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ENHANCED IN SITU ANAEROBIC BIOREMEDIATION OF FUEL-CONTAMINATED GROUND WATER

by

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Enhanced anaerobic biodegradation of ground water contaminated by fuel hydrocarbons has been evaluated at a field demonstration conducted at the Naval Weapons Station, Seal Beach, California. This demonstration included establishing three different remediation zones in situ: one zone was augmented with sulfate, one was augmented with sulfate and nitrate, and the third was not augmented. 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 demonstration are: (1) Certain fuel hydrocarbons were removed preferentially over others, but the order of preference depends on 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. 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. 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.

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EXECUTIVE SUMMARY

Enhanced anaerobic biodegradation of groundwater contaminated by fuel hydrocarbons has been evaluated at a field demonstration conducted at the Naval Weapons Station, Seal Beach, California. This demonstration included establishing three different remediation zones in situ: one zone was augmented with sulfate, one was augmented with sulfate and nitrate, and the third was not augmented. This enables a comparison of hydrocarbon biodegradation under sulfate-reducing, sequential denitrifying/sulfate-reducing, and methanogenic conditions, respectively. The results from the field demonstration are: (1) Certain fuel hydrocarbons were removed preferentially over others, but the order of preference depends on 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. 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. 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.

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1. Introduction

1.1 Background Information

Ground water contamination is a significant problem at thousands of Department of Defense (DOD) installations and formerly used defense sites. Particularly common is ground water contamination from fuel hydrocarbons, which often leak from underground storage tanks. The primary contaminants of concern in fuel hydrocarbon spills are 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 parts per billion (ppb). Toluene, ethylbenzene, and xylene have MCLs of 1 part per million (ppm), 0.7 ppm, and 10 ppm, respectively; exposure above these levels might impair human health. Therefore, the widespread contamination from BTEX represents a significant environmental problem, both at DoD sites and at privately owned properties throughout the United States.

The commonly used pump-and-treat method for removing contamination from ground water has proven to be much more expensive, time consuming, and inefficient than first envisioned. As a result, in situ bioremediation is now being investigated, evaluated, and, in some cases, applied as a remediation technology at contaminated ground water sites. The principle of in situ bioremediation is that certain bacteria, found naturally in ground water aquifers, can metabolize particular contaminants, thereby reducing the contaminant concentrations without extracting the water from the aquifer. These metabolic processes, coupled with other naturally occurring processes that can reduce the contaminant concentrations in ground water, are referred to as natural attenuation. When naturally occurring metabolic processes are used to remediate a contaminated site without any additional alteration of site conditions, the process is referred to as intrinsic in situ bioremediation. When conditions at the site are engineered or altered in order to stimulate or accelerate the biological destruction of contaminants, the process is referred to as enhanced in situ bioremediation. The United States Environmental Protection Agency (EPA) has recently outlined its policy on the use of monitored natural attenuation (i.e., intrinsic bioremediation, with appropriate monitoring) for the remediation of contaminated soil and ground water at sites regulated under Office of Solid Waste and Emergency Response programs [U.S. EPA, 1997b].

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, but is not as well understood. Nitrate, sulfate, ferric iron, or carbon dioxide can replace oxygen as the terminal electron acceptor (oxidant). Because of its relative simplicity and low cost, intrinsic bioremediation is often the method of choice for remediating fuel hydrocarbons [U.S. EPA, 1997b]. However, intrinsic bioremediation can be slow, is often unpredictable, and may be inadequate for many sites, particularly under anaerobic conditions. Thus, engineered intervention (enhancement) might be necessary in order for the oxidation to proceed at acceptable rates.

Enhanced bioremediation might consist of augmenting the contaminated zone with electron acceptor(s), removing products that inhibit the oxidation reaction, or both.

The potential benefits from this technology are large. The principal advantage of bioremediation over conventional pump-and-treat remediation is that the contaminants are destroyed in situ and no secondary waste streams are produced needing further treatment. In situ bioremediation might be susceptible to some of the same impediments that limit the efficiency of the pump-and-treat method; for example, slow contaminant leaching of adsorbed contaminants from aquifer solids can present a continuing source of contamination. However, maintaining the presence of electron acceptors in the contaminated aquifer via periodic injection should be far less expensive than continuous ground water extraction. Therefore, for sites where it can be shown that enhanced in situ bioremediation is technically feasible, it should be less expensive to implement than a pump-and-treat system.

This report describes the results of a project that was intended to meet two aims:

(1) Demonstration of a technology by which contaminants (principally BTEX) are removed from ground water via enhanced in situ anaerobic biodegradation
(2) Investigation of the factors that govern anaerobic biodegradation of fuel hydrocarbons (principally BTEX) under three different types of geochemical conditions.

In order to achieve both of these goals, a system of injection and extraction wells was installed at a fuel-contaminated site. Because one of the goals of this project was quantification of the efficacy of anaerobic bioremediation under particular geochemical conditions, the system was equipped with more observation points and greater operational controls than necessary for a typical application. Throughout the remainder of this report, we note the particular aspects in which our operation differed from the recommended operation at other contaminated sites. Here we also note that this project was not intended to address the removal of methyl *tert*-butyl ether (MTBE), a gasoline additive that is commonly found in ground water at sites contaminated with BTEX.

1.2 Official Department of Defense (DOD) Requirement Statement

The work accomplished under this effort responds to the following DOD environmental technology requirements:

Army: A (1.2.b), Enhanced Alternative and In-Situ Treatment Technologies for Organics (Non-Halogenated) in Groundwater (96–97)

Navy: (1.I.1.e), Improved remediation of groundwater contaminated with non-chlorinated hydrocarbons.

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1.3 Objectives of the Demonstration

This project was intended to meet two broad objectives:

(1) Demonstration of a technology by which fuel hydrocarbons are removed from ground water via enhanced in situ anaerobic biodegradation
(2) Evaluation and quantification of the efficacy of three different anaerobic processes.

The focus of the technology demonstration was on the aromatic hydrocarbons benzene, toluene, ethylbenzene, and xylene (BTEX). Previous laboratory and pilot-scale research had shown that BTEX compounds, especially toluene, can be mineralized under anaerobic conditions using nitrate or sulfate as an electron acceptor [Haag et al., 1991; Edwards et al., 1992; Ball and Reinhard, 1996; Reinhard et al., 1997]. This demonstration involved application of the previous results to a large field site. The site chosen for the demonstration was a contaminated portion of an aquifer at the Naval Weapons Station, Seal Beach, in southern California (Figures 1 and 2). This site was contaminated with fuel hydrocarbons from a leaking underground storage tank, as described in the report "Delineation of a hydrocarbon (weathered gasoline) plume in shallow deposits at the U.S. Naval Weapons Station, Seal Beach, California" [Schroeder, 1991].

With respect to objective (1), the technology demonstration, there were two specific goals:

(i) Validation of the technical viability of enhanced in situ anaerobic bioremediation, i.e., demonstration that anaerobic microbial processes can potentially be used for clean-up of sites contaminated with gasoline and/or other fuel hydrocarbons
(ii) Development of cost data for implementing the technology.

With respect to objective (2), the evaluation of anaerobic in situ bioremediation under different geochemical conditions, three different treatment zones (denitrifying, denitrifying and sulfate-reducing, and methanogenic) were established at the contaminated site. These zones are described in more detail in section 2.1.2, below.

The information developed in this project should allow responsible parties, consultants, remediation contractors, and the general public to better evaluate enhanced anaerobic in situ bioremediation as an option for treating sites contaminated with fuel hydrocarbons, and should contribute to the regulatory acceptance of this technology.

1.4 Regulatory Issues

There are three main regulatory issues with regard to the implementation of the technology described in this report:

(1) Nitrate, which has a drinking water maximum contaminant level (MCL) of 45 mg/L, is injected into the ground water as an electron acceptor. In order for the technology to operate at maximum efficiency, nitrate should be injected at a concentration higher than its MCL. Although nitrate is consumed rapidly after its injection, and therefore does not

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represent a significant contamination risk, regulatory approval is required for injection of nitrate at high concentrations.

(2) Contaminated ground water is extracted, treated, augmented with electron acceptors, and then re-injected into the aquifer. The re-injection of treated ground water requires regulatory approval.

(3) It must be satisfactorily demonstrated that sufficient hydraulic control is established, so that the plume can be contained if any problem arises during implementation of the remediation technology.

Regulatory concern about these three issues is likely to vary from state to state and from region to region. Some methods for working with regulators to gain regulatory acceptance of this technology include the following.

(1) Using contaminated soil and water from the site in question, develop a laboratory microcosm experiment from which the utilization rate of nitrate can be estimated. The laboratory-derived utilization rate may differ from the actual utilization rate in a full-scale implementation, but the demonstration of rapid nitrate utilization would likely help to gain regulatory acceptance.

(2) Before re-injecting treated water into the aquifer, run the system in a pump-and-treat mode only, in order to demonstrate that the water treatment system is capable of removing contaminants of concern. This will provide regulators with the opportunity to evaluate the quality of the treated water that is to be re-injected.

(3) Before injection of electron acceptors (nitrate in particular), inject a tracer such as bromide, and then run the system in a pump-and-treat mode only, in order to demonstrate that the bromide can be fully recovered. This will provide regulators with the opportunity to evaluate the hydraulic control at the contaminated site.

(4) When nitrate is first injected, it should be injected at concentrations below its MCL of 45 mg/L -- perhaps about 15 mg/L. The next injection of nitrate can be at a higher concentration, but still below the MCL. That will give the microbial population a chance to adapt to the presence of nitrate, and will provide regulators with the opportunity to evaluate nitrate utilization in situ. If nitrate is consumed rapidly at concentrations below the MCL, regulators will be more likely to allow injection at concentrations which exceed the MCL.

1.5 Previous Testing of the Technology

Despite an abundance of laboratory data suggesting the probable success of enhanced in situ bioremediation, there have been relatively few field-scale demonstrations. In a few instances, technologies similar to the one described in this report have been tested elsewhere. Generally, the comparable technologies have been implemented in the following manner: water containing an electron acceptor (nitrate) is ponded in ditches, applied to the ground surface via sprinkler, or injected via wells into the vadose zone above a contaminated area. The water then infiltrates vertically into the contaminated zone, where the electron acceptor can stimulate biological transformation of BTEX. An extraction well is placed downgradient of the infiltration area to

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collect any migrating contaminants that are not biodegraded. This technology has been applied at:

(1) U.S. Coast Guard station where JP-4 jet fuel was spilled [Hutchins, et al., 1991]
(2) Abandoned refinery [Battermann and Meier-Lohr, 1995]
(3) Pipeline spill in Park City, Kansas [Hutchins, et al., 1995]
(4) U.S. Air Force base where JP-4 jet fuel leaked from underground piping [Thomas, et al., 1995; Sweed, et al., 1996; Wiesner, et al., 1996]
(5) Military storage facility for jet fuel [Vroblesky, et al., 1997].

Many of these cases have reported partial or complete removal of BTEX compounds from the contaminated area. However, in these instances, it is not clear how much of the BTEX removal was due to anaerobic degradation, and how much was due to other processes such as aerobic degradation or extraction in the downgradient well. Furthermore, these studies were performed with augmentation of only a single electron acceptor, namely nitrate. One of the objectives of the demonstration described in this report is to investigate anaerobic bioremediation of BTEX with nitrate and sulfate as electron acceptors, and to evaluate nitrate- and sulfate-reducing conditions vis-a-vis methanogenic conditions. These different geochemical conditions are explained further in Section 2.1.1, below.

In another relevant field-scale study performed in the Borden aquifer in Ontario, Canada [Barbaro et al., 1992], it was found that toluene, ethylbenzene, and xylene could be partially degraded when the aquifer was enhanced with nitrate as an electron acceptor. In the absence of nitrate, contaminant degradation was considerably slower. Regardless of the nitrate concentration, benzene was recalcitrant, exhibiting very little transformation if any at all.

In general, studies have produced conflicting results regarding the likelihood for benzene removal via anaerobic biodegradation. Although benzene has sometimes been found to degrade under sulfate-reducing conditions [Edwards and Grbic-Galic, 1992; Lovley et al., 1995], nitrate-reducing conditions [Burland and Edwards, 1999], and methanogenic conditions [Grbic-Galic and Vogel, 1987], the degradation process appears to be very sensitive to experimental conditions. In other cases, benzene has been found recalcitrant under nitrate-reducing conditions [Acton and Barker, 1992; Ball and Reinhard, 1996; Reinhard et al., 1997] and under sulfate-reducing conditions [Edwards et al., 1992; Thierrin et al., 1995]. Therefore, one particularly interesting aspect of the project described in this report is the opportunity to evaluate benzene degradation under different anaerobic conditions (described below) at an actual field site.

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2. Technology Description

2.1. Description

One of the two primary objectives of this project is to demonstrate a technology by which BTEX compounds are removed from ground water via enhanced in situ anaerobic biodegradation. This technology requires that we perform the following tasks:

(1) Determine the capacity of anaerobic bacteria for mineralizing fuel hydrocarbons under in situ conditions
(2) Establish a procedure for supplying electron acceptors to an aquifer contaminated with fuel hydrocarbons
(3) Establish a procedure for removing metabolic products that inhibit or interfere with the bioremediation process. In this regard, sulfide is probably of the greatest concern, because sulfate reduction is an important process in this technology, and anaerobic BTEX oxidation is known to be inhibited by high sulfide concentrations [Beller and Reinhard, 1995].

