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Enhanced anaerobic bioremediation of groundwater contaminated by fuel hydrocarbons at Seal Beach, California

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Abstract

Enhanced anaerobic biodegradation of groundwater contaminated by fuel hydrocarbons has been evaluated at a field experiment conducted at the Naval Weapons Station, Seal Beach, California. This experiment included the establishment of three different remediation zones *in situ*: one zone was augmented with sulfate, one was augmented with sulfate and nitrate, and the third was unaugmented. This enables a comparison of hydrocarbon biodegradation under sulfate-reducing, sequential denitrifying/sulfate-reducing, and methanogenic conditions, respectively. In general, the results from the field experiment are: (1) Certain fuel hydrocarbons were removed preferentially over others, but the order of preference is dependent upon the geochemical conditions; and (2) In the zones that were augmented with sulfate and/or nitrate, the added electron acceptors were consumed quickly, indicating that enhancement via electron acceptor injection accelerates the biodegradation process. More specifically, in the sulfate-reducing zone, sulfate was utilized with an apparent first-order rate coefficient of approximately 0.1 day^{-1}. In the combined denitrifying/sulfate-reducing zone, nitrate was utilized preferentially over sulfate, with an apparent first-order rate coefficient of 0.1–0.6 day^{-1}. However, the data suggest that slow sulfate utilization does occur in the presence of nitrate, i.e., the two processes are not strictly sequential. With regard to the aromatic BTEX hydrocarbons, toluene was preferentially removed under intrinsic conditions; biodegradation of benzene was slow if it occurred at all; augmentation with sulfate preferentially stimulated biodegradation of *o*-xylene; and ethylbenzene appeared recalcitrant under sulfate-reducing conditions but readily degradable under denitrifying conditions.

Introduction

Recent estimates place the number of contaminated groundwater sites in the United States as high as 300,000 (National Research Council, 1994). Most of these contaminated sites are the result of leaking underground storage tanks, particularly from fuel hydrocarbon storage. The fuel hydrocarbons of greatest concern are the aromatic compounds benzene, toluene, ethylbenzene, and xylene, collectively known as BTEX. Benzene is a known human carcinogen with a drinking water maximum contaminant level (MCL) of 5 μg/l. Toluene, ethylbenzene, and (total) xylene have MCLs of 1, 0.7, and 10 mg/l, respectively; exposure above these levels might impair human health. Thus, it is extremely important to develop a practical, reliable, and economical method of cleaning up groundwater sites contaminated with fuel hydrocarbons, and particularly those sites contaminated with BTEX. Because the pump-and-treat method for remediation has proven to be less practical, reliable, and economical than first envisioned (Mackay & Cherry 1989; Travis & Doty 1990; National Research Council 1994), much attention has been focused recently on biodegradation as a groundwater clean-up method (e.g., National Research Council 1993). In this paper, the term 'intrinsic biodegradation' is used to indicate *in situ* bioremediation via naturally-occurring metabolic processes without

any additional alteration of site conditions; the term 'enhanced biodegradation' is used to indicate that conditions at the site are engineered or altered in order to stimulate or accelerate the biological destruction of contaminants.

In the case of contamination by fuel hydrocarbons, it is now well known that many microorganisms indigenous to soil can oxidize (mineralize) the contaminants to harmless carbon dioxide and water. This process can occur rapidly under aerobic conditions, i.e., in the presence of oxygen. Under anaerobic conditions (in the absence of oxygen), the process can still occur through the utilization of an alternate terminal electron acceptor (e.g., sulfate, nitrate), but it is not as well understood. This is particularly true under field conditions: over the last decade, numerous experiments have elucidated the conditions under which BTEX compounds can be biodegraded in the laboratory, but comparatively few experiments have evaluated BTEX biodegradation under anaerobic conditions in the field. With regard to applying anaerobic biodegradation as a remediation method for field sites contaminated by fuel hydrocarbons, it is still necessary to determine important factors such as the degradation rates for the target contaminants, the utilization rates of the relevant electron acceptors, and the sequence in which the various BTEX compounds are degraded. Anaerobic degradation of BTEX compounds has been reviewed elsewhere (e.g., Frazer et al. 1995; Chapelle 1999; Phelps & Young 1999).

The study reported here was conducted in a contaminated portion of a shallow groundwater aquifer at the Naval Weapons Station, Seal Beach, in southern California. Laboratory experiments using aquifer sediments and groundwater from the Seal Beach site (Haag et al. 1991; Edwards et al. 1992; Edwards & Grbic-Galic 1992; Ball & Reinhard 1996) had suggested that anaerobic degradation of BTEX was likely to occur there, and a previous field study at the site (Reinhard et al. 1997) supported the laboratory observations. The previous field study consisted of injecting into the aquifer a slug of water containing known concentrations of BTEX and electron acceptors, allowing the injected solution to incubate *in situ* for a specified period of time, and then extracting the water to observe if the BTEX compounds had been degraded (Reinhard et al. 1997). The new experiment, here reported for the first time, can be viewed as an extension of the previous work, but with several important differences from the previous field experiment: it involves significantly larger volumes of groundwater to be treated; it was conducted over longer time in order to observe slower reaction rates; it includes simultaneous addition of sulfate and nitrate as potential electron acceptors; and it incorporates an injection-extraction well design that is potentially applicable to full-scale site remediation.

The field experiment discussed herein is also unique in two other important respects: (1) During the course of the 17-month experiment, more than 9000 samples were collected from 105 different sampling locations, and were analyzed for 15 different analytes, thereby providing detailed spatial and temporal resolution of the subsurface concentrations; and (2) The experiment included the establishment of three different remediation zones *in situ*, each with different geochemical conditions, to allow comparison of hydrocarbon biodegradation under these different conditions.

As with nearly all contaminated field sites, it is impossible to determine the exact amount of contamination present at the beginning of the remediation. This uncertainty prevents both a quantitative mass balance on the target contaminants and a rigorous modeling of the contaminant fate and transport. Thus, in this paper, we have relied heavily on the response of electron acceptors (nitrate and sulfate) which were injected into the contaminated aquifer at known concentrations. We have quantified the rate of disappearance of the electron acceptors – which, plausibly, can be attributed only to biological utilization – and have coupled this quantification with qualitative observations of the temporal changes in BTEX concentrations.

