In Situ Biofilm Barriers: Case Study of a Nitrate Groundwater Plume, Albuquerque, New Mexico

Lomesh Dutta

H. Eric Nuttall

Al Cunningham

Garth James

Randy Hiebert

A new use for biofilm barriers was developed and successfully applied to treat nitrate-contaminated groundwater down to drinking water standards. The barrier was created by stimulating indigenous bacteria with injections of molasses as the carbon donor and a combination of yeast extract and trimetaphosphate as nutrients. This injection of amendments results in bacterial growth in the aquifer, which attaches to the sand grains to create a reactive semipermeable biofilm. The biofilm barrier presented in this article reduced the migration of contaminants and provided an active zone for remediation. The cylindrical biobarrier was constructed using eight wells on the perimeter forming a 60-foot-diameter reactive biodenitrification region. Another well at the center was installed to continuously extract the treated water. The intent was to produce a continuous source of nitrate-free water. The system operated for over one year, and during this period, the biobarrier was revived multiple times by reinjecting molasses in the perimeter wells. Nitrate concentrations of treated water decreased from 275 mg/L (as nitrogen) to < 1 mg/L. © 2005 Wiley Periodicals, Inc.

BACKGROUND

Biofilm barriers are a promising new technology for the containment and remediation of groundwater. These barriers are formed by subsurface injection of bacteria and/or nutrient solutions to stimulate the growth of bacteria that produce extracellular polymeric substances (EPS) and also may degrade contaminants (Costerton et al., 1995). The engineered accumulation of biomass and EPS in the subsurface is used to control groundwater flow and enhance treatment strategies. For example, a biofilm barrier can be used as a "funnel" to channel contaminated groundwater to a treatment zone. Unlike many other barrier technologies, biofilm barriers cause minimal surface disturbance and have no obvious depth limitation.

MSE Technology Applications (MSE), in collaboration with the Center for Biofilm Engineering (CBE), has developed a biological process that combines containment and treatment of contaminated groundwater. The process involves the creation of a subsurface biofilm barrier to destroy nitrate *in situ* while keeping the contaminated plume from reaching fresh water sources. Containment is achieved by selectively plugging permeable strata with a microbial biofilm (Cunningham et al., 1997). As the biofilm develops, it reduces the subsurface hydraulic conductivity, attenuating the transport of contaminants. Several pilot-scale tests conducted by MSE and CBE at the demonstration site in Butte, Montana (Exhibit 1), demonstrated that biobarriers act as an effective alternative to conventional barrier technologies. Both laboratory and pilot tests confirmed that

Published online in Wiley Interscience (www.interscience.wiley.com). DOI: 10.1002/rem.20063

