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DEPLOYING IN-SITU BIOREMEDIATION AT THE HANFORD SITE

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ABSTRACT

An innovative in-situ bioremediation technology was developed by Pacific Northwest Laboratory (PNL) to destroy nitrate and carbon tetrachloride (CCl₄) in the Hanford ground water. The goal of this in-situ treatment process is to stimulate native microorganisms to degrade nitrate and CCl₄. Nutrient solutions are distributed in the contaminated aquifer to create a biological treatment zone. This technology is being demonstrated at the U.S. Department of Energy's Hanford Site to provide the design, operating, and cost information needed to assess its effectiveness in contaminated ground water. The process design and field operations for demonstration of this technology are influenced by the physical, chemical, and microbiological properties observed at the site. A description of the technology is presented including the well network design, nutrient injection equipment, and means for controlling the hydraulics and microbial reactions of the treatment process.

INTRODUCTION

At the U.S Department of Energy's (DOE) Hanford Site located in southeast Washington State, a contaminant plume containing primarily CCl₄ and nitrate is present in the unconfined aquifer. As part of the DOE's effort to develop cost effective technologies for cleanup of their sites, an innovative in-situ bioremediation technology was developed by PNL is being demonstrated at Hanford. The goal is to provide the design, operating, and cost information needed to assess its

effectiveness in remediating CCl₄- and nitrate-contaminated ground water.

The In-Situ Bioremediation Concept

In-situ bioremediation is based on the principal of supplying nutrients to indigenous microorganisms to stimulate their metabolic activity and subsequent degradation of contaminants. For in-situ remediation, easily transported species are delivered to the contaminated zone where biodegradation reactions can occur at the location of the contamination. The demonstration at the Hanford Site will implement in-situ bioremediation of carbon tetrachloride and nitrate. In this process, acetate is utilized as an energy and carbon source by indigenous bacteria that employ nitrate as a terminal electron acceptor. While the primary cellular metabolism is directed at acetate utilization through denitrification, CCl₄ is concurrently dechlorinated by active cellular macromolecules that function in the electron transport system of the bacteria. This dechlorination results primarily in the formation of carbon dioxide and chloride ions, while biomass, water, carbon dioxide, and nitrogen gas are produced from acetate utilization and denitrification. Under some conditions of cellular activity, chloroform is produced as a byproduct. Chloroform production can be controlled in the laboratory, and implementation of byproduct control in-situ will be an objective of this demonstration.

Approach for the Technology Demonstration

Demonstration objectives were established to focus efforts on providing key data for the technology developers, technology users, stakeholders who have a direct interest in the future use of the Hanford Site, and the public. The demonstration objectives are focused primarily on the problem of in-situ bioremediation for chlorinated solvents rather than for nitrate because remediation of the solvent (CCl₄) is the controlling factor for most of the design parameters. The primary results needed to successfully test the technology and establish that it can be applied at full

scale can be summarized in two overall objectives. (1) To establish the effectiveness of the technology, the data obtained must demonstrate that a bioactive zone can be developed and maintained in the aquifer and destroys the contaminants. (2) To transfer the results to future application of the technology, a design methodology for applying the technology at full scale must be produced and validated in the field test. Embodied in this second overall objective is the need to obtain process scale-up information and the need for the design method and technology to address the hydrogeology of remediation sites.

The primary objectives for the demonstration are formally stated as:

1. Demonstrate in-situ biological destruction of CCl_4 and nitrate in the Hanford ground water with sufficient control such that byproducts are either non-hazardous or, for potential byproducts such as chloroform and nitrite, are below the regulatory drinking water limit.
2. Demonstrate nutrient addition strategies that provide effective aqueous nutrient injection to remediate the contamination while minimizing the effects of biofouling around the injection well.
3. Demonstrate a design methodology for deploying, controlling, monitoring, and determining the rate of in-situ bioremediation to restore contaminated aquifers.

This paper will describe the site characterization, design, and deployment processes used to test in-situ bioremediation in the field based on these objectives.