The principal objectives of the field experiment were (1) to demonstrate that enhancing the natural biodegradation process with sulfate and/or nitrate addition is a feasible engineering approach; and (2) to compare the *in situ* biodegradation rate of fuel hydrocarbons under three different sets of geochemical conditions, namely methanogenic, sulfate-reducing, and denitrifying. The results of the experiment suggest that injection of sulfate and/or nitrate does accelerate the *in situ* biodegradation processes, and that the established geochemical conditions can affect the rate and the order of preference for biodegradation of the BTEX compounds.

Field site: Naval Weapons Station, Seal Beach

The Naval Weapons Station (NWS), Seal Beach, is located in southern California. A gasoline station loc-

ated on the premises of the weapons station is contaminated with fuel hydrocarbons that leaked from a steel underground storage tank, as described in detail elsewhere (Schroeder 1991). The gasoline leak was discovered in 1984. The weapons station also contains the Seal Beach National Wildlife Refuge, a wetlands marsh. Investigation by the U.S. Geological Survey (Schroeder 1991) found that the contamination from the leaking tank had migrated to the groundwater underlying the Refuge. The Navy's concern about the possible adverse effects of the contamination on the Wildlife Refuge made the Seal Beach facility a potential site for this field experiment.

Also, the following conditions made the NWS Seal Beach site especially well-suited for this experiment. (1) The groundwater in the contaminated zone had been anaerobic for at least a decade. Both laboratory and field studies had demonstrated the presence of anaerobic bacteria that are capable of degrading fuel hydrocarbons. (2) Previous studies at the site (e.g., Reinhard et al. 1997) indicate that the supply of electron acceptors and/or the removal of inhibitors are limiting, suggesting the need for enhancement of intrinsic biodegradation processes. (3) The aquifer solids are sufficiently permeable to allow pumping of at least a few gallons per minute, i.e., transmissivity is higher than 0.2 m^2/day. (4) Laboratory and field data from previous studies (Haag et al. 1991; Edwards et al. 1992; Edwards & Grbic-Galic 1992; Ball & Reinhard 1996; Reinhard et al. 1997) had suggested the probability of success of enhanced biodegradation at the Seal Beach site.

The groundwater velocity in the region is low, approximately 0.7 cm/day (Reinhard et al. 1997). The groundwater flow rate and direction might fluctuate somewhat with the season and with the tides.

Materials and methods

Experimental configuration

The enhanced biodegradation system installed at the field site consisted of one extraction well and three injection wells, with relative locations as indicated in Figure 1. Each injection well was fully screened across the saturated zone of the aquifer and was located 10 m away from the extraction well. The extraction well was also fully screened across the saturated zone. The rate of injection in each well was approximately 1.5 l/min; the rate of extraction was approximately 4.5 l/min. Different electron acceptors were added at each injection well, creating three different treatment zones with different geochemical sequences. These three zones are labeled Zones 2, 3, and 4, according to the number of the injection well pertaining to each zone (see Figure 1). Zone 3 received no augmentation of electron acceptors, and was therefore expected to develop methanogenic conditions; Zone 2 was augmented with sulfate, and was expected to develop first sulfate-reducing and then methanogenic conditions; Zone 4 was augmented with nitrate and sulfate, and was expected to develop first denitrifying, then sulfate-reducing, then methanogenic conditions. The three zones were established in this manner specifically because one of the objectives of this experiment was to evaluate the efficacy of anaerobic biodegradation under different geochemical conditions. A full-scale implementation of this technology might employ a different number of injection/extraction wells, and might inject different levels of electron acceptors in order to optimize the biodegradation.

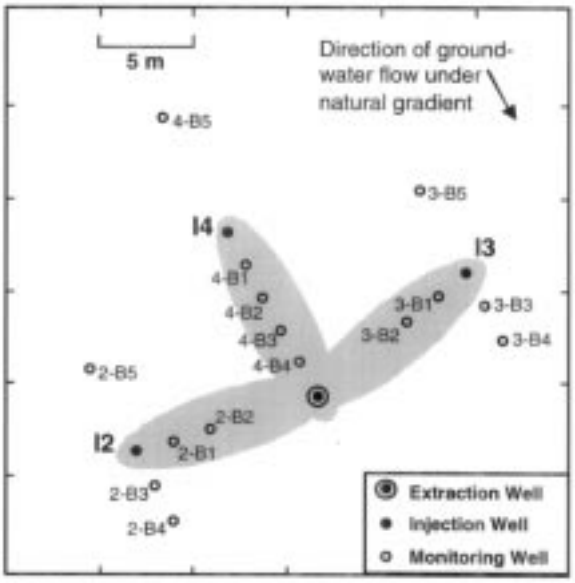

Figure 1. Plan view of the well installation at the field site. Three treatment zones were established, and are labeled Zone 2, Zone 3, and Zone 4, according to the number of the injection well pertaining to that zone. The shaded region represents approximate areas of the treatment zones. Zone 2 was augmented with sulfate; Zone 3 received no electron acceptors; and Zone 4 was augmented with sulfate and nitrate.

Figure 2 shows a schematic of the injection/extraction well system used to enhance the biodegradation process. Samples were taken at monitoring wells located between the injection and the

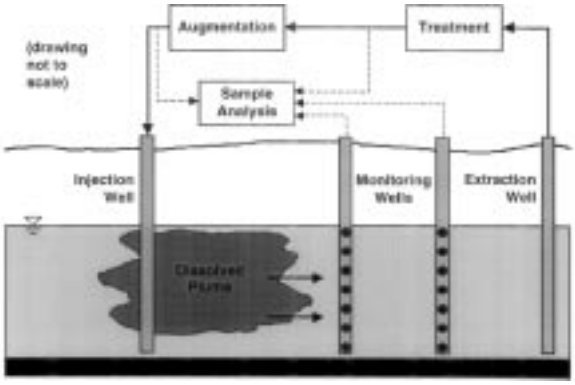

Figure 2. Schematic of the injection/extraction well system used for enhanced biodegradation. Target contaminants and inhibitory by-products are removed by the water treatment system, and the water is augmented with electron acceptors prior to re-injection. Monitoring wells are placed between the injection well and the extraction well (see also Figure 1) to track contaminant concentrations.

extraction wells in order to measure the concentrations of the electron acceptors and of the target contaminants. Extracted water was treated to remove the target contaminants (BTEX and other fuel hydrocarbons) and compounds that can inhibit the bioremediation process (e.g., sulfide). After treatment, the extracted water was augmented with electron acceptors in order to stimulate *in situ* biodegradation of the target contaminants, then was re-injected into the contaminated region of the aquifer.