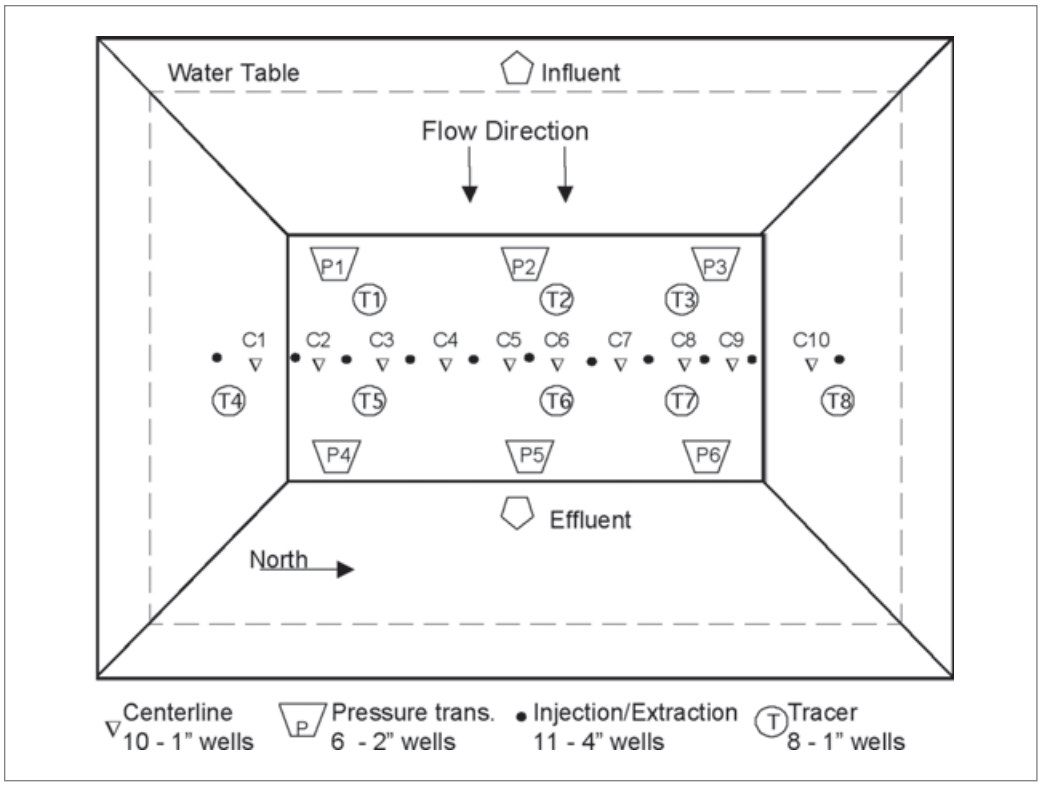


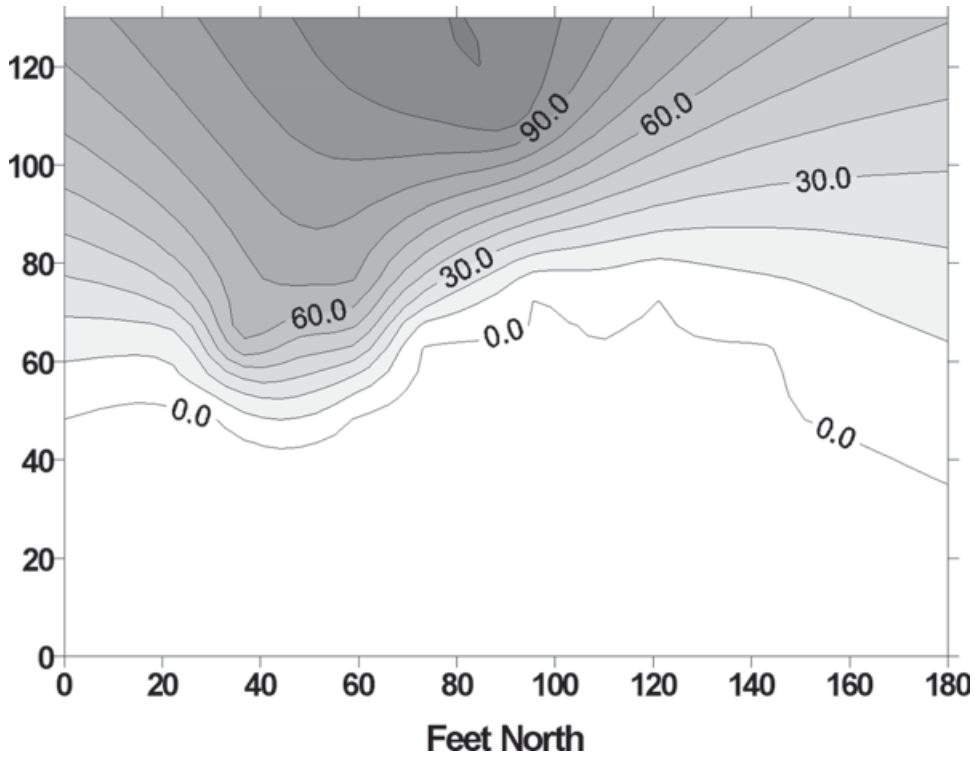
Exhibit 1. Phase 1 study test cell schematic showing monitoring well locations in Butte, Montana

although the biobarrier greatly reduced the hydraulic conductivity, it never completely blocked the flow of water (Cunningham et al., 1991; James et al., 1995).

The current study is a collaborative project between MSE, CBE, and the University of New Mexico (UNM). The study focuses on the denitrification of groundwater using the biofilm biobarrier technology. Phase 1 of this project was conducted at a demonstration site in Butte, Montana (Cunningham et al., 2004) using an artificial sand lagoon. A biobarrier was established across the center of the test cell that was 130 feet wide, 180 feet long, and 20 feet deep by injecting denitrifying bacteria as well as a molasses- and nitrate-based nutrient solution.

The bacterial strain CPC211A of starved cell suspensions of *Pseudomonas fluorescens* were injected to produce abundant amounts of extracellular mucoid exopolymers able to grow well under simulated field conditions and metabolically aid in the denitrification reaction (Hiebert et al., 2001; James et al., 1995, 2000). Microbial counts, including enumeration of heterotrophic bacteria and denitrifying bacteria, were performed using samples collected from the monitoring wells throughout the experiment from December 1999 to October 2001. Near the end of the experiment, counts of heterotrophic bacteria were higher in samples from the centerline (C0–C10) wells than from the upstream (T1–T3) and downstream (T4–T8) monitoring wells. The results indicate that the majority of the biomass forming the biobarrier was located along the center of the test cell.

