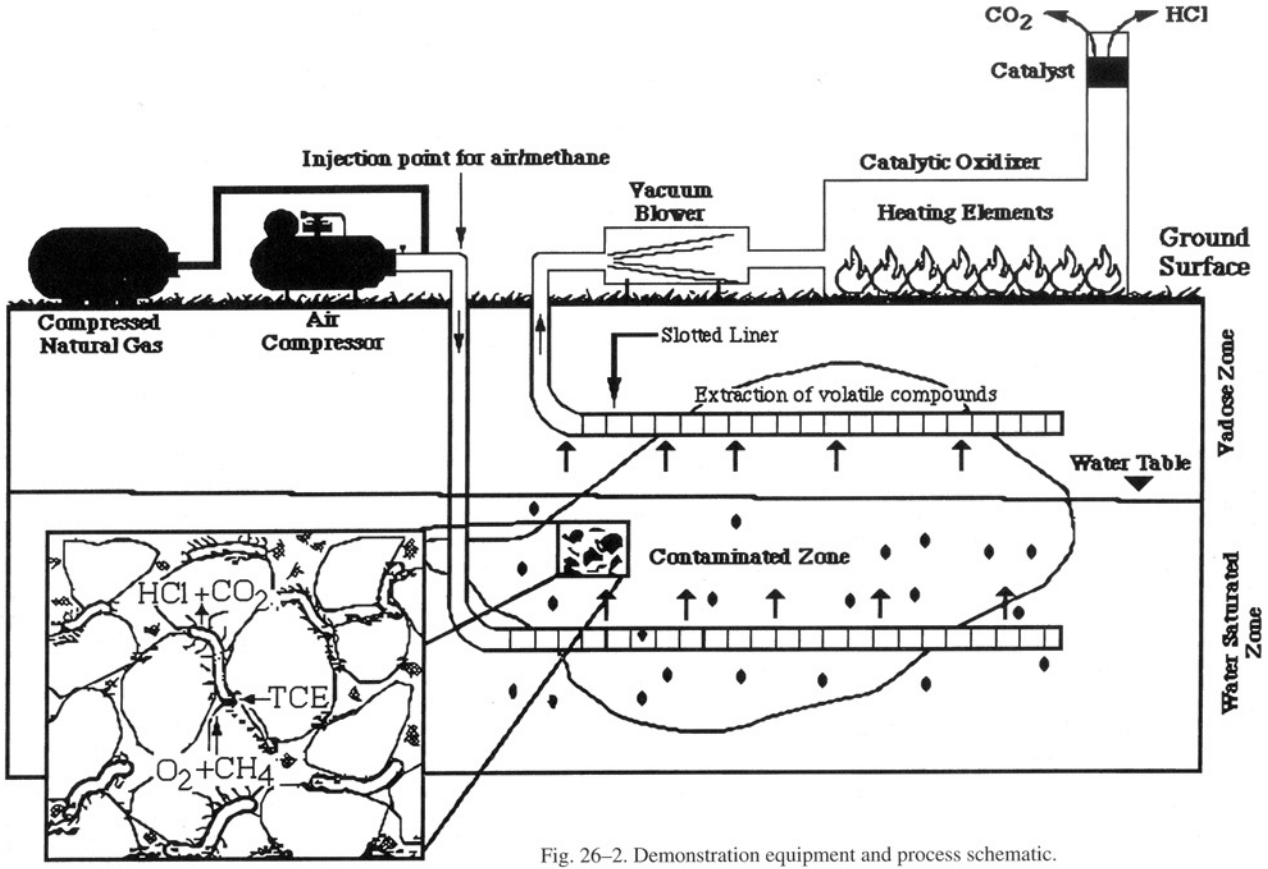

Fig. 26–2. Demonstration equipment and process schematic.

Eight additional boreholes (MHT-10B, MHT-9B, MHT-11C, MHT-5V, MHT-7T, MHT-3T, MHT-1V, MHT-2T) were drilled and sediment samples taken as described above in Section 4.2 of the test plan. Data analysis for these samples is complete and is reported in Eddy Dilek et al. (1993). This work established that methanotrophs were present in the subsurface and that the microbial community in the sediment was under nutrient stress as measured by PLFA analyses.

In situ bioremediation pre-test monitoring from groundwater began 15 Aug.1991. Water samples were collected using dedicated submersible pumps according to documented SRS well sampling protocols (WSRC 3Q5). Based upon previous sampling (Looney et al., 1991) the following wells were sampled every 2 wk for the duration of the project: MHT-1C, MHT-2C, MHT-3C, MHT-4C, MHT-5C, MHT-6C, MHT-7C, MHT-8C, MHT-9C, MHT-11C, MHT-9B, and MHT-10B. All sampling and analysis are as described in Section 4.3.3 of the test plan (Hazen, 1991). Pre-test monitoring of groundwater continued until 20 Apr. 1992 and established, extraction and air injection only baseline necessary for the test. All data analysis for pre-test groundwater monitoring indicated that the subsurface microbial community was (i) under nutrient stress, (ii) had the capability to degrade TCE/PCE, and (iii) had low numbers of methanotrophs. Thus maximal stimulation potential existed prior to methane injection.

26–2.3.4 Operating Campaigns

The BTSG agreed that 3-mo operating campaigns were needed in order to see significant effects (Table 26–1). At the end of each operating campaign sediment samples were taken, in order to better understand effects in the subsurface that may be missed by groundwater sampling alone, e.g., epilithic community changes. During all campaigns the injection rate and the extraction rate of all gases was the same, i.e., 200 scfm injection, 240 scfm extraction. This was done to minimize the effect that rates of injection-extraction were having when the injection composition was changed. It was felt that only by varying one parameter at a time could we best understand the effect that it was having on the subsurface. The strategy for the next operating campaign was dependent upon the recommendation of the BTSG after evaluation of the data from the current and previous campaigns.