Sampling and analysis

Sampling was performed automatically via an Automated Sampling and Analysis Platform (ASAP) from Analytic and Remedial Technology (Milpitas, CA). The automated on-line sampling manifold consisted of 111 sample ports, of which 105 ports were connected directly to the multi-level sample bundles of the monitoring wells. The remaining six ports were connected to the treatment system and to the three injection wells. The ASAP provided samples directly to the instrumentation without operator intervention and was operated continuously from August 1997 until November 1998.

Each of the three treatment zones had five monitoring wells, as shown in Figure 1. Each monitoring well consisted of seven ports spaced vertically 14 inches apart, thus providing seven discrete sample locations covering the length of the injection well screens at each monitoring well location. In each monitoring well, the top port was placed very close to the water table; the bottom port was located about 7 feet below the water table, with the middle five spaced evenly in between. For each treatment zone, one multilevel sample well was located approximately 7 m upgradient of the injection well, and two multilevel sample wells were located 2 and 4 m in the direction of the extraction well. In Zones 2 and 3, additional monitoring wells were placed 2 and 4 m downgradient from the injection well. In Zone 4, where the injection well was directly upgradient of the extraction well, monitoring wells were placed 2, 4, 6, and 8 m from the injection well. This is illustrated in Figure 1.

Each sampling point had a unique name that is given in three parts: the first part indicates in which treatment zone the monitoring well is located; the second part indicates the number of the well bundle within the zone (see Figure 1); and the third part indicates the vertical location of the sampling point, where 1 indicates the uppermost sampler and 7 indicates the lowest sampler. For example, a designation '2-B1-4' means Zone 2, well bundle 1, the fourth sampler from the top. The injection wells are designated I2, I3, and I4, as indicated in Figure 1.

Connections between the monitoring wells and the ASAP were stainless steel tubing. After flushing the sample lines, the ASAP extracted a sample and prepared separate aliquots for analysis of: (1) concentrations of volatile organic compounds (including BTEX) via a modified purge-and-trap method with gas chromatography (GC), photo-ionization detection (PID), and flame ionization detection (FID); (2) concentrations of anions (including bromide, sulfate, and nitrate) via ion chromatography; and (3) pH, dissolved oxygen, and concentration of sulfide via specific probes. The gas chromatograph was not able to resolve *m*- and *p*-xylene, so the concentrations of these two compounds were measured as a sum, *m* + *p*-xylene. Results from the ASAP analyses were automatically logged in a computer database. All samples were stamped with date and time, and had unique names for sample locations.

System operation

The system shown in Figures 1 and 2 can be operated in three modes: (1) injection/extraction with no augmentation of electron acceptors, i.e., flushing of the treatment zones with unaugmented treated water; (2) injection/extraction with augmentation of electron acceptors in the injection wells; and (3) no pumping, i.e., both injection and extraction wells are off. During the

field experiment, one ‘treatment evaluation’ consisted of operating in these three modes sequentially.

First, the three treatment zones were flushed with water that had been treated to remove hydrocarbons, gases (including oxygen), and anions (including nitrate and sulfate), but had not been augmented with electron acceptors. This served to remove inhibitory products, to remove background concentrations of the electron acceptors, and to reduce the initial BTEX concentration in each zone. The flushing stage was implemented mainly to establish base-line conditions in the treatment zones for evaluation purposes; at a full-scale implementation of this technology, the flushing stage might be omitted depending on whether or not inhibitory by-products are present. Furthermore, at a site characterized by extreme heterogeneity of hydraulic conductivity, the flushing might remove inhibitory by-products ineffectively from some regions of the aquifer.

The second stage of a treatment evaluation consisted of injecting the zones with treated water that had also been augmented with the appropriate electron acceptor(s). The augmentation stage lasted for about 4–5 weeks, which was sufficient time to develop treatment zones of about 180 m^3 in size. The third stage was a no-pumping stage, in which both injection and extraction wells were shut off. During this time, the treatment zones were monitored to determine how the BTEX concentrations in each zone responded to the established geochemical conditions. During the no-pumping stage, the treatment zones were under the influence of the natural groundwater flow, which is very slow; during the flushing and augmentation stages, the hydraulics are controlled by the pumping, with negligible influence from the natural gradient.

The field experiment lasted about 17 months and consisted of three treatment evaluations. The first augmentation period ran from 14/9/97–16/10/97; the second augmentation ran from 24/5/98–23/6/98; the third augmentation period ran from 2/9/98–14/10/98. The third augmentation was not preceded by a flushing stage. Each augmentation consisted of different concentrations of electron acceptors being injected, as summarized in Table 1.

The concentrations of the injected electron acceptors were increased from one augmentation to the next in order to slowly build up the proper microbial population. During the first treatment evaluation, all of the injected sulfate and nitrate was consumed very rapidly. During the second treatment evaluation, the electron acceptors were injected at higher concentrations, but were still completely utilized by the end of the treatment evaluation. During the third treatment evaluation, the injected concentrations were again increased, such that some of the electron acceptors were still present in the treatment zones at the end of the third treatment evaluation.

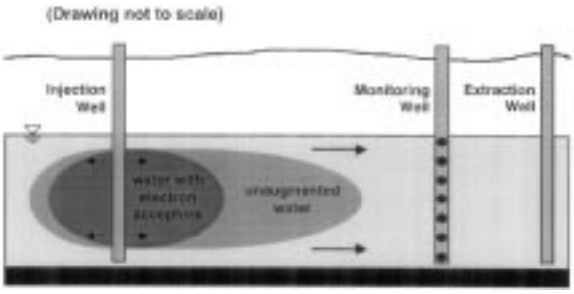

Figure 3. Because the injection and extraction wells are operated in three modes (flushing, augmentation, and no-pumping) sequentially, a volume of unaugmented water from the flushing mode is pushed in front of the water containing electron acceptors.