The barrier was maintained for over two years through the periodic injection of small quantities of nutrient solution. Despite the high concentration of nitrate added to the test cell, nitrate-nitrogen levels in the test cell effluent did not exceed the federal maximum contaminant level (MCL) of 10 mg/L during the project (Exhibit 2).



Note: The biofilm barrier (located ca. 60 feet west) was effective in removing nitrate from the groundwater.

Exhibit 2. Contour map of nitrate concentrations determined at monitoring wells located throughout the test cell near the end of the experiment (September 24, 2001)

FIELD DEMONSTRATION

Phase 2 of this project was conducted to demonstrate the field-scale application of permeable biofilm barriers for the denitrification of groundwater.

Objective

The objective was to develop a successful biobarrier at the field site and determine its effectiveness in containment and remediation of nitrate in groundwater. The ability to develop an effective biobarrier by stimulating the indigenous species was an important consideration to determine the commercial applicability of the technology.

Site Description

The test site chosen for Phase 2 was the Mountain View Subdivision located in the South Valley of Albuquerque, New Mexico. The nitrate contamination of groundwater was caused by overfertilization of a vegetable farm in the 1950s. The nitrate plume covers an area of about 550 acres and has a volume of approximately 6.4 billion liters (Deng, 1998; Nuttall, 2000). The nitrate concentrations at the site were measured to be on the

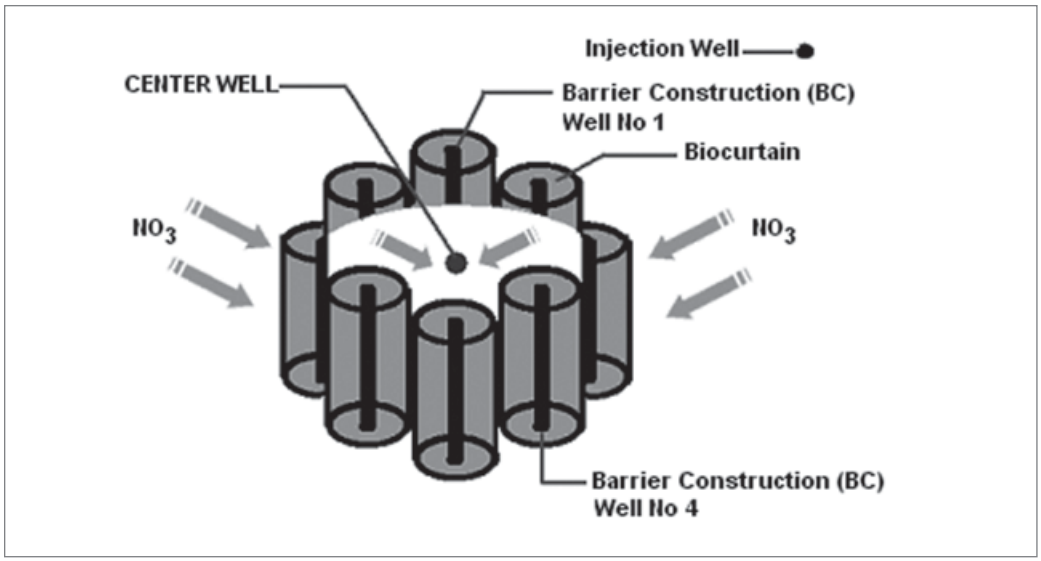


Exhibit 3. The circular biobarrier design

order of 300 mg/L, which is 30 times more than the federal MCL of 10 mg/L. A methemoglobinemia incident was reported at this site in the 1980s where an infant was hospitalized in the Mountain View community due to consuming water from a private well that contained high concentrations of nitrate.

Test Cell Preparation

A cylindrical zone of 3,000 square feet as shown in Exhibit 3 was designed. The zone was based on the requirements to use both the containment capability and the reactivity of the biofilm barrier to produce a system capable of efficiently remediating the nitrate-contaminated plume. The test pattern was composed of eight barrier construction wells (BC-1–8) installed uniformly on the perimeter of the circular zone, nine monitoring wells set up inside (CMW-1, CMW-2, and MW-E1), on (BMW-1 and BMW-2), and outside (OMW-1, OMW-2, and MW-C1) the circular pattern, and a Center Well to continuously produce clean water. An additional set of five wells (five spot [NE, NW, SE, SW, and Center5]) located more than 300 feet from the circular zone, was used to supply nutrient-free water to the cell. The five spot wells were previously used for denitrification experiments at this field site (Chen, 2000; Deng, 1998; Nuttall et al., 1997).