SITE CHARACTERIZATION

Microbiological Background

The current understanding of microbial degradation of CCl_4 is limited. However, CCl_4 biodegrada-

tion has been demonstrated with a number of different bacteria. The conditions that favor biodegradation of CCl_4 are anaerobic. For example, Bouwer and McCarty observed that cultures of sewage treatment bacteria biodegraded CCl_4 to CO_2 and other metabolites under methanogenic (1983a) and denitrification (1983b) conditions. Sulfate-reducing microorganisms have also demonstrated the ability to destroy CCl_4 (Cobb and Bouwer, 1991; Egli et al., 1988). In addition, Semprini et al. (1991) speculated that sulfate-reducing bacteria were responsible for the CCl_4 degradation they observed during a field test of in-situ bioremediation. Biodegradation of CCl_4 under denitrification conditions is of particular interest at Hanford because both CCl_4 and nitrates occur in the unconfined aquifer. Hansen (1990), Criddle et al. (1990), Lewis and Crawford (1993), Bae and Rittmann (1990), and Bouwer and Wright (1988) demonstrated CCl_4 biodegradation with acetate as the electron donor and nitrate as the terminal electron acceptor. In addition, Bae and Rittmann (1990) speculated that CCl_4 competes as an electron acceptor with nitrate. This information, coupled with preliminary results for CCl_4 destruction by other subsurface microbes obtained from the Hanford Site (Brouns et al., 1990; Koegler et al., 1989) led to the speculation that it may be possible to introduce the appropriate nutrients to the subsurface and induce the native bacteria to biodegrade both the nitrate and CCl_4 contamination in-situ.

Aseptically recovered sediment core samples were used to determine the abundance of denitrifying microorganisms in the nitrate-contaminated region of the Hanford unconfined aquifer. Numbers of denitrifiers in sediment samples are in the range of 10^5 to 10^6 colony-forming units/g dry weight of soil. Sediment cores were also used to provide inoculum for laboratory tests.

Site Physical and Chemical Characterization

Figures 1 and 2 illustrate the conceptual model of the site and the primary data from which this model was generated. These data include sediment core analyses, hydrologic pumping tests,

geophysical logs, and tracer tests. Three wells penetrate the uppermost part of the unconfined aquifer and are completed with stainless steel casing and screen. Two of the wells (numbers 29 and 30) have 4-in. inside diameter (ID) casings and are screened between 243 and 279 ft. The third well (number 32) has an 8-in. ID casing and was installed with three separate screened intervals at depth intervals of 243 to 258 ft, 273 to 278 ft, and 293 to 298 ft.

Stratigraphy

The stratigraphy of the saturated zone consists of alluvial sediments, primarily sandy gravels and muddy sandy gravels, of the Middle Ringold Formation. The particle size distribution of the clay, silt, and sand size fractions below the water table are relatively uniform with depth. However, the sediments contain varying degrees of cementation and weathering. The clay size fraction makes up only 5% to 10% of the sediments, with half this fraction actually containing clay minerals. A 9-ft-thick caliche zone lies just above the water table between 238 and 247 ft. A lithology column in Figure 1 summarizes the lithology between 240 and 310 ft.

Hydrology

The characterization data summarized in Figures 1 and 2 indicate two distinct permeable units separated by a low-permeability unit. The high-permeability units lie at depths of approximately 245 to 255 ft and 286 to 300 ft, with an intervening low-permeability unit at a depth of 255 to 286 ft. The variation in hydraulic conductivity with depth is attributed primarily to variations in the degree of cementation of sediment clasts.

A series of constant-rate pumping tests, slug tests, laboratory hydraulic conductivity tests, and tracer tests were performed at the site to estimate hydraulic properties of the formation. The constant-rate pumping tests and slug tests indicated a range of approximately 10^{-2} and 10^{-4} cm/sec for hydraulic conductivity. The laboratory hydraulic conductivity values, measured with a falling

head permeameter, range between 10^{-3} and 10^{-7} cm/sec. The point dilution tracer tests, performed in the fully screened wells (numbers 29 and 30), indicate higher flow in the upper 13 to 18 ft of the test interval versus the lower part of the test interval. These tracer test profiles and the equivalent hydraulic conductivity estimated for each test interval are summarized in Figure 2. The hydraulic conductivity ranges shown adjacent to the lithologic log in Figure 1 are estimates for each equivalent lithologic unit. These ranges were estimated primarily from the field and laboratory hydraulic tests and from supporting characterization data, including lithology encountered during drilling and geophysical logging. The specific capacity for the aquifer units corresponding to the upper and lower screen intervals of the multiscreened well are also shown on Figure 1.

The neutron porosity log, shown in Figure 2, provides an indication of the relative porosity profile with depth. The log shows that the highest porosity is in the upper zone, between a depth of about 247 and 258 ft, and in the lower zone, between 287 and 306 ft. The highest porosity values correspond to high permeable zones as indicated from the hydrologic tests.