26–2.3.4.1 Extraction Only and Extraction with Air Injection. From 26 Feb. 1992 to 18 Mar. 1992 air extraction alone from the upper, vadose zone, horizontal well, AMH-2. Air was extracted constantly at 240 SCFM (range 239–246, average 245 SCFM). On 18 Mar. 1992, we began injection of air at 200 SCFM (range 195–230, average 204 SCFM) via the aquifer horizontal well AMH-1. Increases in total bacteria density and methylotrophs were observed almost immediately after air injection started. The VOC water concentrations in the monitoring well samples remained the same or increased slightly in all 12 wells sampled from 26 Feb. 1992 to 18 Mar. 1992, i.e., air extraction alone. Six of the 12 wells decreased slightly from 18 Mar. 1992 to 20 Apr. 1992, i.e., air extraction and air injection.

26–2.3.4.2 One Percent Methane Injection. Methane injection at 1% began 20 Apr. 1992. The VOC concentration in 8 of the 12 wells decreased from 20 Apr. 1992 to 5 Aug. 1992. During this period, four of the wells in the zone

most affected decreased by 50 to 99% during the first 4 wk following methane injection. Methanotrophs also immediately began increasing after methane injection was started. All five vadose zone piezometers in the zone of influence showed decreases in soil gas concentrations. At the beginning of July methanotroph growth stopped and densities began declining in response to N limitations. Sediment analyses from the end of this campaign indicated increases in methanotrophs in the sediment closest to the injection well and significant decreases in the TCE/PCE concentration in the sediment. Several other measures of microbial activity and abundance also increased dramatically, concomitantly with the start of methane injection and decreased as nitrates decreased. Stimulation of biodegradation activity by the indigenous microflora appears to stimulated dramatically during the initial injection but decreased as available N was used up.

26–2.3.4.3 4% Methane Injection. On 5 Aug. 1992 the second quarter operating campaign began with injection of 4% methane-air. Helium was injected into AMH-1 for 15 wk. Helium was not detected for >24 h, but after 15 wk was detected where methane was not. The conservative nature of the helium suggests that as much as 50% of the methane is being consumed. This is complemented by the observation that CO_2 concentrations increased in these same soil gas samples, a further indication of increased biological activity. Because of the poor effect that 4% methane had on the stimulation, the BTSG decided to eliminate the sediment sampling at the end of this campaign. It was felt that the great expense of this activity could not be justified in terms of the results seen in the groundwater during this campaign. The BTSG elected to immediately begin pulsing the methane in a long pulse interval, dictated by getting to extraction well concentrations that were below 0.5% or several days of air only, whichever came first.

26–2.3.4.4 Pulsed Methane Injection. On 23 October, the third quarter operating campaign began with pulsing of methane-air and initially consisted of air injection alone for 5 to 14 d, followed by injection of 1% methane for 4 to 5 d. It was believed this would reduce competitive inhibition of the methane and TCE for the same enzyme and reduce the inhibition of N fixers shown to be stimulated by air injection alone. After pulsing began, N transforming bacteria increased as the concentration of nitrates stopped decreasing; however, nitrate concentrations were still well below original concentrations. Densities of methanotrophs declined rapidly during the long pulse intervals used during the first part of the pulsing campaign. The BTSG met on 10 Dec. 1992, and given the rapid decline in methanotrophs, observed during the long pulse interval, elected to immediately go to a short pulse interval of 8 to 12 h of 4% methane followed by 36 h of air only. When a shorter pulse interval was used during the last part of the pulsing campaign the densities began to increase again. Given the seeming effect of N and P limitation on the microbial community the BTSG elected to inject both N and P for the final test. The form to be injected was based upon enrichment-mineralization assays on sediment and water samples from the site. Two laboratories ran separate assays using combinations of inorganic phosphate, ammonia, organic phosphate, and nitrous oxide with methane. Based upon these analyses the BTSG elected to use a combination of triethyl phosphate and nitrous oxide injected continuously at low concentrations with short pulse periods of 4% methane.

26–2.3.4.5 Pulsed Methane Injection with Nitrogen and Phosphorus Injection. On 25 January 1993 the fourth and final quarter operating campaign began with the pulsing of 4% methane-air and the continuous addition of 0.07% nitrous oxide-air and 0.007% triethyl phosphate-air. After the N and P injection started the VOC concentration in the groundwater declined a little more rapidly than it had during the previous campaign. Methanotroph densities increased by one to two orders of magnitude during this campaign. The most dramatic change, however, was seen in the mineralization rate. Nearly all water samples showed >90% mineralization of both TCE and PCE by methanotrophs immediately after multiple nutrient injection began.

26–2.3.5 Post-Test

Post-test sediment characterization began 30 Apr. 1993 and was complete 5 May 1993. Post-test groundwater monitoring continued until 25 Sept. 1993. Methane was detectable in the groundwater for 2 mo after methane injection stopped. Methanotroph densities declined very slowly after methane injection stopped, and after 5 mo were still two to three orders of magnitude greater than pre-test densities.