Groundwater Flow Modeling

Modeling was performed to determine the flow rates required to construct the *in situ* biofilm barrier. Visual MODFLOW^{TM} v.2.8.2 was used based on the site characterization data. A 300-square-foot area was modeled in which the well system was designated as the center of the system. The center zone containing the wells and the outer zone of the barrier had cell sizes of 1 square foot and 10 square feet, respectively. The aquifer zone was divided into two vertical layers. The upper one extended from the ground surface to 32 feet below ground level (bgl), and the lower one ranged from 32 feet to 50 feet bgl. The conductivity of the upper layer was (K_{upper}): 1×10^{-3} and the lower one

was (K_{lower}): 1×10^{-2}. The Specific Yield (S_y) and the Specific Storage (S_s) were 0.3 and 0.0001, respectively, and remained the same for both the layers. The groundwater gradient was 0.00001, resulting in h of 0.03 feet across the 300-foot modeled area. The nominal groundwater level (GWL) was 34 feet bgl. For modeling, the GWL and gradient constant head boundaries (CHBs) of 34.03 and 34.00 feet bgl were used at the left and right edges of the modeled area. To provide initial wetting of the upper layer, an initial head of 30 feet bgl was set for the modeled area and transient analysis was chosen (Dutta, 2004).

Microbes and Nutrients

Groundwater samples were collected across the span of wells and were assayed for populations of bacterial species present. The data were used to determine potential nutrients required to stimulate indigenous bacterial species. Specifically, microbiological analyses were performed at the site to evaluate heterotrophic plate counts (HPCs) (i.e., bacterial colonies per mL of sample); direct counts (DCs) (i.e., direct counting of each cell under microscope); and denitrifying bacteria (DNB) most probable number (MPN) estimates (i.e., statistical estimation of bacteria growing in the aqueous media). Bacteria capable of utilizing nitrate and nitrite as terminal electron acceptors and also producing copious amounts of extracellular polymers were desired for this project. Denitrifying bacteria are known to be present ubiquitously, and thus producing a biofilm barrier without the inoculum would result in considerable cost savings as well as prevent significant regulatory hurdles. Previous tests at the site (Nuttall, 1997) had demonstrated instances of occurrence of both denitrification and biofouling, which indicated that bacteria capable of producing the desired results were already present.

Of the many possible carbon nutrients, including acetate, citrate, lactate, ethanol, and molasses, the latter was selected based on the ability to stimulate production of an effective biofilm as confirmed during the laboratory column experiments, previous experience of MSE in stimulating rapid bacterial growth with the production of mucoid extracellular polymers using molasses solutions, and economic considerations.

Bacteria capable of utilizing nitrate and nitrite as terminal electron acceptors and also producing copious amounts of extracellular polymers were desired for this project.

Phosphate and yeast extract were mixed with molasses to produce the appropriate nutrient solution to favor bacterial growth. The groundwater would supply the terminal electron acceptor (nitrate) to complete the bacterial metabolism.

Biobarrier Formation

After installation and development of the wells, the nutrient solution was injected to form the biobarrier. A total of 5 1/2 batches were prepared, with each batch having the composition shown in Exhibit 4.

The barrier construction wells BC-1–8 and Center Well were used to create the biobarrier in the aquifer. The injection process was performed June 4–June 10, 2003. A schematic of the injection process is shown in Exhibit 5.

Initially, the barrier construction wells BC-2, BC-4, BC-6, and BC-8 were injected with the nutrient mixture in 1:40 parts of molasses to water using a peristaltic pump set to obtain an approximate molasses concentration of 4.0 g/L. The feed tank was used to store the prepared batches of nutrient mixture, and the surge tank supplied clean water to the injection operation from a location on the site as shown in Exhibit 5. A peristaltic

Exhibit 4. Concentrations of batch solutions during nutrient injection

Ingredient	Quantity Per batch	Total
Molasses	47 gallons	258.5 gallons
Trisodium phosphate	13 lbs.	71.5 lbs.
Yeast extract	2–3 lbs.	11–16.5 lbs.
Water	Filled up to a total of 400 gallons	Total of 2,200 lbs.