Sub-section 2.1.1, below, describes some of the fundamentals of the microbial degradation processes utilized in this demonstration. Sub-section 2.1.2 describes the engineering process utilized.

2.1.1 Microbial Degradation: Microorganisms are able to use BTEX contaminants as substrates for energy and growth, and electron acceptors such as oxygen, nitrate, or sulfate to convert the contaminants into harmless products (principally carbon dioxide and water), cell mass, and inorganic salts. In almost all cases, oxygen is consumed preferentially over alternate electron acceptors like sulfate or nitrate; after oxygen is consumed, anaerobic microorganisms use a series of alternate electron acceptors. With nitrate as the electron acceptor, nitrogen gas will be produced; with sulfate as the electron acceptor, hydrogen sulfide will be produced. Under methanogenic/ fermentative conditions, methane gas will be produced. In designing an enhanced anaerobic bioremediation system, the formation of these products must be considered.

Factors affecting the rate of BTEX biodegradation include:

(1) Abundance and nutritional status of the organisms
(2) Type and quantity of electron acceptors present
(3) Catabolic inhibition
(4) Toxicity
(5) Temperature

These factors are often difficult to quantify. The most commonly occurring alternate electron acceptors other than oxygen include nitrate, sulfate, ferric iron, and carbon dioxide. The stoichiometry of BTEX oxidation is considered below for aerobic, nitrate-reducing, sulfate-

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reducing, and fermentative/methanogenic conditions, using toluene as a representative BTEX compound.

The aerobic biological degradation of toluene, ignoring cell growth, can be expressed as

$$C_7H_8 + 9\ O_2 \text{ --> } 7\ CO_2 + 4\ H_2O \qquad (1)$$

In this equation, toluene is completely mineralized to carbon dioxide and water, and the formation of biomass is not considered. This stoichiometry shows that the biodegradation of 1 mg/L toluene requires the presence of 3.1 mg/L dissolved oxygen in the ground water. When water and air are in equilibrium at 20°C, oxygen has a solubility of about 9 mg/L in water, which can degrade about 2.9 mg/L toluene. If toluene is present at higher concentrations, its aerobic degradation might be limited by the rate at which oxygen can be supplied to the contaminated zone. Liquid oxygen or hydrogen peroxide can be used to provide oxygen at concentrations higher than 9 mg/L, but these chemicals are expensive; also, as the system re-equilibrates to a dissolved oxygen concentration of 9 mg/L, oxygen bubbles will form in the aquifer, potentially causing hydraulic problems. Therefore, although aerobic BTEX degradation is a relatively rapid process, its rate can be limited by a low supply of oxygen.

Under nitrate-reducing (denitrifying) conditions, the stoichiometry for complete biodegradation of toluene to carbon dioxide is

$$C_7H_8 + 7.2\ H^+ + 7.2\ NO_3^- \text{ --> } 3.6\ N_2 + 7.6\ H_2O + 7\ CO_2 \qquad (2)$$

where cell growth has again been ignored. According to this stoichiometry, the biodegradation of 1 mg/L toluene requires the presence of 4.8 mg/L nitrate, i.e., more nitrate than oxygen is required to oxidize the same amount of toluene. However, nitrate salts are far more soluble than oxygen, so nitrate can be added to ground water in high concentrations.

The concentration at which nitrate can be added is limited by the facts that (1) nitrate has a drinking water MCL of 45 mg/L, so that regulatory approval is required for its injection at high concentrations, and (2) nitrogen gas is a product of the toluene degradation and has limited solubility in water. The stoichiometric equation above has assumed that the only nitrogen-containing product of the reaction is N_2, nitrogen gas. Actually, the dissimilative reduction of nitrate to nitrogen by anaerobic bacteria involves a series of intermediate nitrogen-containing compounds, including nitrite (NO_2^-), nitrogen oxide (NO), and nitrous oxide (N_2O) [Brock et al., 1997]. Under different conditions, the rate of utilization of these intermediates can differ [cf., Kuhn, et al., 1988; Major et al., 1988; Dolfing et al., 1990; Flyvbjerg et al., 1993; Elmen et al., 1997]. Although there are some organisms for which N_2O is the end product of nitrate reduction, in general the final product will be N_2 [Brock et al., 1997]. Therefore, equation (2) above is representative. Under this condition, a practical limit for nitrate introduction into ground water might be around 80 mg/L; above about 88 mg/L. The stoichiometry above suggests that nitrogen gas would be formed in excess of its aqueous solubility, and nitrogen gas bubble formation could potentially alter the hydraulic character of the aquifer. Also, it might be difficult to obtain regulatory approval of nitrate injection at concentrations higher than 80 - 100 mg/L.

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Toluene, ethylbenzene, meta-xylene, and para-xylene have been observed to degrade under denitrifying conditions [e.g., Ball and Reinhard, 1996]. However, ortho-xylene might degrade only due to cometabolism with toluene or another primary substrate [Evans et al., 1991; Alvarez and Vogel, 1995; Ball and Reinhard, 1996], and benzene might be recalcitrant under denitrifying conditions [Kuhn et al., 1988; Evans et al., 1991; Barbaro et al., 1992; Ball and Reinhard, 1996]. Degradation of the BTEX compounds under denitrifying conditions is often observed to occur sequentially, generally with toluene degraded first and the other compounds following.

Sulfate is commonly found in shallow ground water aquifers, especially those influenced by marine geochemical conditions. Under sulfate-reducing conditions, the stoichiometry for the biodegradation of toluene is

$$C_7H_8 + 4.5\ SO_4^{2-} + 3\ H_2O \rightarrow 2.25\ HS^- + 2.25\ H_2S + 7\ HCO_3^- + 0.25\ H^+ \qquad (3)$$

where cell growth has again been ignored. According to this stoichiometry, the biodegradation of 1 mg/L toluene requires the presence of 4.7 mg/L sulfate. Like nitrate, sulfate can be added to ground water in high concentrations. However, the oxidation of toluene via sulfate reduction results in the production of sulfide, which is known to inhibit the reaction at sufficiently high concentrations [Beller and Reinhard, 1995]. Therefore, there may be a practical limitation to the amount of sulfate that can be added as an alternate electron acceptor. It is known that benzene, toluene, and the three xylene isomers can be degraded under sulfate-reducing conditions [Edwards and Grbic-Galic, 1992; Edwards et al., 1992; Lovley et al., 1995], although the benzene degradation appears to be inhibited by the presence of other BTEX compounds [Edwards et al., 1992; Edwards and Grbic-Galic, 1992; Ball and Reinhard, 1996]. It does not appear that organisms found in Seal Beach aquifer materials are able to degrade ethylbenzene under sulfate-reducing conditions [Edwards et al., 1992; Ball and Reinhard, 1996]. As with denitrifying systems, degradation of the BTEX compounds under sulfate-reducing conditions is often observed to occur sequentially, generally with toluene degraded first and the other compounds following.

Aromatic hydrocarbons including benzene and toluene have also been observed to degrade under fermentative/methanogenic conditions [Wilson et al., 1986; Grbic-Galic and Vogel, 1987]. However, BTEX fermentation is poorly understood. Under fermentative conditions, no external electron acceptor is required, because microorganisms use the substrate (i.e., one or more of the BTEX compounds) as both an electron donor and an electron acceptor. The products of fermentation can include carbon dioxide, organic acids (e.g., acetic acid, propionic acid), alcohols (e.g., ethanol), and/or hydrogen gas [National Research Council, 1993]. Fermentation products are biodegraded by other species of bacteria, ultimately resulting in the production of carbon dioxide, methane, and water. The net stoichiometry for the degradation of toluene under these conditions is

$$C_7H_8 + 5\ H_2O \rightarrow 2.5\ CO_2 + 4.5\ CH_4 \qquad (4)$$

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where cell growth has again been ignored. Methanogenesis is inhibited by the presence of other electron acceptors or oxidants (e.g., oxygen, nitrate, or sulfate) and so only occurs in very reduced environments. During some parts of the fermentation/methanogenesis process, carbon dioxide or bicarbonate might be consumed as an electron acceptor; however, the net reaction results in production of carbon dioxide. According to this stoichiometry, the degradation of 1 mg/L toluene results in the production of about 0.78 mg/L methane. Methane gas has limited solubility in water (about 20 mg/L), so degradation of large amounts of contaminant via fermentation/methanogenesis could lead to the formation of methane gas bubbles, thus affecting the hydraulic characteristics of the aquifer.

Because of the biological and physico-chemical limitations associated with individual electron acceptors, one possibility for enhanced bioremediation is to augment the contaminated zone with the maximum capacity of several electron acceptors simultaneously. Under such conditions, the electron acceptors would be used sequentially according to decreasing energy yield. If there is ground water flow, the result will be a spatial variation in geochemical conditions downgradient of the point of electron acceptor injection. Because oxygen is the preferred electron acceptor, the aerobic zone will develop first, followed by a nitrate-reducing zone, a sulfate-reducing zone, and finally a fermentative/methanogenic zone. When using multiple electron acceptors, the limitations of each individual electron acceptor must be considered with respect to solubility limitations and potential chemical and biological interactions. Furthermore, it is difficult to accelerate methanogenic degradation of BTEX compounds via injection of electron acceptors, because the contaminant serves as both electron donor and acceptor. Methanogenic degradation would be expected to be a significant process only where other electron acceptors (oxygen, nitrate, sulfate) have already been depleted.

In the project described in this report, our goal was not only to demonstrate an enhanced bioremediation technology, but also to quantify anaerobic degradation processes and to evaluate the efficacy of augmentation with multiple electron acceptors. In order to properly control the geochemical conditions, we did not employ the maximum capacity of multiple electron acceptors. Rather, we established three distinct treatment zones, each with its own type of geochemical conditions. This is discussed further in Section 2.1.2, below.

2.1.2 Engineering Process: In order to achieve both of the objectives of this project (see Section 1.3), a system of injection and extraction wells was installed at a contaminated site. Because one of the goals of this project was to quantify the efficacy of individual anaerobic degradation processes, the system was operated in a manner different from how it would likely be operated at a "typical" contaminated site. In this section, we note some of the particular aspects in which our operation differs from the recommended operation at other contaminated sites.

In order to compare anaerobic degradation under three different types of geochemical conditions, one extraction well and three injection wells were installed in the most highly contaminated region of the site. Each injection well was used to set up a subsurface treatment zone with particular geochemical conditions. In each of the three zones, four monitoring wells were installed. An additional monitoring well was placed approximately 7 m upgradient of each of the three zones. Figures 3 and 4 show a plan view of the contaminated site and show the positions of

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the extraction well, the injection wells, and the monitoring wells. Slug tests indicated a slow ground water velocity, about 0.7 cm/day, in the direction indicated on Figure 4.

Figure 5 shows a schematic of the injection/extraction well system. Samples were taken at monitoring wells located between the injection well and the extraction well in order to measure the concentrations of the electron acceptors and of the target contaminants.The extraction well was used to remove water that contained either the target contaminants (in this case, BTEX) or compounds that inhibit the bioremediation process (e.g., sulfide). The extracted water was treated to remove the compounds of concern, then augmented with electron acceptors in order to stimulate biodegradation of the target contaminants, then re-injected into the contaminated region of the aquifer. In the demonstration described in this report, we utilized treatment and augmentation systems that were unusual in the following respects:

(1) The treatment system was designed to remove not only target contaminants and inhibitory products, but also excess electron acceptors (oxygen, nitrate, and sulfate). This was done in order to provide careful control over the composition of the re-injected water so that we could investigate the effects of particular geochemical conditions on the bioremediation process. Under typical operating conditions at a full-scale remediation site, excess electron acceptors would not be removed prior to augmentation and re-injection.
(2) The augmentation system did not always provide the optimum mixture of electron acceptors for bioremediation. For instance, no oxygen was added during this demonstration. This was done because one of the goals of the project was to compare bioremediation under controlled anaerobic conditions (denitrifying, sulfate-reducing, and methanogenic).
(3) During the early portions of the demonstration, a tracer (bromide) was injected in order to establish the hydraulic conditions and in order to estimate travel times between the injection wells and the monitoring wells.

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 about 1.5 L/min; the rate of extraction was about 4.5 L/min. Extracted water went through a treatment system to remove hydrocarbons, gases (including oxygen, methane, and nitrogen), and anions (including nitrate, sulfate, and sulfide). After treatment, the water was split into three streams. Each stream was sent to the one of the injection wells, where it was augmented with the appropriate electron acceptor(s): sulfate for injection well 2, sulfate and nitrate for injection well 4, and neither for injection well 3. In this manner, three different treatment zones with different geochemical conditions were established between the injection wells and the extraction well. These three zones are labeled zones 2, 3, and 4, according to the number of the injection well pertaining to each zone (see Figure 4). Zone 3 was expected to develop fermentative/methanogenic conditions; zone 2 was expected to develop first sulfate-reducing and then fermentative/methanogenic conditions; zone 4 was expected to develop first denitrifying, then sulfate-reducing, then fermentative/methanogenic conditions. We chose to install three injection wells and one extraction well in order to compare anaerobic degradation under these three different types of treatments; if this technology were

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employed at a full-scale remediation site, either more or fewer injection and extraction wells could be installed, and the geochemical conditions could be optimized for the particular site.

