

Enhancement of Microbial Sulfate Reduction for the Remediation of Hydrocarbon Contaminated Aquifers - a Laboratory and Field-Scale Project – Final Report Executive Summary

Period Covered by the Report: October 1, 2000 to September 30, 2001 (N/C Ext. May 31, 2002)

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Title: Enhancement of Microbial Sulfate Reduction for the Remediation of Hydrocarbon Contaminated Aquifers – a Laboratory and Field-Scale Project.

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Research Category: Groundwater remediation

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Objectives of the Research Project: The primary goal of this project was to design, implement, and monitor an effective enhanced anaerobic bioremediation technology for treating a hydrocarbon impacted aquifer. The specific goals of this project were to:

1. Use laboratory experiments coupled with geochemical, hydrological, and contaminant characterizations to design a sulfate amendment process for stimulating BTEX biodegradation rates in-situ.
2. Establish baseline contaminant, geochemical, and microbiological conditions prior to sulfate injection.
3. Monitor the performance of the enhanced anaerobic biodegradation process primarily for BTEX remediation.
4. Identify the applicability and limitations of the process.

Progress Summary/ Accomplishments: Geochemical and microbiological characterization of the aquifer justified the use of sulfate amendments as bioremediation strategy: sulfate-reduction activity was limited by sulfate and could be stimulated by its addition. We detected required biodiversity of sulfate-reducing bacteria. Also, addition of sulfate to anoxic sediments stimulated ¹⁴C-benzene biodegradation in laboratory incubations. Based on this knowledge we designed a sulfate amendment process for stimulating BTEX biodegradation rates and successfully implemented it in the field. Benzene decreases have been observed in the wells that were influenced by sulfate injection. The benzene plume decreased in size and concentration after injecting sulfate. Benzene concentrations did not decrease in the “control” monitoring well located downgradient of the three injection wells in which produced water (supplemented with tracers but not sulfate) was injected.

INTRODUCTION: Intrinsic bioremediation has recently emerged as strategy for reducing the concentration of contaminants in subsurface environments. In the 1990s, remediation via natural attenuation has received notoriety for being the most cost-effective solution to remediate a petroleum-contaminated subsurface environment. While proven a viable strategy to remediate hydrocarbon contamination at many sites, intrinsic bioremediation is not a panacea for aquifer remediation. The time required for natural attenuation to effectively degrade hydrocarbon contaminants in-situ is unknown, although hundreds of years are likely. Given this large time frame, the cost effectiveness of monitored intrinsic bioremediation should be in question. Cost effective methods to enhance natural attenuation rates need to be identified.

It has been well documented that aquifers contaminated by organic pollutants become rapidly depleted of oxygen due to the activities of aerobic heterotrophic microorganisms. Once oxygen is consumed, other electron acceptors must be readily available and requisite populations capable of utilizing these electron acceptors must be present, else further natural biodegradation processes cannot proceed. The depletion of electron acceptors is generally considered to be the primary factor that limits BTEX biodegradation in-situ. To overcome these limitations, scientists have been exploring the use of “enhanced” intrinsic bioremediation to speed site clean-up (Morin, 1997). This technology involves supplementing subsurface populations with nutrients and/or electron acceptors, which may be limiting in order to stimulate biodegradation of contaminants. The key to this strategy is to stimulate the activities of naturally occurring microorganisms.

Oxygen has most often been added as the terminal electron acceptor in traditional *in situ* treatment schemes for BTEX-contaminated plumes. Air sparging uses injected oxygen to facilitate aerobic degradation and potentially volatilization (light hydrocarbons only) of contaminants. The limiting factor of this technology is the low solubility of oxygen in groundwater and the ability to achieve good distribution thereof in the subsurface. In addition, the performance of this technology in lower permeability formations or in formations with interbedded clays is highly questionable. Several studies show that this process is ineffective even with a high density of injection wells and constant injection, due to the inability to distribute oxygen into aquifers. Hydrogen peroxide is frequently added because it degrades to form H₂O and O₂, providing a cheaper and more soluble source of oxygen than pure O₂ (40-50 ppm) or air (8-10 ppm in water) (Lee et al., 1987). However, concentrations of greater than 200 ppm H₂O₂ can be toxic to microorganisms and concentrations of 100 ppm may result in the formation of O₂ bubbles which can effectively plug the injection well (Lee et al., 1987; Wilson and Ward, 1987). Adding oxygen to highly reduced, anaerobic aquifers, as an electron acceptor is not effective due to the presence of oxygen scavenging compounds, such as reduced iron and sulfides that are common in BTEX impacted aquifers.