Groundwater Chemistry

The ground water chemistry at the demonstration site is typical for the unconfined aquifer conditions at the Hanford Site. Redox potential (Eh) ranges between 235 and 357 mV, indicating oxidizing conditions. The dissolved oxygen content is depressed to approximately half of saturation. Total dissolved solids averaged about 440 mg/L, and pH ranged between 7.1 and 7.8. Sulfate and chloride ranged between 50 and 67 ppm and between 20 to 26 ppm, respectively. Metal ion concentrations detected in the ground water are dominated by calcium, sodium, potassium, and magnesium.

Contaminant Distribution

Sediment samples from all boreholes showed a relatively uniform CCl_4 concentration profile with

depth and concentrations ranged between 10 and 300 ppb. Detectable CCl_4 concentrations in ground water ranged from 586 to 2197 ppb in the upper and middle zones (247 to 278 ft) and between 1900 and 3789 ppb in the lower zone (293 to 309 ft). The lower concentrations detected in the sediment samples indicate that either volatile organics sorb little to the sediment particles or that some of the organic constituents were lost (volatilized) during sample collection. Nitrates are present throughout the formation, with ground water concentrations ranging from 190 to 310 mg/L. Chloroform was detected in both the solid and aqueous phases in the majority of the samples taken. The concentration of up to 200 ppb chloroform in sediments is relatively constant with depth. The aqueous phase chloroform concentrations ranged up to 540 ppb.

SYSTEM DESIGN

Developing a viable system design for in-situ bioremediation requires addressing complex technical issues and performance evaluation goals associated with the objectives of the demonstration. Therefore, an organized design methodology was developed and implemented. (Figure 3). As shown in the figure, site characterization information provides a basis for subsequent laboratory experiments and flow and transport modeling. Kinetic expressions for the microbial reactions are developed in laboratory experiments for use as the reaction component of the process model. Because the process is *in-situ*, flow cell experiments in porous media are used to examine relevant bacterial transport and to confirm the process model. Experiments and model refinement are iterated until an acceptable description of the process is obtained. This final process model is both a process simulator and a design tool that can be employed for field design. Data from the field demonstration can be analyzed with the help of the process simulator. One of the primary final products of this demonstration will be a design tool, refined and validated with field data.

Design Methodology Components

The following sections describe activities associated with the primary components of the design methodology after site characterization information is obtained. These descriptions are specific to design of the in-situ bioremediation demonstration at the Hanford Site. Much of the information generated from these activities would be transferrable to design of in-situ systems at other sites in the form of the design tool. However, site-specific information is necessary as input to the design tool for use in determining the specific site design. The overall design methodology provides the framework for the design process at other sites.

Kinetic Studies

Extensive kinetic studies were performed using a microbial consortium indigenous to the demonstration site. Batch CCl_4 degradation experiments were conducted employing a balanced 2^{3-1} fractional factorial experimental design as described previously (Petersen et al., 1994). Three replicates of four experimental cases were tested and used as a basis for a comprehensive kinetic model (Hooker et al., in press). The factorial design was comprised of high and low initial concentrations for acetate, nitrate, and nitrite for the separate batch tests. Periodic additions of acetate and nitrate were made to all experiments to maintain levels near these desired concentrations. Periodic additions of nitrite were also made to one set of tests to maintain its concentration near 250 mg/L. Experimental measurements of biomass, acetate, nitrate, nitrite, and CCl_4 obtained from batch studies were used to determine values of kinetic parameters. These constants were chosen based on two criteria: (1) estimates of parameter standard deviation calculated by the parameter estimation program, Simusolv® (Dow Chemical Co., Midland, MI), and (2) a lack of statistically valid interactions between experimental conditions and parameter values. The resulting kinetic model was then tested against batch experiments completed independently of the factorial design and used in a transport code developed as a design tool to simulate the in-situ process.

Simulator

The numerical simulator used in this work is a modification of the multidimensional ReActive Flow and Transport code (RAFT) developed by Wheeler et al. (Wheeler et al., 1992; Chiang et al., 1990; and Chiang et al., 1989). This code uses a mixed finite-element method to calculate pressure, velocity, and concentration profiles in the flow field for convection dominated transport problems. A time-splitting procedure is used to provide a stable solution of the transport and biodegradation equations. Transport equations are solved by the modified method of characteristics, while microbial reactions are described in a system of ordinary differential equations solved using a 4th-order Runge-Kutta technique. This simulator was employed specifically to (1) estimate the degree of hydraulic control afforded by each of the proposed well designs and (2) determine optimal nutrient feeding strategies that minimize biofouling near the well bore and maximize CCl_4 destruction. Use of this comprehensive design tool, which combines flow and transport expressions with microbial kinetic and bacterial attachment/detachment equations, allows for *a priori* performance evaluation of proposed field test site designs.

