REMEDIATION OF NITRATE-CONTAMINATED GROUNDWATER USING A BIOBARRIER

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ABSTRACT

A biobarrier system has been developed for use in remediating shallow alluvial groundwater. This barrier is made from highly porous materials that are relatively long-lasting, carbon-based (to supply a limiting nutrient in nitrate destruction, in most cases), extremely inexpensive, and easy to emplace. In a series of laboratory studies, we have determined the effectiveness of this barrier at destroying nitrate and perchlorate in groundwater from Mortandad Canyon at Los Alamos National Laboratory (LANL). This groundwater was obtained from a monitoring well, MCO-5, which is located in the flowpath of the discharge waters from the LANL Radioactive Liquid Waste Treatment Facility (RLWTF). Water with elevated nitrate levels was discharged from this plant for many years. Recently, the nitrate levels have been brought under the discharge limits. However, the historical discharge has resulted in a nitrate plume in the alluvial groundwater in this canyon. The LANL Multi-Barrier project was initiated in 1999 to develop a system of barriers that would prevent the transport of radionuclides, metals, colloids and other contaminants, including nitrate and perchlorate, further down the canyon in order to protect populations down-gradient. The biobarrier will be part of this Multi-Barrier system. We have demonstrated the destruction of nitrate at levels up to 6.5-9.7 mM nitrate (400-600 mg/L), and that of perchlorate at levels of about 4.3 µM perchlorate (350 ppb). We have quantified the populations of microorganisms present in the biofilm that develops on the biobarrier. The results of this research will be discussed along with other potential applications of this system.

INTRODUCTION

Background.

Aquatic ecosystems and human populations worldwide are affected by contaminated water supplies; one of the most frequent contaminants is nitrate. Remediation of nitrate in groundwater and drinking water by biodegradation is an obvious solution to this problem, and technologies are being developed to provide this solution. One such technology involves the use of a biobarrier system where the barrier materials are placed in the path of a nitrate plume, and the contaminated water is cleaned as it passes through the barrier. The catalyst for the destruction of the nitrate is the indigenous microbial community, which uses nitrate as both a

nitrogen source, and as an electron acceptor under denitrifying conditions. Frequently, the only missing element for optimal performance is carbon, which is supplied by the biobarrier material.

Microbial processes play an extremely important role in *in situ* groundwater treatment technologies. The assumption of carbon limitation is the basis for addition of carbon-based substrates to a system, in the development of bioremediation schemes for nitrate-contaminated groundwater. Although many materials have been proposed for this use, we are aware of only one system being tested at field scale in a barrier-style test cell using sawdust waste from the lumber industry, with zero-valent iron as the primary reactant for the nitrate destruction. It is unclear how microbial processes are involved in this system. The materials under investigation in our work have advantages over such a system in that they are much less likely to become hydrologically restrictive to groundwater flow because of the material size and high porosity. In addition, these materials are very slowly degraded, they avoid the use of a potentially toxic heavy metal, and replacement is likely to be unnecessary over a long time period.

Conceptual basis for biobarrier technology.

The biobarrier concept typically involves construction of a wall of porous material that is placed in a trench perpendicular to the direction of groundwater flow, and extending at least the width and depth of the contaminant plume. A biobarrier can be used as a stand-alone system, when biodegradable materials are the only contaminants, or it can be used along with other barriers as has been done in the LANL Multi-Barrier project. The groundwater system must be reasonably well characterized in terms of direction of flow, width and depth of plume, concentrations along the plume, flow velocity and hydraulic conductivity. Barrier technology is largely applicable to shallow, alluvial plumes (less than 20 feet deep). Under these conditions, a barrier could be placed across the plume downstream from the source. Frequently the source of a nitrate plume is not well characterized, or the flow path of the plume has not been fully characterized, so the optimal placement of a barrier is difficult to assess. Placement close to the source would be most desirable in order to prevent the spread and dilution of the contaminant that is typical of groundwater plumes.

It is most cost-effective to utilize organic waste materials that are relatively resistant to degradation over the long term. If this is not possible, resources may be consumed replacing the barrier materials. Pecan shells are a significant waste problem for the pecan industry, the current solution is land disposal. Their use in biobarriers would be a desirable alternative to this costly form of disposal. Pecan shells are composed primarily of cellulose and lignin; thus, they degrade very slowly, and can provide a "time-release" carbon source. If left uncrushed, they can provide a material with high porosity. Fishbone hydroxyapatite is a waste product of the fishing industry. Fish bones are made of calcium phosphate, or apatite, and are very resistant to deterioration. Apatite has the ability to remove dissolved metals and radionuclides from groundwater simultaneously (1). The precipitates formed with these metals and radionuclides are highly insoluble and very unlikely to be leached subsequently from the barrier. The organic matter (residual tissues of the fish) associated with the apatite provides nutrient materials that

could result in formation of a microbial population capable of denitrification of groundwater as well.