In the last decade, it has been proven that oxygen is unnecessary for the biodegradation of BTEX hydrocarbons. These contaminants have now been found to be biodegraded under nitrate-reducing, iron-reducing, sulfate-reducing, and/or methanogenic conditions (Krumholz et al., 1996). The discovery that BTEX hydrocarbons are biodegraded under

anaerobic conditions has permitted alternate remedial strategies, in which anaerobic electron acceptors have been considered as additives to stimulate *in situ* bioremediation. Electron acceptors that are highly water-soluble and that have an increased capacity to transfer electrons are most desirable. An estimate of the electron-accepting capacity of an injected electron acceptor is a function of the maximum concentration that can be injected and the number of electrons that can be transferred by the electron acceptor. The injection of nitrate could potentially oxidize a large quantity of hydrocarbons because it can be injected at very high concentrations and has a relatively high electron transfer capacity (5 electrons per NO_3^- molecule). However, an important drawback of using nitrate is that it is a regulated drinking water contaminant because it results in detrimental health effects to infants (Thomas and Ward, 1989). Another potential drawback with nitrate is that it is not naturally abundant in geologic formations, thus in many aquifers, microbial populations may not be adapted to utilizing nitrate as an electron acceptor. Fe(III), commonly present in aquifer environments as insoluble, amorphous iron oxides, may also serve as an electron acceptor for hydrocarbon degradation *in situ* given its widespread abundance (Anderson et al., 1998). The potential for adding Fe(III) chelated to nitrilotriacetic acid to stimulate benzene and toluene degradation has been explored at the bench-scale level (Lovley et al., 1994) but adding organic chelators alongside electron acceptors would only exacerbate organic carbon load in the subsurface.

Sulfate can also serve as an electron acceptor for BTEX biodegradation *in situ*. The advantage of injecting sulfate rather than oxygen to stimulate biodegradation is that high concentrations of dissolved sulfate provide a substantially higher electron-accepting capacity relative to oxygen. A recent study showed that intrinsic bioremediation of BTEX hydrocarbons *in situ* occurred predominantly under sulfate-reducing conditions, as sulfate was the only available electron acceptor present in sufficient quantity in the contaminated groundwater (Gieg et al., 1999). Nevertheless, even the supply of sulfate, which was abundant in the native groundwater, was limiting due to the high concentrations of hydrocarbon that served as electron donor in the aquifer. In a field study where BTEX hydrocarbons were injected into a groundwater system with nitrate or sulfate added as electron acceptors, biodegradation was only slightly slower with sulfate than with nitrate (Reinhard et al., 1997). Unlike nitrate, sulfate is not toxic. Sulfate is also abundant in geologic formations where it occurs either dissolved in groundwater or as mineral forms including barium sulfate and gypsum.

One disadvantage to adding sulfate is that biological sulfate reduction can lead to toxic concentrations of sulfide. This occurs once reactive forms of iron, including iron-oxyhydroxides that rapidly precipitate sulfide (Canfield, 1992), become depleted. Indeed dissolved sulfide has been detected in hydrocarbon contaminated aquifers (Gieg et al., 1999). Relatively high concentrations of hydrogen sulfide have been shown to be inhibitory to BTEX biodegradation under sulfate-reducing conditions (Reinhard et al., 1997). An important goal of the laboratory research proposed below will be to assess the potential for dissolved sulfide accumulation during sulfate injection and to obtain a method to mitigate this potential problem. Research conducted to address souring of natural oil reserves has shown that nitrate addition can curb souring by stimulating

sulfide oxidizing bacteria and by inhibiting sulfate reduction (McInerney et al., 1992; Reinsel et al., 1996). On the basis of these studies, the periodic injection of nitrate may be useful to prevent sulfide accumulation.