Each of the monitoring wells was in fact a well bundle consisting of seven monitoring points. The wells were composed of seven 0.1875-inch stainless steel tubes, spaced vertically at distances of 14 inches apart. In each monitoring well, the top tube was placed very close to the water table; the bottom tube was located about 7 feet below the water table, with the middle five tubes spaced evenly in between. As shown in Figure 4, each of the three treatment zones had five multilevel sample bundles associated with it. For each zone, one multilevel sample well was located approximately 7 m updgradient 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.

Sampling was performed automatically via an Automated Sampling and Analysis Platform (ASAP) from Analytic and Remedial Technology (Milpitas, CA). 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
(3) pH, dissolved oxygen, and concentration of sulfide via specific probes.

Results from the ASAP analyses were automatically logged in a computer database. Additional details about the ASAP are provided in the Technology Demonstration Plan [Appendix B] and in the Quality Assurance Project Plan [Appendix C].

The system could 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 flow, i.e., both injection and extraction wells are off. A treatment cycle generally consisted of operating in these three modes sequentially. First, the treatment zones were flushed with water that had been treated to remove hydrocarbons, gases, and anions, but that 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 were present. The second stage of the treatment consisted of injecting the zones with water that had also been augmented with the appropriate electron acceptor(s), in order to stimulate biological activity. Third, the injection and extraction wells were shut off, and the

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system was monitored while in a no-flow mode to see how the target contaminants responded to the established geochemical conditions. Like the flushing stage, the no-flow stage might be omitted in a full-scale implementation of the technology; here it was necessary for evaluation purposes. Over the course of a 4-week augmentation stage, about 180,000 L water was extracted, and about 60,000 L water was injected into each treatment zone. This was sufficient to establish treatment zones of about 150–200 m^3 in size (assuming an aquifer porosity of 0.3–0.4). A conservative tracer, such as bromide, could be added during the flushing period or during the augmentation period to ensure good hydraulic connection between the injection wells and the monitoring wells.

Some of the key design criteria for this technology are:

- Number and location of extraction and injection wells
- Injection and extraction flow rates
- Method of treating extracted water
- Choice of electron acceptors to be injected
- Concentration of electron acceptors injected
- Duration of flushing, augmentation, and no-flow periods

Values chosen for these design parameters will vary depending on site-specific conditions.

2.2 Strengths, Advantages, and Weaknesses of this Technology

Compared to conventional pump-and-treat remediation, the main advantages to in situ bioremediation (both aerobic and anaerobic) are:

(1) Contaminants are mineralized in situ, thereby avoiding the physical removal of the solids laden with toxic contaminants and the above-ground treatment of large volumes of water.
(2) In situ bioremediation produces little or no volume of secondary waste streams that need to be treated and disposed of.
(3) Contaminants are transformed to harmless products, not just transferred to a different phase (e.g., activated carbon).
(4) By maintaining a low contaminant concentration in the ground water, high rates can be maintained for contaminant desorption from aquifer solids and contaminant dissolution from residual non-aqueous liquid phases, thereby shortening overall clean-up times.

The main advantages of anaerobic bioremediation over aerobic bioremediation are:

(1) Alternate electron acceptors such as nitrate or sulfate are more water soluble than oxygen, and consequently allow more rapid supply of electron acceptors to the aquifer.

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(2) Anaerobic bacteria produce less biomass than aerobic bacteria, and therefore are expected to cause fewer problems with aquifer clogging, especially near injection wells.
(3) The use of anaerobic bacteria takes advantage of intrinsic processes which occur naturally without intervention.
(4) Factors (1)–(3) should result in considerable cost savings.

Challenges to the application of anaerobic biodegradation include:

(1) The process is still not thoroughly understood, especially under field conditions, making clean-up times difficult to predict.
(2) Because there is little operational experience with anaerobic bioremediation, the process is not always considered acceptable by regulators.
(3) Benzene, the most toxic of the BTEX compounds, has not conclusively been shown to degrade under all anaerobic conditions that exist in the field.
(4) The efficacy for some gasoline additives (e.g., MTBE) has yet to be determined.

Anaerobic biodegradation is also subject to some impediments that affect other treatment technologies:

(1) Contaminants that are sorbed to aquifer solids or are present in a non-aqueous liquid phase must first be transferred to the aqueous phase before they can be treated. This mass transfer process can limit the rate of nearly all types of treatment technologies.
(2) Electron acceptors that are introduced via injection wells will tend to flow preferentially into regions of high conductivity, leaving contamination in low-conductivity regions untreated. The presence of contamination in low-conductivity regions is also an impediment to pump-and-treat remediation, aerobic bioremediation, air sparging, and other remediation techniques.
(3) When electron acceptors are introduced via injection well, the injected water can push out the contaminated water, preventing adequate mixing of contaminants and electron acceptors. This can also be an impediment to any remediation technique that uses aqueous-phase chemical injection (e.g., aerobic bioremediation).
(4) Contaminants that are commonly found in conjunction with fuel hydrocarbons, such as the gasoline additive methyl *tert*-butyl ether (MTBE), often biodegrade very slowly under either aerobic or anaerobic conditions.

Currently, other anaerobic in situ bioremediation techniques are frequently considered for the remediation of fuel hydrocarbons. One of these is intrinsic bioremediation, as discussed in Section 1.1. Intrinsic bioremediation relies on the natural ground water flow to supply sufficient electron acceptors for mineralization of contaminants, and to remove any inhibitory metabolic products that are formed. The main advantage of intrinsic bioremediation over enhanced bioremediation is that it is less expensive, since injection wells are not needed and no chemicals need to be supplied. However, intrinsic bioremediation might not be sufficient to degrade the target contaminants in all cases, and is almost always slower than enhanced biodegradation,

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especially at sites where the ground water flow is slow, such as the Naval Weapons Station Seal Beach site.

2.3 Factors Influencing Cost and Performance

Table 1 shows the design and operational parameters that affect the cost and performance of the technology, as explained in the Federal Remediation Technologies Roundtable (FRTR) *Guide to Documenting and Managing Cost and Performance Information for Remediation Projects* [1998]. These parameters were also identified in Table 5 of the Technology Demonstration Plan [Appendix B].

Table 1: Operating Parameters Affecting Treatment Cost and Performance

Parameter	Range of Values
Injection Well Flow Rate	1.5 L/min in each zone
Extraction Well Flow Rate	sum of injection rates
pH of Ground Water	6–9 pH units
Nitrate Concentration in Feed Water	15–120 mg/L
Sulfate Concentration in Feed Water	15–120 mg/L

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3. Site/Facility Description

3.1 Background

The site for this demonstration was the Naval Weapons Station (NWS) Seal Beach, located in southern California (Figures 1, 2). The NWS Seal Beach is located on the transition of geologic formations called Landing Hill and the Sunset Gap. The physiographic and hydrogeologic setting are described in detail by Schroeder [1991]. A gasoline station located on the premises of the weapons station is contaminated with fuel hydrocarbons that leaked from a steel underground storage tank. The leak was discovered in 1984. The weapons station also contains the Seal Beach National Wildlife Refuge, a wetlands marsh located in the Sunset Gap formation. An investigation by the U.S. Geological Survey [Schroeder, 1991] found that the contamination from the leaking tank had migrated to the ground water 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 at which to demonstrate this remediation technology.

Furthermore, the following conditions made the NWS Seal Beach site especially well suited for this demonstration:

(1) The ground water 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) The aquifer is shallow and the cost of placing wells is relatively low.
(3) At some sites where the ground water is contaminated with fuel hydrocarbons, the prevailing ground water flow may supply sufficient doses of electron acceptors and remove inhibitory products at rates that render engineered simulation unnecessary. At the Seal Beach site, however, the regional ground water velocity is very small. Previous studies at the site [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 bioremediation processes.
(4) The aquifer solids are sufficiently permeable to allow pumping of at least a few gallons per minute, i.e., transmissivity is higher than 2 ft^2/day. The interbedding of clay lenses within silty sand (possibly sandy silt) is typical for alluvial deposits along the California Coast.
(5) The regulatory agency, the California Regional Water Quality Control Board (CRWQCB), has been supporting research at this site for many years, including controlled release experiments.
(6) Laboratory and field data from previous studies [Haag et al., 1991; Edwards et al., 1992; Edwards and Grbic-Galic, 1992; Ball and Reinhard, 1996; Reinhard et al., 1997] had suggested the probability of success of such a demonstration at the Seal Beach site.

Table 2 summarizes some of the characteristics of the site, in the format suggested by the Federal Remediation Technologies Roundtable (FRTR) *Guide to Documenting Cost and Performance*

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for Remediation Projects [1995], and as documented previously in Table 3 of the Technology Demonstration Plan [Appendix B].

Table 2: Standard Terminology for the Demonstration Site
Site Background:
Historical Activity that Generated Contamination:
Leaking underground storage tank
Management Practices that Contributed to Contamination:
Underground storage tank (fuel)
Site Characteristics:
Media Treated:
Ground water
Soil (in situ)
Light nonaqueous-phase liquids (LNAPL)
Contaminants Treated:
Organic Compounds, Volatile, Non-halogenated: BTEX
Organic Compounds, Petroleum Hydrocarbons
Treatment System:
Primary Treatment Technology:
Soil (in situ): Bioremediation
Ground water (in situ): Bioremediation
Supplemental Treatment Technology:
Pre-treatment: Nutrient (electron acceptor) injection
Post-treatment: Carbon adsorption, Ion exchange, Gas stripping

3.2 Site/Facility Characteristics

The hydrogeology of the NWS Seal Beach has been described in detail by Schroeder [1991]. About 5800 gallons of fuel leaked from an underground storage tank and contaminated the soil and ground water in the region, as shown in Figure 3. The leak was discovered in 1984 when the steel storage tank was being replaced by a pair of fiberglass tanks. Between 1984 and 1996, observation wells monitored by the Orange County Water District indicated that the contaminant plume in the ground water was retracting, and by the beginning of this demonstration, the plume was concentrated around the source area.

In June 1995, a water sample was taken in this region from a well screened near the water table. The sample produced the following concentrations for BTEX compounds: 14.1 mg/L benzene, 13.9 mg/L toluene, 2.7 mg/L ethylbenzene, 8.3 mg/L combined meta- and para-xylene, 4.5 mg/L ortho-xylene. Some monitoring wells showed the presence of a non-aqueous liquid phase. Because the non-aqueous phase is less dense than water, it tends to spread on top of the water

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table, and saturated-zone BTEX concentrations tend to be higher near the water table than they are several feet below the water table.

The ground water velocity in the region is low, approximately 0.7 cm/day. The ground water flow rate and direction might fluctuate somewhat with the season and with the tides.

Table 3 shows the site characteristics that affect the treatment cost and performance, as specified by the Federal Remediation Technologies Roundtable (FRTR) *Guide to Documenting and Managing Cost and Performance Information for Remediation Projects* [1998].

Table 3: Matrix Characteristics Affecting Treatment Cost and Performance

Parameter	Value	Measurement Procedure
Soil Types (Soil Classification and Clay Content)	See well logs in Appendix D. Here we give the extraction well as an example. 0–2 feet below ground surface (BGS): silty, fine to coarse sand 2–4 ft BGS: silty clay 4–5 ft BGS: silty, medium to coarse sand 5–8 ft BGS: coarse sand, some gravel 8–10 ft BGS: silt 10–12 ft BGS: silty fine sand 12–14 ft BGS: fine to medium sand 14–14.5 ft BGS: silty clay 14.5–15 ft BGS: medium sand	visual inspection by well logger, reviewed by registered geologist
Hydraulic Conductivity	0.0005–0.002 cm/sec	pump tests on 6 wells
pH	6.9–8.2 (usually 7.1–7.8) pH units	pH probe
Total Organic Carbon	0.015%–0.028%, bulk value 0.024%	
Non-aqueous phase liquids (NAPL)	Yes, light (less dense than water)	Found in monitoring wells

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4. Demonstration Approach

4.1 Performance Objectives

This project was intended to meet two broad goals:

(1) Demonstration of a technology by which contaminants are removed from ground water via enhanced in situ anaerobic biodegradation
(2) Detailed documentation of the anaerobic biodegradation of fuel hydrocarbons under three different types of geochemical conditions.

In keeping with these two primary goals, the demonstration focused on the use of alternate electron acceptors (i.e., electron acceptors other than oxygen) for in situ bioremediation of hydrocarbon fuel contaminants. It is important to note that, because one of the goals of this project was a detailed evaluation, the remediation technology was operated under carefully controlled conditions, sometimes at conditions that were known to be less than optimal. This was done in order to collect data for the evaluation of anaerobic biodegradation processes. The performance objectives by which the success of this project is measured must take into consideration the dual goals of the project.

The technology design used an injection/extraction well doublet (Figure 5). The extraction well is used to remove compounds that inhibit the bioremediation; it is also used as a source of water that can be treated, augmented with electron acceptors, and then supplied to the contaminated zone via the injection wells.

The principal performance criterion for this demonstration is the historical change in contaminant concentrations as measured in the monitoring wells, which are located between the injection well and the extraction well (Figures 4 and 5). The target compounds for this demonstration are benzene, toluene, ethylbenzene, and xylene, collectively known as BTEX. The drinking water maximum contaminant levels (MCLs) for the BTEX compounds are, respectively, 5 parts per billion (ppb), 1 part per million (ppm), 0.7 ppm, and 10 ppm.

The secondary performance criterion for this technology is based on the measured concentrations of electron acceptors and of metabolic products. This secondary criterion is used to further validate the degradation of the contaminants.