Nitrate-related health effects.

Nitrate is a contaminant that is widespread in the industrialized world and agricultural areas. Nitrate in high concentrations can lead to depletion of oxygen in the bloodstream resulting in methemeglobinemia, often referred to as "blue baby syndrome," which can be fatal. Nitrate intake from drinking water also has been linked to increased rates of mortality from gastric cancer (2). Nitrate is a highly oxidized molecule. Thus it is very reactive and can lead to production of reactive species, such as free radicals, in the body with reasonably well known carcinogenic effects. The other important aspect of nitrate in the environment is the rate at which human inputs of fixed nitrogen have grown in recent years. Vitousek et al. (3) has shown that anthropogenic nitrogen fixation now exceeds all natural sources. Biobarrier technology provides a potential effective, viable solution to protect surface waters and drinking waters from contamination without major cost or disturbance of the environment.

Biofilms and denitrification.

Although microbiologists have been studying the denitrification process under laboratory conditions using pure species and controlled populations for a long time, the study of biofilms, mixed populations of unknown microorganisms growing together on the surface of a solid material, is a relatively new field. The species composition of the microbial population that develops in a given biofilm will be dependent on a number of different factors, including, but not limited to: water availability, presence/absence of oxygen, nutrient and contaminant concentrations, pH, Eh, location of the biobarrier, presence of co-contaminants and water flow rate.

Nitrogen cycling and denitrification pathways.

The biogeochemical cycling of nitrogen is highly dependent on microbial processes (2,4,5). Two primary nitrate reduction processes are termed assimilatory and dissimilatory nitrate reduction. Assimilatory nitrate reduction (ANR) is a process that provides nitrogen to the microbial cell in the form of ammonia and other reduced organic nitrogen end products (i.e. amino acids, nucleic acids), which allow the cells to grow and reproduce. The enzymes involved are not inhibited in the presence of oxygen, but are regulated by ammonia concentration. High levels of ammonia in the environment do not occur due to the rapid incorporation of ammonia into the cell, and feedback inhibition by ammonia on the assimilatory nitrate reductase enzyme. Dissimilatory nitrate reduction (DNR), or nitrate respiration, occurs in the absence of oxygen. Nitrate acts in place of oxygen as a terminal electron acceptor. The presence of nitrate in contaminated water along with oxidized metals such as iron [Fe(III)] or uranium [as uranyl, U(VI)] has been shown to inhibit reduction of these metals until the nitrate has been reduced completely (6,7). This is because nitrate is second only to oxygen in the amount of energy that is

derived by the microbial cell as it is reduced, and this reduction occurs at a higher redox potential than any of these metals or radionuclides.

There are two types of dissimilatory nitrate reduction. Only one is termed "true" denitrification. The first process is carried out by many different species of facultatively anaerobic bacteria found in soil and sediments, where nitrate is reduced to nitrite only. The resulting nitrite is either excreted, or is reduced via hydroxylamine to ammonia (the nitrate ammonification process) under appropriate conditions. However, it is an environmentally less significant process for the reductive removal of nitrate. Its importance appears to be limited by the number of reducing equivalents that must be consumed in the system. True denitrification, the second process, is carried out in soil primarily by *Pseudomonas* and *Alcaligenes* spp., although many other genera (e.g. *Rhizobium*, *Azospirillium*) are known to denitrify under certain conditions. Nitrate is sequentially converted as shown in Equation 1 in this process, where a different specific enzyme catalyzes each step in the chain.

The microbial conversion of nitrate to reduction products by the dissimilatory process is illustrated as follows with the oxidation state of nitrogen shown:

$$\underset{+5}{NO_3^-} \text{ ---> } \underset{+3}{NO_2^-} \text{ ---> } \underset{+2}{[NO]} \text{ ---> } \underset{+1}{N_2O} \text{ ---> } \underset{0}{N_2} \qquad \text{(Eq. 1)}$$

NO is shown in parentheses because there is some controversy concerning the formation of NO in this process. In any case, it is rapidly converted to N_2O, making it difficult to detect, and to determine what role it plays in the process. Many organisms of the first type have the enzyme systems (i.e., the first two in the chain, nitrate reductase and nitrite reductase) to produce only NO (which is reduced to N_2O abiotically). Other organisms, the "true" denitrifiers, have enzyme systems to carry the reduction to completion (i.e. nitric oxide reductase and nitrous oxide reductase), producing N_2 as the final gaseous product. In organisms that produce N_2 from nitrate, N_2O will be an intermediate in the denitrification process. Under typical environmental conditions, denitrification is the only process whereby nitrate is reduced to N_2O or N_2.