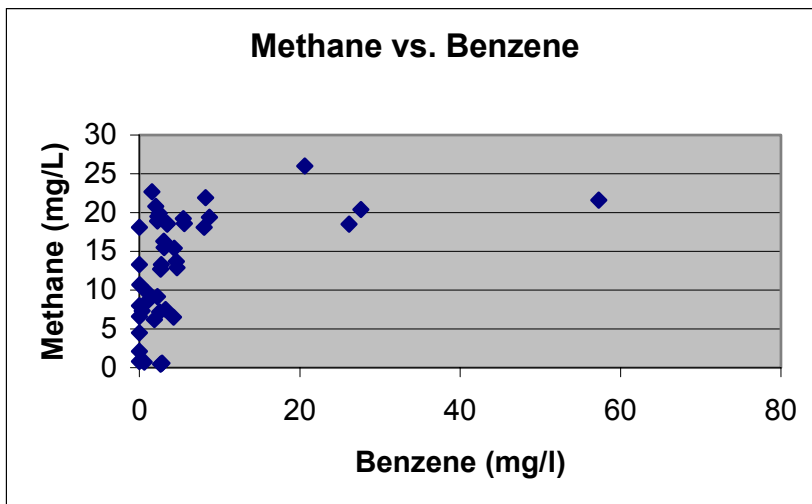
Based on these findings, it seems very promising that a relatively simple bioremedial system relying only on the injection of sulfate and other nutrients can be developed. However, this remedial strategy must be optimized and better understood before it can be implemented at full-scale. Some of the key issues that needed to be addressed are reflected in the “Objectives”.

RESEARCH RESULTS:

Field Sampling and Laboratory Research

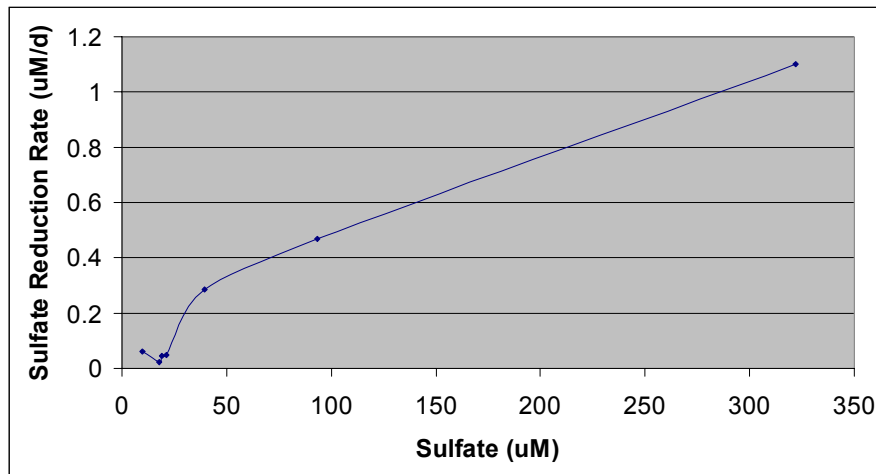
Laboratory experimentation and groundwater geochemical surveys were used to assess whether sulfate amendment is an acceptable bioremediation strategy at the study location. Groundwater throughout the BTEX impacted regions of the site is depleted of oxygen, nitrate, and sulfate. High concentrations of methane were detected particularly in areas that contain increased levels of benzene and other hydrocarbons (Figure 1). Thus, the geochemical signatures attest to the electron acceptor depleted conditions within impacted areas of the aquifer.

Figure 1. Dissolved methane vs. dissolved benzene.



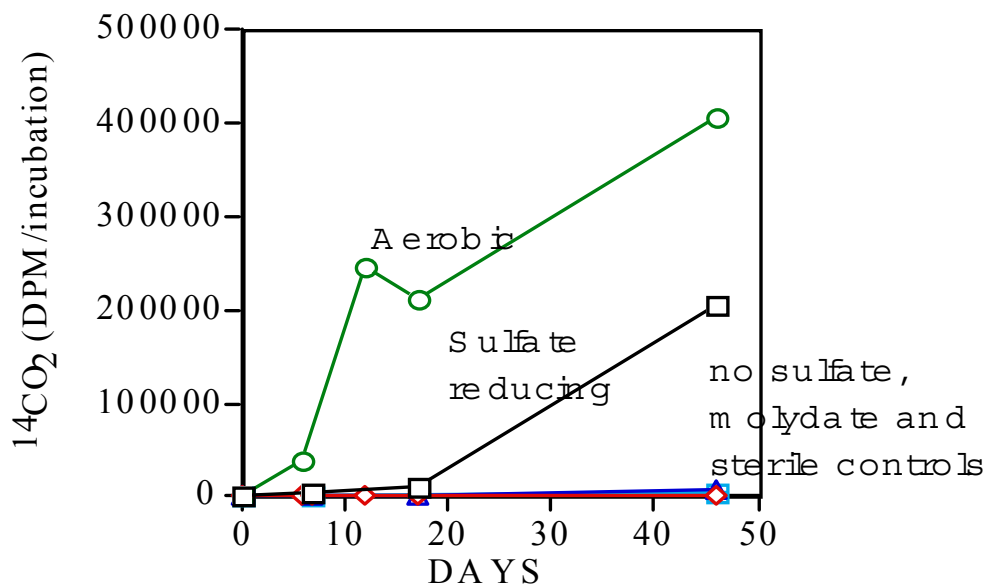
Sulfate reduction rates in groundwater samples obtained prior to amending the aquifer with sulfate correlated positively with the in-situ concentration of sulfate (Figure 2). In consistent fashion, after the aquifer was supplemented with sulfate, sulfate reducing activity in groundwater samples increased (data not shown). Several weeks of exposure to sulfate was required to stimulate sulfate reduction activity in some regions.