A conservative tracer was injected during the flushing period of the first treatment evaluation in order to establish that the monitoring wells were hydraulically connected to the injection wells. The results of the tracer study coupled with analytical groundwater modeling confirm that the approximate treatment zones represented on Figure 1 are accurate.

Effect of system operation on anticipated results

The operation of the system in the three different modes (flushing, augmentation, and no-pumping) has a strong effect on both the spatial and the temporal concentration behavior of the contaminants and the electron acceptors. Figure 3 illustrates how the sequential operation in the flushing mode and the augmentation mode leads to a volume of unaugmented water which is ‘in front’ of the water containing the electron acceptors. Thus, this unaugmented water reaches the monitoring wells first, followed by the water containing the electron acceptors.

Figure 4 shows the expected response at the monitoring wells, in terms of the time evolution of the observed concentrations of electron acceptors. For illustration purposes, Figure 4 assumes that the electron acceptors are injected at a concentration of 50 mg/l. For comparison, Figure 4 also shows the expected behavior of a conservative tracer. During the flushing stage, only unaugmented water is pumped through the aquifer, and the monitoring well does not detect any tracer or any electron acceptors. After the aug-

Table 1. Injected electron acceptor concentrations during treatment evaluations

Evaluation #	Zone 2	Zone 3	Zone 4
1	15–20 mg/l sulfate	No electron acceptors added	15 mg/l nitrate, 15 mg/l sulfate
2	70–90 mg/l sulfate	No electron acceptors added	45–55 mg/l nitrate, 70–80 mg/l sulfate
3	40–50 mg/l nitrate, then 75–95 mg/l sulfate	No electron acceptors added	85–125 mg/l nitrate 70–100 mg/l sulfate

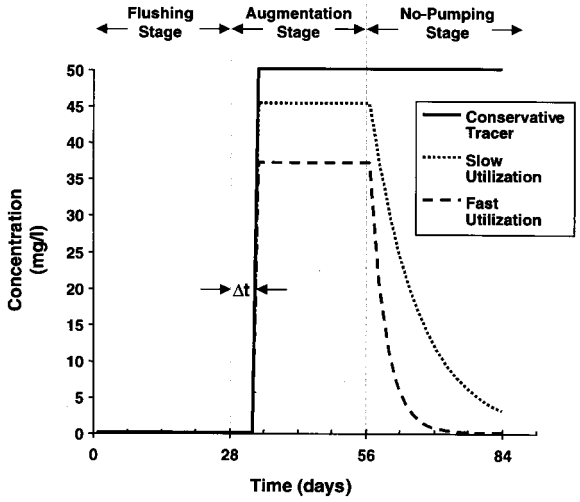

Figure 4. Expected time evolution of measured concentrations of electron acceptors and conservative tracer at the monitoring wells, assuming an injected concentration of 50 mg/l. The time interval Δt represents the travel time from the injection well to the monitoring well. Concentration curves shown assume first-order utilization kinetics for the electron acceptors.

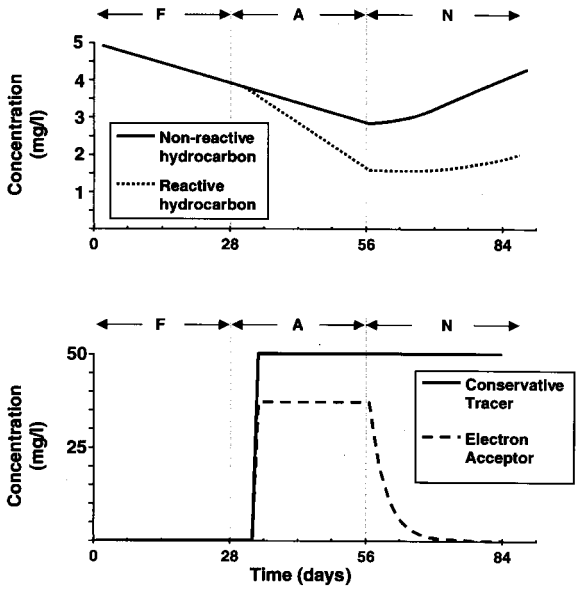

Figure 5. Expected time evolution of measured concentrations of petroleum hydrocarbons (upper graph). The intervals are denoted 'F' for the flushing stage, 'A' for the augmentation stage, and 'N' for the no-pumping stage. The lower graph shows the expected time evolution of the electron acceptor concentration, as shown also in Figure 4.

mentation stage begins, it takes a time Δt for the augmented water to travel from the injection well to the monitoring well. In each of the three zones, the first monitoring well is about 2–3 days' travel time downgradient of the injection well. During this travel time, the electron acceptors are utilized biologically, so they break through at less than the injected concentration of 50 mg/l. Rapidly-utilized electron acceptor (e.g., nitrate) breaks through at a lower concentration than slowly-utilized electron acceptor (e.g., sulfate), because more is consumed during the travel time. The conservative tracer is not utilized and breaks through at its injected concentration of 50 mg/l. Once the no-pumping stage begins, the biological utilization of the electron acceptors continues, but they are no longer replenished, and their concentrations decay towards zero. The concentration curves shown in Figure 4 assume first-order utilization kinetics for the electron acceptors, and ignore the effects of local dispersion.