Denitrification enzymatic processes.

In a recent review of the literature on denitrifying enzyme processes (8), the author has demonstrated that the processes are not as simple as might be expected. For instance, there is a group of microorganisms that use nitrous oxide in a respiratory process as a terminal electron acceptor, without the presence in the cell of the other enzymes in the denitrification pathway. The end products of denitrification are usually a mixture of nitrous oxide and nitrogen gases, which are lost to the atmosphere, resulting in depletion of combined nitrogen in the soil environment.

It is the "true" denitrification process that is most relevant to bioremediation processes that can occur in a biobarrier. Conditions must be established to enhance the development of the denitrifying population, and to maintain this population at a level that will not result in loss of permeability of the barrier (i.e. plugging). It is critical to the success of such a barrier that the processes and the populations of microorganisms be well understood. Although the denitrification process has been studied for years, a whole-system approach to the study of microbial processes and the compartmentalization of the original nitrate is important to demonstrate the fate of the contaminant. Simple microbial reduction to ammonia in a flowing water system will not remove the contaminant from the system. It remains available for nitrification processes to re-oxidize the nitrate as oxygen again becomes available downstream of the barrier.

Whole-system studies of denitrifying populations.

A whole-system study of microbial populations is described by Lemmer et al. (9) using a denitrification system that provided methanol as a substrate. Denitrification using methanol as a substrate is not a recent development. It has been used in wastewater treatment systems for several decades. These systems rely on the development of a microbial biofilm, and usually involve the use of a fixed-bed reactor or sand filter to support the biofilm (9,10). The populations of bacteria present in such systems were recently characterized by Lemmer et al. (9) and Neef et al. (11) and have been shown to include Gram-negative bacteria from the α, β and γ subclasses of the Proteobacteria class. Enterobacteriaceae and Moraxellaceae, members of the γ subclass were present, but represented only a few species and a small fraction of the population. Contrary to the common denitrifying microbial species in soil, most of the denitrifiers in this engineered system turned out to be members of the β subclass, primarily members of the genera *Hydrogenophaga* and *Comamonas*, and the α subclass, primarily *Paracoccus* and *Hyphomicrobium*. Carbon substrate fed into the system had a large effect on selection of the microbial populations that were established in this ecosystem. Many of the members of the α subclass were facultative methylotrophs, which use reduced carbon substrates without C-C bonding such as methanol, methane, and methylamine or methylated sulfur compounds. The ability to use methanol as a carbon source appears to provide a selective advantage to these species.

We anticipate that only part of the total microbial population will be active in the primary role of nitrate reduction in our systems. Other species, primarily the common aerobic soil bacteria, will positively impact the system by utilizing the dissolved oxygen in the influent water, and providing anaerobic conditions under which the denitrifying bacteria are able to function. These aerobes can also negatively impact it by using up nutrients and reducing the conductivity of the system. Because this research involves real environmental systems, it is important to understand the effects of both the environmental factors and the nutrient conditions present in the system on the microbial populations. There are numerous studies of bioremediation systems for many different contaminants; however, few have studied the catalysts for the processes, i.e. the microbial populations, in any great detail as was done by these researchers. Further studies

using our system are planned to identify the major microbial populations, to determine the eventual fate of the nitrogen in these systems, and to determine the effect of environmental parameters such as pH, Eh, DO, and concentrations of nutrients (C, N, P).

Biobarrier investigations completed.

We have investigated denitrification processes in a biobarrier system that uses highly porous waste materials to provide support for microbial populations. We have performed both batch and column studies. The batch studies involved comparison of two potential carbon-based biobarrier support materials, pecan shells alone, and pecan shells mixed with solid nutrient material (dog food) in a 10:1 ratio. The results of the column studies have been reported elsewhere (12,13). The column studies were performed using a selected biobarrier support material and were used to confirm that the microbial reactions occurring under denitrifying conditions in the batch studies are not altered dramatically under flowing conditions with the subsequent introduction of oxygenated groundwater. The objectives of the batch studies were to: 1) determine the effectiveness of the support materials in development of a biofilm, and in destruction of nitrate, 2) quantify the microbial populations that were present in the batch systems, 3) determine the amounts of nitrite and ammonia produced by each system, and 4) determine the conditions produced with respect to pH by the microbial activity in the biobarrier. An additional objective of these experiments was to determine if these systems would be effective at reducing perchlorate in the groundwater, although all of the analytical interferences for perchlorate in these systems have not been elucidated.