Figure 2. Correlation of sulfate reduction rates measured in groundwater samples with sulfate concentration.



Laboratory experiments were also conducted to identify the effect of sulfate addition on benzene biodegradation. As illustrated in figure 3, the addition of sulfate to anoxic sediment slurries stimulated ^{14}C -benzene decomposition relative to slurries that were not amended with sulfate. Benzene biodegradation was not observed in incubations that were treated with molybdate to inhibit sulfate reduction, thereby providing additional evidence for the importance of sulfate. Sulfate addition stimulated benzene biodegradation in samples obtained from many, but not all, of the sampling locations within the study site (not shown).

Figure 3. ^{14}C -benzene decomposition under various conditions as evidenced through the production of $^{14}\text{C}\text{-CO}_2$.

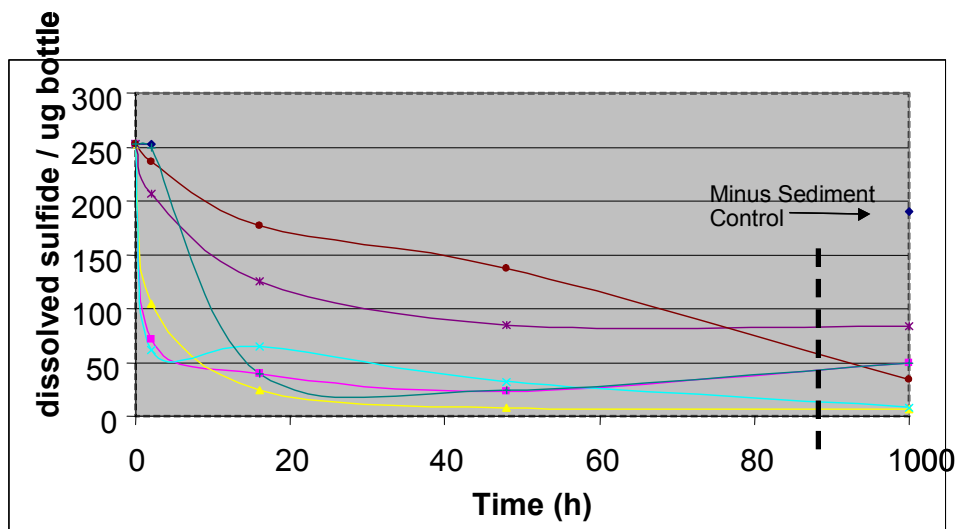


Environmental factors other than sulfate availability were evaluated for their potential impact on benzene biodegradation. For instance, toxicity experiments indicated that benzene concentrations over 100 mg/L did not inhibit sulfate reduction. Therefore, benzene toxicity is likely not an important variable governing hydrocarbon biodegradation linked to sulfate reduction at this site.

The results of the geochemical surveys and laboratory experiments provided ample information arguing that sulfate amendment should be a viable strategy to treat benzene at the site. The groundwater is depleted of sulfate, sulfate amendment stimulated sulfate reduction and benzene biodegradation in sediment slurries, and the benzene concentrations at the site were not inhibitory. To further assess this technology, the potential adverse consequences of injecting sulfate were explored.

