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Subsurface Biofilm Barriers for the Containment and Remediation of Contaminated Groundwater

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ABSTRACT: An engineered microbial biofilm barrier capable of reducing aquifer hydraulic conductivity while simultaneously biodegrading nitrate has been developed and tested at a field-relevant scale. The 22-month demonstration project was conducted at the MSE Technology Applications Inc. test facility in Butte, Montana, which consisted of a 130 ft wide, 180 ft long, 21 ft deep, polyvinylchloride (PVC)-lined test cell, with an initial hydraulic conductivity of 4.2×10^{-2} cm/s. A flow field was established across the test cell by injecting water upgradient while simultaneously pumping from an effluent well located approximately 82 ft down gradient. A 30 ft wide biofilm barrier was developed along the centerline of the test cell by injecting a starved bacterial inoculum of *Pseudomonas fluorescens* strain CPC211a, followed by injection of a growth nutrient mixture composed of molasses, nitrate, and other additives. A 99% reduction of average hydraulic conductivity across the barrier was accomplished after three months of weekly or bi-weekly injections of growth nutrient. Reduced hydraulic conductivity was maintained by additional nutrient injections at intervals ranging from three to ten months. After the barrier was in place, a sustained concentration of 100 mg/l nitrate nitrogen, along with a 100 mg/l concentration of conservative (chloride) tracer, was added to the test cell influent over a six-month period. At the test cell effluent the concentration of chloride increased to about 80 mg/l while the effluent nitrate concentration varied between 0.0 and 6.4 mg/l.

Keywords: biodegradation, biofilm, groundwater, remediation, subsurface biotechnology.

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Introduction

This article summarizes the field-scale testing and evaluation of a subsurface biofilm barrier for the containment and remediation of nitrate-contaminated aquifers. Biofilm barrier technology involves the injection and subsurface transport of starved bacterial cultures followed by resuscitation with injected growth substrates. This process results in the production of copious amounts of extracellular polymer (EPS), which plugs the free pore space of the aquifer, thereby reducing porosity and hydraulic conductivity. This zone of reduced hydraulic conductivity serves as a novel barrier technology for controlling off-site plume migration while also providing a mechanism for *in situ* bioremediation. Formation of a denitrifying biofilm barrier, resulting in a 99% reduction in hydraulic conductivity, was demonstrated at the field scale as reported herein.

Porous Media Biofilm Processes

As bacterial cells are transported in porous media flow, some cells will adhere to the surface of media particles. If supplied with sufficient nutrients (i.e., substrate, electron acceptors, trace nutrients), attached cells will grow, reproduce, and form a biofilm. A biofilm is generally defined as a matrix of microbial cells and EPS (Characklis and Marshall, 1990). In porous media, biofilm growth is complicated by complex fluid dynamics and nutrient transport. Biofilm growth is removed by natural decay or physical detachment. Biofilm morphology in porous media systems can be highly

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variable, ranging from patchy, discontinuous colonies to thick, continuous films. For example, if high concentrations of easily biodegradable substrates (e.g., glucose) are injected into a packed bed reactor, thick biofilms may develop that are hundreds of microns in thickness (Rittmann, 1993). At the other extreme, subsurface biofilms capable of degrading trace organic compounds generally consist of isolated micro colonies of soil bacteria in the range of 10^5 to 10^7 CFU/g of soil.

Hydraulic Conductivity Reduction

If biofilm accumulation significantly reduces the free pore space available for groundwater flow, a significant reduction in porous media porosity and hydraulic conductivity will result (Taylor *et al.*, 1990; Cunningham *et al.*, 1991; Gerlach *et al.*, 1998; Sharp *et al.*, 1999a). Cunningham and colleagues (1997) reported experiments conducted to evaluate hydraulic conductivity reduction due to biofilm accumulation using rectangular test cells (sand bed 91.4 cm wide, 30.4 cm deep, and 122 cm long). *Klebsiella oxytoca* in starved mode were injected into the test cell and resuscitated using sodium citrate growth medium and oxygen as the primary electron acceptor. Resulting biofilm accumulation caused a reduction in hydraulic conductivity to less than 0.0001% of the initial value of approximately 7×10^{-2} cm/s. Biofilm barrier integrity was unaffected by exposure to heavy metals (strontium and cesium) at 1 ppm levels, as well as 300 mg/l of carbon tetrachloride.

The ability of thick biofilm to reduce hydraulic conductivity provides the basis for developing subsurface biofilm barriers for plume containment. If the biofilm is also capable of contaminant biotransformation, then the potential exists for a subsurface biofilm barrier to simultaneously provide for containment as well as *in situ* bioremediation. The concept of growing dual-species biofilms in porous media to reduce media permeability while simultaneously mineralizing trichloroethylene is the subject of recent and ongoing investigations (Komlos, 2001).

Subsurface Biofilm Barrier Concept

The formation of biofilm containment barriers requires that selectable bacteria be injected and transported through the subsurface between adjoining injection wells. After the formation has been inoculated with starved bacteria, suitable growth substrate and nutrients are injected to stimulate microbial growth and biofilm formation (Figure 1). The resulting zone of reduced hydraulic conductivity serves as a novel barrier technology for controlling off-site migration and facilitating *in situ* biodegradation of mobile contaminants. Biofilm barrier technology may also be useful as a means of funneling contaminated groundwater through abiotic subsurface treatment systems (i.e., zero valent iron systems) as shown in Figure 1. The main advantages offered by biofilm barrier technology are: (1) biofilm barrier construction will be achieved without excavation and therefore may be potentially useful at locations with restricted access to the subsurface, and (2) there is no obvious depth limitation with biobarrier technology.

Scale-Up Issues

The type of biofilm barrier configuration shown in Figure 1 requires that uniform columns of biofilm be developed in a radial direction around the injection wells. The biofilm columns must be large enough in radius to overlap with each other to form a continuous barrier. Although numerous laboratory studies (cited

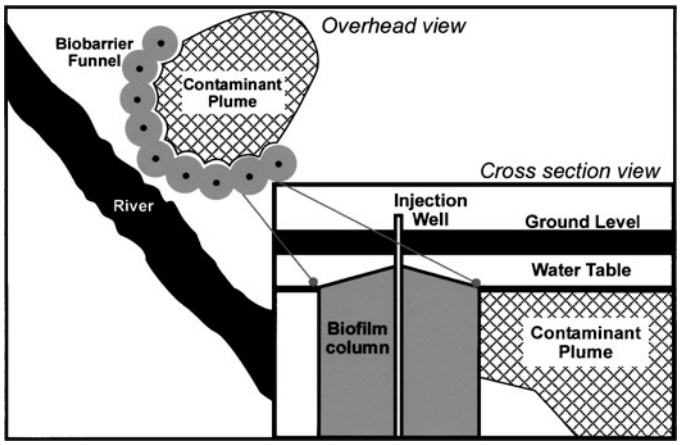

Figure 1. Visualization of a subsurface barrier composed of thick biofilm which plugs aquifer pore space while enhancing contaminant biodegradation. Biofilm barriers could also serve as a means of funneling groundwater though zones of active treatment (e.g., a zero-valent iron funnel-and-gate system).