EXPERIMENTAL METHODS

Biobarrier support materials.

This project evaluated two different sets of materials to identify the most appropriate configuration for the biobarrier component of the Multi-Barrier system. The biobarrier support materials evaluated consisted of 1) pecan shells, and 2) pecan shells and dog food. The water used in each experiment was obtained from the LANL groundwater monitoring well MCO-5, located in Mortandad Canyon, Los Alamos, NM.

Degradation experiments.

Two sets of experiments were performed to determine the amount of bacterial growth and contaminant degradation in nitrate (NO_3)-contaminated water using these components. Each experiment contained four different sets of samples: 1A/B) the water and the support material were both sterilized (sterile control), 2A/B) the support materials were sterilized, the water was non-sterile (i.e. provided the bacteria), 3A/B) the water was sterilized, the support material was not, and 4A/B) both the water and the support material were non-sterile. Duplicate samples were run under each set of conditions in each experiment. The filtered water was also analyzed with each experiment as sample 5A/B.

For each experiment, pecan shells (2 g), or pecan shells and dog food (2 g/0.2 g) were loaded into polycarbonate test tubes and 20 mL of well water was added for a solution/solid ratio of 10:1 (and a pecan shell/dog food ratio of 10:1). Larger scale experiments were also performed using 100 ml of water and 10 g (or 10 g/1 g) solids in culture bottles. Reaction containers were incubated at room temperature on a shaker. Successive samples were taken on the day after the tubes were loaded (Day 1), and on Day 2, Day 7, Day 14 and Day 21 in most cases. Samples were taken from several experiments for analysis of microbial populations, nitrate, nitrite, ammonia, perchlorate and pH.

The concentration of contaminants in Mortandad Canyon groundwater is about 4.3 µM (or 350 ppb) perchlorate and around 0.5 mM nitrate (~0.1 mM NO_3-N). This water was used in various experiments unsupplemented, or supplemented with higher concentrations of nitrate, up to 9.7 mM nitrate (2.2 mM NO_3-N).

Microbial cell counts.

Detection of active microorganisms can be difficult since no single analytical method will identify all physiological types (5). Since the denitrifying population is most relevant to the destruction of nitrate in our system, we chose to use an assay for viable, culturable organisms that relies on the ability of the cell to use nitrate under slightly anaerobic conditions. The Most Probable Number (MPN) analysis used involves the use of a nitrate-reducing MPN method developed from methods found in Microbiological Methods 7th edition (14).

Populations of bacteria in our system consist of both suspended and attached populations. The initial series of experiments were designed to determine if we were enhancing the growth of either population, so only the suspended cell counts were determined. Microbiological media selected for cultivation of the microorganisms utilized a formulation for the detection and enhancement of nitrate-reducing organisms (denitrifiers) as developed by Atlas (15). We chose to use a denitrifying MPN counting method to provide an estimate of the viable and culturable denitrifying cell counts in this suspended population.

Nitrogen compound analysis.

Nitrate and nitrite were determined using an ion chromatographic method (EPA method 300.0). The detection limit for nitrate is 0.5 µM (NO_3-N) and for nitrite is 0.9 µM (NO_2-N). A Dionex DX-800 chromatograph equipped with an AG14/AS14 anion column with a suppressed conductivity detector was used for these analyses. The system is a component of an on-line monitoring system used for monitoring of the discharge water from the Radioactive Liquid Waste Treatment Facility (RLWTF) at LANL (16). Ammonia was determined similarly with a CG12A/CS12A cation-exchange column, and conductivity detection, with a detection limit of around 5.6 µM NH_4^+ (17).

Perchlorate analysis.

Perchlorate was measured as ammonium perchlorate using ion chromatography. The EPA does not currently regulate perchlorate in drinking water, however, the State of California has adopted 0.22 µM (18 ppb or µg/L) as an acceptable level for drinking water. The EPA is expected to adopt this level as the regulatory limit for drinking water in the US. The ion chromatographic method uses an IonPac AS11 column and suppressed conductivity detection to quantify perchlorate down to a detection limit of about 0.03 µM perchlorate.

