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Project Summary

Biotransformation of Gasoline-Contaminated Groundwater Under Mixed Electron-Acceptor Conditions

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Introduction

The main objective of the research was to evaluate nitrate-based bioremediation as an enhanced bioremediation technology in a gasoline source area under controlled, experimental conditions. Because the quantity of mass in a source is typically very large in relation to the groundwater plume, the source area exerts an influence on plume longevity, and therefore on plume remediation strategies, including natural attenuation. Consequently, hydrocarbon source remediation remains an important groundwater quality issue. In this study the soluble, plume-forming aromatic hydrocarbons, benzene, toluene, ethylbenzene, xylene isomers, trimethylbenzene isomers, and naphthalene (referred to as BTEXTMB) were designated as the target compounds.

Electron-acceptor mixtures were evaluated in an attempt to maximize the biotransformation of these target compounds. In addition to NO_3^-, microaerophilic dissolved O_2 (2 mg/L or less) was present in most experimental treatments to potentially enhance mass losses of the soluble compounds such as benzene that are recalcitrant under denitrifying conditions. Multiple lines of evidence were gathered to evaluate this mixed electron-acceptor approach in the Borden aquifer. These included a series of laboratory microcosm experiments, microbial characterization studies, and a field demonstration. The *in situ* demonstration under highly-controlled, dynamic conditions provided the most realistic and useful assessment of this technology.

This Project Summary was developed by EPA's National Risk Management Research Laboratory's Subsurface Protection and Remediation Division, Ada, OK, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Experimental Approach

The enhanced bioremediation of aromatic hydrocarbons in the presence of NO_3^- and mixtures of NO_3^- and O_2 was investigated at both the laboratory and field scales. Prior to the field demonstration, a series of preliminary laboratory microcosm experiments were performed to determine the effects of O_2, NO_3^- concentration, and inorganic nutrients on the biotransformation of neat BTEX in pristine Borden sand. Following these experiments, another series of microcosm experiments was performed with gasoline-contacted groundwater to determine whether BTEX biotransformation would be enhanced under mixed electron-acceptor conditions (microaerophilic / denitrifying). This was done by comparing mixed-electron-acceptor microcosms with microaerophilic only and anaerobic, denitrifying microcosms. These initial laboratory experiments were performed with substrate concentrations that corresponded to a 10x dilution of gasoline-saturated water (10-15 mg/L total aromatics). However, after the gasoline was spilled in the field it became apparent that aromatic-hydrocarbon concentrations

would greatly exceed these levels throughout the treatment cells. An additional series of microcosm experiments was therefore performed to evaluate aromatic-hydrocarbon biotransformation under conditions more similar to the field. These experiments included treatments with gasoline-saturated water (ca. 100 mg/L total aromatics), and were performed with the American Petroleum Institute gasoline (API 91-01) used in the field.

The field experiment was performed in the Borden aquifer. Seventy liters of API 91-01 gasoline were first released into two sealed, sheet-piling treatment cells (2m by 2m) to create gasoline-contaminated source areas below the ambient water table. A schematic of the treatment cells is shown in Figure 1. Six months after the spills, clean groundwater amended with different combinations of electron acceptors was flushed vertically through the cells to stimulate microbial activity. Water was flushed continuously through the cells under steady flow conditions. The "Nitrate Cell" received a mixture of microaerophilic O_2 and NO_3^-, and the "Control Cell" received microaerophilic O_2 only. Groundwater samples were then analyzed for aromatic-hydrocarbons, added electron-acceptors, metabolites, and other geochemical indicators of biotransformation during both flushing and static periods over a 13 month period. After this experiment was completed, cores were collected from the treatment cells to perform a follow-up microcosm experiment to confirm the results obtained in the Nitrate Cell, and to investigate changes in microbial biomass and dehydrogenase activity in response to nearly two years of gasoline exposure.