Soil Columns

Treatability tests were performed in a soil column apparatus to observe biomass accumulation rates and contaminant degradation and to verify attachment/detachment functions used in the process simulator. Since actual field biodegradation rates cannot be measured until after the final design has been built and operated, soil columns were determined to be the next best source of information. The soil columns allow for the flow of contaminants, microbes, and nutrients in a simulated ground water through columns of packed soil. Although this type of treatability test is significantly more difficult to run, it gives information on biomass growth and contaminant degradation that is impossible to obtain in the batch studies.

Results indicate that biomass growth and accumulation occurs near the injection point of the soil columns. Pulsed addition of nutrients can delay or prevent biofouling in the soil columns,

indicating that this strategy will extend the useful lifetime of nutrient injection wells in the field. Computer simulations were calibrated from the data obtained in the soil column tests and used to develop nutrient addition strategies and the field design.

Field Design

The generic field design for in-situ bioremediation consists of a well network used to recirculate ground water with an injection well where small volumes of nutrients are introduced to the aquifer. The technology relies on being able to create a biologically active zone in the aquifer around this injection well. Figure 4 illustrates an injection well and the resulting cylinder-shaped bioactive zone in the aquifer. Extraction wells are placed as necessary to recycle ground water through this bioactive zone based on the site hydrological properties. Ground water recycle can be accomplished completely within the aquifer for some sites. Others sites may have properties that require ground water to be pumped to the surface and then reinjected through the injection well. Though ground water is pumped to the surface in this scenario, it is not treated at the surface, only transferred to the injection well. All treatment occurs within the aquifer. For any of the possible well configurations, the characteristics of the bioactive zone are the key parameter in the performance of the bioremediation technology. Therefore, the field demonstration is focused on determining the reaction rate within this zone and how it is established and maintained. Reaction rates in the bioactive zone are determined by measuring the concentration of contaminants and nutrients at the inlet, the injection well, and comparing these to the concentrations at the outlet, a monitoring well placed outside the bioactive zone. These reaction rates are referred to as the one-pass reaction rate because they are based on one pass of the ground water through this zone. Overall reduction in contaminant concentration and destruction of contaminant mass are functions of the one-pass reaction rate, the amount of ground water being mixed by the wells, and the number of times ground water is recycled through the reaction zone.

Design Parameters

Data from laboratory tests, site characterization, and the results of process simulation is used to select the appropriate field design. These data are used to assess various well configurations to select the design that best meets the demonstration objectives. Important data compared for each well configuration include (1) recirculation rate, (2) hydraulic control, (3) well spacing, screen location, and process monitoring locations, and (4) predicted biomass distribution, one-pass reaction rates, and overall contaminant degradation for the given properties of the configuration.

Design Selection Criteria

Designing the demonstration is based on meeting two primary criteria associated with the demonstration objectives: (1) Obtain measureable responses in test parameters to demonstrate contaminant remediation; (2) Apply field data from the demonstration to scale-up of other sites.

Overall, criterion 1 addresses the need to measure changes in contaminant and nutrient concentrations as a way to quantify the contaminant destruction reactions. The primary measured parameter is the reaction rate for one pass through the biologically active zone created in the aquifer. Thus designs are assessed with respect to parameters that affect the one-pass reaction rate. Configurations are preferred if a measureable decrease in contaminant concentration occurs over time at the extraction screen. This overall contaminant concentration decrease is important data to augment one-pass reaction rate data. However, the amount of decrease at the extraction screen over a specific time period will change for different well configurations because it is a function of both the amount of hydraulic control and the one-pass reaction rate. In addition to parameters that affect reaction rate, the number and type of monitoring points must be sufficient to provide data with acceptable confidence to determine whether demonstration objectives have been achieved. For example, a design with more or better monitoring points to collect data is judged as preferable with respect to this criterion.

Criterion 2 is focused on the need to demonstrate and collect data for the technology that is relevant to full-scale deployment. Thus, issues such as the adaptability of the design to different hydrogeologic settings, the ability to scale the design to larger well spacings, the applicability of the demonstration equipment to a full-scale design, the process monitoring and control requirements for the design, and the ability to treat layers within a heterogeneous aquifer are important considerations.

DEPLOYING THE SYSTEM

The equipment and operating strategy that will be used to implement in-situ bioremediation of CCl₄ and nitrate in the unconfined aquifer at the 200 West Area of the Hanford Site are described below.