Other analyses.

The pH of samples was determined using a three-point calibration at 4, 7 and 10, and an Orion pH meter.

RESULTS AND DISCUSSION

Nitrate degradation.

Experiments with levels of nitrate from ~0.5 to 9.7 mM of nitrate produced effective degradation to below detection using both the pecan shell and pecan shell/dog food biobarrier systems. The primary difference in all of these experiments lies in the length of time required in the batch experiments to fully degrade the nitrate. The batch experimental degradation rate can be used as a predictor of the rate expected in the field system. However, the ratio of water to solids (10:1) in the batch system was selected in order to have adequate solution for analysis. The actual liquid/solid ratio in the field will be much lower, therefore the batch results can be viewed as a conservative estimate of the degradation rates since the amount of nitrate present at a given time will be smaller. A second difference between the two systems was an increase in the levels of ammonia produced in the process in the presence of dog food.

Figures 1 and 2 show the results using pecan shells alone and pecans shells with dog food respectively, with Mortandad Canyon groundwater supplemented with 9.7 mM of nitrate. Experimental results (not shown) using the unsupplemented water indicate that 2 days is sufficient to destroy the ~0.5 mM of nitrate present using either support material system.

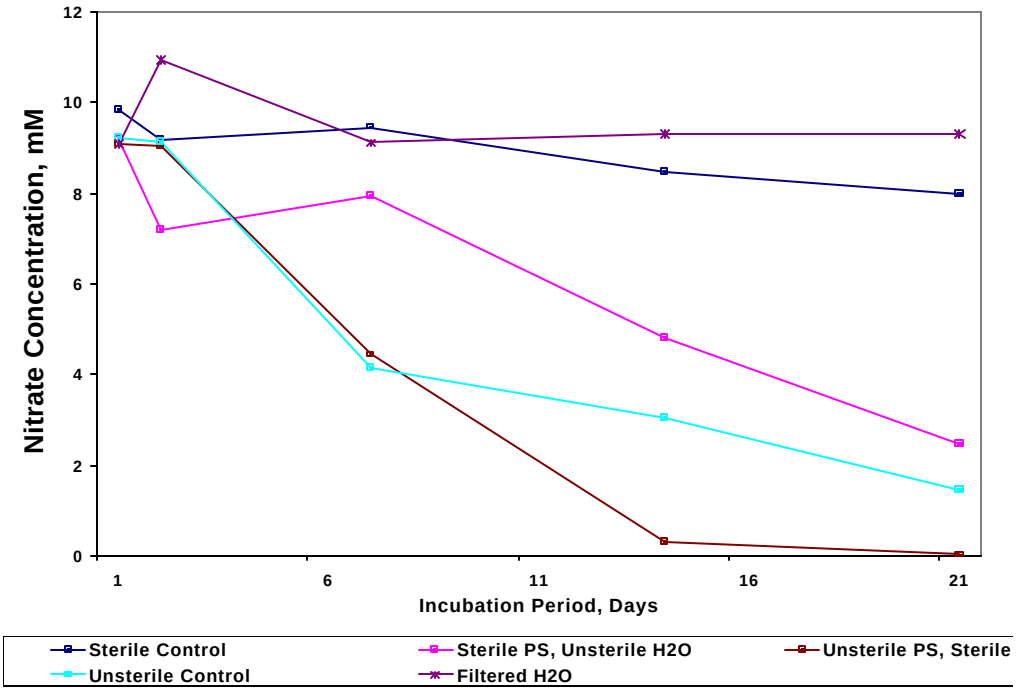

Fig. 1. Biodegradation of nitrate in Mortandad Canyon groundwater supplemented with 9.7 mM nitrate using a pecan shell biobarrier support material.