Experimental Results

Laboratory Experiments

In the preliminary experiments with low concentrations of benzene, toluene, ethylbenzene, and the xylene isomers, mass losses under aerobic and denitrifying conditions were generally consistent with results from previous investigations of Borden aquifer material. All of these compounds were degradable under aerobic conditions, but microbial activity was limited by inorganic nutrients. Under anaerobic, denitrifying conditions, both toluene and ethylbenzene biotransformed most consistently, while the other aromatic compounds appeared to be recalcitrant. We did not observe nutrient limitations under denitrifying conditions; mass losses were generally small and the minor assimilatory requirement for N may have been satisfied by NO_3^-, eliminating the need for an additional source of supplied N.

In the laboratory, the effect of microaerophilic O_2 was found to depend on the concentrations of aromatic hydrocarbons and other carbon compounds present in the microcosm. When aqueous concentrations of the aromatics were low (10x dilution of gasoline-saturated water), and there were no other sources of labile carbon, the mass of O_2 in a microcosm was fairly large relative to the mass of carbon, and aerobic mass losses were observed. Notably, however, benzene mass losses were typically minimal under these conditions. On the other hand, in gasoline-contaminated microcosms less extensive

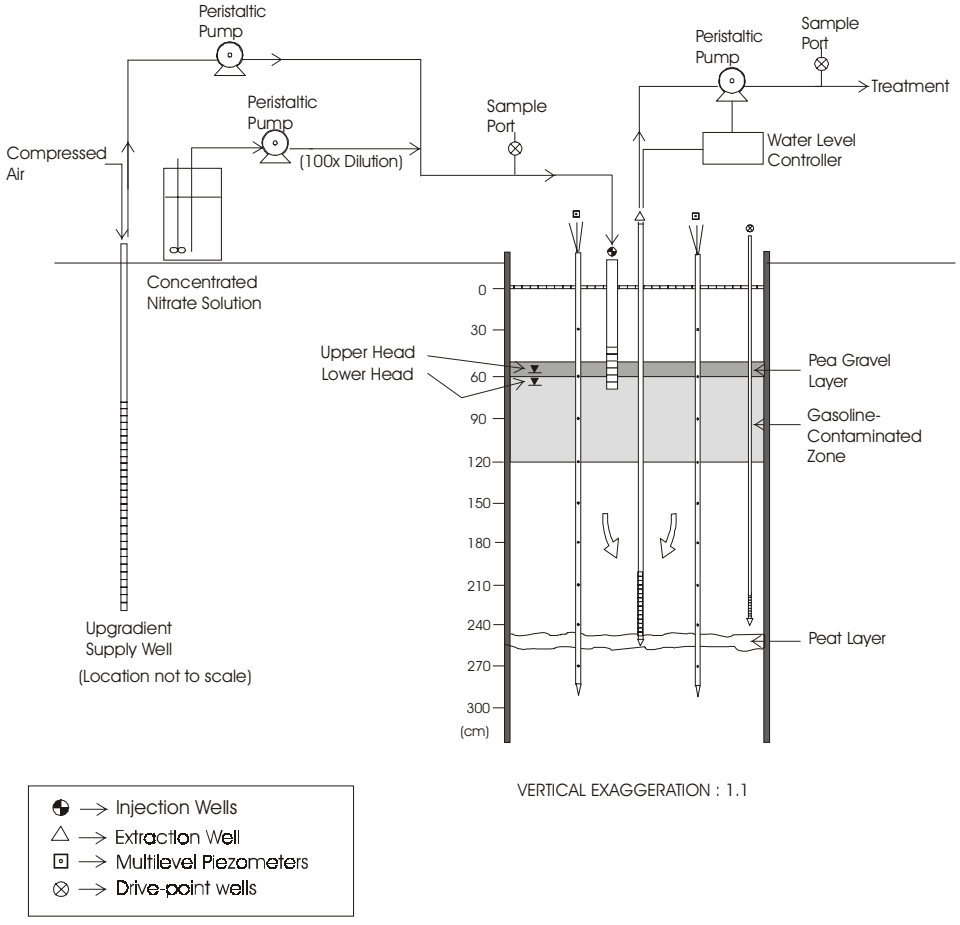

Figure 1. Injection-system schematic for the Nitrate Cell with selected instrumentation to illustrate positions of injection/extraction wells and multilevel piezometer ports. The system for the Control Cell was identical except for NO_3^- addition equipment.

mass losses were observed, presumably as a result of O_2 consumption by other gasoline hydrocarbons. When aqueous concentrations of the aromatic hydrocarbons were increased to gasoline-saturated levels to better reflect field conditions, negligible losses of the aromatics were observed despite rapid consumption of the microaerophilic O_2. Under these conditions, the mass of O_2 was probably too low to observe any losses, even if the aromatics were utilized in preference to other gasoline hydrocarbons, and abiotic demands were minimal.