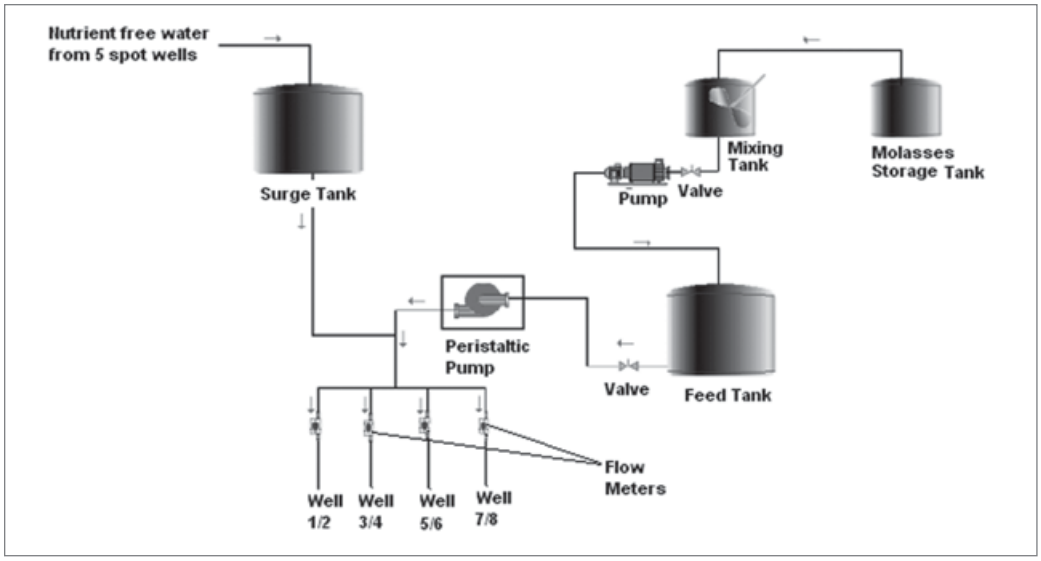


Exhibit 5. Flow diagram of nutrient injection in the field

pump was set at the rate of 0.6 gallons per minute (gpm), and the total feed rate injected into each of the wells was maintained at approximately 5.5 gpm. During this step, the barrier construction wells BC-1, BC- 3, BC-5, and BC-7 and the Center5 spot well were constantly pumped and fed to the surge tank to maintain the mass balance of water in the tank (flow in = flow out), thereby maintaining the tank volume.

After injecting the desired amount of nutrients into the wells BC-2, BC-4, BC-6, and BC-8, the extraction pumps were pulled out and nutrients were then injected into wells BC-1, BC-3, BC-5, and BC-7. In this case, the surge tank was filled using only the water from the five spot wells. Hence, lower flow rates for injection had to be used. The injection process was followed by injecting "clean" water from the five spot wells into all BC wells at ~4.5 gpm for 30 minutes to drive the nutrients from the inside and surroundings of the well to prevent biofouling. Water at 290 mg/L from the five spot wells and free from nutrients was continuously injected into the Center Well during all injection operations at a rate equivalent to the injection rate into the other wells. The process prevented the nutrients from coming toward the Center Well, resulting in a biobarrier with the desired circular pattern. At the end of the injection phase, the system was given a two-week incubation period to allow the bacteria to establish the biobarrier.

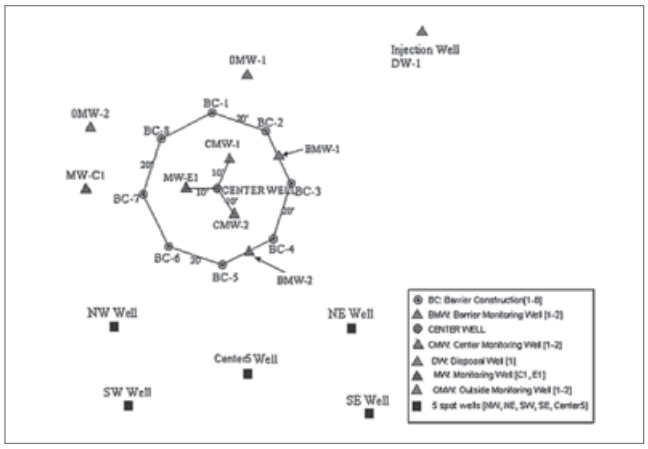


Exhibit 6. Schematic diagram showing location of wells

Operating and Monitoring System

The Center Well was continuously used to produce treated water at 1 gpm. The treated water was returned to the aquifer using injection well DW-1, located more than 300 feet away from the region of the barrier as shown in Exhibit 6. This setup resulted in a hydraulic gradient between the biobarrier zone and the surrounding aquifer that allowed the continuous inflow of contaminated water across the denitrifying biobarrier.

Monitoring wells were installed inside (CMW-1, CMW-2, and MW-E1), on (BMW-1 and BMW-2), and outside (OMW-1, OMW-2, and MW-C1) the circular biobarrier, as shown in Exhibit 6. Groundwater samples from the Center Well and the monitoring wells were assayed using YSI 6920 groundwater environmental monitoring system and Dionex ion chromatography equipment over a period of several months for nitrate (as nitrogen) concentrations.