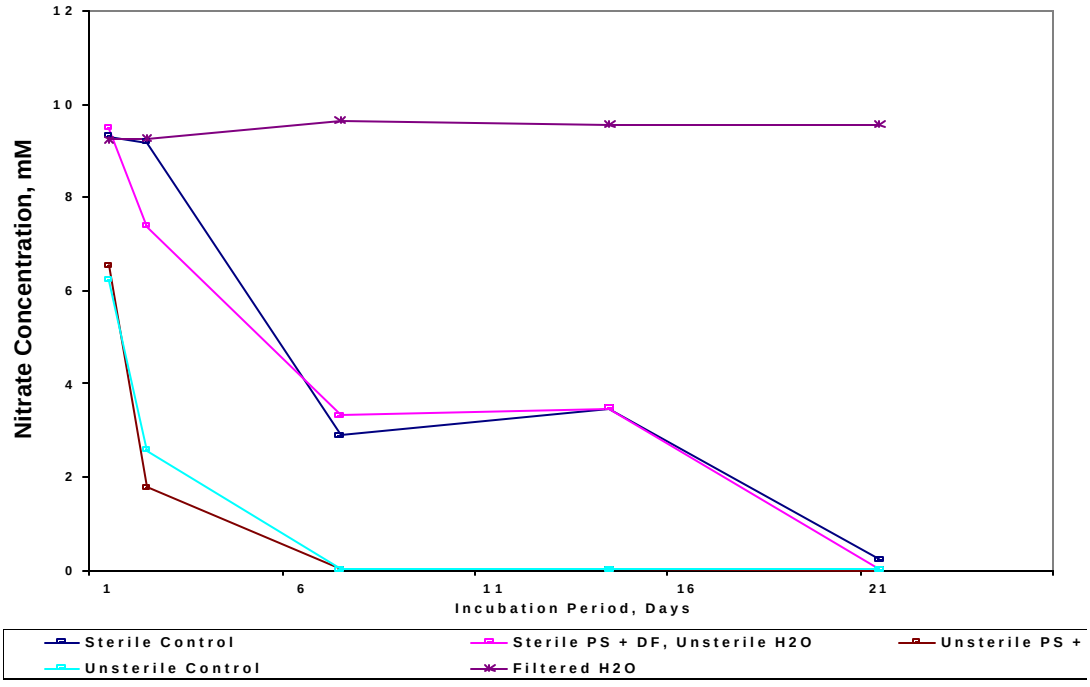

Fig. 2. Biodegradation of nitrate in Mortandad Canyon groundwater supplemented with 9.7 mM nitrate using a pecan shell and dog food biobarrier material.

The development of nitrate degradation products within the biobarrier is an important consideration for this technology, especially if they are not completely degraded. Therefore, we measured the concentrations of nitrite and ammonia in each system as part of the experiments with 9.7 mM nitrate. The results for the unsterilized sample (i.e. 4A/B) from both the pecan shell and the pecan shell/dog food systems are presented in Tables I and II.

Table I. Accumulation of nitrate degradation products in a pecan shell biobarrier system.

Pecan Shell – 9.7 mM nitrate in MCO-5 Water			
Day	**Nitrate (mM)**	**Nitrite (mM)**	**Ammonia (µM)**
1	9.20	0	11
2	9.10	0	11
7	4.10	1.3	100
14	3.00	0.7	133
21	1.50	1.3	106

Table II. Accumulation of nitrate degradation products in a pecan shell/dog food biobarrier system.

Pecan Shell and Dog Food – 9.7 mM nitrate in MCO-5 Water			
Day	**Nitrate (mM)**	**Nitrite (mM)**	**Ammonia (µM)**
1	6.22	2.3	156
2	2.58	4.1	217
7	0.01	0.9	778
14	0.02	1.5	794
21	0.02	1.9	806

The nitrate in both of these systems was reduced to low levels, but the addition of dog food to the pecan shells provided a much faster rate of reduction. Figure 2 shows that the reduction of the nitrate was complete in the companion control with unsterile pecan shells and sterile water. It is not clear why we did not get full reduction in the sample with both unsterile pecan shells and water, however, we are confident that this is a result of experimental variation only. In both systems, nitrite accumulated, but eventually also was reduced to approximately one-tenth of the original nitrate concentration, showing that it is not accumulating to high concentrations. With time, it is expected that all of the nitrite would be reduced to background levels in these systems.

There is a difference between the pecan shell system and the pecan shell/dog food system in the amount of ammonia that is generated and that accumulated in the water. The addition of dog food increases about 7-fold the amount of ammonia generated in comparison to the pecan shells alone. However, the levels that accumulated in these batch tests were an extremely small fraction of the original nitrate added, and should present no problems in the

treated groundwater, as this amount would be rapidly used up by the indigenous soil bacteria when the water moves out of the biobarrier.

It is clear that the addition of dog food enhances the rate of degradation of the nitrate in this groundwater. However, it is not clear that the benefits in terms of the degradation rate increase are great enough to warrant the extra difficulty involved in mixing the dog food in with the pecan shells prior to emplacement. The nitrogen degradation product accumulation results determined at the highest level of nitrate tested demonstrate that using pecan shells alone produces far less nitrite and ammonia, and therefore should be a better selection for the biobarrier material in an *in situ* system.