In addition to these two performance criteria, the Technology Demonstration Plan [Appendix B] identified the following technical issues to be evaluated with regard to the technology's performance:

(1) Disposal of a process waste stream
(2) Reliability of anaerobic bioremediation, particularly with regard to

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(a) Presence and viability of anaerobic bacteria capable of growing on BTEX compounds under field conditions, which might include a limited supply of nutrients or the build-up of toxic by-products
(b) Reliability of hardware used for recirculating water through the treatment zone, which might be susceptible to plugging caused by excessive biomass formation, precipitation, or gas formation

(3) Ease of use, i.e., how much individual training, preliminary investigation, or special precautions are needed to implement this technology
(4) Versatility, i.e., how easily the technology can be applied to remove contaminant groups other than BTEX or fuel hydrocarbons
(5) Off-the-shelf procurement, i.e., are the hardware and software for this technology easily obtained
(6) Maintenance, especially of

(a) Injection and extraction flows
(b) Water quality in the feed stream
(c) Treatment system for extracted water
(d) Electron acceptor feed solution

(7) Scale-up issues, i.e., how easily this technology might be applied to larger-scale sites.

4.2 Physical Set-up and Operation

The treatment system consisted of one extraction well and three injection wells, with relative locations as indicated in Figure 4. Different electron acceptors were added at each injection well, creating three different treatment zones with different geochemical sequences. 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 injection wells were installed in locations of moderate BTEX contamination; the extraction well was installed in a region of relatively high contamination. Details of the well design are provided in the Technology Demonstration Plan [Appendix B]. As noted previously, the three zones were established in this manner specifically because one of the objectives of this project 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 bioremediation.

A treatment evaluation consisted of three stages. First, the three treatment zones were flushed with water that had been treated to remove hydrocarbons, gases, and anions, 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

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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. 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-flow 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.

This technology demonstration lasted about 17 months and consisted of three treatment evaluations. The first augmentation period ran from 9/14/97–10/16/97; the second augmentation ran from 5/24/98-6/23/98; the third augmentation period ran from 9/2/98–10/14/98. For all three treatment evaluations, the injection flow rate was about 1.5 L/min in each well, and the extraction flow rate was about 4.5 L/min. The third augmentation was not preceded with a flushing stage. Each augmentation consisted of different concentrations of electron acceptors being injected, as summarized in Table 4. 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.

Table 4: 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 5 shows a schematic for the ground water recirculation system used in this demonstration. Figure 6 shows a schematic for the water treatment system that is used to remove hydrocarbons, gases, and anions from the extracted water before it is augmented with electron acceptors and re-injected. Figure 7 shows a schematic for how the treated water is split into three streams and augmented with the appropriate electron acceptor(s). We utilized treatment and augmentation systems that were unusual in the following respects:

(1) The treatment system was designed to remove not only target contaminants and inhibitory products, but also excess electron acceptors (oxygen, nitrate, and sulfate). This was done in order to provide careful control over the composition of the re-injected water so that we could investigate the effects of particular geochemical conditions on the bioremediation process. Under typical operating conditions at a full-scale remediation site, excess electron acceptors would not be removed prior to augmentation and re-injection.

(2) The augmentation system did not always provide the optimum mixture of electron acceptors for bioremediation. For instance, no oxygen was added during this

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demonstration. This was done because one of the goals of the project was to compare bioremediation under three different types of anaerobic conditions (denitrifying, sulfate-reducing, and methanogenic).

The flushing and augmentation stages typically lasted 4–5 weeks. During these stages, the injection/extraction system was operated all 24 hours per day, and one operator was present at the site at all times to maintain the water treatment system and to ensure that the injection, extraction, and sampling ran properly. During the no-flow stages, only periodic maintenance was required.

During the treatment demonstration, low-volume waste streams were produced from various sources. Ion exchange was used to remove anions from the extracted water prior to augmentation and re-injection; the brine produced from regenerating the ion exchange columns represented one waste stream. Spent activated carbon (used to remove hydrocarbons from the extracted water) represented a second waste stream. In addition, the analytical system and the injection system produced a waste stream of less than 0.5 L/min. In a typical application of this technology, where sampling and analysis would be performed less frequently, this waste stream would be even lower volume.

4.3 Sampling Procedures

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. Connections were 0.1875-inch stainless steel sample lines. The remaining six ports were connected to the treatment system and to the three injection wells. After flushing the sample lines, the ASAP extracts a sample and prepares separate aliquots for analysis of volatile organic compounds, anions, pH, and dissolved oxygen. The ASAP provided samples directly to the instrumentation without operator intervention and was operated continuously from August 1997 until November 1998. Sample waste water was recycled back to the inlet of the water treatment system.

Each of the three treatment zones had five monitoring wells, as shown in Figure 4. Each monitoring well consisted of seven 0.1875-inch stainless steel tubes with the inlets spaced vertically 14 inches apart, thus providing seven discrete sample locations covering the length of the injection well screens at each monitoring well location. The inlet of each sample tube was enclosed in glass wool filter protected by nylon "horse hair" fabric, a double weave knit. The monitoring wells were installed by placing the sample bundles in 2-inch boreholes and backfilling with sand; this minimizes the required flush volumes required to obtain representative samples. Additional details on the sampling procedure can be found in the Technology Demonstration Plan [Appendix B] or in the Quality Assurance Project Plan [Appendix C].

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4.4 Analytical Procedures

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.

Although the modified purge-and-trap system is not of the design used in standard methods, it has been demonstrated successful by the U.S. Environmental Protection Agency [U.S. EPA, 1993]. The gas chromatograph was not able to resolve meta- and para-xylene, so the concentrations of these two compounds were measured as a sum. Photo-ionization detection was used for the measurement of BTEX concentrations; flame ionization detection was used for the measurement of the concentrations of aliphatic hydrocarbons.

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. Additional details about the analytical procedure are provided in the Technology Demonstration Plan [Appendix B] and in the Quality Assurance Project Plan [Appendix C].

Table 5: Compounds Analyzed by Automatic Sampling and Analysis Platform

Compound	Gas Chromatography	Ion Chromatography	Specific Probe
Methane	X		
3-methyl-pentane	X		
Hexane	X		
Benzene	X		
Toluene	X		
Ethylbenzene	X		
Xylenes	X		
Trimethyl-benzenes	X		
Chloride		X	
Bromide		X	
Nitrate		X	
Sulfate		X	
Dissolved Oxygen			X
pH			X
Sulfide			X

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5. Performance Assessment

5.1 Performance Data

As discussed in Section 4.1, above, the evaluation of the technology's performance is based primarily on the historical change in contaminant (BTEX) concentrations, and secondarily on the historical change in electron acceptor concentrations. These concentrations were measured through monitoring wells. Each monitoring well had seven sampling points, spaced vertically about 14 inches apart. 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 4); 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 "4-B1-5" means zone 4, well bundle 1, the fifth sampler from the top. The injection wells are designated "I2," "I3," and "I4," as indicated in Figure 4.

In the subsections below, we present data showing the historical response of the BTEX contaminants and of the electron acceptors. Contaminant histories are presented in Figures 8–24. On many of these figures, 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-flow) stage, in which the injection and extraction wells were shut off such that there was no flow through the treatment zones.

5.1.1 Benzene Concentration History: Figure 8 shows the change in benzene concentration with time, as measured at monitoring points 3-B1-5 and 4-B1-5. These two monitoring wells were chosen for comparison because they are located at approximately the same vertical location, a few feet below the water table, but in different treatment zones. In both monitoring wells, the benzene concentration was initially in the range 3–5 mg/L, but decreased rapidly during the initial flushing stages. At the end of the first augmentation (10/14/97), the concentration in each well was about 1 mg/L. However, during the first no-flow stage, the benzene concentration rebounded in both wells: to about 2 mg/L in 4-B1-5, and to over 5 mg/L in 3-B1-5. This rebound is very commonly seen in pump-and-treat remediation systems when the pumping is temporarily shut down. It can be caused by:

(1) Dissolution of benzene from globules of non-aqueous phase liquid into the aqueous phase
(2) Desorption of benzene from aquifer solids into the aqueous phase
(3) Molecular diffusion of benzene from a region of the aquifer where the aqueous concentration is still very high
(4) Slow flow of ground water into the test zone from a more contaminated region.

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It is very difficult to speculate which of these causes might be primarily responsible. It is perhaps significant that the rebound was much stronger in zone 3 (where no electron acceptors were added) than in zone 4 (which was augmented with nitrate and sulfate); this could be an indication that the addition of electron acceptors suppressed the rebound effect by stimulating biological degradation of benzene. However, there are numerous other reasons why the four rebound mechanisms listed above might be stronger in zone 3 than in zone 4; therefore, the suppressed rebound in zone 4 should not necessarily be interpreted as in indication of biodegradation of benzene.

During the second treatment cycle, similar behavior was observed. The concentrations decreased rapidly during the flushing stage, decreased slowly during the augmentation stage (which could be due either to biological activity or to the continued flushing that occurs during this stage), then rebounded during the no-flow stage. As before, the rebound was stronger in zone 3 than in zone 4. In both zones, the rebound was weaker in the second treatment cycle than in the first treatment cycle. This is not surprising, because the additional flushing in the second treatment cycle would have removed some of the benzene mass, weakening the rebound mechanisms listed above.

In the third treatment cycle, no flushing stage preceded the augmentation. Benzene concentrations dropped during the augmentation stage and did not rebound during a short no-flow stage. The final benzene concentration was higher in zone 3 than in zone 4. In both zones, the final benzene concentration was much lower than initial concentration. In zone 3, the benzene concentration dropped from over 4 mg/L to about 0.3 mg/L, and in zone 4, the benzene concentration dropped from about 3 mg/L to about 0.05 mg/L.

To summarize these observations, the benzene concentrations in the ground water did drop considerably during the 17-month demonstration period. However, it appears that most of the benzene removal was probably due to flushing; there may have been some benzene removal from biodegradation, but the data are inconclusive. Benzene was removed more effectively from zone 4 than from zone 3. This could be an indication that augmentation with electron acceptors improves benzene removal, but it might merely be an indication that zone 3 had a higher residual saturation or was more susceptible to encroachment from highly contaminated regions of the site. Qualitatively, these results are consistent with previous studies [e.g., Acton and Barker, 1992; Edwards et al., 1992; Thierrin et al., 1995; Ball and Reinhard, 1996; Reinhard et al., 1997] that have shown benzene to be relatively resistant to biological degradation under anaerobic conditions.

5.1.2 Toluene Concentration History: Figure 9 shows the change in toluene concentration with time at point 4-B1-5; for comparison, the benzene concentration at 4-B1-5 is also shown. Figure 9 clearly shows that the toluene concentration was orders of magnitude lower than the benzene concentration, even at the start of the first flushing stage at the very beginning of the demonstration . In general, toluene was present at high concentrations only near the water table, where there is more residual non-aqueous phase liquid (NAPL) hydrocarbon. A few feet below the water table, the toluene concentration rarely exceeded 50 μg/L at any of the monitoring wells. It appears that toluene was preferentially degraded by the native microbial population, and that this degradation could take place even without augmentation of electron acceptors. This is

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consistent with the results from previous laboratory studies with aquifer materials from Seal Beach [Haag et al., 1991; Ball and Reinhard, 1996].

5.1.3 Ethylbenzene Concentration History: Figure 10 shows the change in ethylbenzene concentration with time at location 2-B1-4. For comparison, the benzene concentration at 2-B1-4 is also shown. Figure 10 shows that in zone 2, which was augmented with sulfate, ethylbenzene responded to the treatment in a manner almost identical to benzene, i.e., removal appears to be primarily via flushing. Thus, it appears that augmentation with sulfate did not enhance ethylbenzene degradation. However, an interesting result was seen during the third augmentation (9/2/98–10/14/98): during this augmentation, in which nitrate was added prior to sulfate (see Table 4), the ethylbenzene concentration dropped rapidly. This might be an indication that, whereas augmentation with sulfate had little effect on ethylbenzene concentration, addition of nitrate enhanced ethylbenzene degradation.

Figure 11 is consistent with the hypothesis that addition of nitrate enhances ethylbenzene degradation. Figure 11 shows the benzene and ethylbenzene concentrations at 4-B1-4. Note that there are two scales for the y-axis in Figure 11; the scale on the left-hand side of the graph is for ethylbenzene, and the scale on the right-hand side of the graph is for benzene. Figure 11 shows that, in zone 4, which was augmented with nitrate as well as sulfate, the ethylbenzene concentration steadily declined, reaching a final concentration of less than 50 µg/L. Ethylbenzene appears to have experienced significantly less rebound than benzene, perhaps indicating greater biodegradation of ethylbenzene than of benzene under denitrifying conditions. These results are consistent with previous laboratory studies, which have shown that ethylbenzene can be degraded under denitrifying conditions but not under sulfate-reducing conditions [e.g., Edwards et al., 1992; Ball and Reinhard, 1996].

In zone 3, where fermentative/methanogenic conditions were expected to develop, the ethylbenzene concentration was low even at the beginning of the demonstration. It is therefore difficult to assess the effectiveness of methanogenesis with regard to ethylbenzene removal.