26–3 SYSTEM PERFORMANCE

26–3.1 Criteria for Success-Performance Measures

As decided upon by the Bioremediation Technical Support Group (Expert Panel) and stated in the beginning of the Test Plan (Hazen, 1991), there are four primary criteria by which the overall success of this demonstration will be evaluated:

1. **Biostimulation-Biodegradation**
   Evidence of biological destruction (biodegradation) of TCE from the contaminated soils and water. Since a major advantage of bioremediation is destruction, it is important and significant to demonstrate that biodegradation is occurring. The evidence is expected to come primarily from comparison of the compositions of the off-gases before and after addition of methane to stimulate biodegradation, and from laboratory studies in soil columns using soil cores from the site. In the latter case we expect to show that radiolabeled TCE is degraded under conditions similar to those in the field.
2. **Bioremediation**
   Increased reductions of TCE in soil and water samples from the site during periods of biostimulation. The technology is expected to accelerate the removal of TCE over in situ air stripping alone, which is the focus of the first phase of the integrated demonstration.
3. **Cost Effectiveness**
   Reduced cost over comparable conventional technologies. Comparison of costs of air stripping currently in use at the site and cost of in situ air stripping from the first demonstration. Costs of air stripping, in situ air stripping operations, and the bioremediation can be compared with rates

of removal and/or degradation to arrive at normalized costs for all three processes for the same site.

4. **Ease of Use and Operation**
   Relatively simple and trouble-free operation. These characteristics contribute to favorable economics. A critical assumption for the successful demonstration is that gases can be successfully injected via the lower horizontal well and recovered via the upper well. This ability has been demonstrated in Phase 1 of the integrated demonstration project. The wealth of data from in situ air stripping demonstration can be compared and used as a control for the bioremediation project.

26–3.2 Biostimulation

Densities of methanotrophs, the functional group that the process was trying to stimulate, increased in the groundwater by as much as seven orders of magnitude. Representative data is given in well MHT-2C for groundwater close to the injection source, while MHT-9C represents data for groundwater at the most distal end of the injection well (Fig. 26–3). Due to the skewed orientation of the horizontal wells, see Fig. 26–2, well MHT-9C is not influenced by the extraction process, only the injection process. Biostimulation occurred first in the wells that were closest to the injection point and later moved farther and farther away (Fig. 26–3). During the previous in situ air stripping demonstration and during the air injection only phases, concentrations of TCE in MHT-9C actually increased; however as the methanotroph density increased in this well during the methane injection campaigns, for the first time we recorded a significant decrease in TCE concentration. Densities of methanotrophs in the sediment closest to the injection well increased from rarely detectable to more than a million cells per gram of dry weight. The methanotroph enumerations were done by three different laboratories using three different methods and all obtained nearly identical results. In addition, increases in methanotroph densities were only observed after methane injection started. Overall bacterial density as indicated by Acridine Orange Direct Counts (AODC) increased by only two orders of magnitude, while viable counts (1% PTYG) increased by two to four orders of magnitude. All increases in all of the enumeration parameters in both the sediment and groundwater were directly associated with methane-air injection, with the greatest increases occurring first at collection sites closest to the injection well. Other microbial community components increased only slightly or not at all, e.g., fungi, actinomycetes, and protozoa.

Phospholipid fatty acid (PLFA) analyses also indicated biostimulation of methanotrophs and that methanotrophs were being stimulated to become the dominant population in the total microbial community. Phospholipid fatty acid analyses provide a direct measurement of cell wall components that are found in all microorganisms and specific markers for certain functional groups, e.g., Methanotrophs. Thus this method shows by direct analyses of the water and the sediment the total biomass present and the amount of that biomass that is a specific group like the methanotrophs. These assays provide a more direct assay of the microbial community and verify that biomass increased in the groundwater and that the major portion of the biomass increase was due to increases in

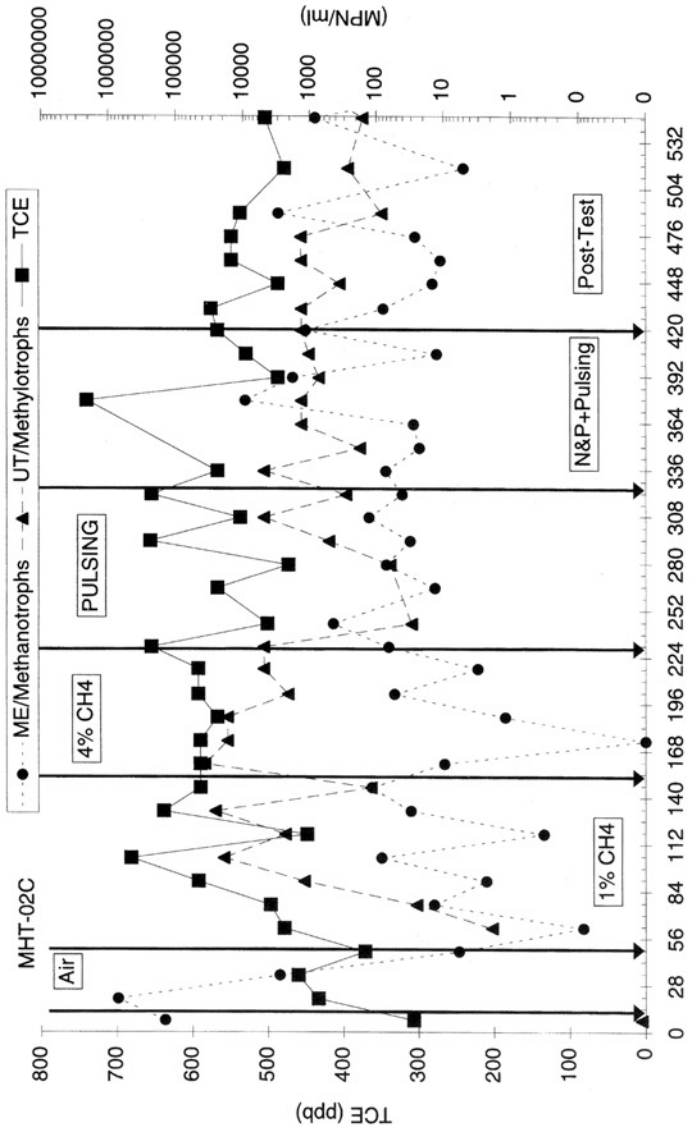

Fig. 26–3. Densities of methanotrophs and methylotrophs vs. trichloroethylene concentration in Well MHT-2C and MHT-9C over time.