The potential for dissolved sulfide formation was evaluated by identifying the capacity of the sediments to precipitate sulfide. Sediments collected from several regions were found to have a high capacity to precipitate sulfide thereby minimizing the potential for dissolved sulfide accumulation. Figure 4 illustrates decreases in sulfide concentration over time in sediment slurries that were amended with sodium sulfide. These analyses revealed that a significant quantity of sulfide can be precipitated in the sediment thereby minimizing the potential for dissolved sulfide accumulation. Nevertheless, we explored whether nitrate amendment

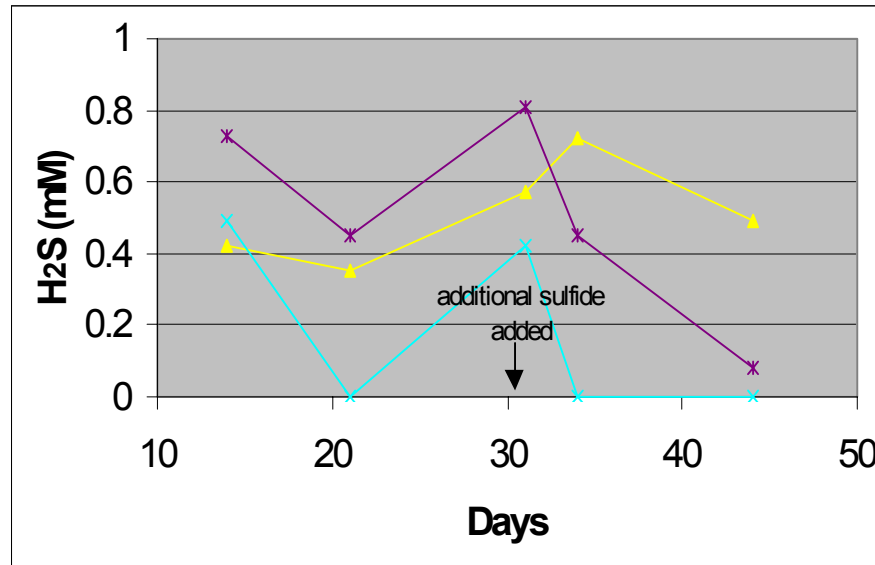
Figure 4. Decrease in dissolved sulfide over time in anoxic sediment slurries due to precipitation with iron.



could be used to oxidize sulfide, should it be produced over extended periods. As shown in figure 5, the dissolved sulfide added to sediment slurries decreased in concentration more rapidly in incubations that were treated with nitrate (blue lines) relative to incubations that were not supplemented with nitrate or sterilized (with nitrate). Sulfate

accumulated in the nitrate amended incubations indicating that sulfide was oxidized completely to sulfate. Iron sulfide minerals were also oxidized in the sediment slurries containing nitrate. These results suggest that the periodic input of nitrate could be used as a means to oxidize sulfide should it be produced. This oxidation process could supply additional sulfate as electron acceptor and produce iron phases that are more reactive towards sulfide.

Figure 5. Decrease in dissolved sulfide concentration in incubations amended with nitrate (blue lines).