5.1.4 Xylene Concentration History: Figure 12 shows the change in xylene concentration with time at location 2-B1-4. The meta- and para-isomers of xylene were measured as a sum concentration because they co-eluted from the gas chromatograph; ortho-xylene is measured separately because it could be resolved on the gas chromatograph. For comparison, Figure 12 also shows the benzene concentration at 2-B1-4. The m+p-xylene isomers responded in a manner almost identical to benzene, i.e., the sum concentration of m+p-xylene was not effectively reduced by sulfate augmentation. This means that at least one of the meta- and para-isomers was resistant to degradation under sulfate-reducing conditions, and perhaps both isomers were. In contrast, the o-xylene concentration dropped sharply during the first augmentation stage (9/14/97–10/16/97) and never experienced any rebound. It appears that the o-xylene was almost completely transformed due to the addition of sulfate. Previous studies with Seal Beach aquifer material had produced conflicting results regarding the degradation of o-xylene under sulfate-reducing conditions:

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(1) Edwards et al. [1992] observed o-xylene mineralization after a lag period and after toluene and p-xylene were degraded.
(2) Ball and Reinhard [1996] observed o-xylene degradation to occur under sulfate-reducing conditions only via co-metabolism with toluene, which is believed to result in (2-methyl-benzyl)-succinate as a dead-end metabolic product [Beller et al., 1995, 1996].
(3) 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 demonstration, 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. Samples were not analyzed for the presence of (2-methyl-benzyl)-succinate during this demonstration, so it is difficult to determine if o-xylene was a primary or secondary substrate.

Figure 13 shows the change in xylene and benzene concentrations with time at point 4-B1-5. There are two scales for the y-axis in Figure 13; the scale on the left-hand-side of the graph is for xylene, and the scale on the right-hand-side of the graph is for benzene. Figure 13 shows that, in zone 4, which was augmented with nitrate as well as sulfate, the xylene concentrations steadily declined. Xylene appears to have experienced significantly less rebound than benzene, perhaps indicating greater biodegradation of xylene than of benzene under denitrifying conditions. As in zone 2, the o-xylene isomer disappeared much more readily and rapidly than the m+p-xylene isomers; o-xylene was almost completely removed by the end of the first flushing stage, prior to any augmentation with nitrate or sulfate.

In zone 3, where fermentative/methanogenic conditions were expected to develop, the concentrations of all xylene isomers were very low even at the beginning of the demonstration . It is therefore difficult to assess the effectiveness of methanogenesis with regard to xylene removal. In a previous study, performed with a sediment from a location other than the Seal Beach site, Edwards and Grbic-Galic [1994] found toluene and o-xylene to degrade under methanogenic conditions after lag times of 100–250 days. Other BTEX compounds did not degrade under these conditions.

In general, o-xylene appears to have been the second-most-readily degraded BTEX compound (next to toluene). The m+p-xylene isomers were relatively recalcitrant under sulfate-reducing conditions; under denitrifying conditions, m+p-xylene were removed more quickly than benzene and at about the same rate as ethylbenzene. The preferential degradation of o-xylene prior to m+p-xylene is somewhat surprising in the light of previous results [Haag et al., 1991; Edwards et al., 1992; Ball and Reinhard, 1996; Reinhard et al., 1997], although it might be expected under strictly methanogenic conditions [Edwards and Grbic-Galic, 1994].

5.1.5 Nitrate Concentration History: Nitrate was injected into zone 4 during the three augmentations. The first augmentation period ran from 9/14/97–10/16/97; the second augmentation ran from 5/24/98–6/23/98; the third augmentation period ran from 2 September

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1998 through 14 October 1998. Figure 14 shows the concentration of nitrate injected into well I4, and the concentration measured at 4-B1-5, as functions of time. In all three injections, nitrate broke through at well bundle 4-B1 at a concentration lower than the injected concentration, presumably indicating microbial utilization of some of the nitrate between the injection well and the monitoring well. Bromide, a conservative tracer, broke through at 100% of its injected concentration (data not shown), indicating that the low nitrate breakthrough concentrations were not due to a poor hydraulic connection. Figure 14 also shows that, once the injection of nitrate ceased (indicating the onset of a no-flow stage in the test zone), the nitrate concentration at the monitoring well dropped rapidly. This drop in the nitrate concentration cannot be explained by ground water flow, because the injection/extraction wells were shut off during these periods, and the natural ground water flow is very slow. Once again, the most plausible explanation is that the drop in nitrate concentration was due to biological utilization of nitrate as an electron acceptor.

Figure 15 shows the nitrate breakthrough and decay at 4-B1-5 for the second augmentation only. We fit a first-order decay model to the measured nitrate concentrations assuming a travel time of 3 days between the injection well and 4-B1-5. The assumed 3-day travel time is consistent with the tracer data (not shown). The implied value of the first-order rate coefficient for nitrate utilization is 0.26/day. As can be seen from Figure 15, the results of the model fit are reasonable. The model used to estimate this rate coefficient is quite simplistic (no local dispersion accounted for, first-order kinetics rather than Monod kinetics), but might be useful to get a general idea of the rate of nitrate utilization. Fitting a first-order utilization model to the nitrate concentration data for the third augmentation produces an implied first-order rate coefficient of 0.1/day. The decrease in nitrate utilization rate between the second and third augmentations might indicate that (a) utilization is slower when nitrate is injected at higher concentrations, and/or (b) the rapidly-degradable substrates were consumed in the first two augmentations, such that the nitrate demand in the third augmentation was exerted by more slowly-degraded contaminants.

The rate of water injection during the flushing and augmentation stages is about 1.5 L/min in each well. Using this information, coupled with the measured nitrate concentrations in the zone 4 injection well, we were able to estimate that about 1.1 kg nitrate was added during the first augmentation, 3.3 kg nitrate in the second augmentation, and 9.3 kg nitrate in the third augmentation. Also, 2.2 kg nitrate was added to zone 2 during the third augmentation (see Table 4). Thus, the total amount of nitrate added during the three injections was about 15.9 kg. According to the stoichiometry shown in Equation (2), this would be enough to oxidize 3.3 kg of toluene (about 3.8 L, or 1.0 gal, if toluene were present as a pure non-aqueous phase) to carbon dioxide and water. In reality, much of the nitrate probably went to oxidize fuel hydrocarbons other than BTEX.

5.1.6 Sulfate Concentration History: Sulfate was injected into zones 2 and 4 during the three augmentations. In zone 2, sulfate was the only electron acceptor injected for the first two augmentations, but during the third augmentation, sulfate injection was preceded by nitrate injection. In zone 4, sulfate and nitrate were injected simultaneously for all three augmentations.

Figure 16 shows the concentrations of sulfate and nitrate injected into well I2, and the sulfate concentration measured at 2-B1-4, as functions of time. For all three augmentations, sulfate

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broke through at the monitoring well at a concentration 75–80% of the injected concentration. This probably indicates biological utilization of some sulfate between the injection well and the monitoring well. Bromide, a conservative tracer, broke through at 100% of its injected concentration (data not shown), indicating that the apparent sulfate attenuation is not due to poor hydraulic connection between the injection and monitoring wells. Figure 16 also shows that, after each augmentation stage ended, indicating the onset of a no-flow stage, the sulfate concentration at 2-B1-4 dropped back down to almost zero. This is also evidence of biological utilization of sulfate. The drop in sulfate concentration cannot be explained by advection, because the injection and extraction wells were not running during the no-flow stages, and natural ground water flow is very slow: bromide persisted at nearly 100% of its injection concentration during the no-flow period. Therefore, biological utilization of sulfate is the most plausible explanation for the observed behavior.

Figure 17 shows the sulfate breakthrough and decay at 2-B1-4 for the second augmentation only. We fit a first-order decay model to the measured sulfate concentrations assuming a travel time of 2.5 days between the injection well and 2-B1-4. The assumed 2.5-day travel time is consistent with the tracer data (not shown). The implied value of the first-order rate coefficient for sulfate utilization is 0.1/day. As can be seen from Figure 17, the results of the model fit are reasonable. The model used to estimate this rate coefficient is quite simplistic (no local dispersion accounted for, first-order kinetics rather than Monod kinetics), but might be useful to get a general idea of the rate of sulfate utilization. Sulfate utilization is slightly slower than nitrate utilization for the same injected concentrations of sulfate and nitrate (cf. Figures 14 and 16).

Figure 16 also shows two other interesting features regarding the sulfate history in zone 2. The first of these interesting features is the large spike in observed sulfate concentration between 3/18/98 and 4/15/98. This was a rainy time period, and we believe the spike to be the result of rainwater infiltration from the surface. The flushing stage for the second augmentation began on 4/7/98, which is when the sulfate concentration very rapidly dropped from 180 mg/L back down to less than 20 mg/L.

The second interesting feature that can be seen in Figure 16 is an increase in the observed sulfate concentration at 2-B1-4 upon injection of nitrate into well I4 during the third augmentation. Figure 18 shows an interesting detail of the third augmentation. It appears that the introduction of nitrate was able to oxidize sulfide back to sulfate. It is presumed that sulfide had accumulated in zone 2 as a result of sulfate reduction from the first two augmentations. The ability of nitrate to oxidize sulfide to sulfate has been observed previously [Ball and Reinhard, 1996], and might be significant as a means of controlling sulfide inhibition.

Figure 19 shows the concentrations of sulfate and nitrate injected into well I4, and the nitrate and sulfate concentrations measured at 4-B1-5, as functions of time. The nitrate data at I4 and 4-B1-5 were also shown in Figure 14. In zone 4, sulfate broke through at the monitoring well at 100% of its injected concentration. This is in contrast to zone 2, where some sulfate utilization was observed between the injection well and the monitoring well. Also, in zone 4, when each augmentation ended, indicating the onset of a no-flow stage, the sulfate concentration at 4-B1-5 decayed at a much slower rate than was observed in zone 2. It appears that sulfate utilization is

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slowed considerably by the presence of nitrate. This is not surprising, given that nitrate reduction is more energetically favorable than sulfate reduction for microorganisms [Borden, 1994]. It is unclear whether or not nitrate utilization and sulfate utilization are strictly sequential, i.e., whether sulfate utilization begins only after nitrate has been completely consumed. Figure 20 shows a detail of the second augmentation in zone 4. From Figure 20, it appears that some sulfate decay does occur while nitrate is still present, indicating that the two utilization processes can occur simultaneously and are not strictly sequential. However, the sulfate decay is rather slow, so this conclusion cannot be drawn definitively.

Figures 16 through 20 have shown the sulfate histories at monitoring well bundles 2-B1 and 4-B1, which are the well bundles closest to the respective injection wells (see Figure 4). At the well bundles further away from the injection well, the sulfate history does not correlate as strongly with the injected concentrations. Figure 21 shows the sulfate history at monitoring point 2-B2-4, which is located 4 m away from injection well I2. At well bundle 2-B2, the sulfate concentration appears to fluctuate almost randomly, and does not appear to depend on whether the system is being flushed or augmented. This might be an indication of a poor hydraulic connection between the injection well and well 2-B2. The results of a tracer test with bromide are inconclusive on this point; bromide injected into well I2 was seen to break through at nearly 100% at 2-B2-4 and 2-B2-6, but the implied travel time was about 3 weeks, which is much longer than would be expected based on the distance of 4 m between wells (Figure 22). Therefore, it is possible that the observed behavior at 2-B2-4 is due to natural fluctuations in the background sulfate concentration, and that 2-B2 is not hydraulically connected to injection well I2. However, even if this is the case, it is somewhat surprising that the background sulfate concentration would remain in the range 20–40 mg/L, given that there is BTEX contamination present at 2-B2. We would expect that the sulfate would be reduced biologically for BTEX oxidation, which would presumably result in background sulfate concentrations of less than 20 mg/L, as observed in well 2-B1. It is also possible that there is enough sulfide present at 2-B2 to inhibit the sulfate reduction; if 2-B2 is not hydraulically connected to I2, then this sulfide would not be removed during the flushing stages. This combination of factors could explain the continuously high sulfate concentration observed at 2-B2.

Based on injection flow rates of 1.5 L/min and the observed sulfate concentrations in the injection wells, we calculated that zone 2 received 1.3 kg sulfate in the first augmentation, 5.3 kg sulfate in the second augmentation, and 4.5 kg sulfate in the third augmentation, for a total of 11.1 kg. Zone 4 received 1.3 kg sulfate in the first augmentation, 5.3 kg sulfate in the second augmentation, and 6.9 kg sulfate in the third augmentation, for a total of 13.5 kg sulfate. The total amount of 24.6 kg sulfate added would, according to the stoichiometry shown in Equation (3), be enough to oxidize 5.2 kg of toluene (about 5.8 L, or 1.5 gal, if toluene were present as a pure non-aqueous phase) to carbon dioxide and water. In reality, much of the sulfate probably went to oxidize fuel hydrocarbons other than BTEX.

5.1.7 Methane Concentration History: Figure 23 shows the history of methane concentration in zones 2, 3, and 4. In all three zones, the initial methane concentration was greater than 5 mg/L, but dropped rapidly during the initial flushing and augmentation stages. During the first no-flow stage, the methane concentration in all three zones rose considerably, indicating the occurrence of

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methanogenesis. The observed methanogenesis was weakest in zone 2. This is probably because zone 2 had the lowest total dissolved BTEX concentration; as shown by Equation (4), a lower BTEX concentration results in a lower produced methane concentration. The BTEX concentrations in zones 3 and 4 were comparable to each other, and so were the produced methane concentrations. Zone 3 exhibited somewhat more methanogenesis than zone 4, which might be an indication of a higher total concentration of fuel hydrocarbons. However, the lower methane concentrations in zone 4 might also be caused by the augmentation of nitrate and sulfate in that zone; nitrate and sulfate are both known to inhibit methanogenesis. In any event, the relatively high concentrations of methane produced in all three zones strongly suggests that fuel hydrocarbons were being degraded by fermentation and methanogenesis. According to the stoichiometry in Equation (4), an observed methane concentration of 5 mg/L would indicate the degradation of over 6 mg/L toluene. The high methane concentrations seem to indicate that other compounds in addition to BTEX were being degraded via methanogenesis.