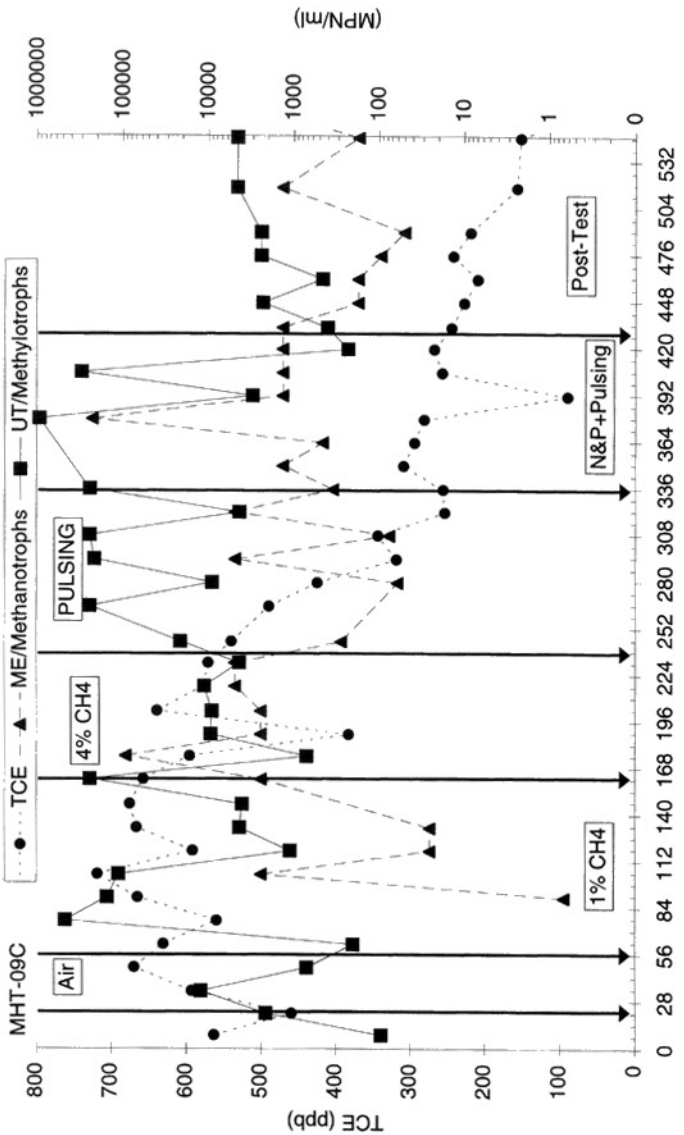

Fig. 26–3. Continued.

methanotroph biomass. This has important implications, since it shows that the methane stimulation strategy is specific to methanotrophs, the TCE-degraders. The PLFA analyses have demonstrated their utility in the direct determination of microbial composition and biomass of functional groups of concern, e.g., degraders, especially relative to the total biomass. The PLFA analyses could become an important characterization and monitoring tool for bioremediation.

Nucleic acid probe analyses by two different laboratories also showed very specifically that methanotrophs were being stimulated in the sediment. For more complete results and discussion see Bowman et al. (1993). Brockman et al. (1993) showed that sediment samples taken after stimulation had greater concentrations and frequency of detection of probes to soluble methane monooxygenase (MMO), methanol dehydrogenase (MDH), toluene monooxygenase (TMO), and dehalogenase (DH). One probe measured showed no change, toluene diooxygenase (TDO). Thus those enzyme systems that might be induced to produce more of the gene products capable for TCE-oxidation, were. Well water isolates analyzed with nucleic acid probes demonstrated that viable populations of methanotrophs able to produce soluble MMO and degrade TCE are extensively distributed throughout the M area aquifer and also suggest that sMMO can be expressed in the M Area groundwater and other groundwaters (Bowman et al., 1993). Samples from pristine and TCE contaminated areas in Tennessee showed that the SRS, M Area samples had a higher average sMMO-specific activity than either of the other sites (Bowman et al., 1993). The nucleic acid probe tests have demonstrated that nucleic acid probes can indicate the presence and relative concentrations of microbes with enzymes that specifically degrade the contaminants of concern. This method allows for the direct monitoring of functional groups, a potentially much better way to characterize and monitor and control in situ bioremediation. Culture and direct staining assays have had the extreme drawback of not indicating the microorganisms of interest and/or those that the process is trying to stimulate or control.

Biostimulation was indirectly shown by the depletion of nitrate (a limiting nutrient) in the groundwater as stimulation continued. Biostimulation also was indirectly indicated by the increase in CO_2 observed in the extraction air after injection was started and by the consumption of methane (50%) calculated via measurements of methane and helium tracer in injection well and extraction well. The evidence for biostimulation is unequivocal. Pulsing and multiple nutrient were found to give the greatest biostimulation. The continuous 4% methane injection was not as stimulatory as continuous 1% methane injection or pulsing of 4% methane.