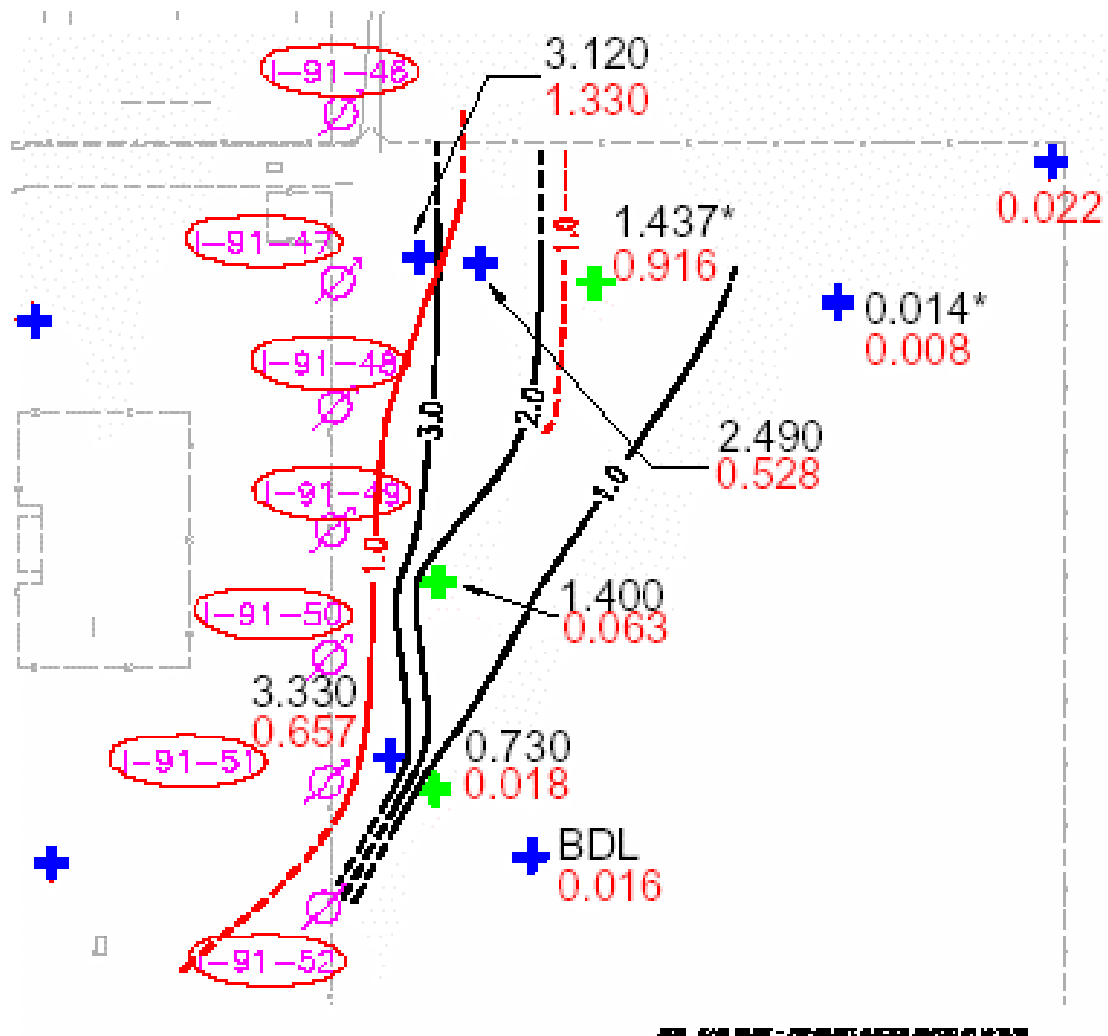


Field Implementation

Beginning in October of 2001, Surbec-ART and Conoco Inc. implemented an enhanced anaerobic BTEX bioremediation project in the vicinity of the South Plant at the Conoco refinery in Ponca City. Several groundwater parameters have been monitored to evaluate the effectiveness of the system. These include 1) the distribution of sulfate and groundwater tracers (bromide and fluorescein), 2) geochemical indications of sulfate reduction, 3) the concentrations of BTEX hydrocarbons, and 4) potentiometric surface maps relative to baseline conditions.

Benzene decreases have been observed in several wells that were influenced by sulfate injection. Figure 6 illustrates the benzene contours prior to (black contours), and approximately four months post (red contours), sulfate injection in the southeastern portion of the treatment area. The benzene plume decreased in size and concentration after injecting sulfate. Benzene biodegradation was initially observed mainly in the southeastern portion of the treatment area and expanded over time to the north where higher levels of BTEX were present.