Process Equipment

Deploying the in-situ bioremediation system involves specifying and setting up the appropriate process equipment, determining the process control and data acquisition needs, and determining the sampling needs. The objectives of this demonstration are the drivers for the data needs and the requirements of the system. A general in-situ bioremediation system can be assembled without knowledge of the final, specific design. However, the specific design dictates the monitoring points, the feeding strategy, the sampling plan, and the number of wells, injection lines, pumps, etc. There are three main subsystems of the bioremediation system: the process trailer, the sampling equipment, and the process control/data acquisition system. Each of these subsystems and the components of the subsystems are described below.

This is a stand-alone technology that does not require interfaces with other remediation technologies because the two contaminants present in the aquifer can be fully remediated using in-situ bioremediation. The only waste stream that will be generated is from groundwater sampling for use in process monitoring.

Process Trailer

The process trailer contains the nutrient injection equipment and the process control/data acquisition systems. These systems were designed to fit into a semitruck trailer to provide a mobile unit that is easy to set up and use.

The nutrient solutions (acetic acid and nitric acid) are contained in bulk storage tanks in the process trailer. Nutrient feedstock concentrations are greater than the toxicity limit for bacteria so that no growth occurs in the tanks. The process control computer controls nutrient injection intervals, duration, and flow rate into the well by control of in-line solenoid valves and a variable-speed gear pump. Input to the computer for control purposes includes line pressure and flow rate. A gear pump was selected for the design to provide the positive pressure necessary to open a down-well backpressure valve to avoid siphoning the contents of the injection line during static periods. The injection point for nutrients is down-well within the well screen so that nutrients are not diluted to nontoxic concentrations until they are pumped into the aquifer. This configuration is necessary to avoid bacterial growth in the injection system where it may cause fouling.

Sampling Equipment for Demonstration Parameters

Sampling equipment was designed to obtain representative samples of groundwater for analysis of volatile organic compounds (VOC), anions, microbe numbers, temperature, pH, and redox potential. These parameters are needed to help determine what is occurring in the aquifer with respect to transport of nutrients and tracer, destruction of CCl_4 , and growth of microorganisms.

In-situ probes will be used down-well to measure the temperature, pH, and redox potential in water at the injection and extraction well screens and in monitoring wells. These measurements are collected by the data acquisition system and are described below.

In-well pumps will be used to pump water to the surface for obtaining groundwater samples (for VOC, anions, cations, and microbes). Bladder pumps will be used for sampling because of the depth of the sampling locations. Two sampling schedules will be used: (1) an intense sampling period, when samples will be taken at frequent intervals and (2) routine sampling, with less frequent sampling for establishing long-term trends. The intense sampling periods will be used to monitor the progression of nutrient or tracer pulses within the recirculation area. Several sampling pumps will be directly connected to an automated sampler to collect samples as required during the intense sampling periods. The autosampler will consist of a fraction collector and the appropriate piping system. All sampling locations will be sampled manually during routine sampling procedures. Manual samples will be collected from each sampling location using a syringe and specially designed sampling port on the effluent line of the in-well pump. Manual sampling will be used for VOC analyses during intense sampling events and for all analyses during routine sampling. The manual sampling method is designed to obtain samples without exposure to the atmosphere to avoid volatilization of VOC and to collect anaerobic samples.

Process Control

Operation of the in-situ bioremediation demonstration requires process control for nutrient injection and sample collection, as well as data management for process monitoring equipment. An IBM compatible personal computer (PC) with appropriate input/output equipment is used for the control system and is housed in the process trailer. Commercially available software capable of multiple control and data acquisition functions controls the bioremediation nutrient injection and data acquisition systems. The software monitors all the signals collected by a network of input/output signal processors and stores the collected data in files on the PC.

There are three main subsystems where process control is important: the nutrient injection system, the autosampler, and the sampling pump system. In the process trailer, the feed pumps, the feed line pressure, the feed line flow rate, and the feed tank liquid level are controlled or monitored.

The feed pumps are turned on or off and their flow rate adjusted as appropriate to deliver nutrients to the well according to a defined schedule. The feed line pressure and flow rates are monitored to determine that (1) the proper amount of nutrients is being delivered to the well, (2) there is no plugging of the feed lines, and (3) there are no leaks in the feed lines. If an adverse condition is encountered an alarm will be activated on the PC and the feed pump will be shut down (if appropriate).

The autosampler system is controlled by the control system. The fraction collector is manually programmed but is activated at the start of an intense sampling period by the PC. The solenoid valves that control which line is being sampled are cycled by the control system to allow proper purging and sampling.

The sampling pumps are manually controlled during routine sampling periods, but are controlled by the PC during intense sampling periods. A cleaning system will be used to automatically clean down-well bladder pumps that are used as part of intense sampling events.