26–3.3 Biodegradation

Evidence for increased biodegradation was demonstrated by increases in TCE and PCE mineralization potential, by measurements of nucleic acid probes, and by correlations between contaminants, degraders, and daughter products. The nucleic acid probe analyses demonstrated that the methanotrophs being stimulated were those possessing soluble methane monooxygenase (sMMO), the form of MMO most active in TCE oxidation. Specific sMMO activity also was shown to be higher than a pristine site and a TCE contaminated site in Tennessee

(Bowman et al., 1993). Methanotroph isolates from the water that were positive for sMMO were tested for their ability to oxidize both TCE and naphthalene and mineralize TCE (Bowman et al., 1993). Mineralization of TCE and PCE was always detectable in both groundwater and sediments; however, rates declined slowly with each successive operating campaign, until multiple nutrient injection started. Immediately after beginning multiple nutrient injection the mineralization rate went to >90% for both TCE and PCE. These results especially when combined with the history-matching numerical simulation model (Travis & Rosenberg, 1994) are strongly suggestive that native nutrient depletion can have a strong effect on the rates of contaminant degradation by methanotrophs. Enzien et al. (1994) also showed with sediment column simulations of the M area demonstration site that even though PCE is not directly oxidized by methanotrophs, significant amounts of reduction dechlorination (anaerobic process) can occur in the sediment, even in a bulk aerobic environment. This explains field data showing mineralization of PCE and reduction of PCE in the groundwater and sediment that was related to biostimulation operating campaigns. Those isolates from wells most affected by the injection process were shown to have rates of TCE oxidation that were more than two times greater than the rates for Methylosinus trichosporium OB3b, the type culture for methanotrophs and supposed best TCE oxidizer (Tsien et al., 1989).

Indirect measures of biodegradation were indicated by the strong inverse correlations between well water densities of methanotrophs and TCE concentrations. Two laboratories measuring methanotrophs by different MPN methods showed the same trend, e.g., SRTC $r = -0.398$ ($P < 0.0001$) and UT $r = -0.385$ ($P < 0.0001$). Thus as the methanotroph densities increased in the well water samples the concentration of TCE decreased concomitantly. Given our knowledge that methanotrophs cannot oxidize PCE, and that PCE reductive dechlorination can occur in the background, albeit slowly, we would expect that the correlations between methanotrophs and PCE would be weak or insignificant. Indeed, correlations between the SRTC methanotroph MPN and PCE were –0.096 ($P > 0.05$) while the UT methanotroph MPN vs. PCE correlation was –0.170 ($P < 0.01$). Though these environmental data correlation analyses are inconclusive, as to cause and effect, they do suggest that the reductions of TCE in the environment could have been caused by methanotrophs.

Accumulation of anaerobic daughter products were not observed in the soil gas, water, or sediment at any time during the demonstration. This suggests that an aerobic methanotrophic mechanism of biodegradation was dominant. The formation of epoxides (oxidation) by methanotrophs has a rate of VC>DCE>TCE (Wackett et al., 1989), while the reductive dechlorination has an opposite rate of PCE>TCE>DCE. Thus if an aerobic methanotrophic process of biodegradation was dominant, the accumulation of VC and DCE would be practically impossible, since the epoxide formation would favor their disappearance first. Indeed, epoxide formation is not a stepwise reductive dechlorination, so the formation of DCE and VC never occurs when TCE is being oxidized, unlike the reductive dechlorination process.

Helium tracer tests also indicated biodegradation by the loss of methane. By calculating the amount of methane that should have been observed in the injection well using the helium breakthrough curves we can see that much of the

methane being injected was being consumed-adsorbed in the subsurface. More than 50% of the methane being injected was being removed before it reached the extraction well. Combined with increases in methanotrophs and CO_2 concentrations in the soil gas and extraction well gas, this is strongly suggestive of significant microbial community biodegradation of methane and fortuitously TCE.

Using sediment and groundwater from the demonstration site, Andrews and Hansen (1994) used a differential soil bioreactor (DSBR) to simulate processes that might occur during the demonstration. They showed that the combination of low flow rates and high numbers of methanotrophic bacteria densities cause all of the methane fed into the DSBR to be consumed. Densities of methanotrophs in the DSBR increased very rapidly after the addition of methane. Two control experiments where no methane was added showed no degradation of TCE, while two experiments where methane was added showed TCE biodegradation. The rate of TCE degradation observed in these experiments was 28 mg d^{-1} m^{-3}. The DSBR simulations indicated that N and P is very important to degradation rates (as observed with the field measurements of mineralization rates), but not necessarily to the numbers of methanotrophs. These experiments also suggested that there may be an optimum dissolved methane concentration by TCE degradation by methanotrophs. This also was indicated by the apparent detrimental effect of the continuous 4% methane injection campaign on the methanotrophs.

Water analyses also indicted a strong inverse correlation ($r = -0.321$, $P < 0.00001$) between TCE concentration and chloride concentration in the water. Thus as TCE concentration declined in the groundwater, the chloride concentration increased. The only mechanism known that could result in this correlation is the biodegradation of TCE to CO_2 and chloride. Though much of the evidence for biodegradation of TCE-PCE is indirect, this last evidence for biodegradation is convincing and very direct.

26–3.4 Bioremediation

The evidence for bioremediation is linked by necessity to changes in TCE-PCE inventories in the soil gas, sediment, and groundwater, and the evidence for biodegradation and biostimulation discussed above. Concentrations of TCE and PCE declined in all well samples coincident with the onset of injection (Fig. 26–3). Water concentrations of TCE-PCE decreased by as much as 95%, reaching concentrations below detectable limits, i.e., <2 ppb. Those wells closest to the injection well showed the greatest decline, though as the test progressed even wells that showed no effect during the previous in situ air stripping demonstration showed significant decline. Soil gas TCE-PCE declined by >99%, with the piezometers closest to the injection well having consistent undetectable concentrations by the end of the demonstration (Fig. 26–3). Sediment concentrations were significantly lower after only 3 mo of 1% methane injection. Total sediment inventories of TCE-PCE declined by only 24%; however, this is quite significant considering that this decline represented a decline from 100 ppb to nondetectable concentrations, i.e., well below drinking water standards (Fig. 26–4 and 26–5). Densities of methanotrophs also were inversely correlated with the concentration of TCE in groundwater, i.e., as densities of methanotrophs increased, the concentration of TCE decreased; see discussion in Section Biodegradation. Chloride, the