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above) have been carried out to investigate hydraulic conductivity reduction caused by biofilm accumulation in porous media, several scale-up issues must be addressed in order to successfully transfer these laboratory results to the field scale. For example, it is mandatory that bacterial injection and transport result in a relatively uniform distribution of inoculum across the formation between points of injection. In addition, a strategy for injecting bacterial cells, substrate, nutrients, and electron acceptor must be developed to facilitate a uniform temporal and spatial growth of the bacteria.

Starvation-Facilitated Bacterial Transport and In Situ *Resuscitation in Porous Media*

The starvation, transport, and *in situ* resuscitation of biofilm producing bacteria are the key processes used to facilitate uniform distribution of bacterial inoculum across field-relevant length scales in porous media. These concepts are based on observations by numerous investigators including MacLeod and colleagues (1988), Lappin-Scott and colleagues (1988), Cunningham and colleagues (1997), Gerlach and colleagues (1998), and Sharp and colleagues (1999b), which show that starvation of bacterial inoculum substantially improves bacterial transport in porous media while encouraging spatially uniform biomass growth. Results show that starvation can reduce bacterial cell volume by as much as 75% and these cells can be resuscitated to their vegetative state at recovery rates of 40–80%, depending upon bacterial type and length of starvation. An important consequence of the starvation process is a prolonged lag growth period experienced by the starved bacteria during resuscitation. The lag period can last 48–120 hours and is a function of bacterial strain, starvation time, and type of resuscitation nutrient (Calatozza, 2000). The lag period allows nutrient injected into the aquifer to penetrate a significant distance before resuscitation begins.

Results by Sharp and colleagues (1999a), presented in Figure 2, illustrate starvation-facilitated bacterial transport and resuscitation in porous media. Figure 2 shows results from a series of 50-ft long, packed (f-70 Ottawa sand) column studies which were used to demonstrate the enhanced transport characteristics of starved cells at field relevant scales. Eight columns were used to compare transport, breakthrough and *in situ* biofilm production between starved and

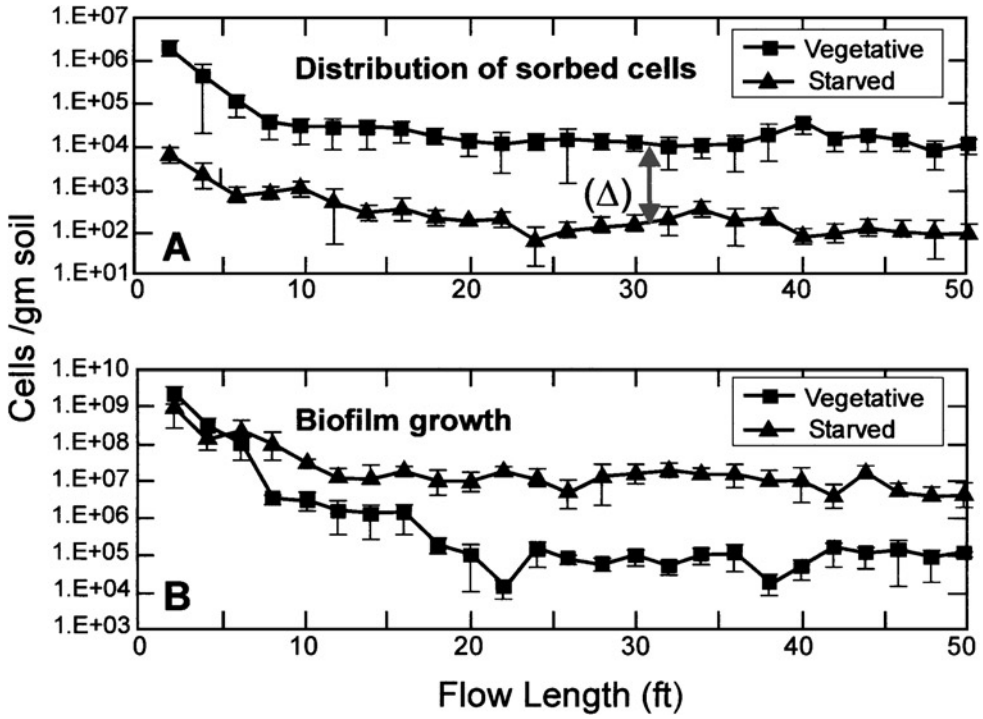

Figure 2. Transport and resuscitation of starved and vegetative bacteria (*Klebsiella oxytocca*) along a 50-ft packed column: (a) the distribution of sorbed vegetative and starved cells immediately after column inoculation; and (b) the distribution of biofilm cells after resuscitation and growth over a three day period.

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vegetative cultures of the biofilm producing bacteria *Klebsiella oxytocca.* The porous media columns were inoculated with equal amounts of either vegetative or 16-week starved cultures of *Klebsiella oxytocca*. After inoculation, the columns were rinsed with sterile buffer to wash out unadsorbed cells in the columns. Column effluent from inoculation and buffer rinse were collected and analyzed for number of cells (starved or vegetative) that broke through each column. Results showed that 10% of the injected starved cells broke through the 50-ft columns, compared to less than 0.4% of the vegetative cells. After rinsing, two vegetative and two starved columns were destructively sampled Camper *et al*. (1985) to determine the numbers of cells adsorbed along each column length (cells/g soil). As shown in Figure 2a, the vegetative cells adsorbed onto the sand in much higher numbers than the starved cells, especially at the head of the column. The difference between the vegetative and starved distribution curves is a result of two primary effects: (1) there are fewer starved cells in the columns because 10% of starved cells broke through; and (2) due to the effects of starvation, the starved cells were recovered (culturable) at about 60% compared to 90% for the vegetative cells. However, these results indicate that starved cells penetrated farther (i.e., higher breakthrough concentration) and adsorbed more uniformly along the flow path than vegetative cells.

After rinsing, the four remaining columns (two vegetative and two starved) were fed a continuous supply of citrate-based media for three days to support *in situ* resuscitation and biofilm growth. The four columns were destructively sampled to determine spatial distribution of biofilm (cells/g soil) along the 50-ft columns (Figure 2b). These results show that both sets of columns had a maximum, and essentially equal, biofilm density at the head of the column. However, the vegetative biofilm column dropped off significantly after 5 ft, while the starved column shows a higher and relatively constant biofilm distribution through out the column length. The high density, uniform biofilm growth in the starved cell columns is a result of the prolonged lag phase (approximately 50 hours) exhibited by the starved cells, which allowed the nutrient to penetrate the entire column length before significant biofilm growth began. The shorter lag phase experienced by the vegetative cells resulted in rapid biofilm growth at the head of the column, severely reducing the amount of nutrient available to the cells downstream in the column. Gerlach and colleagues (1998) and Calatozza (2000) have presented similar results demonstrating the enhanced transport characteristics of starved cells using numerous bacterial strains and various resuscitation media. The enhanced transport characteristics and prolonged lag phase associated with cell starvation allows for a greater radius of influence from an injection well, which decreases the number of wells needed to produce a biofilm barrier wall. Starvation-facilitated bacterial transport and resuscitation were used in the field demonstration described below.