In pristine aquifer material, NO_3^- utilization was frequently observed under anaerobic conditions, but consumption was slow relative to O_2, occurring over time periods on the order of months to years, and losses were limited to toluene, ethylbenzene, and less consistently, *m*-xylene. When substrate concentrations were increased to gasoline-saturated levels, negligible NO_3^- utilization was observed in pristine aquifer material. This suggested that the indigenous denitrifying population was inhibited by high aqueous substrate concentrations. In contrast, after *in situ* exposure, denitrifying activity was apparently not inhibited by gasoline constituents; NO_3^- utilization was observed in microcosms prepared with gasoline-contaminated aquifer material and gasoline-saturated groundwater, but consumption of the labile aromatic hydrocarbons was not evident. These compounds did not appear to be the preferred substrates in this carbon-rich environment. For example, in microcosms amended initially with microaerophilic O_2, NO_3^-, and gasoline-saturated water, the conditions most similar to those established *in situ*, there were negligible aromatic-hydrocarbon losses under anaerobic, denitrifying conditions until pure O_2 was added to microcosm headspaces on incubation day 154 (Figure 2). Overall, laboratory results with gasoline-contaminated material showed that benzene, toluene, ethylbenzene, *m*-xylene, *p*-xylene, 1,2,4-trimethylbenzene, and naphthalene would biotransform readily at the expense of O_2.

In general, under mixed electron-acceptor conditions the patterns of O_2, and NO_3^-, and aromatic-hydrocarbon concentrations suggested that O_2 and NO_3^- were used sequentially; most aromatic-hydrocarbon biotransformation occurred within the first few days of incubation, likely at the expense of microaerophilic O_2, with additional losses of toluene and ethylbenzene occurring under denitrifying conditions over longer time periods. When the initial concentrations of the aromatic

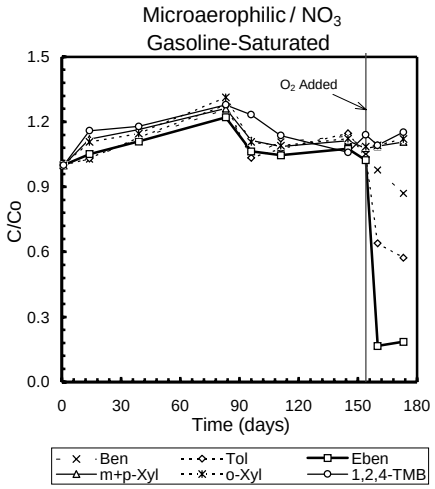

Figure 2. Normalized concentrations of selected aromatic hydrocarbons in gasoline-contaminated aquifer material amended with microaerophilic O_2, NO_3^-, and gasoline-saturated water. Lines join means of replicate microcosms.

hydrocarbons were low, there was a beneficial effect of dual electron acceptors in laboratory microcosms: mass losses in microaerophilic / NO_3^- microcosms were more extensive than in comparable microaerophilic and anaerobic, denitrifying microcosms. This showed that under certain conditions the extent of mass loss could be maximized by the presence of these two electron acceptors. However, as mentioned above, in experiments with gasoline-contaminated aquifer material, which were more representative of *in situ* conditions, mass losses were either very small or negligible under mixed microaerophilic / NO_3^- conditions. These microcosm results were consistent with field observations.