RESULTS AND DISCUSSION

The nitrate values in the Center Well dropped from 275.1 mg/L nitrate (as nitrogen) at the start of the experiment to a value of < 0.05 mg/L (MCL $=$ 10mg/L) during the course of the operation. The concentrations of nitrate detected in samples from the Center Well are shown in Exhibit 7.

As shown in Exhibit 7, the initial nitrate reduction was rapid. Nitrate-nitrogen values dropped from 275 mg/L to 64mg/L in the first 57 days. The subsequent rise may be due to an organic carbon limitation in the biofilm barrier. Nutrients were reinjected after intervals of 86 and 196 days from the start of the operation, also shown in Exhibit 7. The experiment had a total runtime of 401 days, 74 days of which the system remained shut down. The cease in operations was due to two nutrient reinjection operations and biofouling in the Center Well, resulting in buildup of biomass on the Center Well's pump, which caused it to stop functioning.

The nitrate (as nitrogen) concentrations in the monitoring wells with time are shown in Exhibit 8. The concentrations of nitrate detected in monitoring wells inside (CMW-1, CMW-2, and MW-E1) the biofilm barrier dropped significantly, while the

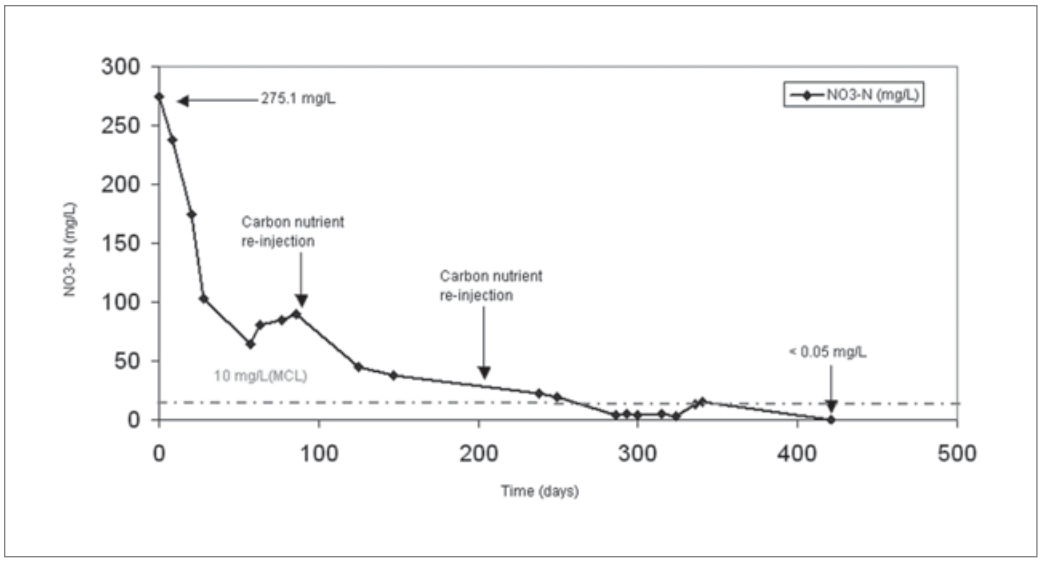


Exhibit 7. Nitrate (as nitrogen) concentration in the Center Well with time

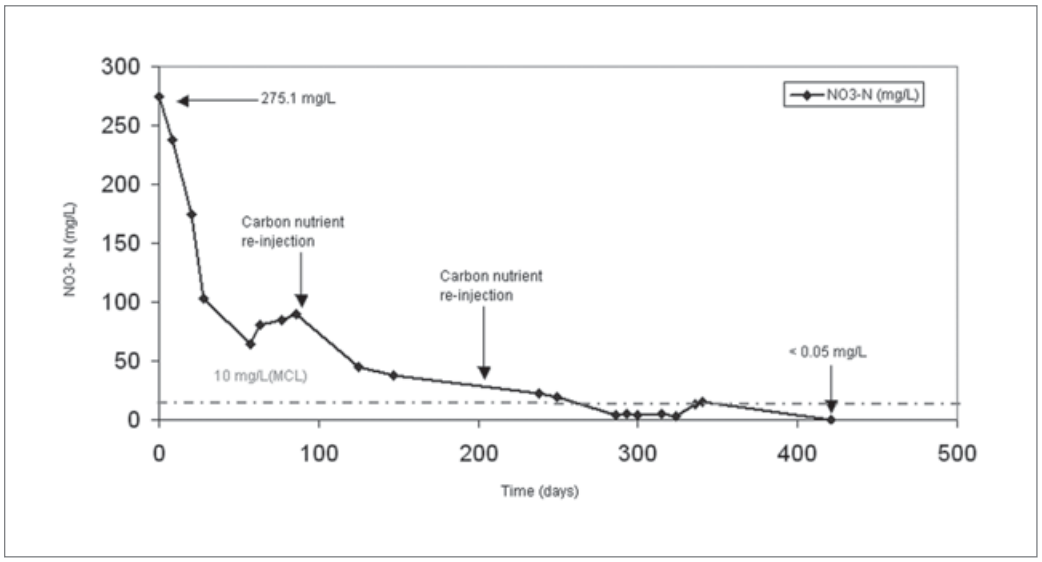


Exhibit 8. Nitrate (as nitrogen) concentration in monitoring wells BMW-1, BMW-2, CMW-1, CMW-2, MW-E1, OMW-1, OMW-2, and MW-C1

concentrations in the wells outside the barrier (OMW-1, OMW-2, and MW-C1) remained consistent with the initial values. Thus, these results demonstrate the biofilm barrier's ability to denitrify groundwater.

OPERATIONAL EXPERIENCE/ISSUES

Biofouling

During the process, biofouling was seen as one of the major challenges to the operation. The accumulation of biomass occurred around well screens as well as in the pipeline

from the Center Well. Coating and clogging of surfaces resulted in an overflow of the disposal well and temporary suspension of the operation of the pump used to extract water from the Center Well. These fouling problems began occurring around four months after the start of operation. Excess biomass was removed from the disposal well and associated piping by using air pressure to lift the fouled water column out of the well, then blowing the biomass out of the well. After blowing as much biomass as possible, the well was surged several times with water and bleach solution to destroy biomass around the well bore. Biweekly additions of 1.5 gallons of bleach to both the disposal and the Center Well were scheduled thereafter.

Nutrient Reinjection