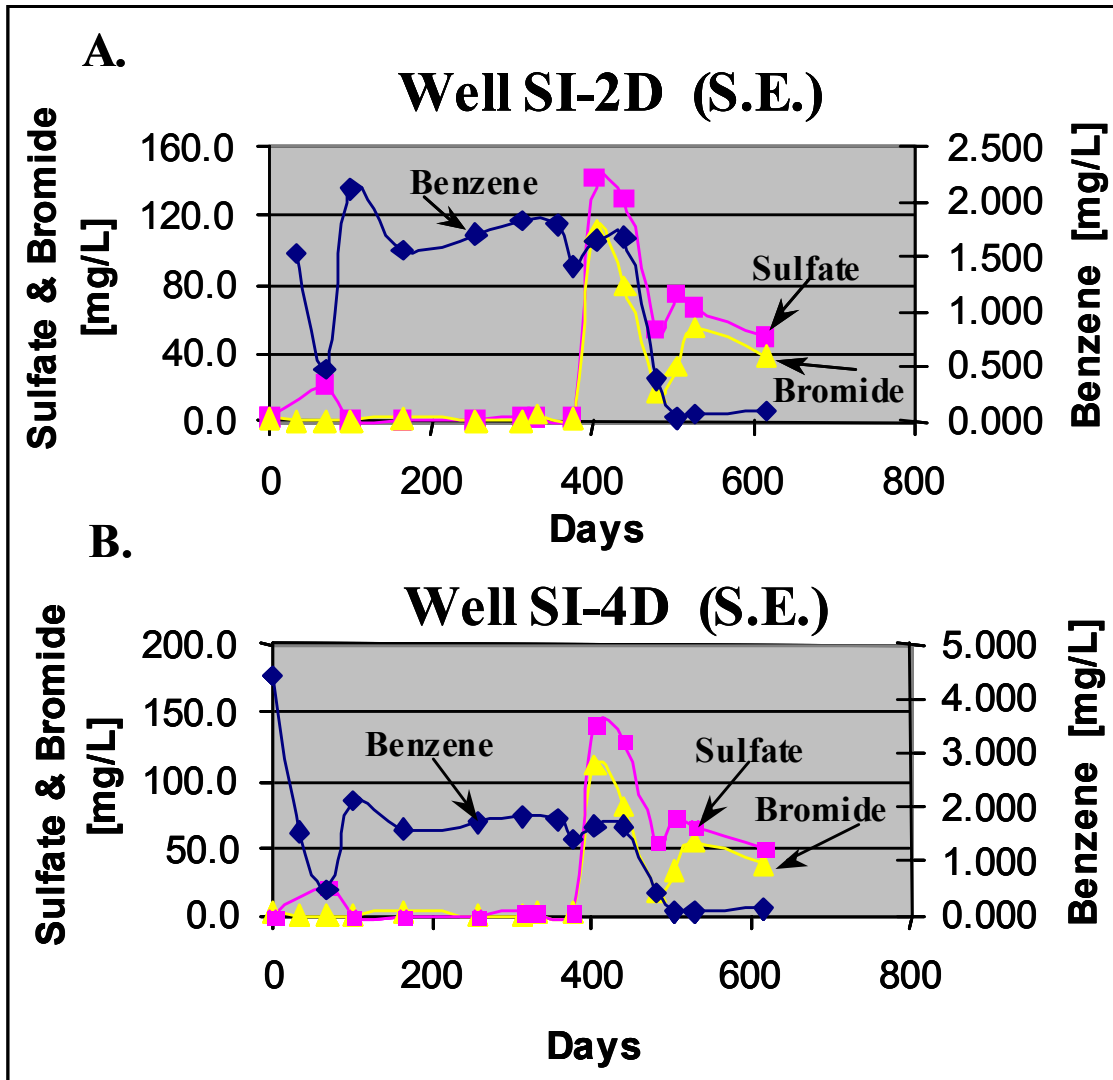
Figure 6. Benzene concentrations prior to (black contours) and approximately 4 months after (red contours) injecting sulfate.



Several lines of evidence indicate that the decreases in benzene were attributed to sulfate amendment and not to dilution or natural BTEX trend declines. Close inspection of figure 6 reveals that dissolved benzene concentrations did not decrease immediately after sulfate breakthrough. This delay is consistent with a lag period associated with the initiation of benzene biodegradation. In addition, the benzene concentrations in SI-2D and SI-4D are below the concentration of benzene in the produced water that was used for injecting the sulfate. The degree of natural fluctuations in benzene concentrations in