Borden Field Experiment

Mean injection flow rates were 250 ml/min and 237 ml/min for the Nitrate and Control Cells, respectively. The length of the vertical flow path was about 2 m, and these rates corresponded to cell residence times of approximately 9 days. Roughly 20 cell pore volumes were flushed over six months of continuous operation. The mean electron-acceptor concentrations in injected water were 2.3 mg/L O_2 and 116 mg/L NO_3^- in the Nitrate Cell, and 2.3 mg/L O_2 in the Control Cell.

Data from multilevel piezometers showed that dissolved O_2 was consumed rapidly to a non-zero threshold concentration in both treatment cells. Because dissolved O_2 was depleted at sampling ports located 60 cm below ground surface (bgs), and water was injected at about 50 cm bgs, O_2 was apparently consumed within the first 10 cm of the vertical flowpath. On the basis of the laboratory data, the O_2 was utilized primarily by microbial activity, but it could not be determined whether the aqueous aromatic hydrocarbons or other gasoline constituents were serving as substrates. Utilization in abiotic reactions may also have occurred in the field. In contrast to the rapid O_2 consumption, NO_3^- utilization was low, but the production of NO_2^- suggested that some biological NO_3^--reduction had been induced. Nitrate concentrations in injection water and piezometer ports at 60-cm and 180-cm depths are shown in Figure 3. A mass balance indicated that only 12% of added NO_3^- was consumed over the six-month flushing experiment. Nitrate concentrations were also monitored during a period when the cells were static and the residence time was much larger. Under these conditions, complete NO_3^- depletion was observed over a time period on the order of 100 days, but accompanying losses of labile compounds such as toluene and ethylbenzene were not evident.

Dissolved aromatic hydrocarbon concentrations remained near gasoline-

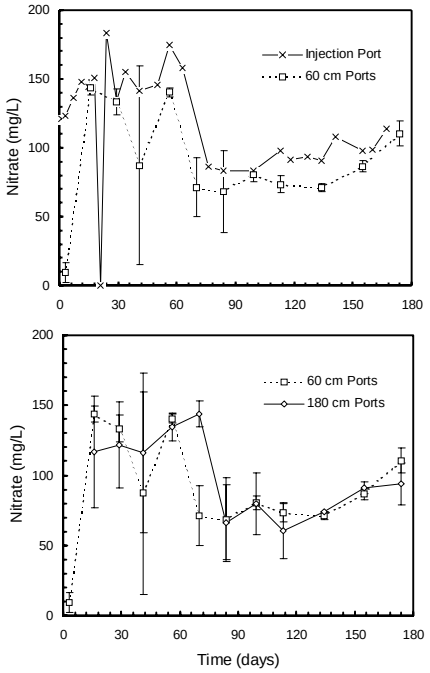

Figure 3. Nitrate concentrations in injection water and 60- and 180-cm bgs piezometer ports during six months of continuous injection into the Nitrate Cell. Plotted values for 60- and 180-cm depths are means and standard deviations from five piezometers.

saturated levels in both cells throughout the experiment. Concentration trends were generally consistent with the dissolution of a multicomponent liquid (i.e., relatively rapid depletion of soluble compounds such as benzene), and there was no clear evidence of preferential removal of labile compounds from denitrifying activity. These trends can be seen in the extraction-well breakthrough curves for the two treatment cells (Figure 4). After the experiments were completed cores were collected from the cells to measure the mass of BTEXTMB remaining in the residual gasoline and mass balances were completed. In terms of total BTEXTMB, 81% and 83% of the initial mass was recovered in the Control and Nitrate Cells, respectively, which correspond to roughly 2,500 g of unrecovered mass per cell. These losses probably resulted from a combination of physical losses and systematic error associated with the mass balance procedure.

Mass balance results for the added electron acceptors were used to estimate the amount of aromatic-hydrocarbon mass loss that could reasonably be attributed to biotransformation. The results suggested that the mass of microaerophilic O_2 injected into the treatment cells was too low to observe any losses even if all of the O_2 was consumed in mineralization reactions with aromatic compounds. Similarly, given the limited NO_3^- utilization, the mass loss of compounds that are labile under denitrifying conditions was likely very small relative to the amount of mass in the Nitrate Cell. Consequently, although there was evidence from metabolite formation that some aromatic-hydrocarbon biotransformation had occurred, the bulk of the experimental data indicated that mass losses from biotransformation were quite small in both cells. These observations indicated, therefore, that abiotic gasoline dissolution was the dominant mass removal mechanism in both treatment cells.