Given this expected behavior of the electron acceptors, Figure 5 shows a qualitative description of the expected petroleum hydrocarbon concentration histories. During the flushing stage (denoted 'F' in Figure 5), the hydrocarbon concentrations at the monitoring wells decrease because the clean injected water flushes the hydrocarbons towards the extraction well. Despite the fact that the uncontaminated water is displacing the native groundwater, the hydrocarbon concentrations might not reach zero during the flushing stage. This is because hydrocarbon sources can exist *in situ*,

as residual non-aqueous-phase liquid (NAPL), or as sorbed to the aquifer solids. As the contaminated water is flushed out by the clean injected water, hydrocarbons dissolve from the NAPL and/or desorb from the aquifer solids, keeping the concentration above zero. Once the augmentation stage (denoted 'A') begins, a reactive hydrocarbon will respond with a more rapid decrease in concentration. Reactive hydrocarbons are removed by two mechanisms during the augmentation stage: via continued flushing, and via biodegradation. However, non-reactive hydrocarbons are removed only via the flushing mechanism, and they are expected to behave the same during the augmentation stage as during the flushing stage. Once the no-pumping stage (denoted 'N') begins, it is possible that the hydrocarbon concentrations will experience some 'rebound'. This is again due to the dissolution of hydrocarbons from a non-aqueous phase, or due to desorption from the aquifer solids, or perhaps even due to slow migration of native groundwater under the influence of the natural gradient. A reactive hydrocarbon is expected to experience only a slow rebound while there is electron acceptor still present; once the electron acceptor has been totally consumed, the rebound is expected to accelerate. Non-reactive hydrocarbons are expected to rebound quickly as soon as the no-pumping stage begins.

Results

Because over 9000 samples were collected from 105 monitoring locations during the course of the field experiment, it is impossible to present here more than a small fraction of the data collected. Therefore, only those results that are representative of the most important findings are presented below. In many of the figures that follow, different periods in time are labeled with the letters F, A, or N. These letters correspond to the three stages of a treatment evaluation: 'F' indicates the first (flushing) stage, in which the treatment zone was flushed with treated but unaugmented water; 'A' indicates the second (augmentation) stage, in which the treatment zone was augmented with electron acceptors; and 'N' indicates the third (no-pumping) stage, in which the injection and extraction wells were shut off such that there was no flow through the treatment zones. In Zone 3, where no electron acceptors were added, the treatment evaluations are divided only into flushing (F) and no-pumping (N) stages.

Electron acceptor utilization

As described in the Introduction, one of the objectives of this field project was to demonstrate that enhancing the natural biodegradation process with sulfate and/or nitrate addition is a feasible engineering approach. In order to demonstrate this, it is necessary to examine the utilization of the injected sulfate and nitrate.

Figure 6 shows the utilization of sulfate in Zone 2 (which was augmented with sulfate only) during the second treatment evaluation. Figure 7 shows the utilization of nitrate in Zone 4 (which was augmented with both nitrate and sulfate) during the second treatment evaluation. In both cases, the electron acceptor broke through at less than 100% of the injected concentration, and exhibited exponential-like decay once the augmentation stage ended, indicating that the electron acceptors were most likely being utilized biologically for hydrocarbon transformation. Nitrate was fully consumed within about three weeks after the augmentation stage ended, and sulfate was fully consumed within about six weeks. The fact that the electron acceptors are consumed so rapidly after their injection indicates that the supply of electron acceptors is probably a limiting factor in the intrinsic hydrocarbon biodegradation at this site. Thus, the injection of electron acceptors would be expected to accelerate the biodegradation process. However, Figures 6 and 7 do not indicate which hydrocarbons in particular are being transformed by the processes of denitrification or sulfate reduction.

In both Figure 6 and Figure 7, the behavior of the injected electron acceptor is very similar to the expected behavior described in Figure 4. The observed breakthrough curves were fit with a model which assumes first-order utilization of the electron acceptor. Based on these curve fits, the apparent utilization rate of sulfate in Zone 2 was approximately 0.1 day^{-1} for the second treatment evaluation; the apparent utilization rate of nitrate in Zone 4 was approximately 0.22 day^{-1} for the second treatment evaluation. Nitrate was most rapidly utilized during the first treatment evaluation, and was least rapidly utilized during the third treatment evaluation. Presumably, this is because the most-readily degradable hydrocarbons were consumed during the first treatment evaluation, leaving the more recalcitrant hydrocarbons to be degraded more slowly during the subsequent augmentations. Overall, the apparent first-order utilization rate of nitrate in Zone 4 was about 0.1–0.6 day^{-1}. The apparent

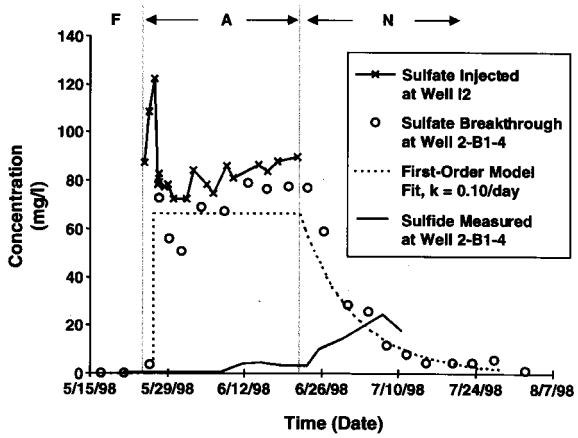

Figure 6. Breakthrough of sulfate at well 2-B1-4 following injection at well I2, during the second treatment evaluation. Sulfate broke through at less than 100% of the injected concentration, and exhibited exponential-like decay once the augmentation stage ended, indicating biological utilization for hydrocarbon degradation. Production of sulfide is further evidence of biological activity. Apparent first-order utilization rate for sulfate was 0.10 day^{-1}. The flushing stage is denoted 'F', the augmentation stage is denoted 'A', and the no-pumping stage is denoted 'N'.

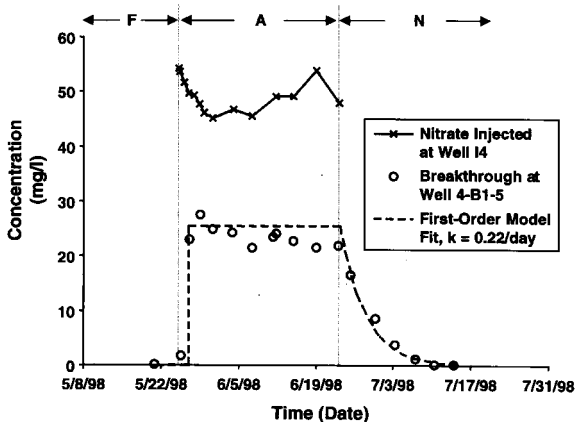

Figure 7. Breakthrough of nitrate at well 4-B1-5 following injection at well I4, during the second treatment evaluation. Nitrate broke through at less than 100% of the injected concentration, and exhibited exponential-like decay once the augmentation stage ended, indicating biological utilization for hydrocarbon degradation. Apparent first-order utilization rate was 0.22 day^{-1}. The flushing stage is denoted 'F', the augmentation stage is denoted 'A', and the no-pumping stage is denoted 'N'.

first-order utilization rate of sulfate in Zone 2 was close to 0.1 day^{-1} for all three treatment evaluations.