5.1.8 Summary and Implications of Observed Concentration Histories: It appears that nitrate and sulfate were both utilized biologically for the oxidation of fuel hydrocarbons, including the BTEX compounds. This indicates that the enhancement of a contaminated aquifer via addition of electron acceptors can increase the rate of bioremediation of the aquifer. However, when nitrate and sulfate are not present, methanogenesis occurs, which should also lead to the bioremediation of the contaminated aquifer, albeit perhaps at a slower rate. Augmentation with electron acceptors appeared to increase the rate of degradation of ethylbenzene and the xylene isomers. However, augmentation did not appear to have much effect on the removal of benzene. Toluene was degraded under background conditions, i.e., even without the introduction of additional electron acceptors. The amount of nitrate and sulfate added during the three augmentations was enough to degrade about 8.5 kg BTEX. In reality, much of the sulfate and nitrate probably went to degrade fuel hydrocarbons other than BTEX. It is important to note, however, that during this project, we did not attempt to optimize the types and amounts of electron acceptors injected (e.g., no oxygen was added); rather, we were attempting to investigate anaerobic degradation under three different types of conditions.

5.2 Data Assessment

In general, we believe the reported data to be of high quality and to allow assessment of the demonstration's objectives. The Automated Sampling and Analysis Platform (ASAP) enabled us to compile a very large database of concentration histories at 105 different sampling point locations throughout the contaminated site. As a result, we have a very complete picture of how particular compounds responded to the treatment in time and in space.

Furthermore, the large database allows us to compare particular recorded measurements in order to provide an internal check on the consistency of the data. For instance, the BTEX concentrations were measured using gas chromatography with detection via photo-ionization (PID) and flame ionization (FID). The concentrations reported in this section are those that were measured via PID, which is believed to be more reliable for aromatic hydrocarbons; however, the FID measurements are available as a qualitative cross-check that the PID measurements are reasonable.

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Also, the availability of seven sampling points spaced vertically at each monitoring well allows for another useful internal check on the data. We would not expect two different sampling points in the same well bundle to record exactly the same concentrations of a particular compound, but because there is not extreme physical heterogeneity at this site, we would expect the two sampling points to record similar trends. For example, Figure 24 shows the measured benzene concentration at two different vertical points in the same well bundle: points 2-B1-4 and 2-B1-6. Although the two monitoring points seldom produced identical concentration measurements for benzene, it is clear from Figure 24 that the two monitoring points were qualitatively in agreement with each other. This is precisely what we would expect from different points along the same vertical sampler.

As discussed in Section 5.1.6, above, this is not necessarily the case between different monitoring wells. For instance, Figure 21 showed that wells 2-B1 and 2-B2 produced very different measurements for sulfate concentration. However, we do not believe this to be an indication of poor data quality; rather, we believe that it indicates the spatial variability present at most field sites.

Despite our general confidence in the quality of the data, there are certain factors that confound the analysis. At any field site it is quite difficult to produce carefully controlled conditions. For instance, at the Seal Beach site, it is known that non-aqueous phase liquids (NAPLs) were present during this project. However, the exact amount and exact composition of the NAPL are unknown. Because of this, it is impossible to compute even a reasonable estimate for a mass balance on the BTEX compounds. The NAPL phase is believed to have acted as a source of BTEX throughout the duration of the demonstration, but the rate of BTEX dissolution from the NAPL phase into the aqueous phase is unknown. (Because gasoline is less dense than water, the NAPL tends to spread on top of the water table, such that BTEX concentrations in the saturated zone near the water table are quite high throughout the duration of the demonstration. In this report, we have chosen to focus on BTEX concentrations a few feet below the water table, where concentrations are low enough that the effects of the electron acceptors can be detected.) Other field processes also confound a quantitative analysis of the mass of BTEX degraded. Desorption of BTEX from aquifer solids into the aqueous phase, diffusion of BTEX from regions of high concentration into the treatment zones, and slow encroachment of native ground water can all lead to the introduction of additional BTEX into the aqueous phase of the treatment zones. Because of all these factors, it is impossible to use the data, regardless of their quality, to perform a quantitative analysis of BTEX degradation. A semi-quantitative analysis can be made on the basis of the electron acceptors added, because the composition of the injected water was carefully controlled via the above-ground water treatment system (Figures 5–7). Thus, a mass balance on the nitrate and/or sulfate injected is more reasonable than a mass balance on the actual fuel hydrocarbon contaminants.

5.3 Technology Comparison

At sites similar to the Seal Beach site, where fuel hydrocarbons contaminate the ground water, there are two primary alternatives to enhanced in situ bioremediation: pump-and-treat, and intrinsic bioremediation. Below, we compare each of these alternatives to enhanced in situ

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bioremediation, using the results of this project as a guide. However, here it is important to recall that the technology implemented in this project was operated in a manner that differs from the recommended operation at a full-scale remediation site. For instance, because we were interested in evaluating three different types of anaerobic biodegradation, no oxygen was added to the injected feed water; at a full-scale implementation, oxygen could be added along with nitrate and sulfate in order to speed up the remediation.

Pump-and-treat remediation consists of extracting contaminated ground water from the site, treating the water above-ground to remove the contaminants of concern, and then either re-injecting the water into the aquifer or else disposing of it suitably. Essentially, the methodology used in this project is a pump-and-treat system with the added feature of augmenting the re-injected water with electron acceptors in order to stimulate in situ bioremediation. For a relatively minor modification, it is possible to derive great benefits in terms of the speed of aquifer remediation. Compare, for example, benzene to o-xylene. It was found (Section 5.1.1) that benzene was removed from the aquifer primarily via the extraction well, and subsequent adsorption onto activated carbon (Figures 5, 6). That is, benzene is removed primarily via pump-and-treat remediation. In contrast, o-xylene was rapidly degraded upon the addition of sulfate into zone 2 (Figure 12), and removal of o-xylene appears to be primarily via anaerobic biodegradation. Any contaminant mass that is degraded in situ does not need to be removed from the water in the above-ground treatment system. Thus, the in situ degradation of o-xylene extends the life of the activated carbon beds and shortens the amount of time that the extraction system must be run; both of these considerations indicate a savings in time and cost. Thus, as compared to a standard pump-and-treat remediation system, the demonstrated system has distinct advantages (shorter clean-up times, probably lower overall cost) and very few disadvantages (cost of electron acceptors, cost and maintenance of the chemical delivery system). The main question is whether or not the extra benefits are worth the additional cost of the electron acceptors and the injection system (Figure 7). This question will be considered in Section 6, below.

The other competing technology is intrinsic bioremediation. Intrinsic bioremediation consists of monitoring the site to ensure that no hazardous contaminants are reaching any receptors (e.g., drinking water wells, or, in this case, the Seal Beach National Wildlife Refuge), but does not employ injection of additional electron acceptors. Intrinsic bioremediation is therefore simpler and less expensive, because it does not require any injection wells, extraction wells, above-ground treatment system, or chemicals. However, there are some drawbacks to intrinsic bioremediation. It is likely to take much longer than enhanced bioremediation, especially if methanogenesis is much slower than sulfate reduction or nitrate reduction. This is especially true at a site like Seal Beach, where the very slow ground water flow limits the supply of electron acceptors and limits the removal of methane and inhibitory products. Methanogenesis is likely a more significant removal mechanism than either sulfate reduction or nitrate reduction at many fuel-contaminated ground water sites [Miller et al., 1995], and can lead to the generation of explosive methane gas. Furthermore, if a potential receptor is very close to the contaminated site, as in the case of Seal Beach, where the leaking tank was located very near the boundary of the wildlife refuge, intrinsic bioremediation might be insufficient to prevent the contamination from reaching the receptor. If a contaminated site is fairly isolated, and if the time for remediation is

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not of major concern, and if all of the contaminants are known to degrade under the conditions naturally present, then intrinsic bioremediation is preferable to the enhanced bioremediation technology demonstrated here; however, under many circumstances, intrinsic bioremediation is probably inadequate.

5.4 Technology Evaluation

In Section 1.3 of this report, we stated that this project was intended to meet two broad objectives:

(1) Demonstration of a technology by which fuel hydrocarbons are removed from ground water via enhanced in situ anaerobic biodegradation
(2) Evaluation and quantification of the efficacy of three different anaerobic processes (methanogenic, sulfate-reducing, and combined sulfate- and nitrate-reducing).

Most of this report has been dedicated to a discussion of objective (1), the demonstration of the technology. However, objective (2) is also important, and in this section we address the quantification of the efficacy of the anaerobic processes.

If possible, the best way to quantify the efficacy of the three treatment processes would be to estimate the rate at which BTEX compounds are degraded under each of the three conditions. However, during the demonstration, it was possible to monitor only the aqueous BTEX concentrations, and not the amount sorbed or the amount present as residual NAPL. Therefore, it is impossible to estimate a rate at which the BTEX compounds were degraded.

However, as discussed above, it is possible to estimate the rate at which the electron acceptors nitrate and sulfate were utilized. Specifically, we found that nitrate was utilized with an apparent first-order rate constant of 0.1–0.26 day^{-1} when injected at concentrations of about 50–100 mg/L (see Figure 15). Sulfate was utilized with an apparent first-order rate constant of about 0.1 day^{-1} when injected (without nitrate) at a concentration of about 80 mg/L (Figure 17).

What is significant about these estimated rates is that they indicate that the injected electron acceptors would be nearly completely utilized during the augmentation and no-flow stages of a treatment cycle. This conclusion is supported by Figures 14–18, although Figures 19 and 20 suggest that sulfate might not be completely utilized when nitrate is present. If we assume that the electron acceptors are completely utilized during the augmentation an no-flow stages, then the efficacy of nitrate- and sulfate-reduction is essentially 100%, i.e., the rate of BTEX removal via anaerobic biodegradation is limited not by the kinetics of the biodegradation reaction, but rather by the rate at which nitrate and sulfate can be introduced into the contaminated region.

For Zone 2, we assume an injection rate of 1.5 L/min, an injection concentration of 80 mg/L sulfate, a 30-day augmentation period followed by a 30-day no-flow period, and the stoichiometry shown in Section 2.1.1. This results in the degradation of 1.1 kg of fuel hydrocarbons as a result of the sulfate augmentation.

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For Zone 4, we assume an injection rate of 1.5 L/min, an injection concentration of 80 mg/L nitrate and 80 mg/L sulfate, a 30-day augmentation period followed by a 30-day no-flow period, and the stoichiometry shown in Section 2.1.1. This results in the degradation of 2.2 kg of fuel hydrocarbons as the result of sulfate and nitrate augmentation.

In Zone 3, where methanogenic conditions are established, it is impossible to quantify the efficacy of the anaerobic process without a method for measuring the sorbed BTEX concentrations and the amount present as residual NAPL.

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6. Cost Assessment

6.1 Cost Performance

Tables 6, 7a, and 7b provide an assessment of the expected operational costs for the technology when implemented. The tables do not necessarily represent the actual cost of the particular demonstration described in this report; rather, they are a prediction of how much it should cost to implement this technology at a typical site. An example of typical site conditions is given below. These tables have been prepared in the format specified by the 1998 *Guide to Documenting and Managing Cost and Performance Information for Remediation Projects*, which was prepared by the member agencies of the Federal Remediation Technologies Roundtable (FRTR). The tabulation specified by the 1998 Guide differs from that specified by the previous version of the Guide [FRTR, 1995].

As has been noted throughout this document, certain aspects of the demonstration project were performed under different operating conditions than would be expected at a full-scale implementation of this technology. Because the goal of this section is to provide information about the cost of a full-scale implementation of the technology, not the cost of the demonstration, it is important to specify the assumed parameters of the full-scale implementation. For the cost assessment, we make the following assumptions, which are all reasonable for a typical fuel-contaminated site:

- About 5,000 gallons of fuel leaked from an underground storage tank, contaminating the soil and shallow ground water. About 95% of the fuel (4750 gallons) was recovered via excavating the vadose zone down to the water table, or via some other removal mechanism. The remaining 250 gallons are present below the water table in three forms: as globules of non-aqueous phase liquid, dissolved in the ground water, and sorbed to aquifer solids.
- The spill has impacted a volume below the water table of about 3,000 m^3, of which approximately 2,000 m^3 is aquifer solids and 1,000 m^3 is contaminated ground water (based on a porosity of 0.33). The contaminated portion of the aquifer extends to about 3 m below the water table, and the areal extent of the contamination is about 1000 m^2, which is roughly one quarter of an acre.
- Five treatment zones are developed at the contaminated site, each consisting of an injection well and an extraction well. The injection rate and extraction rate in each zone are 4.6 L/min.
- A treatment cycle consists of a one-month flushing period, a one-month augmentation period, and a one-month no-flow period. Four treatment cycles are performed per year. Based on these parameters, about 200,000 L water are injected into each of the five treatment zones during the augmentation stage, for a total of 1,000,000 L injected during each augmentation stage.
- During the augmentation stages, nitrate is injected at 80 mg/L, sulfate at 80 mg/L, and oxygen at 9 mg/L.