Field Demonstration Goals

The field-scale demonstration project reported herein represents the initial attempt to develop a field-scale subsurface biofilm barrier that will result in significant reduction in hydraulic conductivity while simultaneously denitrifying a dissolved nitrate plume. For this project it was determined that a 99% reduction in hydraulic conductivity in the field demonstration test would be sufficient to demonstrate the barrier technology.

Denitrification refers to the reduction of nitrate or nitrite to a gaseous product (nitric oxide, nitrous oxide, or dinitrogen: $NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$). In most cases, denitrifying bacteria are capable of aerobic respiration but use nitrate as an alternative electron acceptor when oxygen is limiting. The first step in the denitrification process, nitrate (NO_3^-) reduction to nitrite (NO_2^-), can be accomplished by a variety of bacteria. Denitrifying bacteria can complete the sequence through nitric oxide (NO) and nitrous oxide (N_2O) to produce dinitrogen gas (N_2). Because nitric and nitrous oxide are considered air pollutants (greenhouse gases) the most desirable denitrification product is dinitrogen gas. Certain anaerobic bacteria can also obtain energy through the reduction nitrate to ammonium (NH_4^+) in a process termed "dissimilatory nitrate reduction to ammonium." This process is not desirable for treatment of nitrate-contaminated groundwater because of the potential for the ammonium to be re-oxidized to nitrate. The biofilm barrier described here was formed using a denitrifying species, *Pseudomonas fluorescens* strain CPC211A, which is also capable of copious EPS production.

Materials and Methods

Field Demonstration Facility

Field-scale testing and evaluation of subsurface biofilm barrier technology began in December 1999. The field demonstration test cell was designed to develop a comprehensive data set for evaluation of biofilm barrier performance. The test facility, constructed by MSE Technology Applications Inc. in Butte, Montana, consisted of a test cell 130 ft (40 m) wide, 180 ft (56 m) long, 21 ft (6.1 m) deep, lined with impermeable plastic, as

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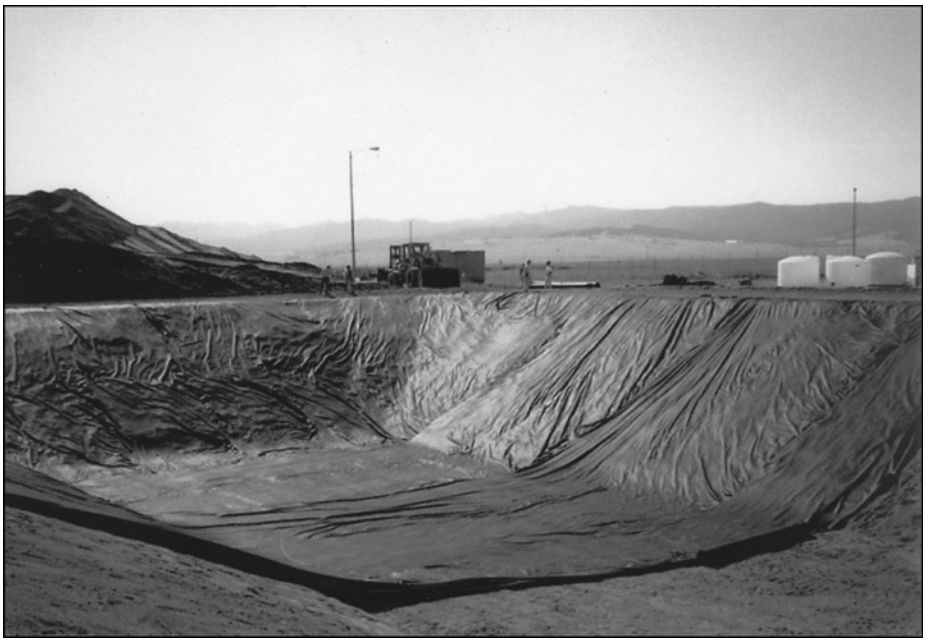

Figure 3. Test cell construction showing installation of the impermeable liner.

shown in Figure 3. A plan view schematic of the test cell is shown in Figure 4.

Test cell construction was initiated in the autumn of 1999 by excavating a 21-ft deep main basin with horizontal dimensions of 100 × 50 ft. Side slopes with a ratio of 2:1 were excavated from the bottom of the main basin to surface grade, resulting in a surface footprint of approximately 180 × 130 ft. Following excavation, the cell was lined with a 30 mm polyvinylchloride (PVC) liner. A network of six-inch diameter perforated plastic

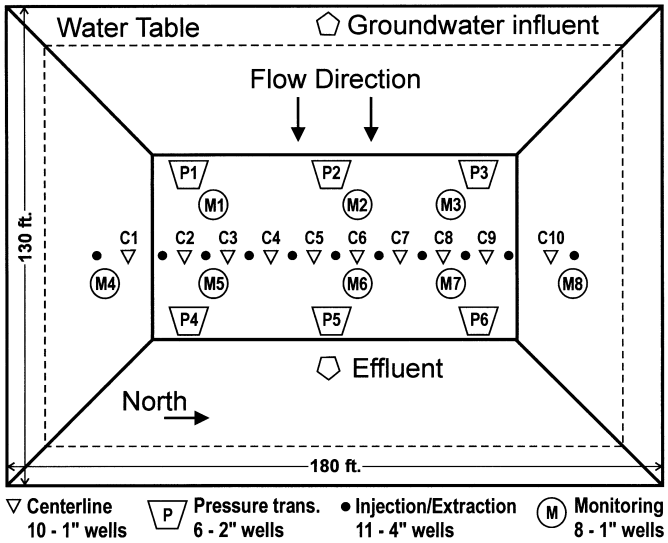

Figure 4. Plan view schematic showing the relative location and orientation of test facility including location of injection/extraction and monitoring wells. The exterior dimensions of the test cell are 180 ft × 130 ft, as shown. The centerline injection/extraction and monitoring wells are located approximately 55 feet down gradient from the point of groundwater influent. The monitoring wells are located 10 feet from the centerline wells on the up-gradient side and 20 feet from centerline on the down-gradient side. Pressure transducer wells are located 25 feet on either side of the centerline and the groundwater effluent well is located 27 feet down gradient from centerline.

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pipe was placed on the western side slope of the test cell and a vertical standpipe on the eastern edge to allow for injection and extraction of groundwater in preparation for establishment of a cross flow gradient. A pneumatic submersible pump was placed into the eastern standpipe.