In Zone 4, where both sulfate and nitrate were injected, nitrate was utilized preferentially over sulfate. In this Zone, for a given treatment evaluation, sulfate utilization generally began after the nitrate was consumed. However, the data suggest that slow sulfate

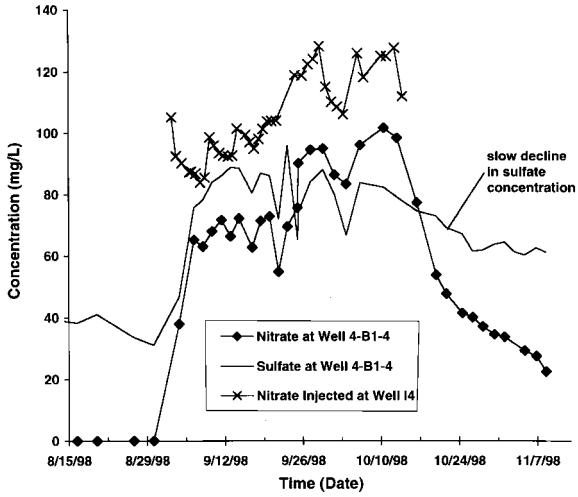

Figure 8. Detail of the nitrate and sulfate concentrations at well 4-B1-4 during the third augmentation in Zone 4. The sulfate concentration measured at 4-B1-4 declined slowly but steadily between 6 October 1998 and 10 November 1998, perhaps indicating some sulfate utilization in the presence of nitrate.

utilization can occur in the presence of nitrate, i.e., the two processes are not strictly sequential. Figure 8 shows a detail of the third augmentation in Zone 4. Between 6 October 1998 and 10 November 1998, the sulfate concentration at well 4-B1-4 decreased slowly but significantly in the presence of nitrate. Thus, it appears that some sulfate utilization occurred when nitrate was present. However, sulfate utilization occurred much more rapidly in Zone 2, where sulfate was injected without nitrate (cf. Figures 6 and 8).

A final interesting result with regard to the electron acceptors was observed in Zone 2, where the third augmentation consisted of nitrate followed by sulfate (see Table 1). As shown in Figure 9, the sulfate concentration in Zone 2 increased in response to the injection of nitrate at injection well I2 (from 30 August 1998 to 7 September 1998). We believe the increase in sulfate concentration to have been caused by the oxidation of sulfide to sulfate in the presence of nitrate. Some sulfide had accumulated in Zone 2 as a result of sulfate reduction in response to the first two sulfate augmentations (Figure 6). The ability of nitrate to oxidize sulfide to sulfate has been observed previously (Ball & Reinhard 1996). This is potentially quite useful as a means of *in situ* control of sulfide inhibition.

BTEX removal under methanogenic conditions

Figure 10 shows the concentration histories of benzene and methane at well 3-B1-4. Because Zone 3

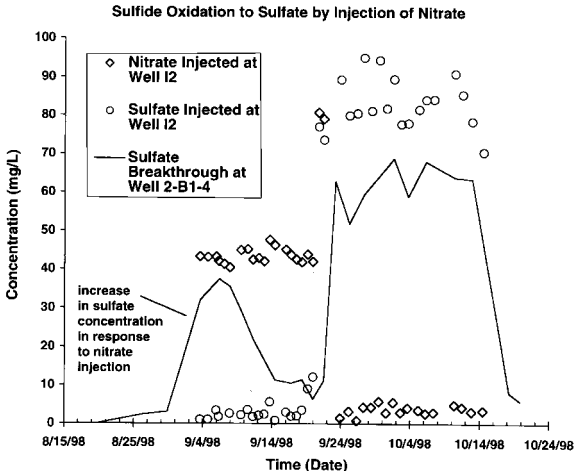

Figure 9. Nitrate and sulfate concentrations injected at well I2 and measured at well 2-B1-4 during the third augmentation in Zone 2. Injection of nitrate at well I2 leads to an increase in the sulfate concentration at 2-B1-4, probably indicating the oxidation of sulfide to sulfate upon nitrate addition.

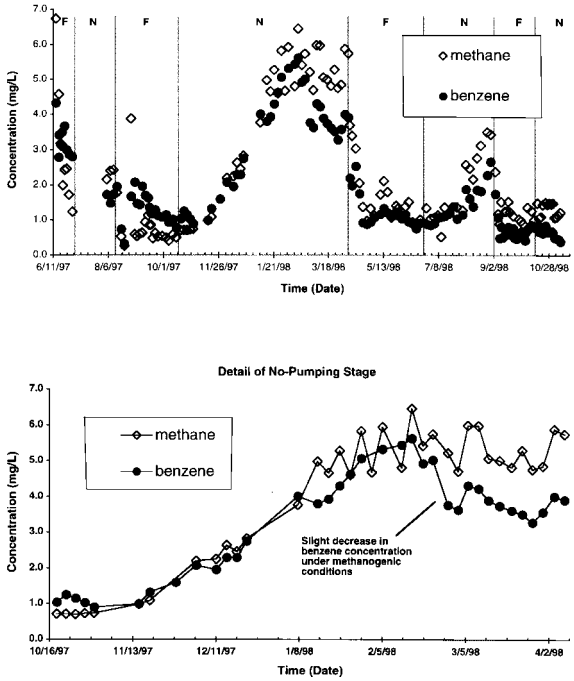

Figure 10. Concentration histories of benzene and methane in Zone 3, which received neither sulfate nor nitrate augmentation. Upper graph: increase in methane concentration during no-pumping stages indicates methanogenic hydrocarbon degradation. Lower graph: slight decrease in benzene concentration might be indication of methanogenic biodegradation of benzene. However, the large 'rebound' in the benzene concentrations during no-pumping stages (upper graph) indicates that benzene biodegradation is slow if it occurs at all.

received neither nitrate nor sulfate augmentation, it was expected that Zone 3 would develop fermentative-methanogenic conditions. The concentrations of toluene, ethylbenzene, and xylenes are not shown in Figure 10 because those concentrations were very low in Zone 3 even at the beginning of the experiment, making it impossible to assess whether or not methanogenesis is an effective removal mechanism for those compounds.