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- The reaction stoichiometries presented in Section 2 are valid. Therefore, the injection of 320 kg/year nitrate, 320 kg/year sulfate, and 36 kg/year oxygen degrades 145 kg/year fuel hydrocarbons (as toluene). This is equivalent to about 44 gallons/year.
- The above-ground treatment system for extracted water consists of granular activated carbon for hydrocarbon removal, followed by aeration for the removal of nitrogen gas (via stripping) and sulfide (via oxidation to sulfate). The treatment system operates eight months out of each year, i.e., during the flushing stages and during the augmentation stages, but not during the no-flow stages. Treated water is augmented with electron acceptors and is re-injected into the aquifer.
- The hydrocarbon concentration in extracted ground water averages 3 mg/L, and is effectively removed by the granular activated carbon. This is consistent with observations from the NWS Seal Beach demonstration. Therefore, about 24 kg/year hydrocarbons are removed via adsorption onto activated carbon, which is equivalent to about 7.2 gallons/year.
- The activated carbon system has an empty-bed volume of 230 L and an empty-bed contact time of 10 minutes. The system is loaded with 92 kg activated carbon (400 kg/m^3). During each treatment cycle, about 6 kg hydrocarbons are removed, such that the loading at the end of the treatment cycle is 65 g hydrocarbon per kg activated carbon. The activated carbon is replaced after each treatment cycle, i.e., four times annually.
- The overall hydrocarbon removal rate is 169 kg/year, equivalent to 51 gallons/year. About 85% of the removal is from in situ biodegradation, and about 15% is from activated carbon adsorption. The projected clean-up time is 4.9 years.

In addition, we assume that sampling and analysis are required on a monthly basis during the first year of operation, on a bi-monthly basis during the second and third years, and on a quarterly basis during the fourth and fifth years. This schedule is realistic in the sense that, as the remediation proceeds and clean-up is demonstrated, sampling and analysis can be performed on a less frequent basis. Thus, the operation and maintenance (O&M) costs for the project vary from year to year depending on the frequency of sampling and analysis. We assume that ten sampling locations are monitored over time. Finally, we assume that O&M costs are subject to an annual increase in price of 5% due to inflation, but that the present value of future costs is represented by an 8% annual discount rate.

Under these conditions, Table 6 summarizes the anticipated capital costs, while Tables 7a and 7b summarize the present value of the operating and maintenance (O&M) costs. The estimated capital cost is $470,000. The estimated present value of the O&M costs is $615,000 over 5 years. The total present value of the clean-up costs is $1,085,000, or $4,340 per gallon of fuel hydrocarbon recovered.

These cost estimates are based on an assumed set of conditions, as described above. The assumed conditions are reasonable, and are representative of many of the sites at which this technology might be applied. However, actual conditions can vary widely from site to site, and the cost estimates provided here should not be considered to apply exactly to all sites. For instance, if the contaminated area is larger than that assumed here, then more than 5 injection-extraction well pairs might be required, and more than ten monitoring wells might be required. This would increase both the capital cost (more wells needed, higher mobilization costs, etc.) and the O&M

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costs (more samples requiring analysis, more rapid activated carbon use, etc.). Of course, a larger contaminated area would also mean that competing technologies would probably be more expensive, as well, but the costs might not increase in equal proportions for all technologies under consideration. Therefore, even though the cost estimates in this report are believed to be quite reasonable and accurate for the representative site conditions considered, each contaminated site should be considered individually when a remediation technology is being selected.

6.2 Cost Comparisons to Conventional and Other Technologies

As discussed in Section 5.3, above, the two most common alternatives to the technology described here are (1) pump-and-treat, and (2) intrinsic bioremediation. Below, we estimate the clean-up times and the costs if the conventional pump-and-treat method were applied to the "typical" spill described in Section 6.1, above. We also consider the conditions under which intrinsic bioremediation might or might not be preferable to the Enhanced In Situ Anaerobic Bioremediation method. Finally, we consider when enhanced aerobic biodegradation (e.g., via air sparging) might be a viable alternative.

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Table 6: Capital Cost for Enhanced In Situ Bioremediation Technology

Capital Cost Element	Cost	Sub-Cost
Site Characterization:		
Hydrogeologic characterization:	$100,000	
Wells for estimating hydraulic head and gradient:		$60,000
Pump tests to estimate hydraulic conductivity:		$20,000
Cores and core analysis to estimate hydraulic conductivity:		$20,000
Microcosm studies to test biological activity:	$10,000	
Technology mobilization, set-up, and demobilization:		
Transportation/delivery of equipment, facilities, and personnel:	$20,000	
Set-up of temporary facilities (e.g., trailer) and utilities:	$20,000	
Demobilization:	$10,000	
Planning and preparation:		
Engineering design and modeling:	$50,000	
Permits and licenses, including air emission and water discharge:	$20,000	
License fees associated with use of a technology:	$0	
Regulatory interaction:	$5,000	
Written plans:	$35,000	
Work plans:		$10,000
Sampling and analysis plans:		$10,000
Health and safety plans:		$5,000
Community relations plans:		$5,000
Site management plans:		$5,000
Site work:		
Establish physical infrastructure for technology application:	$15,000	
Activities necessary to restore site to pre-remediation conditions:	$15,000	
Activities necessary to meet specifications of site restoration plan:	$15,000	
Preparing specific site of the technology:	$15,000	
Clearing and grubbing:		$5,000
Earthwork:		$5,000
Construction of utilities, culverts, treatment pads, foundations, etc.:		$5,000
Installation of treatment system (equipment and appurtenances):		
Extraction wells (5 wells, $5000 each):	$25,000	
Injection wells for augmentation (5 wells, $5000 each):	$25,000	
Monitoring wells (10 multi-level samplers, $5000 each):	$50,000	
Above-ground water treatment system:	$10,000	
Activated Carbon column (1 m length, 0.5 m diameter):		$2,000
Trickling Filter for aeration:		$6,000
Chemical Injection System:		$2,000
Startup and testing:		
Establishment of operating conditions:	$5,000	
Shakedown:	$5,000	
Training of O&M personnel:	$5,000	
Other capital costs:		
Data processing and computer equipment:	$5,000	
Safety equipment:	$5,000	
Vehicles:	$5,000	
Total Capital Cost:	$470,000	

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Table 7a: Annual Operating and Maintenance (O&M) Costs for Technology

O&M Cost Element	Cost	Sub-Cost
Labor:		
Maintenance of technology and associated equipment:	$25,000	
Labor supervision:	$5,000	
Payroll expenses:	$5,000	
Materials:		
Consumable supplies:	$5,000	
Activated carbon (replaced quarterly; includes disposal):		$5,000
Process materials:	$0	
Bulk chemicals:	$13,000	
Nitrate (320 kg/year):		$7,000
Sulfate (320 kg/year):		$6,000
Raw materials:	$0	
Utilities and fuel		
Fuel:	$500	
Electricity: (primarily for running pumps)	$1,000	
Natural gas:	$0	
Water:	$500	
Equipment ownership, rental, or lease:	$0	
Performance testing and analysis:		
Monitoring, sampling, analysis ($1000 per well per round):	see Table 7b	
Other O&M Costs:		
Maintenance and repair of office/administrative equipment:	$5,000	
Health and safety costs:	$5,000	
Personal protective equipment:		$2,000
Monitoring of personnel for health and safety:		$3,000
Total Annual O&M Costs (not including monitoring):	$65,000	
Total Annual O&M Costs (including monitoring):	see Table 7b	

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Table 7b: Present Value of O&M Costs, Including Monitoring
Enhanced In Situ Anaerobic Bioremediation Method

Year	Sampling Frequency	Monitoring Cost (Unadjusted)	Total O&M Cost (Unadjusted)	O&M Cost (with inflation)	O&M Cost (present value)
1	Monthly	$120,000	$185,000	$185,000	$185,000
2	Bi-Monthly	$60,000	$125,000	$131,250	$121,528
3	Bi-Monthly	$60,000	$125,000	$137,813	$118,152
4	Quarterly	$40,000	$105,000	$121,551	$96,491
5	Quarterly	$40,000	$105,000	$127,628	$93,811
Total O&M Cost, Present Value					$615,000

Notes: Sampling frequency is assumed to follow the schedule above
Costs based on assumption of ten (10) monitoring wells and cost of $1000 per well per round of sampling/analysis
Inflation rate assumed 5% annually, discount rate assumed 8%
Clean-up time for this method estimated to be 5 years
Other site characteristics described in Section 6.1

6.2.1 Comparison to Pump-and-Treat (Conventional Clean-up)

Our cost estimate for the pump-and-treat remediation is based upon the following assumptions:

- Site conditions are the same as those described in Section 6.1, above, but with the following differences.
- Five extraction wells are installed, each with a flow rate of 4.6 L/min. This is the same specification that was made in the cost estimate for the proposed technology in Section 6.1, above. The wells are operated continuously, such that a total of 12,100,000 L are extracted in one year.
- Extracted water is treated with granular activated carbon for hydrocarbon removal. Air stripping might also be possible, but to facilitate comparison with the proposed technology as described in Section 6.1, we assume that activated carbon is suitable.
- The hydrocarbon concentration in extracted ground water averages 3 mg/L, and is effectively removed by the granular activated carbon. This is consistent with observations from the NWS Seal Beach demonstration. Therefore, about 36 kg/year hydrocarbons are removed via adsorption onto activated carbon, which is equivalent to about 11 gallons/year.
- The activated carbon system has an empty-bed volume of 230 L and an empty-bed contact time of 10 minutes. The system is loaded with 92 kg activated carbon (400 kg/m^3). The carbon is replaced every two months, such that the hydrocarbon loading just before replacement is about 65 g hydrocarbon per kg of activated carbon.

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- Some in situ biological removal is expected, either aerobic or via methanogenesis, even without addition of sulfate or nitrate as electron acceptors. We assume that about 20 kg/year are degraded biologically.
- The total hydrocarbon removal rate is 56 kg/year, which is equivalent to about 17 gallons/year. The total clean-up time is 14.7 years.
- There are some costs that are expected to be lower for the pump-and-treat technology than for the proposed enhanced in situ bioremediation technology. Specifically,
- Assuming that the treated water is discharged, no injection wells are required for re-injection of treated water.
- Because pump-and-treat is an established technology, fewer samples are required during the monitoring program. We assume seven monitoring locations for the pump-and-treat method, as opposed to ten monitoring locations for the enhanced in situ bioremediation method.
- The above-ground treatment system does not require aeration or chemical injection.
- Because less equipment is required, design and delivery costs are somewhat lower.
- No sulfate or nitrate is required.

In addition, we assume that sampling and analysis are required on a bi-monthly basis during the first 9 years of operation, and on a quarterly basis during the final 6 years. This schedule is realistic in the sense that, as the remediation proceeds and clean-up is demonstrated, sampling and analysis can be performed on a less frequent basis. We assume that 7 sampling locations are monitored (as opposed to 10 locations for the enhanced in situ bioremediation scheme described above). As before, we assume that O&M costs are subject to an annual increase in price of 5% due to inflation, but that the present value of future costs is represented by an 8% annual discount rate.

Under these conditions, Table 8 summarizes the anticipated capital costs, while Tables 9a and 9b summarize the present value of the O&M costs. The estimated capital cost is $397,000. The estimated present value of the O&M costs is $1,143,000 over 15 years. The total present value of the clean-up costs is $1,540,000, or $6,160 per gallon of fuel hydrocarbon recovered.

Based on this analysis, the proposed technology, enhanced in situ bioremediation, is preferable to conventional pump-and-treat remediation. Although there are some additional costs associated with implementing a relatively new technology, the overall cost is lower, and the associated clean-up time is much shorter (about 5 years instead of about 15 years). However, it is important to note that the extent of biodegradation for the conventional pump-and-treat remediation is extremely uncertain. We have assumed 20 kg/year biodegradation, but if the actual rate is much faster or much slower, then the cost for the pump-and-treat remediation could be affected significantly. Table 9b gives an indication how the estimated cost could be adjusted for shorter or longer clean-up times.