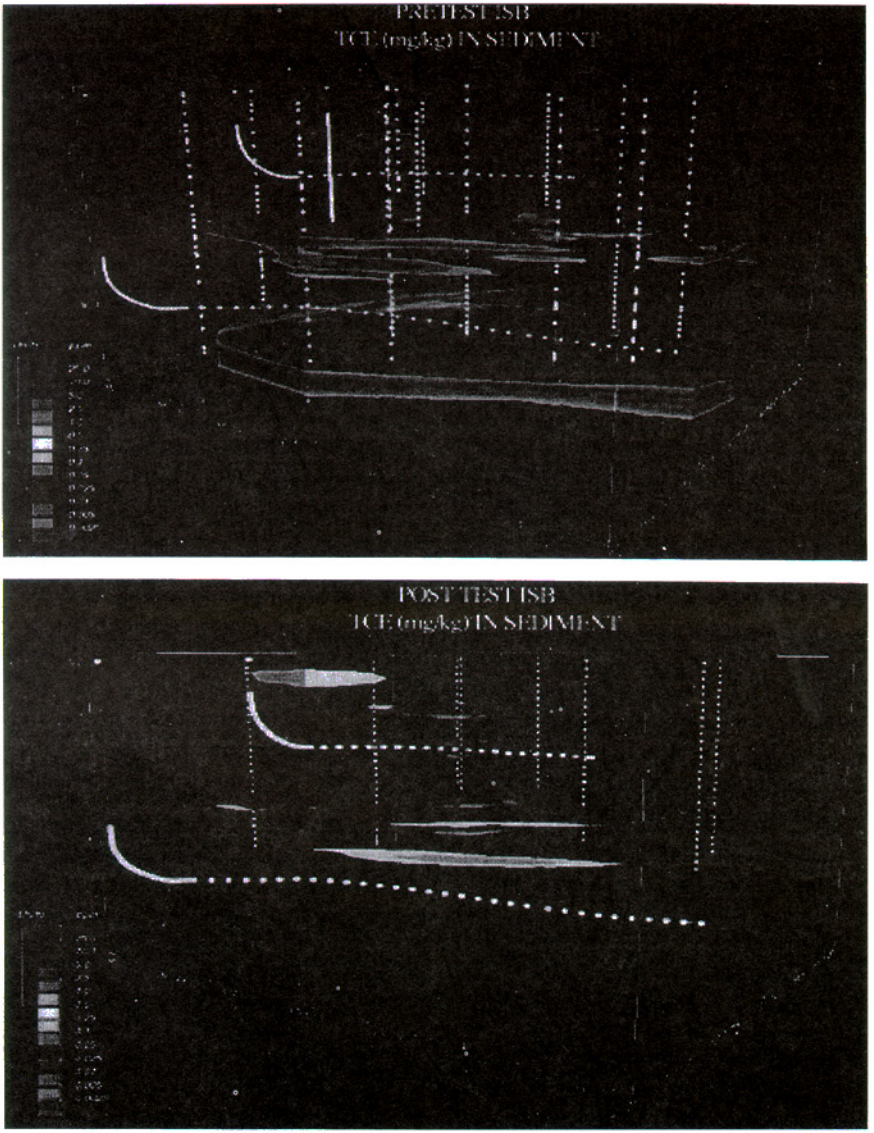

Fig. 26–4. Concentration of trichloroethylene (TCE) in sediment before and after demonstration (Earthvision three-dimensional depiction of >800 sediment samples).

end product of TCE-PCE biodegradation, in the groundwater also was inversely correlated to TCE concentration in the same sample. This again directly indicates that bioremediation was occurring during the demonstration since chloride is the end-product of TCE oxidation.

It also is important to note that no toxic daughter products were produced by this process. The VC and DCE are common daughter products produced by