The increase in methane concentrations during the no-pumping periods is probably due to methanogenic degradation of short-chain organic acids, which are produced by fermentation of the petroleum hydrocarbons. The relatively high methane concentrations produced suggest that a large quantity of petroleum was being degraded via fermentation/methanogenesis, but it is impossible to determine which hydrocarbons in particular were degraded. We suspect that many petroleum hydrocarbons other than BTEX contributed to the methanogenesis.

The benzene data show that benzene concentrations decreased during the flushing periods, but rebounded during no-pumping periods, in a manner qualitatively similar to that predicted in Figure 4. The rebound in benzene concentrations could be due to benzene dissolution from a non-aqueous phase, benzene desorption from aquifer solids, and/or slow flow of benzene-laden groundwater under the natural gradient. The bottom graph of Figure 10 shows that, towards the end of the longest no-pumping stage, there was some decrease in the benzene concentration, suggesting that perhaps there was some benzene degradation via fermentation/methanogenesis. A similar trend was observed during the last no-pumping stage (upper graph). However, overall, the benzene concentration remained far above its MCL throughout the duration of the experiment, suggesting that methanogenesis is not an effective removal mechanism for benzene at this site. If any benzene degradation occurred, it was slow, and most benzene removal appears to have been caused by flushing rather than biodegradation.

BTEX removal under sulfate-reducing conditions

Figure 11 shows the concentration histories of benzene, *o*-xylene, and ethylbenzene at well 2-B1-4. Zone 2 was augmented with sulfate during the first two augmentations, and augmented with nitrate then sulfate during the third augmentation. The toluene concentration is not shown in Figure 11 because it was very low throughout the entire experiment; the *m* +

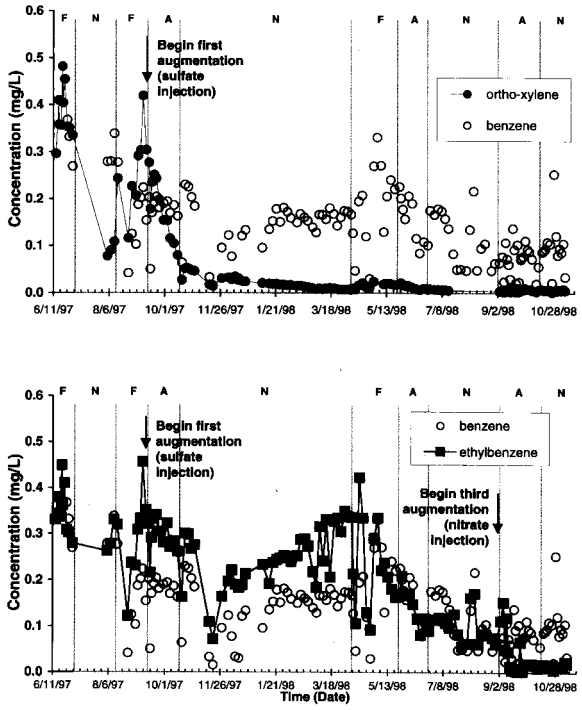

Figure 11. Concentration histories of benzene, *o*-xylene, and ethylbenzene at well 2-B1-4. Upper graph: augmentation with sulfate preferentially stimulates removal of *o*-xylene. Lower graph: ethylbenzene is recalcitrant under sulfate-reducing conditions, but is readily removed under nitrate-reducing conditions.

p-xylene concentration is not shown because it was nearly identical to that of benzene throughout the entire experiment. Thus, if we assume that benzene was removed from Zone 2 only via flushing, and not via biodegradation, then the same would be true for *m* + *p*-xylene, because the concentrations were so close to identical.

Figure 11 shows that the benzene, ethylbenzene, and *o*-xylene concentrations in Zone 2 all decreased over the course of the experiment. However, only *o*-xylene appears to have been removed as a result of sulfate augmentation: note the rapid decrease in *o*-xylene concentration during the first augmentation period, beginning immediately after the introduction of sulfate. The *o*-xylene concentration did not rebound during the no-pumping periods, probably indicating that all *o*-xylene in the vicinity of well 2-B1-4 had been consumed. Previous studies with Seal Beach aquifer material had produced conflicting results regarding the degradation of *o*-xylene under sulfate-reducing conditions. Edwards et al. (1991) observed *o*-xylene mineralization after a lag period, and after toluene and *p*-xylene were degraded. Ball and Reinhard (1996) observed *o*-xylene degradation to occur under sulfate-reducing conditions apparently via co-metabolism with toluene. Reinhard et al. (1997) observed toluene and *m* + *p*-xylene removal under unamended conditions, and *o*-xylene removal upon the addition of sulfate. In this experiment, toluene concentrations were low at all times, such that it is doubtful that *o*-xylene transformation was due to co-metabolism with toluene. It is possible that *o*-xylene was degraded as a primary substrate, or that it was degraded co-metabolically with a primary substrate other than toluene. No data are available for the presence of (2-methyl-benzyl)-succinate, which is an indicator for *o*-xylene cometabolism (Beller et al. 1995, 1996), so it is difficult to determine if *o*-xylene was a primary or secondary substrate.

In contrast to *o*-xylene, the removal of benzene, ethylbenzene, and *m* + *p*-xylene (data not shown) was slow, apparently due more to flushing than to biodegradation. The ethylbenzene concentration did drop sharply during the third augmentation, when nitrate was added prior to sulfate, suggesting that nitrate addition can stimulate removal of ethylbenzene. It has been observed previously that ethylbenzene is recalcitrant under sulfate-reducing conditions, but is degraded under denitrifying conditions (e.g., Ball and Reinhard 1996). Augmentation with sulfate does not appear to enhance the biodegradation of benzene or *m* + *p*-xylene. Toluene concentrations were so low throughout the entire experiment that it is impossible to assess the effect of sulfate augmentation on toluene.