To maintain the barrier, it had to be revived multiple times by adding the amendment mixture into the eight perimeter wells. The amount of amendment injected and the duration before each reinjection was calculated based on stoichiometric calculations. The reinjection procedure was similar to the biobarrier formation process, except that the continuous injection of nutrient-free water into the Center Well was not performed.

SUMMARY AND CONCLUSIONS

An *in situ* biobarrier was developed at a site located in the South Valley of Albuquerque, New Mexico. The curtain was developed by stimulating the indigenous bacteria using molasses as the carbon substrate. The results of the study indicate that the biobarrier was able to contain and remediate nitrate concentrations 30 times over the MCL, thus demonstrating the technology as a promising approach for the treatment of contaminated groundwater. The ability to direct groundwater flow using a biobarrier could be used to channel contaminated groundwater to an active treatment zone while also contributing to bioremediation of the water. In situations where groundwater flow is minimal, pumping strategies to draw the contaminated groundwater into an active treatment zone could be enhanced with biobarrier technology. This technology has commercial value for assisting agricultural businesses, such as feedlots, hog farms, and fertilizer suppliers in reducing their environmental impact and ensuring the availability of safe drinking water. Since the barrier is constructed of a very low-cost carbon source (i.e., molasses at $0.17/pound) and given that the barrier can be rejuvenated many times, this technological approach to *in situ* bioremediation will have significant cost advantages.

REFERENCES

Chen, J. (2000). Continuous biodenitrification of groundwater: In situ field test. Unpublished master's thesis, Chemical and Nuclear Engineering Department, University of New Mexico.

Costerton, J. W., Lewandowski, Z., Caldwell, D. E., Korber, D. R., & Lappin-Scott, H. M. (1995). Microbial biofilms. Annual Review of Microbiology, 49, 711–745.

Cunningham, A., Sharp, R., Hiebert, R., & James, R. (2004). Fundamentals and applications of starved cell sub-surface biological barriers. Journal of Bioremediation. 7(4-3), 1–13.

Cunningham, A., Warwood, B., Sturman, P., Horrigan, K., James, G., Coesterton, J. W., et al. (1997). Biofilm processes in porous media—Practical applications. In P. S. Amy & D. L. Haldesman (Eds.), The microbiology of the terrestrial deep subsurface (pp. 325–344). Boca Raton, FL: Lewis Publishers.

Cunningham, A. B., Characklis, H. M., Abedeen, F., & Crawford, D. (1991). Influence of biofilm accumulation on porous media hydrodynamics. Environmental Science and Technology, 25, 1305–1311.