These batch studies have been validated using both small and large-scale columns run in series with the other materials in the Multi-barrier system under variable flow conditions (12,13). The large-scale column and an additional 2-dimensional box system are currently under investigation. The results will be presented in a future publication.

Perchlorate degradation.

The results of the perchlorate degradation studies are not as clear as are those of the nitrate. There is a great deal of evidence that the perchlorate is degraded in both the pecan shell and pecan shell/dog food systems. There are also problems with interference in the analytical system from the highly colored organic material that is leached from the shells as they are incubated. We have developed a method to remove the organic material using a resin filter for petroleum hydrocarbons, since the leached organic compounds are likely to be similar in composition to many polyaromatic hydrocarbon compounds. More investigation and validation of the analytical results are necessary before we will feel comfortable that the observed pechlorate degradation results are real.

However, an example of the existing evidence for degradation of perchlorate in these systems is shown in Figure 3. This experiment used groundwater supplemented with 6.5 mM of nitrate. The filtered water sample in this figure demonstrates that the water has about 4.3 µM of perchlorate present at each time point. In all of the other samples, it appears that we are making perchlorate at levels up to almost 61 µM in the biobarrier system, something that is most likely analytical interference and not true perchlorate, as it is not logical that bacteria could make perchlorate under nitrate-reducing conditions. However, after 7 days, the three samples with microbial activity have all reduced the perchlorate levels to the detection limit, in this case, 0.004 µM. These results indicate that the biobarrier would be capable of achieving clean up of the water to below the California and EPA regulatory limits. The investigation of the necessary analytical method adjustments to solve the interference problems and produce validated data is continuing.

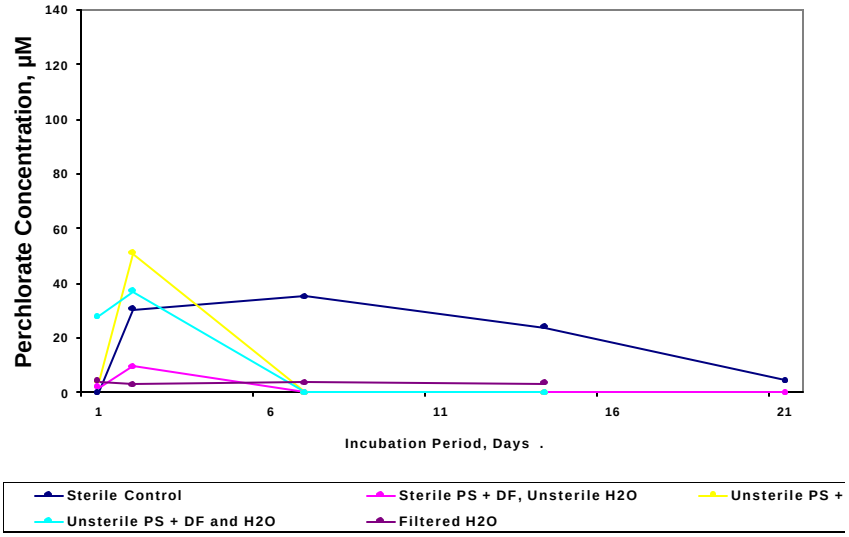

Fig. 3. Degradation of perchlorate in Mortandad Canyon groundwater supplemented with 6.5 mM of nitrate.

Microbial population analyses.

The nitrate-reducing MPN estimates of cell numbers were determined at each time point for each sample during several experiments. The cell numbers in the suspended population were determined, while those that adhered to the pecan shells were not measured. The population of adhering cells will be enumerated following extraction from the shells and the results will be reported separately. MPN results for an experiment in which unsupplemented MCO-5 water was used are presented in Table III. Results are reported for each day when samples were taken. Dilutions were prepared for the MPN analyses in series from 1000- to 100,000-fold or greater, if necessary. Results for the pecan shell/dog food experiments were similar and are not shown.

Table III. Denitrifying Most Probable Number (MPN) cell counts (cells/mL) in unsupplemented nitrate-contaminated Mortandad Canyon groundwater.