It was determined that a 1 ft/day seepage velocity, together with a hydraulic gradient of 0.001 to 0.002, was desirable for the operation of the test cell. To achieve this seepage velocity the excavated soil was sieved to raise its hydraulic conductivity to an appropriate level. The excavated dirt was wet-screened, with very fine material (−600 mesh) being discarded. The soil retained on a #20 sieve and greater (classified as a well-graded sand) was used to backfill the test cell. The fines were placed on the bottom 2 ft and top 5 ft of the test cell, while the coarse fraction was placed in the remainder of the cell. There was not enough coarse material to completely fill the test cell, so the southern third was filled with a well-graded sand from a different location that had similar sieve properties. (It was later determined that the southern zone hydraulic conductivity was about twice that found in the rest of the test cell.) Compaction was accomplished by placing 18-in lifts and driving heavy equipment over the lifts. During placement of the fill dirt, water was removed from the test cell to facilitate compaction. The cell was mounded about one ft in the center and then filled with clean groundwater to a depth of 15 ft above the PVC liner.

Using hollow stem auger equipment, eleven 4-in diameter injection wells were placed across the 180-ft (56-m) dimension as shown in Figure 4. These were spaced at 12-ft intervals and completed with PVC pipe. Using a Geoprobe™ unit, ten 1-in diameter PVC monitoring wells were placed across the centerline of the test cell midway between each pair of injection wells. In addition, three 1-in diameter monitoring wells were located up gradient of the centerline and five 1-in diameter monitoring wells were located down gradient. Pressure transducers were located in six 2-in diameter observation wells to measure the bulk hydraulic gradient across the test cell. All wells were completed with PVC pipe with the injection wells having a 5-ft screened interval ending 4 ft above the PVC liner and all other wells having a 12-ft screened interval ending 2 ft above the PVC liner.

A flow field was established across the 180-ft (56-m) dimension by injecting supply water via a stand pipe at the up-gradient (western) boundary while simultaneously pumping from a recovery well located at the down-gradient (eastern) boundary. The stand pipes for the supply water and the recovery well served as the sample points for the influent and effluent samples respectively. The initial flow rate was established at 3 gpm; however, as the biofilm barrier developed, the flow rate was decreased in order to maintain a constant hydraulic gradient of 0.002 across the barrier. Prior to barrier formation, the effective ground water velocity through the site was established at approximately 1 ft/day (0.305 m/day). The test cell had an average initial hydraulic conductivity of 4.1×10^{-2} cm/sec. The groundwater temperature was 7°C.

Inoculum Preparation

The bacterial inoculum for biofilm barrier formation consisted of a starved cell suspension of *Pseudomonas fluorescens* strain CPC211A. This strain was isolated by MSE from a petroleum-contaminated aquifer and was selected for these experiments based on its growth and copious production of mucoid extracellular polymers under simulated field conditions, as well as its ability to use nitrate as a terminal electron acceptor. The bacterial cells were grown in large quantities and starved using a proprietary starvation procedure developed by MSE.

Application of Bacteria and Nutrient Solution

Biofilm barrier formation was initiated by applying the starved bacterial suspension as well as a nutrient solution containing molasses as a source of carbon and energy, and nitrate to serve as a nitrogen source and terminal electron acceptor for bacterial metabolism. The starved bacterial suspension contained a total *P. fluorescens* population of 2.6×10^8 cells/ml, which was mixed with water from the groundwater source well and nutrients to give an injected concentration of approximately 2×10^6 cells/ml. The injected nutrient concentrations were approximately 10 g/l for molasses, 3.0 g/l for sodium nitrate, and 0.12 g/l for potassium phosphate. Bacterial and nutrient solutions were applied to the odd-numbered injection/extraction wells using a gravity fed system with a hydraulic head of 3–6 ft. Simultaneously, water was extracted from the even-numbered injection/extraction wells. This initial injection procedure was performed for two days, during which time total bacterial counts were monitored from extraction and centerline well samples using membrane filtration and epifluorescent microscopy (Standard Method 9216). Subsequent injections were performed by gravity feed of bacteria and nutrient or nutrient-only solutions to all of the injection/extraction wells. Table 1 shows the dates, durations, and volumes of all injections to the test cell. Three primary injections of starved bacteria were applied to the site. The first and largest injection was followed by two smaller injections to construct the wall. A fourth minute injection to expend excess starved cell culture was applied at

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Table 1
Molasses and bacterial inoculum injection volumes for biofilm barrier formation

Injection	Date		Molasses Liters	Inoc. Liters
	Start	Stop		
1	12/10/99	12/12/99	1880	830
2	12/16/99	12/20/99	1460	210
3	12/27/99	12/30/99	1250	210
4	1/13/00	1/21/00	1050	0
5	1/31/00	2/2/00	314	21
6	2/14/00	2/16/00	314	0
7	2/23/00	2/24/00	418	0
8	8/9/00	8/10/01	209	0
9	5/31/01	6/1/01	209	0
10	8/29/01	8/30/01	209	0

approximately six weeks into the project. After eighteen days, the vast majority of starved cells were applied and only nutrient solution was supplied.

Microbiological Enumeration

Total bacterial populations (reported as cells/ml) were enumerated by epifluorescent direct count (Kepner and Pratt, 1994). Ground water samples were preserved with formaldehyde (final concentration 4.7%) immediately after collection. Samples were serially diluted with buffer water and filtered on 0.45 μm black polycarbonate filters and stained with a 10 μg/ml solution of 4, 6-diamidino-2-phenylindole. For each filter, bacterial cells were then counted in at least ten randomly selected microscopic fields until approximately three hundred cells had been counted. Heterotrophic plate counts (reported as CFU/ml) were performed using R2A agar (difco), following serial dilution with buffered water (APHA, 1995). Analysis of soil samples for denitrifying enzyme activity was accomplished using the acetylene inhibition method described by Smith and colleagues (1978) and later modified by Murray and Knowles (1999).

Hydraulic Conductivity Monitoring

The vacuum slug test method was used to measure values of hydraulic conductivity at the ten 1-in diameter piezometers, installed along the barrier's centerline. The slug tests were conducted using a special well head apparatus, a high-speed data logger and pressure transducer, and a water level indicator. The well head apparatus was designed to seal the piezometer, while at the same time allowing access for water level measuring instruments. To conduct a test, the well head apparatus was placed in a piezometer and secured. Depth to the static water level was measured and recorded. The transducer was then placed down the piezometer near the bottom, followed with the electric water level indicator. The water level indicator probe was set 5 ft above the static water level to indicate that the desired water level was attained. The well head apparatus ports were then either closed or sealed and the vacuum line connected to a vacuum pump. The vacuum inside the well was increased until the water level meter indicated that the water had been raised to the desired level. Once water had risen to the 5-ft level, a small bleeder valve was opened to maintain a constant vacuum, holding the water level relatively steady and allowing the aquifer to equilibrate. The data logger was then activated and the vacuum released through a 2-in ball valve on the well head apparatus, allowing the water level in the well to recover. The data was downloaded from the data logger to a personal computer and was analyzed using the SuperSlug^{TM} software package to determine values of hydraulic conductivity. These values from each of the 10 observation wells were averaged to obtain an average hydraulic conductivity for the entire barrier.