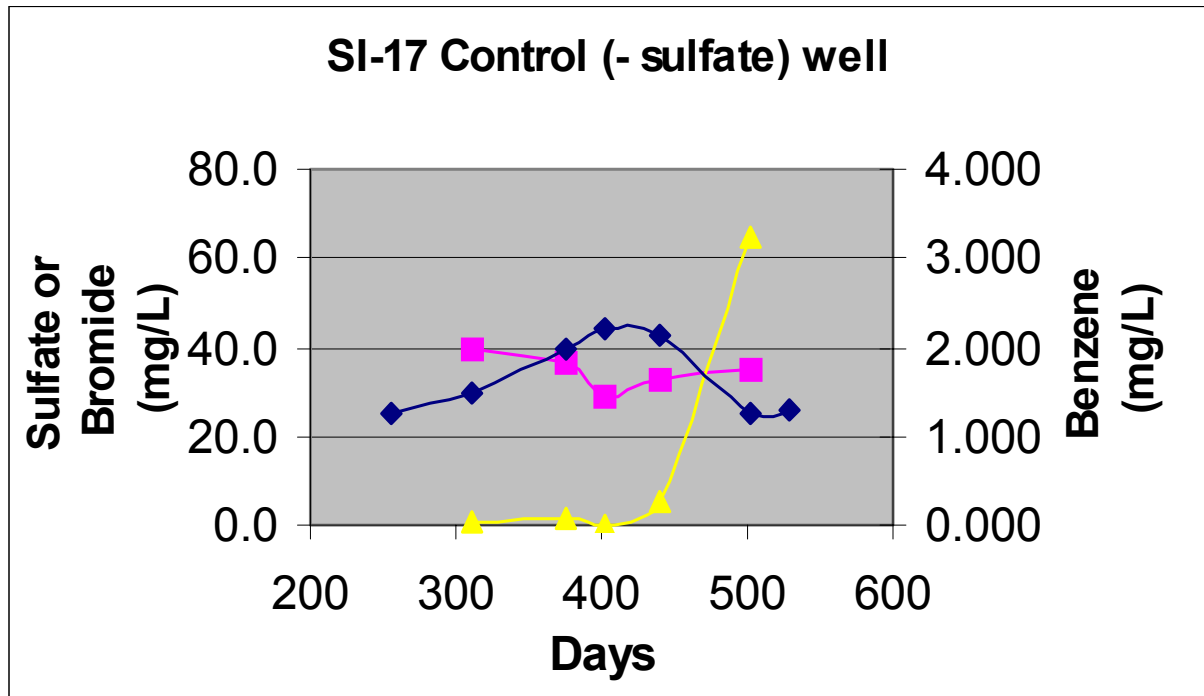
areas not influenced by sulfate injection during the same time frame has been minimal, relative to the decreases observed in the treatment zone.

Figure 7. Groundwater monitoring data illustrating benzene decreases that coincided with sulfate breakthrough.



Benzene concentrations did not decrease in the “control” monitoring well (SI-17) located downgradient of the three injection wells (I91-54, I91-55, I91-56) in which produced water (supplemented with tracers but not sulfate) was injected. Benzene concentrations in SI-17 actually increased slightly from approximately 1.4 mg/L to approximately 2.2 mg/L after sulfate injection was initiated. This slight increase could be a result of the increased flux of benzene from the vicinity of the injection wells.

Figure 8. Groundwater monitoring data collected from the “control” monitoring well where tracers but not sulfate was injected.



Sulfate amendment did not result in a decrease in benzene concentration in all regions of the treatment area during the monitoring period. For instance, benzene concentrations did not decrease an isolated area that contained greater than 50 ppm benzene. This region also contained increased levels of other hydrocarbons including alkanes. Laboratory experiments conducted with sediment from this location indicated that sulfate addition stimulated alkane, but not benzene, biodegradation in these samples. Thus, the preferential biodegradation of labile hydrocarbon in this area may preclude benzene decomposition. It is not known whether continued sulfate input would eventually deplete these labile hydrocarbons thereby allowing benzene decomposition to commence.

Conclusions: Sulfate amendment is a viable bioremediation technology. Sulfate injections into anoxic aquifer contaminated with BTEX resulted in substantial reduction of benzene plume in size and concentrations. Toxicity experiments indicated that benzene concentrations over 100 mg/L did not inhibit sulfate reduction. In case when sediment capacity to precipitate sulfide is limited, the periodic input of nitrate could be used as a means to oxidize sulfide should it be produced.

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Publications/Presentations:

- Ulrich, G.A., Suzuki M., Hollingsworth M., Suflita, J.M., Steger J., Davidova I.A. 2001. Evaluation of enhanced anaerobic biodegradation via sulfate amendment to treat an aquifer at a refinery. The Eighth International Petroleum Environmental Conference, Issues and Solutions in Exploration, Production and Refining. November 6-9, 2001, Houston, Texas.

Ulrich, G.A., Davidova I.A., Steger J., Duncan K.E., and J.M. Suflita. Enhancement of Microbial Sulfate Reduction for the Remediation of Hydrocarbon Contaminated Aquifers. (In prep.)

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groundwater, aquifer, ecological effects, carcinogen, benzene, BTEX, aquatic, bioremediation, environmental chemistry, biology, analytical methods, surveys, measurement methods, south central United States, petroleum refinery, anaerobic, biodegradation, sulfate