Data Acquisition

Data to monitor and analyze the performance of this demonstration are collected both manually and automatically. Sample analysis and data collection for VOC, anions, and microorganisms are performed manually, not within the control system. Data collected by the control system includes down-well measurement of pressure, flow rate, temperature, pH, and redox potential.

Pressure transducers are used to obtain pressure readings from the screened intervals in all wells. A buildup of pressure in the injection well may indicate plugging of the well from biomass buildup. The flow rates of the recirculation pumps are measured using a venturi with pressure transducers to measure the differential pressure.

The temperature, pH, and redox potential are all measured using a down-well multiprobe. These parameters indicate environmental conditions that must be maintained within acceptable ranges.

Field Operating Strategy

The field operating strategy was devised to obtain field data for assessing technology performance in terms of the demonstration objectives. To support these determinations, the parameters listed in Table 1 will be measured. This section describes the primary measure of success for each objective. The means for assessing the performance of the technology with respect to the objectives is also discussed.

(1) Demonstrate In-Situ Bioremediation of Nitrate and CCl₄

This objective will be successfully met if the data indicate that biological activity was responsible for removing nitrate and CCl₄ from the ground water. The evidence required to support this conclusion is a simultaneous reduction in nutrient concentrations, increase in biomass levels, disappearance of the contaminant, and appearance of metabolic intermediates or products (Madsen, 1991).

Loss of CCl₄ due to biotic reactions is determined by comparing the losses measured during operation of the treatment zone in a control mode to losses measured during active bioremediation. The first 3 months of the demonstration will be used to establish abiotic losses of contaminants due to the ground water mixing employed at the site. Ground water will be mixed with no addition of nutrients so that these losses can be quantified. In the subsequent phases of the demonstration, nutrient injection will be used to stimulate bioremediation of the target contaminants. Therefore, by comparing the control and treatment losses, the effectiveness of the bioremediation treatment can be quantified. The one-pass reaction rates for CCl₄ and nitrate are the primary measure of the

technology's effectiveness in destroying contaminant. The overall reduction in contaminant concentration at the demonstration site is a secondary measure of contaminant destruction effectiveness. The overall reduction in contaminant concentration is considered a secondary measure because it is dependent on the well configuration and hydraulic control of the ground water, and these may be different for the configuration used in the demonstration than the configuration used for full-scale applications.

Reduction in nutrient concentration is determined by comparing tracer pulse concentration profiles to the concentration profiles of nutrient species at specific monitoring locations. Any reduction in the concentration profiles of the nutrient species beyond that exhibited by the tracer can be attributed to biological reactions since laboratory experiments with sediment from the test site indicate that there will be almost no sorption of the injected species. These tests will be performed in several ways as described below.

To demonstrate that acetate and nitrate are being biologically destroyed without injection of conservative tracers that will build up in the treatment zone, two types of tests will be performed. The standard injection strategy, where acetate and nitrate are allowed to mix within the treatment zone, will result in the degradation of both acetate and nitrate. In the second type of test, pulses of either acetate or nitrate separately will result in much lower degradation of the injected species because microbial degradation of these compounds is linked through the energy-yielding pathways of the organism. Some loss of acetate and nitrate may occur as a result of endogenous respiration or bioaccumulation related to survival strategies of the microbes. This loss is estimated to be small compared to the degradation of these compounds using the primary metabolic pathways. Thus, responses of the nutrient species at the monitoring points for the standard injection strategy (acetate and nitrate together) can be compared to the response when only one of the nutrient species is injected. For instance, increased concentrations of acetate at a monitoring point compared to concentrations observed when both acetate and nitrate are injected together will demonstrate that

the loss of acetate in the treatment zone is linked to the presence of nitrate. This implies that biological processes are responsible for the loss of acetate and nitrate.

Less frequently, the responses of nutrient species will be compared to the response of a conservative tracer to demonstrate that the nutrients are being consumed within the treatment zone. These tests will be performed in conjunction with hydraulic tracer studies that will be conducted each month. The frequency of these tests is limited by the persistence of the conservative tracer within the treatment zone.

The microbial reactions will also produce unique metabolic products. The reaction intermediates most readily measured are nitrite and chloroform. Thus, the aqueous samples collected to determine if nutrients are being used will also be analyzed for the appearance of nitrite and chloroform. In addition, the pH of the ground water and CO₂ levels just above the water table will be monitored since microbial denitrification will cause an increase in these parameters. To verify acetate-supported microbial growth, the biomass concentration in the ground water at specific monitoring locations will be measured. In addition, a soil sampling well may be installed near the end of the test to collect sediment samples for biomass measurement.