Nitrate and Chloride Tracer Addition

Exposure of the biofilm barrier to nitrate-contaminated groundwater was achieved by adding sodium nitrate to the test cell cross-flow influent to achieve a concentration of approximately 100 mg/l as nitrogen. This concentration is ten times the EPA Primary Drinking Water Standard of 10 mg/l. The nitrate concentration used in this study was similar to nitrate concentrations found at many contaminated sites, including a nitrate plume in Albuquerque, NM and numerous shallow aquifers across the U.S. that are impacted by cattle farming, industries and highly fertilized crops. Chloride (100 mg/l) was also added to the test cell influent

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to serve as a conservative tracer to monitor transport of solutes through the test cell.

Monitoring of Chloride and Nitrogen Species

Groundwater samples were collected from the monitoring wells after three well bore volumes were purged. Samples were collected using a peristaltic pump and sanitized 1/4-in diameter polyethylene tubing. Prior to sampling, three well bore volumes were purged from each monitoring well. All sampling materials and procedures including sample preservation, storage and analysis were conducted in accordance with EPA guidelines. The following EPA methods were used for sample analysis: nitrate and nitrite, EPA 353.2; chloride, EPA 325.2; ammonia, EPA 350.2, total Kjeldahl nitrogen, EPA 351.2.

Results and Discussion

Biofilm Barrier Formation

Total bacterial populations were determined by microscopic direct count in samples of the injected fluid, centerline monitoring wells, and extraction wells during the initial injection (Table 1, Injection 1). The injected fluid contained approximately 2×10^6 cells/ml. Prior to injection, indigenous bacterial populations in samples from the centerline and extraction wells, measured using heterotrophic plate counts, ranged from 3.5×10^5 to 1.7×10^6, with a mean of $5.9 \pm 3.4 \times 10^5$ CFU/ml. Following the initial injection, the bacterial populations were periodically measured and averaged for the ten centerline wells (Figure 5). Bacterial populations increased from $3.4 \pm 2.1 \times 10^6$ CFU/ml at the end of the initial inoculation and nutrient addition in December to $6.8 \pm 2.5 \times 10^7$ CFU/ml by February. The relatively high standard deviations in the bacteria numbers were a result of low numbers in the high permeability zone at the southern portion of the barrier (C1–C5) contrasting with the higher numbers in the northern portion (C6–C10) of the barrier. The bacterial numbers for the middle of the barrier were relatively uniform. Bacterial populations declined after the barrier had been fully established and nutrient additions had ceased, resulting in nutrient limitations within the barrier.

Examination of the enumeration plates from samples collected at the centerline wells during biofilm barrier formation revealed that the predominant colony morphology present was consistent with that of the injected *P. fluorescens* strain (CPC211A). Bacterial isolates were obtained from several of these colonies, which had phenotypic profiles (API20NE) that were identical to *P. fluorescens* CPC211A. These findings indicate that the increase in cell numbers during biobarrier formation was primarily the result of growth of the injected cells.

Hydraulic Conductivity Reduction

Hydraulic conductivity was measured via slug test method at the centerline monitoring wells as discussed previously. The hydraulic conductivity values from each of the ten centerline wells were averaged and plotted in Figure 6 along with the total mass of nutrient feed in pore volumes applied to the system over a 21-month period beginning with initial injection on 10 December 1999. A pore volume represents the approximate amount of pore space the barrier wall would have to occupy to create an effective barrier. For this project, a pore volume is equal to 212,038 liters (7,488 cu ft.) based on a 130-ft long, 16-ft deep, and 12-ft thick barrier wall in an aquifer with an average porosity of 30%. Tests performed seven weeks after the initial injection showed that the average hydraulic conductivity had dropped from an initial value of 4.1×10^{-2} cm/sec to 9.6×10^{-4} cm/sec; a reduction of almost 98%. This reduction was considered sufficient to meet the project goal and, accordingly, emphasis was shifted to developing a nutrient injection schedule, which would maintain hydraulic conductivity at or near this level. From Figure 6 it is evident that nutrient injections can occur as infrequently as three-to ten-month intervals without the average hydraulic conductivity increasing significantly.

Recall that approximately the southern one-third of the test cell was constructed of a coarser grain soil than the center and northern sections. Figure 7 illustrates the corresponding effects on the slug test hydraulic conductivity measurements averaged over southern, middle, and northern one-third zones of the test cell. The southern zone data shows an initial (pre-barrier) hydraulic conductivity of 9.0×10^{-2} cm/sec compared to an initial value of about 4.1×10^{-2} cm/sec for the center and northern sections. Analysis of the 1 February 2000 data showed that the hydraulic conductivity for the northern and center section had dropped to approximately 4×10^{-4} cm/sec. The southern section had a corresponding hydraulic conductivity of about 2×10^{-3}cm/sec. In an effort to make the cell more uniform, the nutrient addition efforts were concentrated in the south zone. During focused maintenance feedings of approximately 0.2 pore volumes, the majority of the nutrient was directed to the four south injection wells. This had the effect of bringing the southern zone conductivity more in line with the center and north sections of the cell as shown in Figure 7.

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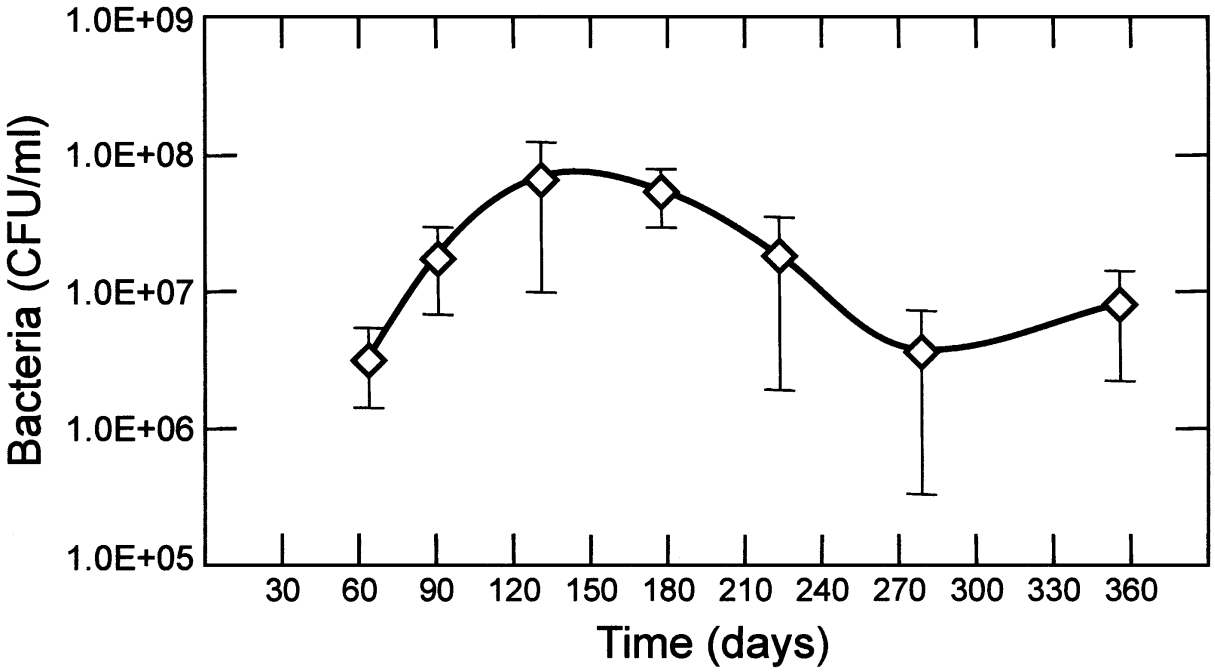

Figure 5. Average bacterial populations for the ten centerline observation wells over a 300-day period following injection in December 1999.