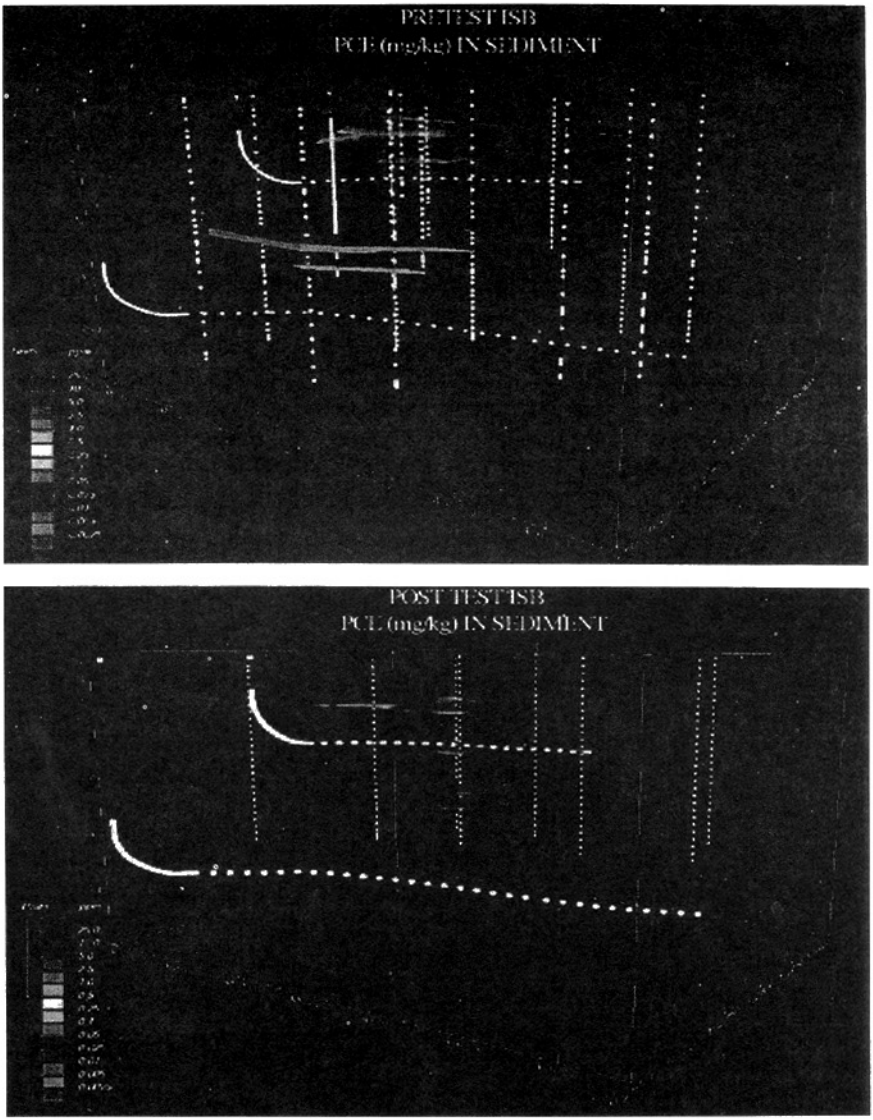

Fig. 26–5. Concentration of tetrachloroethylene (PCE) in sediment before and after demonstration (Earthvision three-dimensional depiction of >800 sediment samples).

the anaerobic reductive dechlorination of TCE and/or PCE. And VC and DCE were not detected in the soil gas, extraction air, water, or sediment at any time during the demonstration. By maintaining an aerobic environment and stimulating oxidizing bacteria, the process demonstrated that it is possible to bioremediate TCE and PCE without the formation of toxic intermediaries. Microtox assays (McGinnis, 1994) also showed that there was no toxicity changes in either the

water or sediment associated with periods of maximum biostimulation-biodegradation (as indicated by increases in methanotrophs and decreases in TCE). This makes this technology safer and more environmentally acceptable than anaerobic types of bioremediation.

As discussed in the section above, PCE is not degraded by methanotrophs but may be affected by the associated anaerobes that increase as a result of biomass increases caused by the biostimulation process. Thus the ratio of TCE to PCE would decrease over time, since TCE would be removed faster than PCE. Indeed, field analyses of the extraction well gas indicated that the ratio of TCE/PCE declined significantly as the demonstration progressed ($r = 0.98$, $P < 0.00001$).

The soil column models using demonstration site sediment and groundwater done by Andrews and Hansen (1994) showed that in experiments where no methane was added that no TCE removal occurred. When methane was added methanotroph densities increased rapidly and TCE removal occurred. Their experiments found rates of TCE degradation of 28 mg d^{-1} m^{-3}. Using this rate, as much as 719 kg of TCE was biodegraded during the demonstration or 1.68 kg d^{-1}.

Travis and Rosenberg's (1994) history matching model was used to do a variety of numerical simulations of this demonstration. Since they used kinetic biodegradation formula in the model they were able to specifically turn off biodegradation and calculate the difference between what was observed vs. what might have occurred if no biodegradation had occurred. These simulations demonstrated that 41% more TCE was removed-biodegraded by the bioremediation process vs. just air stripping alone. They found that as much as 777 kg (2.1 kg $d^{-1}m^{-3}$) would have been biodegraded during the demonstration, which compares favorably to the DSBR bioreactor rates observed by Andrews and Hansen (1994). Using methane consumption rates 350 and 1200 scf methane lb^{-1} TCE biodegraded from previous optimized bioreactor studies (T. Hayes, GRI, 1994, personal communication), the estimated amount of TCE biodegraded was calculated from the total methane that could have been consumed (difference between injection well concentrations and extraction well concentrations of methane). The range was from 318 kg (0.7 kg d^{-1} m^{-3}) to 1091 kg (2.5 kg $d^{-1}m^{-3}$) biodegraded during the demonstration. While this last calculation has a number of problems, it is similar to the calculations based on the history matching model and the DSBR experiments.

The Travis and Rosenberg (1994) simulations also showed that this in situ bioremediation process resulted in lower residual levels of TCE than in situ air stripping, "in places by a factor three to six lower." Their simulations suggested that native nutrient depletion (N and P) would occur towards the end of the 1% injection campaign; the water and sediment data for N and P and changes in bacteria densities support this. They also found that the addition of N and P would greatly increase the rate of TCE biodegradation, and the mineralization measurements suggest that this is what occurred during the final operating campaign. The simulations tested also suggested that in situ bioremediation by methanotroph stimulation is not very dependent on site-specific factors found at Savannah River. This technology should be applicable at a wide variety of other sites. Clearly stripping will initially be more efficient at sites where the VOC concentrations are very high; however, even in this situation in situ bioremediation will

be best when the concentrations become low. Rapid final polishing of many sites may only be possible with in situ bioremediation.

One of the more difficult endeavors of in situ bioremediation of TCE and PCE has been the measurement of bioremediation, due to the lack of a unique, persistent and nontoxic daughter product. Chloride shows promise when natural levels are low and rates of biodegradation are high; however, further proof must rely upon careful simulations (numerical and laboratory) and statistical analyses of environmental data from the demonstration. This demonstration has shown that in situ bioremediation of TCE and PCE is occurring and that various methods of estimating the rates are similar.