(2) Demonstrate Engineering Strategies to Minimize the Effects of Biofouling

This objective will be successfully met if bioremediation of CCl₄ and nitrate is sustained to completion of the remediation goals and biofouling does not become the limiting factor in the life of the remediation process. The experimental evidence required to support this conclusion is the variation of pressure and flowrate profiles for the injection well during the remediation period. In addition, laboratory flow cell experiments using porous media will be used to demonstrate the ability to control biofouling and for comparison to field results.

The primary measure used to demonstrate that biofouling does not limit bioremediation operations

is the pressure and flow data at the injection screen. The groundwater recirculation pump will be designed so that increases in pressure of up to 100% of the initial pressure can be tolerated without a reduction in flow rate. Ideally, the pressure at the injection screen will increase to no more than 200% of the initial pressure during the course of remediation. Greater increases may be acceptable if the remediation process can proceed at these higher pressures and the potentially reduced flow rate. Tracer tests will be used to measure whether hydraulic control of the treatment zone is maintained during the demonstration and, in particular, during changes in the pressure at the well. Significant increases or decreases in conservative tracer travel times to monitoring points will be a qualitative indication that the hydraulic recirculation zone has changed. Unfortunately, models will not be useful for predicting the precise changes in the recirculation pattern due to these changes in tracer travel times. Thus, qualitative decisions will be made with respect to this issue. The primary quantitative measure of biofouling will be the injection pressure coupled with the continued operation of the remediation process.

(3) Demonstrate a Design Methodology for In-Situ Bioremediation

This objective will be successfully met if our design approach leads to a successful implementation of in-situ bioremediation. This is defined, for this objective, as meeting the criteria for objectives 1 and 2. A successful design strategy will be validated by demonstrating that the well placement, hydraulic and reaction modeling, laboratory kinetic analysis, and monitoring strategy were all suitable for developing a successful bioremediation demonstration. In addition, field data during and after the demonstration will be used to demonstrate control of the process. During the demonstration, control will be measured by the ability to retain hydraulic control of the treatment zone and maintain adequate bioremediation rates. After the demonstration ground water will be monitored to determine whether control of the process was sufficient to prevent (or minimize) any byproducts or changes in aquifer characteristics that were hazardous or undesirable. Thus, the design tool will be validated with field data.

SUMMARY

As part of the DOE's effort to develop cost effective technologies for cleanup of their sites, in-situ bioremediation is being demonstrated at the Hanford Site. The in-situ bioremediation technology stimulates indigenous microorganisms to cometabolically destroy CCl_4 as they metabolize acetate and nitrate, a co-contaminant at the site. Specific objectives were outlined for demonstration of the technology: (1) generate a bioactive zone in the aquifer for destruction of the contaminants, and (2) produce a validated design methodology that can be applied to the full scale application of the technology. The design and operating strategy for the demonstration is based on obtaining useful data to measure the performance of the technology with respect to the specific test objectives.

Because of the difficult technical issues associated with the technology, a rigorous design methodology is necessary. Microbial kinetic expressions, site properties, and a transport model are combined as a design tool/process simulator to select an appropriate field design. Concurrent laboratory studies validate the design tool and support field design. The field design is selected using the design tool to assess configurations with respect to their suitability in meeting demonstration objectives and in providing adequate provisions for data collection. The field equipment design must effectively deploy the appropriate process configuration, provide for process control, and collect data of acceptable quality for use in assessing the performance of the technology.

Important products from the technology demonstration include the field data and technology design. The design methodology and design tool/process simulator that has been validated in the field are also important to future application of in-situ bioremediation.

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REFERENCES

Bae, W. and B.E. Rittmann. 1990. Effects of electron acceptor and electron donor on biodegradation of CCl₄ by biofilms. In: *Environmental Engineering, Proceedings of the 1990 Specialty Conference*, C.R. O'Melia (ed.). American Society of Civil Engineers, New York.

Bouwer, E.J. and P.L. McCarty. 1983a. Transformation of 1- and 2- carbon halogenated aliphatic organic compounds under methanogenic conditions. *Appl. Environ. Microbiol.* 45:1286-1294.

Bouwer, E.J. and P.L. McCarty. 1983b. Transformation of halogenated organic compounds under denitrification conditions. *Appl. Environ. Microbiol.* 45:1295-1299.

Bouwer, E.J. and J.P. Wright. 1988. Transformations of trace halogenated aliphatics in anoxic biofilm columns. *J. Contam. Hydrol.* 2:155-169.