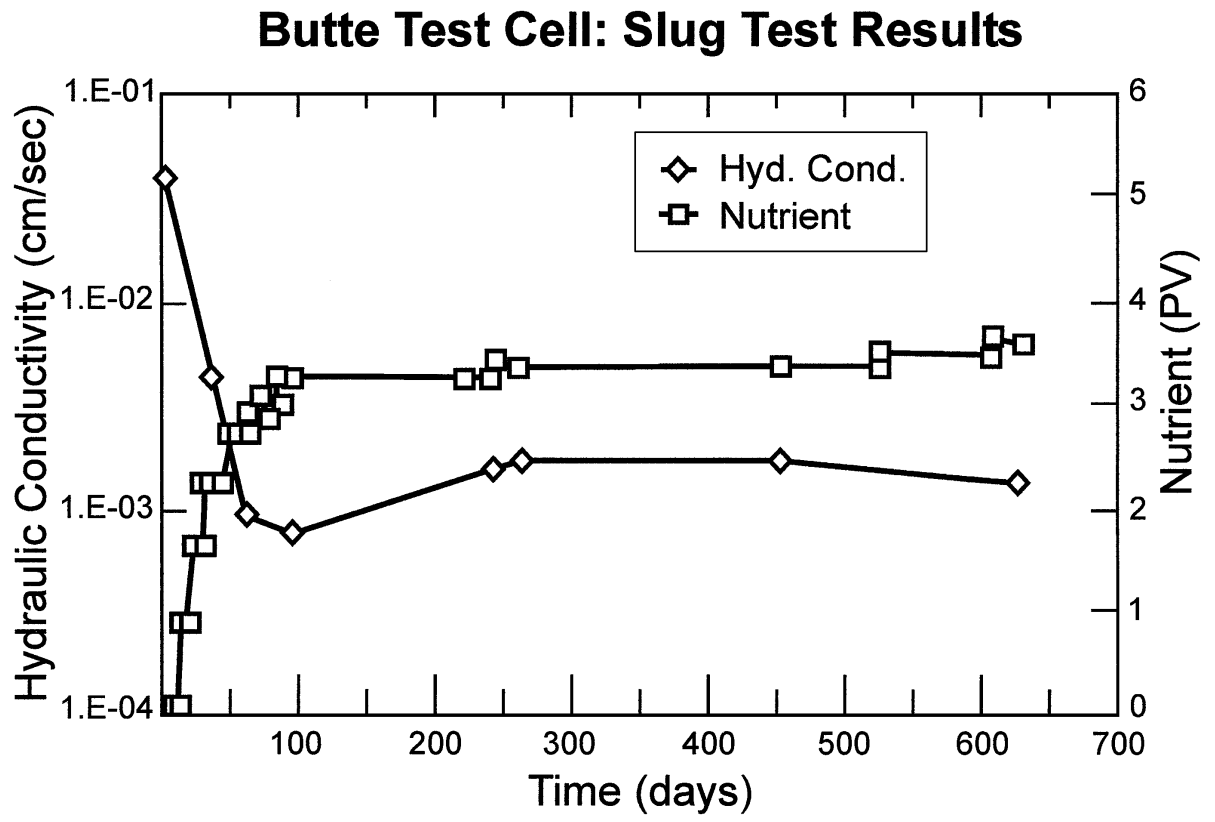

Figure 6. Average test bed hydraulic conductivity and cumulative nutrient addition for 22-month test period. One nutrient pore volume (PV) equals 212,038 liters (7,488 cu ft).

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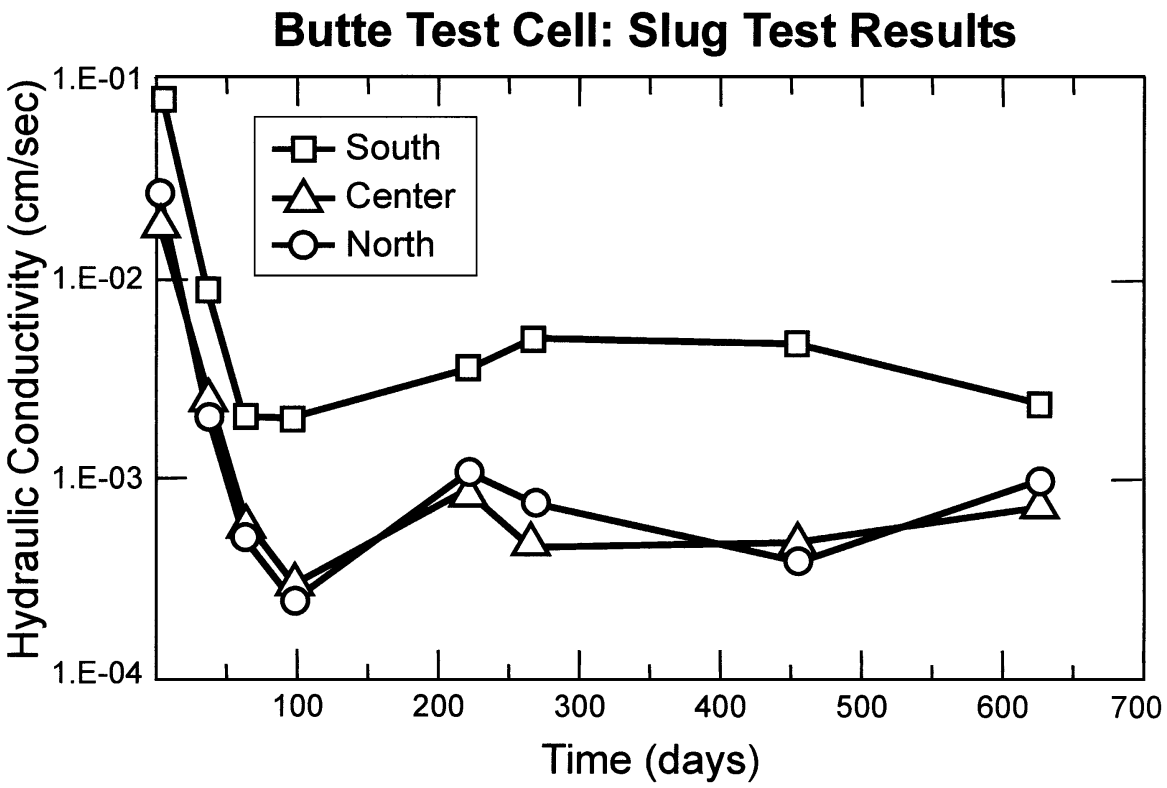

Figure 7. Average hydraulic conductivity results from three different zones of the test cell.