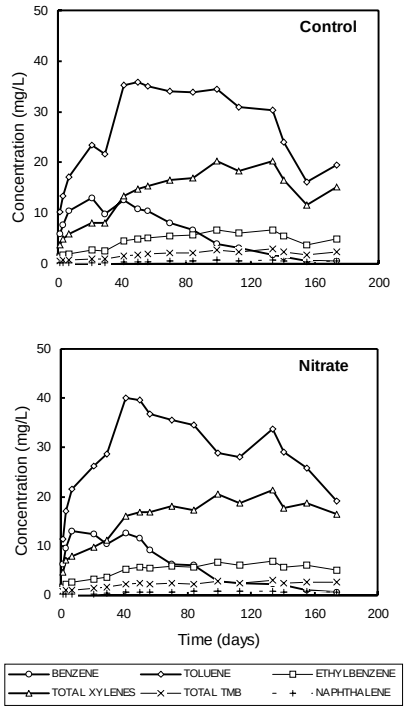

Figure 4. Concentrations of dissolved aromatic hydrocarbons in samples collected from extraction-well ports in the Nitrate and Control Cells.

Conclusions and Implications

Although there was evidence that microbial activity had been stimulated, the field and laboratory data indicated overall that nitrate-based bioremediation was not an effective source-area remedial technology in this aquifer. The field evidence for activity included 1) NO_3^- consumption and NO_2^- production, 2) O_2 consumption, and 3) metabolite production. However, NO_3^- utilization was slow relative to the residence time in the treatment cell, and utilization of labile aromatic compounds was not apparent. It is not clear why a large denitrifying population capable of rapid aromatic-hydrocarbon biotransformation did not develop in the treatment cell in response to extended exposure to abundant substrate and NO_3^-. One possibility is that the denitrifying population remained relatively sensitive to the presence of a gasoline phase and associated high aqueous concentrations. Dissolved O_2 was utilized rapidly in both laboratory and field experiments, demonstrating that aerobic activity was not inhibited, but under microaerophilic conditions mass losses were limited by the quantity of O_2 available for reaction, and possibly by abiotic demand in the field. Based on the laboratory results, dissolved O_2 may have been used to oxidize compounds that otherwise would have been recalcitrant under anaerobic, denitrifying conditions, but *in situ* losses appeared small relative to the mass of gasoline hydrocarbons in the cells. This suggests that the partial oxidation of recalcitrant parent compounds by microaerophilic O_2 was a relatively unimportant process in this system. In addition, there was no evidence that other terminal electron acceptors were being utilized in the treatment cells. As expected from these electron-acceptor trends, mass losses were not enhanced in the cell treated with microaerophilic O_2 and NO_3^- relative to the unremediated control, and effluent breakthrough curves in both cells were consistent with concentration trends expected to result from abiotic gasoline dissolution.

These conclusions pertain to the specific experimental system evaluated in this study (i.e., a recent gasoline spill flushed for a relatively short period of time and monitored over a short flow path). It is conceivable that this approach, particularly with respect to the effects of microaerophilic O_2, would be more effective during the latter stages of an enhanced bioremediation project when source-area concentrations were lower, or for downgradient plume control using a reactive wall or other semi-passive remedial technology. Similarly, although NO_3^- utilization was minor over the flow path evaluated here, adaptation resulting in the development of a substantial population capable of degrading TEX may have occurred with continued exposure. Based on previous studies in this aquifer, it is also possible that substantial NO_3^- utilization would have occurred further downgradient (beyond this experimental system) in the anaerobic core of the plume, providing a benefit to an enhanced or intrinsic remediation strategy.

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The complete report, entitled "Biotransformation of Gasoline-Contaminated Groundwater Under Mixed Electron-Acceptor Conditions," (Order No. PB99-139677; Cost: $36.00, subject to change) will be available only from:
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