BTEX removal under combined denitrifying and sulfate-reducing conditions

Figure 12 shows the concentration histories of methane, benzene, and *m* + *p*-xylene at well 4-B1-4. Zone 4 was augmented with both nitrate and sulfate. The concentrations of toluene, ethylbenzene, and *o*-xylene are not included in Figure 12 because they were low throughout the experiment.

As in Zone 3, the benzene concentration in Zone 4 decreased during the flushing and augmentation stages, but rebounded during the no-pumping stages. The rebound of benzene concentrations was weaker in Zone 4 than in Zone 3, which received no electron acceptors (cf. Figures 10 and 12). It is possible that this indicates some biodegradation of benzene due to nitrate and sulfate augmentation; however, there are many other reasons why the rebound in Zone 4 might have been weaker than that in Zone 3, and there-

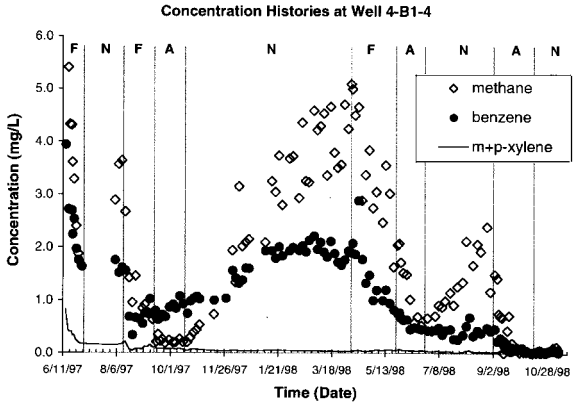

Figure 12. Concentration histories of methane, benzene, and *m* + *p*-xylene at well 4-B1-4. Benzene degradation is slow if it occurs at all, even with the addition of sulfate and nitrate. Methanogenesis appears to occur during the no-pumping stages, even in the presence of sulfate and nitrate. No rebound of *m* + *p*-xylene is observed, perhaps indicating that the injection of nitrate stimulates biodegradation of those compounds.

fore it is very difficult to conclude that any benzene degradation occurred. The rebound of benzene concentrations in Zone 4 indicates that, if any benzene biodegradation did occur in this zone, it must have been very slow. Thus, even though sulfate and nitrate augmentation might accelerate benzene degradation as compared to strictly methanogenic conditions, overall it appears that sulfate and nitrate augmentation are not particularly effective means of removing benzene at this site. Also, we note that the methane concentration in Zone 4 did increase during the no-pumping stages, indicating that methanogenesis was probably occurring despite the injection of sulfate and nitrate.

The concentration of *m* + *p*-xylene in Zone 4 decreased rapidly during the initial flushing stages and did not rebound during the no-pumping stage. This behavior is qualitatively different from the behavior in Zone 2, which received sulfate only, and in which *m* + *p*-xylene rebounded during no-pumping stages (cf. benzene data in Figure 10). It is possible that this indicates stimulation of *m* + *p*-xylene degradation via nitrate augmentation; however, because the concentration was relatively low even at the beginning of the experiment, it is difficult to conclude with certainty. Ethylbenzene behaved in a manner qualitatively identical to *m* + *p*-xylene (data not shown). The results of the third augmentation in Zone 2 (Figure 10) also suggest that nitrate addition stimulates ethylbenzene degradation, a result which has been observed previously (Rabus & Widdel 1995, Ball et al. 1996, Ball & Reinhard 1996).

Toluene and *o*-xylene concentrations were very low in Zone 4 throughout the entire experiment, so it is impossible to assess the effect of combined nitrate and sulfate augmentation on the biodegradation of toluene or *o*-xylene.

Conclusions

Enhanced anaerobic biodegradation of groundwater contaminated by fuel hydrocarbons has recently been evaluated at a field experiment conducted at the Naval Weapons Station (NWS), Seal Beach, California. A portion of the shallow groundwater at NWS Seal Beach had been contaminated by fuel hydrocarbons from a leaking underground storage tank. This experiment included the establishment of three different remediation zones *in situ*: one zone was augmented with sulfate, one was augmented with sulfate and nitrate, and the third was unaugmented. During the course of the 17-month experiment, more than 9000 samples were collected from 105 different sampling locations, and were analyzed for 15 different analytes, thereby providing detailed spatial and temporal resolution of the subsurface concentrations. The principal objectives of the experiment were (1) to demonstrate that enhancing the natural biodegradation process with sulfate and/or nitrate addition is a feasible engineering approach; and (2) to compare the *in situ* biodegradation rate of fuel hydrocarbons under three different sets of geochemical conditions, namely methanogenic, sulfate-reducing, and denitrifying.

In the zones that were augmented with sulfate and/or nitrate, the added electron acceptors were consumed quickly. This indicates that the supply of electron acceptors is a limiting factor for hydrocarbon degradation at the Seal Beach site. Thus, enhancement via injection of electron acceptors is believed to accelerate the biodegradation of the petroleum hydrocarbons. In the zone which received sulfate only, the apparent first-order rate coefficient for sulfate utilization was approximately 0.1 day^{-1}. In the zone which received nitrate and sulfate, nitrate was used preferentially over sulfate, with an apparent first-order rate coefficient of 0.1–0.6 day^{-1}. The data suggest that slow sulfate utilization can occur in the presence of nitrate, i.e., the two processes are not strictly sequential. Injection of nitrate appears to be an effective means of oxidizing sulfide back to sulfate.

Certain fuel hydrocarbons were removed preferentially over others. The order of preference depends

upon the geochemical conditions. With regard to the BTEX compounds, toluene was preferentially removed under intrinsic conditions, as evidenced by its very low concentration in all regions at the beginning of the experiment. Augmentation with sulfate preferentially stimulated the removal of *o*-xylene, but did not appear to significantly accelerate the biodegradation of benzene, ethylbenzene, or *m* + *p*-xylene. Augmentation with nitrate appears to have stimulated the removal of ethylbenzene, and might also have stimulated the biodegradation of *m* + *p*-xylene. Biodegradation of benzene was slow under all geochemical conditions if it occurred at all. Thus, at the Seal Beach site, it is expected that benzene will be the limiting factor in the overall clean-up process.

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