Denitrification

To initiate the denitrification test, nitrate (100 mg/l as nitrogen) and a conservative tracer (chloride, 100 mg/l) were added to the test cell influent as shown in Figure 8. These influent concentrations were sustained over a six-month period, during which no growth nutrient was added to the system. At the test cell effluent, the concentration of chloride gradually increased over the test period while the nitrate-nitrogen concentration varied between 0.0 and 6.4 mg/l. The gradual increase in chloride concentration at the effluent well demonstrates the desired "retardation" effect of the established biofilm wall and the complex three-dimensional nature of the flow at the site, which included areas of high permeability (southern zone) and channeling at the ends of the barrier. The appearance of effluent nitrate-nitrogen in detectable concentrations was likely the result of the channeling around the southern zone of the test cell as previously discussed. Also, soil core samples collected before and after the test period indicated that soil nitrate-nitrogen increased in samples collected along the south end of the barrier. While further reduction of the south zone channeling may have further reduced the effluent nitrate concentrations, they were still well below the EPA Primary Drinking Water Standard of 10 mg/l.

A contour map of nitrate concentrations determined throughout the test cell near the end of the experiment is shown in Figure 9. Again, the higher permeability in the southern section of the barrier is highlighted by nitrate contours penetrating the south-end of the barrier wall. Overall, these results indicate the biofilm barrier was effective for removing nitrate from contaminated groundwater.

Nitrite and Ammonium Concentrations

Throughout the test period nitrite-nitrogen concentrations in the test cell effluent remained below the EPA Primary Drinking Water Standard of 1.0 mg/l with the exception of values measured on 22 August 2001 (2.2 mg/l) and 24 September 2001 (4.3 mg/l). Since nitrite is an intermediate product of denitrification, these results are consistent with the observations for effluent nitrate indicating that complete denitrification of nitrate to nitrogen gas is the primary reaction taking place in the barrier.

Prior to the addition of nitrate to the test cell influent, ammonium concentrations of 4.9 to 10.6 mg/l (as nitrogen) were detected in the test cell effluent. After the addition of 100 mg/l of nitrate to the influent during the denitrification challenge, there was a gradual

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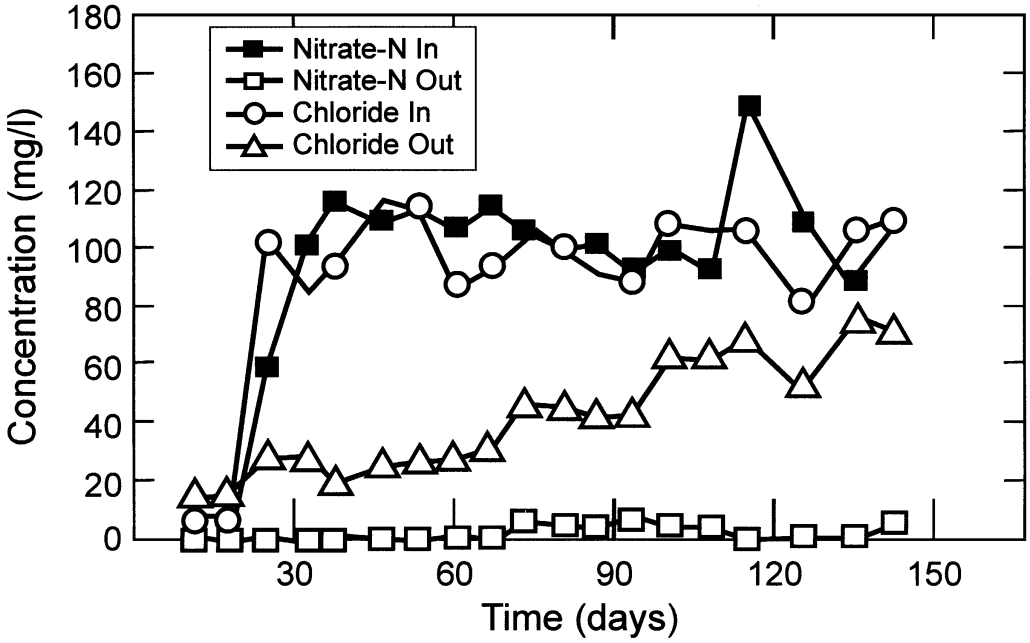

Figure 8. Influent and effluent nitrate and chloride tracer concentrations during a six-month period. Results demonstrate the level of effectiveness of the biofilm barrier at removing nitrate.

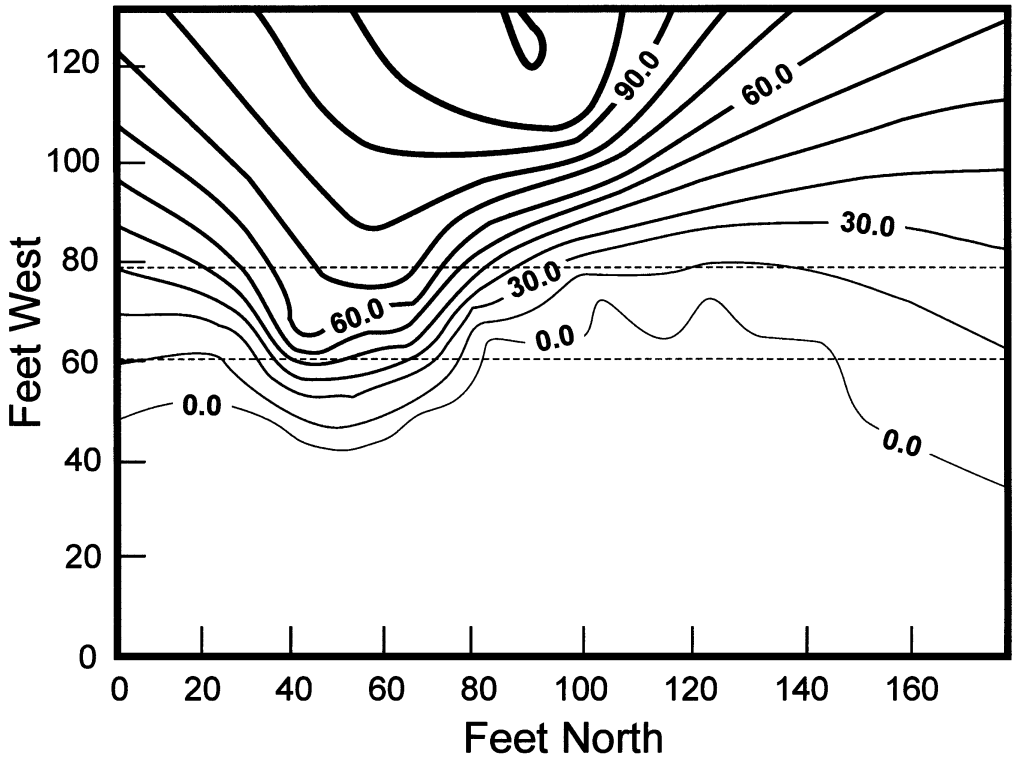

Figure 9. Contour map of nitrate concentrations determined at monitoring wells located throughout the test cell near the end of the experiment (24 September 2001). The dashed lines indicate the approximate location of the biofilm barrier across the test cell. The barrier width extended approximately 15 ft on either side of the centerline.