Deng, L. (1998). In situ biological denitrification of groundwater. Unpublished master's thesis, Chemical and Nuclear Engineering Department, University of New Mexico.

Dutta, L. (2004). Pilot scale testing of in situ biofilm barrier for remediation of groundwater. Unpublished master's thesis, Chemical and Nuclear Engineering Department, University of New Mexico.

Hiebert, R., James, G., Sharp, R., & Cunningham, A. (2001, August). Development and Demonstration of Subsurface Biofilm Barriers Using Starved Bacterial Cultures. Association of Environmental Health and Sciences (AEHS) Contaminated Soil Sediment and Water, International Issue, pp. 45–50.

James, G. A., Warwood, B. K., Cunningham, A. B., Sturman, P. J., Hiebert, R., & Costerton, J. W. (1995). Evaluation of subsurface biobarrier formation and persistence. Proceedings of the 10th Annual Conference of Hazardous Waste Research, Great Plains–Rocky Mountain Hazardous Substance Research Center, Manhattan, KS, pp. 82–91.

James, G. A., Warwood, B. K., Hiebert, R., & Cunningham, A.B. (2000). Microbial barriers to the spread of pollution. In J. J. Valdes, Bioremediation (pp. 1–14). Boston: Kluwer Academic Publishers.

James, G. A., Warwood, B. K, Horrigan, K., Hiebert, R., Cunningham, A. E., & Coesterton, J. W. (1996). Containment of heavy metal and chlorinated organic solvent contamination using microbial barriers formed in packed-sand columns. Presented at the 96th General Meeting of the American Society of Microbiology, New Orleans, LA. Washington, DC: American Society for Microbiology.

Nuttall, H. E. (1997). Biodenitrification of New Mexico ground water. New Mexico Journal of Science, 3, 64–73.

Nuttall, H. E. (2000, June). Research and development. In Interstate Technology and Regulatory Council (ITRC), Emerging technologies for enhanced in situ biodenitrification (EISBD) of nitrate-contaminated groundwater: Technology overview (pp. 17–23). Washington, DC: ITRC.

Nuttall, H. E., Lutze, W., Barton L. L., & Wolfram, J. (1997). In situ bioremediation of contaminated ground water at an UMTRA site. International Symposium In Situ and On Site Bioreclamation Proceedings, pp. 435–440.

Lomesh Dutta works at Kleinfelder Inc. in Sacramento, California. Prior to this, he obtained a master's degree in chemical engineering from the University of New Mexico, where he worked with Dr. Eric Nuttall on biofilm barriers as a part of his master's degree research.

H. Eric Nuttall is a professor in chemical engineering at the University of New Mexico. His areas of research include bioremediation and nanotechnology applications to water cleanup. He is active in the Interstate and Technology Regulatory Council and teaches Internet training. Dr. Nuttall consults in the area of *in situ* bioremediation.

Al Cunningham is a professor of civil engineering and also directs the Research and Education Initiatives program for Montana State University's Center for Biofilm Engineering. He has developed a cross-disciplinary research program in hydrodynamics and microbial process engineering with focus areas that include: bioremediation of contaminated groundwater and soil, bioavailability of organic compounds and metals, microbial transport, and biofilm accumulation in porous media and closed conduits. Current research sponsors include the National Science Foundation, the Environmental Protection Agency, the Department of Energy, the Army Research Office, MSE Technology Applications Inc., Conoco Inc., and the Inland Northwest Research Alliance. Dr. Cunningham has developed graduate- and undergraduate-level projects and courses that investigate environmental microbial process problems of concern to industry and society. Industrial and government participants provide suitable projects and progress reviews.

Garth James was a staff microbiologist at MSE Technology Applications Inc. in Bozeman, Montana, during this project. Dr. James holds a BS degree in microbiology from the University of Saskatchewan and a PhD in microbial ecology from the University of Calgary. Dr. James has extensive experience in environmental microbiology, biofilm microbiology, project management, and working with multidisciplinary teams. Dr. James is currently employed by the Center for Innovation in Bozeman, Montana, and Montana State University.

Randy Hiebert, P.E., is the biotechnology manager at MSE Technology Applications Inc. He has both BS and MS degrees in chemical engineering from Montana State University. Mr. Hiebert has been a project manager for MSE on several projects, including the Mine Waste Technology Program Nitrate Destruction Project. This project consisted of biologically destroying nitrate-contaminated wastewater emanating from the TVX Mineral Hill Mine near Jardine, Montana. Mr. Hiebert was also the project manager for the Department of Energy Biofilm Barrier Projects and several pollution prevention and waste minimization projects for the U.S. Army Construction Engineering Research Laboratory.