26–3.6 Ease of Use and Operation

The total number of days the system could have operated was 429; it actually operated 384, or 90% of the time. Operations during the initial 6 wk of startup-debug were well within expectations with only one down time of 73 h during the first week, when the main controller board failed. The system operated 9206 h of 10 303 h possible, removing 12 096 lbs VOC, destroying >11 370 lbs VOC, and emitting <726 lbs VOC. Since operations commenced 26 Feb. 1992, of the 10 303 h that the system could have been operating, it was down for maintenance, testing, repairs, and experiments for 1097 h. Of this down time 73 h was due to a control board failure, a single occurrence in the first 7 d, which has now been backed up, and 80 h due to a lightning strike that damaged several electrical components of the system. The system was shutdown for 58 h beginning 31 July 1992 for a special series of experiments and again on 19 Jan. 1993 for 100 h. The system was down 72 h in September for electrical system repairs due to failing circuit boards. The system was down for 89 h due to regulator freezing and minor CatOx repairs. The system also was down 72 h the week of 19 Jan. 1993 for modifications to the CatOx. All remaining down time was due to required maintenance of the system (e.g., changing oil on compressors) and 250 h required because of site scheduled power outages for M area. Thus excluding weather, experiments, and scheduled power outages, the system was operational 95% of the time. The electrical repairs all occurred during the first week of operation and after a lightning strike disabled a microprocessor board. All repairs were completed within 72 h. The system was of such ease to use and automated such that one full-time technician that also was responsible for a normal amount of required analytical monitoring could operate at least six of these systems simultaneously.

26–3.7 Performance Summary

The soil column models using demonstration site sediment and ground water done by Andrews & Hansen (1994) showed that in experiments where no methane was added that no TCE removal occurred. When methane was added methanotroph densities increased rapidly and TCE removal occurred. Their experiments found rates of TCE degradation of 28 mg $d^{-1}m^{-3}$. Using this rate as much as 719 kg of TCE was biodegraded during the demonstration or 1.68 kg d^{-1}.

This demonstration represents the first time ever that multiple nutrients (carbon, nitrogen, phosphorus) have all been injected as gases. The horizontal wells that form the basis for the SRS Integrated Demonstration provided significant advantages over conventional bioremediation nutrient delivery techniques. The increased surface area allowed better delivery of nutrients and easier recovery of gas, as well as minimizing formation clogging and plugging phenomena. There was never any indication of reduced flow or plugging during any of the six operational conditions employed. Indeed the zone of effect was far greater than that ever reported for liquid nutrient injection systems.

26–4 ECONOMIC PERFORMANCE

Modeling by Los Alamos National Laboratory for the in situ air stripping demonstration using the same wells for the previous demonstration showed that the in situ air stripping is only 58–60% of the cost of conventional cleanup. Conventional cleanup in this instance is defined as a combination system that would employ both soil vapor extraction and ground water pump-and-treat (Schroeder et al., 1992). They found that a major factor in the cost effectiveness of in situ air stripping is its demonstrated higher removal rate of VOCs. The estimated 5-year cost performance for in situ air stripping was $13/lb VOC removed, while pump and treat and soil vapor extraction are $22/lb VOC removed.

The in situ bioremediation demonstration cost modeling indicates that the cost of adding the methane and nutrients increased the cost by only 8% over the in situ air stripping. Thus even if no biodegradation occurred, the cost of adding the nutrients and associated machinery was only slightly more, and therefore would still make the system at least 30% more cost effective than the conventional technology (Saaty & Booth, 1994). The cost of methane is only $0.47/therm (100 scf). Only 1570 lb of TCE/PCE needs to be biodegraded to offset the additional costs of the nutrients and their associated equipment. The bioremediation modeling suggests that 41% more TCE is biodegraded/removed by the ISB process as compared to the ISAS process, as discussed previously (Travis & Rosenberg, 1994). In addition, their simulations suggest that it would take in situ air stripping more than 10 yr to achieve 95% removal of the contaminants, while the in situ bioremediation process would take as little as 4 yr. Since the bioremediation process also destroys contaminants in situ, it reduces the cost of treatment when combined with the extraction process. Thus they calculate that $1.6 million in cleanup costs may be saved at the demonstration site alone using the in situ bioremediation technology. The normalized process short term costs (1 yr) comparisons for the equipment used in this demonstration were as follows:

Technology	Short term Costs (cost/lb VOC)
Pump and treat with soil vapor extraction	$31.29
In situ air stripping	$13.92
In situ bioremediation (no biodegradation)	$29.05
In situ bioremediation (41% biodegradation)	$20.75

The in situ bioremediation technology provides significant cost improvements over other technologies, especially given that it will achieve cleanup goals faster. It clearly is not cost effective at high concentrations of TCE-PCE, where bulk removal processes like pump and treat-soil vapor extraction and in situ air stripping are cheaper; however, it can cut the final time to complete a remediation in one-half by reaching cleanup goals faster than the physical processes. It also may be the only technology applicable to sites with difficult to extract components (clay lenses, low permeability sediments). Fully optimized, in situ bioremediation will be the fastest and cheapest technology for a large number of sites.

26–5 APPLICATION SUMMARY

This demonstration has shown that (i) bacteria capable of degrading TCE-PCE can be stimulated in situ using relatively simple nutrients, (ii) biostimulation and biodegradation occurred in situ without production of toxic daughter products, (iii) that the process is easy to use and can be automated, (iv) that the cost for adding on the methane injection capability is relatively low and easily recovered, (v) that gaseous nutrient injection represents a significant new delivery technique for in situ bioremediation, and (vi) that combined with in situ air stripping this technology represents a significant improvement in terms of cost and efficiency over conventional baseline technologies used for remediation of chlorinated solvents.

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