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increase in ammonium-nitrogen in the test cell effluent with the average effluent ammonium nitrogen concentration being 12.7 ± 2.5 mg/l. We hypothesize that the ammonium-nitrogen could have been produced either by dissimilatory nitrate reduction to ammonium or mineralization of organic nitrogen due to decomposition of biomass in the biofilm barrier. Ammonium was not present in the natural ground water used as the influent at the site and it could not have been associated with the resuscitation media since the barrier was not being fed directly before or during the denitrification test. Monitoring well samples (T4–T8) showed that increases in ammonium-nitrogen occurred in the center of the flow cell where negligible concentrations of both chloride tracer and nitrate were detected. These results support the hypothesis that the source of the ammonium nitrogen detected in the effluent was likely due to decomposition of the biofilm barrier and not dissimilatory nitrate reduction to ammonium. However, the increase in ammonium nitrogen across the barrier was equivalent to less than 10% of the nitrate nitrogen that was removed within the barrier, indicating that ammonium production within the barrier is not a significant process.

Microbiological Analyses

Microbiological analyses included enumeration of heterotrophic bacteria in water samples from the monitoring wells, as well as an assay of soil core samples for denitrifying enzyme activity. Samples for the enumeration of heterotrophic and denitrifying bacteria were collected from the monitoring wells near the end of the denitrification test. The population of heterotrophic bacteria in samples from the centerline wells ranged from 6.6×10^4 to 1.1×10^7 (mean = 4.4×10^6 CFU/ml). Samples from the upstream and downstream monitoring wells had populations of heterotrophic bacteria ranging from 1.9×10^4 to 4.1×10^5 CFU/ml (mean = 1.1×10^5 CFU/ml). Thus, populations of heterotrophic bacteria were higher in samples from the centerline wells than the upstream and downstream monitoring wells. These results indicate that the biomass forming the barrier was concentrated along a zone of approximately 15-ft in width on either side of the centerline. The presence of concentrated biomass in a distinct region along the center of the barrier demonstrates the effectiveness of utilizing starvation-facilitated bacterial transport to effectively distribute and develop a well-defined biofilm barrier in the subsurface.

Soil samples were collected for analysis of denitrifying enzyme activity (DEA) before nitrate was added to the test cell influent and near the end of the nitrate addition period. Soil core samples were collected from locations upstream of the biofilm, near the centerline of the test cell, and downstream of the biofilm barrier. Samples at each location were taken at both the north and the south ends of the test cell. DEA was not detected in any of the samples collected upstream of the barrier. Prior to nitrate addition, the DEA values along the centerline of the barrier ranged from 0.01 ng/g/h at the north end to 3.9 ng/g/h at the south end. Down stream, DEA was detected at the north end only at 0.12 ng/g/h. After nitrate addition an increase in DEA was observed in all centerline and down stream samples at both the northern and southern regions of the barrier. The highest DEA was found at the centerline of the south end at 12.6 ng/g/h. The highest DEA value on the north end of the barrier was 0.87 ng/g/h at the downstream location. Increases in DEA after nitrate addition indicate stimulation in the activity of denitrifying bacteria. On the south end of the barrier, this stimulation occurred primarily in the middle of the biofilm barrier and was easily detected at the centerline location. On the north end of the barrier, the stimulation of DEA likely occurred on the upstream side or front of the barrier due to the lower permeability of the northern region. Overall, these results indicate that nitrate addition stimulated denitrification activity throughout the barrier, with the highest activity occurring in the areas of highest permeability and highest flow.

Summary and Conclusions

This 22-month field study has demonstrated four key elements of subsurface biofilm barrier technology: (1) the utility and effectiveness of starvation-facilitated bacterial transport as a method for placing and distributing biofilm producing bacteria in the subsurface; (2) the feasibility of injecting a mixture of growth nutrients in such a way as to develop a uniform spatial distribution of biofilm over a radius of at least 15 ft; (3) the effectiveness of subsurface biofilm in reducing hydraulic conductivity by two orders of magnitude (99%) at field-relevant scales; (4) the ability to sustain an effective, long-term biofilm barrier by short-term injections of growth nutrient at intervals ranging from three to ten months; and (5) the development of a denitrifying biofilm barrier capable of effectively removing nitrate (initial concentration 100 mg/l) from contaminated groundwater.

Chloride tracer studies indicated that the south end of the field test cell had an initial hydraulic conductivity approximately twice that of the rest of the site. The presence of this higher hydraulic conductivity zone contributed to some degree of channeling throughout the

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test period. During the last six months of the test period, nutrient injections targeted at the south end of the test cell significantly reduced the local hydraulic conductivity, thereby demonstrating that channeling in zones of relatively high hydraulic conductivity can likely be controlled.

To date, this biofilm barrier wall has been in place for 44 months and has maintained a 99% reduction in hydraulic conductivity with only minimal maintenance nutrient feed. Understanding issues pertaining to the long term integrity and maintenance of the barrier wall is ongoing. We believe that once the barrier is established and nutrient feed is ceased, the biofilm (extracellular polymer) remains intact in the pore space while the actual bacterial cells revert into a starved or semi-starved state due to a lack of nutrients. In this state, the cells will require a pro-longed lag-phase as described earlier. If part of the barrier wall weakens, injected nutrient will transport through the paths of least resistance to the weakened areas. The prolonged lag-phase experienced by the cells near the injection well will allow the nutrient to reach the weakened areas without premature plugging or well fouling. This aspect of the barrier wall allows the wall to self-heal with only minimal nutrient addition. Further monitoring and testing of the barrier wall will evaluate this phenomenon and demonstrate the long-term viability of this barrier technology.

Attractive aspects of biofilm barrier technology include: (1) biofilm barrier construction can be achieved without excavation and therefore may be useful at sites where access to the subsurface is restricted; (2) there is no obvious depth limitation with biofilm barrier technology; and (3) once established, the biofilm barrier requires minimal maintenance for long-term operation. Possible applications of biofilm barrier technology include: (1) containment barriers whose primary purpose is to reduce hydraulic conductivity and contain or redirect a dissolved contaminant plume; and (2) reactive biofilm barriers which can carry out biodegradation of dissolved contaminants while simultaneously reducing formation hydraulic conductivity.

The project team is currently in the process of evaluating a denitrifying biofilm barrier for treatment of a large nitrate plume in New Mexico. Other field applications being considered include the containment of leaky underground storage tank plumes, redirection of groundwater plumes to enhance the effectiveness of existing treatment processes, construction of *in situ* cutoff walls for reducing salt water intrusion into fresh water aquifers, and the plugging of high permeability zones in oil fields to enhance secondary oil recovery operations. Principals employed during this study are also being used in laboratory and pilot scale research to develop microbially mediated redox-reactive biofilm barriers for the reduction of chlorinated organics and precipitation of heavy metals.

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