Brouns, T.M., S.S. Koegler, W.O. Heath, J.K. Fredrickson, H.D. Stensel, D.L. Johnstone, and T.L. Donaldson. 1990. *Development of a Biological Treatment System for Hanford Groundwater Remediation: FY 1989 Status Report*, PNL-7290, Pacific Northwest Laboratory, Richland, WA.

Chiang, C.Y., M.F. Wheeler, and P.B. Bedient. 1989. A modified method of characteristics technique and a mixed finite element method for simulation of groundwater solute transport. *Water Resour. Res.* 25:1541-1549.

Chiang, C.Y., C.N. Dawson, and M.F. Wheeler. 1990. *Modeling of In-situ Bioremediation of Organic Compounds in Groundwater*, Technical Report TR90-31. Rice University, Houston, TX.

Cobb, G.D. and E. J. Bouwer. 1991. Effects of electron acceptors on halogenated organic compound biotransformations in a biofilm column. *Environ Sci. Technol.* 25:1068-1074.

Criddle, C.S., J.T. DeWitt, D. Grbic-Galic, and P.L. McCarty. 1990. Transformation of carbon tetrachloride by *Pseudomonas sp.* strain KC under denitrification conditions. *Appl. Environ. Microbiol.* 56:3240-3246.

Egli, C., T. Tschan, R. Scholtz, A.M. Cook, and T. Leisinger. 1988. Transformation of tetrachloromethane to dichloromethane and carbon dioxide by *Acetobacterium woodii*. *Appl. Environ. Microbiol.* 54:2819-2824.

Hansen, E.J. 1990. Transformation of Tetrachloromethane Under Denitrifying Conditions by a Subsurface Bacterial Consortium and Its Isolates, M.S. Thesis. Washington State University, Pullman, WA.

Hooker, B.S., R.S. Skeen, and J.N. Petersen. (in press). Biological destruction of CCl₄ part II: kinetic modelling, *Biotechnol. Bioeng.*

Koehler, S.S., T.M. Brouns, W.O. Heath, and R.J. Hicks. 1989. *Bioremediation of Hanford groundwater and process effluents: FY 1988 Status Report*, PNL-6917, Pacific Northwest Laboratory, Richland, WA.

Lewis, T.A. and R.L. Crawford. 1993. Physiological factors affecting carbon tetrachloride dehalogenation by the denitrifying bacterium *Pseudomonas* sp. strain KC. *Appl. Environ. Microbiol.* 59:1635-1641.

Petersen, J.N., R.S. Skeen, K.M. Amos, and B.S. Hooker. 1994. Biological destruction of CCl₄ Part I: Experimental Design and Data. *Biotechnol. Bioeng.* 43:521-8..

Semprini, L, G.D. Hopkins, D.B. Jansen, M. Lang, P.V. Roberts, and P.L. McCarty. 1991. *In-situ biotransformation of carbon tetrachloride under anoxic conditions*, EPA Report No. EPA/2-90/060, U.S. EPA, Ada, Oklahoma.

Wheeler, M. F., K. R. Roberson, and A. Chilakapati. 1992. Three-Dimensional Bioremediation Modeling in Heterogeneous Porous Media. In: *Computational Methods in Water Resources IX*, vol 2., pp. 299-315, Elsevier, Oxford, UK.

Table and Figure Captions

Table 1. Summary of process measurements used to collect data for monitoring in-situ bioremediation of carbon tetrachloride and nitrate.

Figure 1. Lithological log and estimated hydraulic conductivity distribution for the portion of the Hanford unconfined aquifer to be used in the demonstration of in-situ bioremediation.

Figure 2. Hydraulic testing results and estimated hydraulic conductivity distribution for the location described in Figure 1.

Figure 3. Design methodology used to develop a field design for in-situ bioremediation of carbon tetrachloride and nitrate contamination at the Hanford Site.

Figure 4. Illustration of an idealized biologically active zone created around a nutrient injection well to degrade aquifer contaminants.

Measurement

VOC

Anions

Cations

Pressure

Flow

Temperature

pH

Redox potential

Aerobic heterotrophs

Denitrifiers

Sulfate reducers

Iron reducers

Coliforms

Device or Assay

Gas chromatography

Ion chromatography

Inductively Coupled Plasma (ICP)

Pressure transducer

Venturi with pressure transducers

Thermocouple on in-situ multiprobe

In-situ multiprobe

In-situ multiprobe

Spread plate enumeration

Most probable number enumeration

Most probable number enumeration

Most probable number enumeration

Membrane filter enumeration