Sample Identification	Day 1	Day 7	Day 14	Day 21
1A-Sterile Control	9.3E+04	9.3E+04	2.4E+05	1.1E+08
1B-Sterile Control	9.0E+03	0.0E+00	0.0E+00	4.6E+07
2A-PS Sterile, H2O not	1.1E+04	>1.1E+07	>1.1E+07	>1.1E+08
2B-PS Sterile, H2O not	3.4E+04	>1.1E+07	>1.1E+07	>1.1E+08
3A-H2O Sterile, PS not	>1.1E+06	>1.1E+07	>1.1E+07	>1.1E+08
3B-H2O Sterile, PS not	>1.1E+06	>1.1E+07	>1.1E+07	>1.1E+08
4A-PS + H2O Unsterile	>1.1E+06	>1.1E+07	>1.1E+07	>1.1E+08
4B-PS + H2O Unsterile	>1.1E+06	>1.1E+07	>1.1E+07	>1.1E+08

In spite of all of our efforts to create sterile controls for each experiment, the presence of bacterial cells in the sterile controls was documented in most experiments by positive MPN counts and nitrate degradation (e.g. see Figure 2, Sterile Control). Attempts to sterilize these systems were largely unsuccessful, even when the materials were autoclaved on three successive days. While this problem makes the experimental results less than desirable, they provide an extremely good indication that the biobarrier will be successful, as there should be no problem in developing a healthy biofilm to destroy contaminants that move through the biobarrier in the groundwater.

Other analyses.

The pH of each culture was taken at each time point during the incubation for most of the experiments. There is a decrease in pH due to microbial metabolism and production of hydrogen ion in the degradation reactions. The pH values measured on the unsterile cultures (i.e. 4A/B) from the experiments supplemented with 9.7 mM nitrate are presented in Table IV.

Table IV. The pH of the Sterile Control (1A/B) and the Unsterile Cultures (4A/B) measured at various time points during incubation of pecan shells and pecan shell/dog food systems in Mortandad Canyon groundwater supplemented with 9.7 mM nitrate.

	Pecan Shells		Pecan Shell + Dog Food	
Day	pH – 1A/B	pH – 4A/B	pH –1A/B	pH – 4A/B
1	**5.7**	**7.3**	**5.7**	**nd**
2	**5.4**	**7.4**	**5.6**	**6.1**
7	**7.3**	**8.0**	**5.5**	**5.8**
14	**7.4**	**8.4**	**5.5**	**5.3**
21	**7.2**	**8.2**	**7.1**	**5.6**

nd = not determined

There is a distinct difference in the pH that is produced upon incubation of the water in the presence of the pecan shells vs. the pecan shell/dog food mixture. The microbial reactions that are occurring are obviously different. The dissimilatory denitrification pathway is known to generate hydroxyl ions thus increasing the pH. One could speculate that this is the dominant pathway in the pecan shell culture, although there are many other reactions that produce hydroxyl ions. An acidity increase is evident in the pecan shell/dog food experiment, due either to production of hydrogen ions or organic acids, both of which are possible products of microbial activity. More information about the actual populations that are enhanced in these systems is necessary in order to elucidate the mechanisms responsible for these pH changes. However, the range of pH that we have found is not outside that typical of ground waters, so it should pose no problem when used with the Multi-Barrier technology.

CONCLUSIONS

The results presented in this paper demonstrate the effectiveness of a biobarrier design using pecan shell waste for reduction and elimination of nitrate in a dilute groundwater contaminated with multiple contaminants, including radionuclides, heavy metals, nitrate and perchlorate. The results indicate that an active biofilm is developing within and on the biobarrier support materials, and that this biofilm is effective in destroying nitrate in the groundwater. A biobarrier can be used in conjunction with other barrier materials and can be configured as a Multi-Barrier (such as that under development at Los Alamos National Laboratory). Thus, a technology is created that can be used to clean up many different mixtures of contaminants in a cost-effective, highly efficient and less-intrusive manner than can be provided by other available technologies.

The addition of dog food to the pecan shells as a source of micronutrients and protein produced a more rapid rate of destruction of nitrate in the Mortandad Canyon groundwater, as expected. The addition of dog food to the Multi-Barrier system would make it much more complex to emplace, but would provide a significant enhancement of the microbial growth and nitrate degradation rates. It appears that this step is probably not necessary, a pecan-shell system alone will produce satisfactory degradation results.

We have produced evidence that the pecan shell biobarrier is able to destroy perchlorate as well, and those studies are continuing in an attempt to eliminate analytical problems and provide valid results. The Multi-Barrier system developed at Los Alamos National Laboratory is capable of removing nitrate, perchlorate, colloids, metals and radionuclides, although the results of experiments with the last three groups of contaminants are discussed elsewhere.

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