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Table 8: Capital Cost for Conventional Pump-and-Treat Technology

Capital Cost Element	Cost	Sub-Cost
Site Characterization:		
Hydrogeologic characterization:	$100,000	
Wells for estimating hydraulic head and gradient:		$60,000
Pump tests to estimate hydraulic conductivity:		$20,000
Cores and core analysis to estimate hydraulic conductivity:		$20,000
Technology mobilization, set-up, and demobilization:		
Transportation/delivery of equipment, facilities, and personnel:	$15,000	
Set-up of temporary facilities (e.g., trailer) and utilities:	$20,000	
Demobilization:	$10,000	
Planning and preparation:		
Engineering design and modeling:	$40,000	
Permits and licenses, including air emission and water discharge:	$20,000	
License fees associated with use of a technology:	$0	
Regulatory interaction:	$5,000	
Written plans:	$35,000	
Work plans:		$10,000
Sampling and analysis plans:		$10,000
Health and safety plans:		$5,000
Community relations plans:		$5,000
Site management plans:		$5,000
Site work:		
Establish physical infrastructure for technology application:	$15,000	
Activities necessary to restore site to pre-remediation conditions:	$15,000	
Activities necessary to meet specifications of site restoration plan:	$15,000	
Preparing specific site of the technology:	$15,000	
Clearing and grubbing:		$5,000
Earthwork:		$5,000
Construction of utilities, culverts, treatment pads, foundations, etc.:		$5,000
Installation of treatment system (equipment and appurtenances):		
Extraction wells (5 wells, $5000 each):	$25,000	
Monitoring wells (7 multi-level samplers, $5000 each):	$35,000	
Above-ground water treatment system:	$2,000	
Activated Carbon column (1 m length, 0.5 m diameter):		$2,000
Startup and testing:		
Establishment of operating conditions:	$5,000	
Shakedown:	$5,000	
Training of O&M personnel:	$5,000	
Other capital costs:		
Data processing and computer equipment:	$5,000	
Safety equipment:	$5,000	
Vehicles:	$5,000	
Total Capital Cost:	$397,000	

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Table 9a: Annual (O&M) Costs for Conventional Pump-and-Treat Technology

O&M Cost Element	Cost	Sub-Cost
Labor:		
Maintenance of technology and associated equipment:	$25,000	
Labor supervision:	$5,000	
Payroll expenses:	$5,000	
Materials:		
Consumable supplies:	$7,500	
Activated carbon (replaced quarterly; includes disposal):		$7,500
Process materials:	$0	
Raw materials:	$0	
Utilities and fuel		
Fuel:	$500	
Electricity: (primarily for running pumps)	$1,500	
Natural gas:	$0	
Water:	$500	
Equipment ownership, rental, or lease:	$0	
Performance testing and analysis:		
Monitoring, sampling, analysis ($7,000 each round):	see Table 9b	
Other O&M Costs:		
Maintenance and repair of office/administrative equipment:	$5,000	
Health and safety costs:	$5,000	
Personal protective equipment:		$2,000
Monitoring of personnel for health and safety:		$3,000
Total Annual O&M Costs (not including monitoring):	$55,000	
Total Annual O&M Costs (including monitoring):	see Table 9b	

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Table 9b: Present Value of O&M Costs, Including Monitoring
Conventional Pump-and-Treat Method

Year	Sampling Frequency	Sampling Cost (Unadjusted)	Total O&M Cost (Unadjusted)	O&M Cost (with inflation)	O&M Cost (present value)
1	Bi-Monthly	$42,000	$97,000	$97,000	$97,000
2	Bi-Monthly	$42,000	$97,000	$101,850	$94,306
3	Bi-Monthly	$42,000	$97,000	$106,943	$91,686
4	Bi-Monthly	$42,000	$97,000	$112,290	$89,139
5	Bi-Monthly	$42,000	$97,000	$117,904	$86,663
6	Bi-Monthly	$42,000	$97,000	$123,799	$84,256
7	Bi-Monthly	$42,000	$97,000	$129,989	$81,915
8	Bi-Monthly	$42,000	$97,000	$136,489	$79,640
9	Bi-Monthly	$42,000	$97,000	$143,313	$77,428
10	Quarterly	$28,000	$93,000	$128,760	$64,412
11	Quarterly	$28,000	$93,000	$135,198	$62,623
12	Quarterly	$28,000	$93,000	$141,958	$60,883
13	Quarterly	$28,000	$93,000	$149,056	$59,192
14	Quarterly	$28,000	$93,000	$156,509	$57,548
15	Quarterly	$28,000	$93,000	$164,334	$55,949
Total O&M Cost, Present Value					$1,143,000

Notes: Sampling frequency is assumed to follow the schedule above
Costs based on assumption of seven (7) monitoring wells and cost of $1000 per well per round of sampling/analysis
Inflation rate assumed 5% annually, discount rate assumed 8%
Clean-up time for this method estimated to be 15 years
Other site characteristics described in Sections 6.1 and 6.2.1

6.2.2 Comparison to Intrinsic Bioremediation. When intrinsic bioremediation is technically feasible, it is almost certain to cost less than the proposed enhanced bioremediation technology. Intrinsic bioremediation does not require the installation of injection wells, extraction wells, or above-ground water treatment equipment. The primary cost for intrinsic bioremediation is the cost of monitoring, sampling, and analysis. Although this cost can be substantial, particularly if the remediation takes several years, the overall cost of intrinsic bioremediation is low in comparison to the other treatment technologies considered here.

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Therefore, the determining factor as to whether or not intrinsic bioremediation is preferable to enhanced bioremediation is the expected effectiveness of intrinsic biological processes. The following issues need to be considered:

1) At sites like Seal Beach, where the very slow ground water flow limits the supply of electron acceptors and limits the removal of methane and inhibitory products, intrinsic bioremediation might be extremely slow.
2) During intrinsic bioremediation, methanogenesis is often the most significant biological removal process [Miller et al., 1995], which can lead to the build-up of explosive methane gas.
3) If the contaminated site is located close to potential receptors, as in the case of Seal Beach, where the leaking tank was very near the boundary of the wildlife refuge, intrinsic bioremediation might be insufficient to prevent the contamination from reaching the receptor.

Therefore, although intrinsic bioremediation is almost certain to be less expensive than the enhanced bioremediation demonstrated here, there are many circumstances in which intrinsic bioremediation is unsuitable. In such cases, enhanced in situ bioremediation is likely to be a viable alternative.

6.2.3 Comparison to Enhanced Aerobic Biodegradation. Another alternative method to that demonstrated in this project is Enhanced Aerobic Biodegradation. Enhanced Aerobic Biodegradation would consist of the introduction of oxygen into the aquifer rather than alternate electron acceptors such as sulfate or nitrate, in an effort to stimulate aerobic organisms to oxidize BTEX to carbon dioxide and water. Aerobic biodegradation of BTEX compounds is generally faster than anaerobic degradation, and can in some cases be considerably faster, particularly for benzene. Therefore, if oxygen can be introduced into the aquifer effectively and inexpensively, then Enhanced Aerobic Biodegradation would probably be preferable to Enhanced Anaerobic Biodegradation.

However, delivery of oxygen into a contaminated aquifer can be difficult and/or expensive. Methods include:

1) Injecting water saturated with dissolved oxygen gas
2) Injecting water with some concentration of peroxide, which quickly reacts to produce molecular oxygen
3) Air sparging, i.e., the introduction of oxygen gas bubbles at the bottom of the contaminated region, which allows the bubbles to flow upwards through the contaminated region, delivering oxygen to the bacteria while simultaneously stripping volatile contaminants out of the water
4) Placing oxygen-releasing compounds (ORCs) in situ to provide a continuous supply of oxygen.

However, each of these techniques has its own limitations. For instance,

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1) the solubility of oxygen in water is low, so that nitrate and sulfate can be introduced at much higher concentrations than oxygen.
2) Peroxide is a very reactive, unstable compound, which reacts unselectively with aquifer materials, e.g., peroxide can oxidize ferrous iron, Fe^{2+}, to ferric iron, Fe^{3+}. This consumes the peroxide and can result in precipitation of ferric compounds.
3) Air sparging must be used in conjunction with a soil vapor extraction (SVE) system, which makes it expensive, and the delivery of oxygen to the necessary locations via air sparging is unreliable and can be limited by the rate of oxygen diffusion.
4) Oxygen-releasing compounds (ORCs) might be the most promising of these oxygen delivery methods, but the technology is still developing. In some cases use of ORCs has been found to alter aquifer pH, or to decrease the permeability of the aquifer matrix, presumably due to precipitation caused by oxidation of minerals in the matrix. Also, ORCs might deliver oxygen to only a relatively small vicinity around the ORC compound, while other regions of the aquifer remain anaerobic.

There may also be other limitations to these methods not considered here. By contrast, delivery of nitrate and sulfate at relatively high concentrations is a relatively simple process.

Finally, we note here that the Enhanced In Situ Bioremediation technology demonstrated in this project is, in fact, a hybrid aerobic/anaerobic treatment method. Because oxygen is consumed rapidly, the anaerobic processes are of extreme importance, and have been emphasized in the demonstration of this technology. However, as noted elsewhere in this report, a full-scale implementation of this project would include the introduction of oxygen into the aquifer as well as the introduction of nitrate and sulfate.

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7. Regulatory Issues: Approach to Regulatory Compliance and Acceptance

Extracting and re-injecting ground water requires permission from the appropriate regulatory agencies. As discussed in Section 1.4, above, there are three main regulatory issues with regard to the implementation of the technology described in this report:

(1) Nitrate, which has a drinking water maximum contaminant level (MCL) of 45 mg/L, is injected into the ground water as an electron acceptor. In order for the technology to operate at maximum efficiency, nitrate should be injected at a concentration higher than its MCL. Regulatory approval is required for injection of nitrate at high concentrations.

(2) Contaminated ground water is extracted, treated, augmented with electron acceptors, and then re-injected into the aquifer. The re-injection of treated ground water requires regulatory approval.

(3) It must be satisfactorily demonstrated that sufficient hydraulic control is established, so that the plume can be contained if any problem arises during implementation of the remediation technology.

For the demonstration at NWS Seal Beach, we first injected nitrate at low concentrations and monitored the subsequent biological utilization of nitrate. When we had demonstrated that nitrate was biologically degraded very rapidly, regulators were amenable to the injection of higher concentrations of nitrate.

We found regulators to be concerned with the re-injection of treated ground water into the aquifer. Demonstration of hydraulic control of the site and formulation of appropriate contingency plans are helpful aides in obtaining regulatory approval for re-injection.

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8. Technology Implementation

8.1 Department of Defense (DOD) Need

In April 1997, the United States Environmental Protection Agency (EPA) published a report that included a survey of the demand for remediation of DOD sites [U.S. EPA, 1997a]. Among the relevant findings of that report were the following:

- Based on 1995 site data, an estimated 8,336 DOD sites would require clean-up.
- Based on 1994 site data, 3,212 sites had identified both the types of contaminated media (ground water, soil, surface water, and/or sediment) and the types of contaminants present (volatile organic compounds, semivolatile organic compounds, metals, fuels, explosives, etc.). Of these 3,212 sites,
    - 2,290 (71%) had contaminated ground water.
    - 2,093 were contaminated with VOCs and 712 were contaminated with fuels. Benzene, toluene, ethylbenzene, and xylene (BTEX) could be categorized in either the VOCs category or the fuels category.
    - 22% were explicitly recognized as containing BTEX contamination. However, the actual percentage of sites with BTEX contamination might be higher if some BTEX sites were classified in the more general VOCs category (44% of sites were identified with non-halogenated VOCs).

Assuming that 71% of all DOD sites have contaminated ground water, and also making a conservative assumption that 22% of ground water sites are contaminated with fuel hydrocarbons, then we would expect fuel contamination at 1,302 of the 8,336 sites identified in 1995. This number is probably a conservative estimate, and the actual number of DOD sites where ground water is contaminated with fuel hydrocarbons might be several thousand. Based on this assessment, we consider the technology described in this report to be of extreme interest to DOD. Not all sites will have conditions amenable to the implementation of this technology, but many sites will.

8.2 Transition and Technology Transfer

The major impediment to full-scale application of this technology is the fact that, despite the significant progress made in this project, questions still remain regarding the efficacy of anaerobic remediation of fuel hydrocarbons under different geochemical conditions. The hydraulic components of the technology, although highly dependent upon specific site conditions, are well established and well understood. This is also true for the above-ground water treatment of the extracted water. The primary difficulty in applying this technology to a full-scale site is the determination of what electron acceptors should be injected, and at what concentrations. Among the BTEX compounds, only toluene has been observed to degrade rapidly under general anaerobic conditions. Biodegradation of the other compounds appears to be highly dependent upon the terminal electron acceptor(s) present and also upon other site-specific conditions that are much more difficult to identify.

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We recommend two ways in which these difficulties can be mitigated:

(1) Further exploration, under carefully controlled laboratory conditions as well as actual field conditions, to better determine what specific factors control microorganisms' ability to degrade BTEX and other fuel hydrocarbons
(2) Whenever this technology is implemented full-scale, installation at the contaminated aquifer should be preceded by laboratory experiments with the aquifer material in order to determine how fast particular contaminants degrade under different geochemical conditions.

Active research on anaerobic biodegradation is underway at many universities and government laboratories, which should improve our understanding and ability to apply this technology in the coming years.

A related issue is whether or not this technology is preferable to monitored intrinsic bioremediation, which is currently favored by many industries. Monitored intrinsic bioremediation alleviates the uncertainty over electron acceptor selection by relying completely on whatever conditions are naturally present. This also makes monitored intrinsic bioremediation less expensive than enhanced bioremediation. The choice between the two technologies is largely one of economics; enhanced bioremediation is expected to lead to shorter clean-up times, especially at sites where sulfate reduction and/or denitrification are known to accelerate biodegradation.

This technology should be readily acceptable by regulatory agencies (see also Section 1.4 and Section 7). Although we did not have an industrial partner during this demonstration, we expect the technology to be rapidly accepted by industry. The equipment employed is readily available commercially, and is commonly installed in other types of treatment systems. Ground water contamination from leaking underground storage tanks is a very large industrial problem [National Research Council, 1994], especially with regard to fuel hydrocarbons. Therefore, we anticipate industry to be extremely interested in the continued development of this technology.

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9. Lessons Learned

Below, we summarize some of the most important scientific and technical findings from this demonstration project. These findings should be useful to those who are planning a demonstration of enhanced anaerobic in situ bioremediation, or those who are attempting to implement this technology at a full-scale remediation site.

- Nitrate and sulfate can be utilized biologically as electron acceptors during the in situ anaerobic oxidation of fuel hydrocarbons (including BTEX compounds) in contaminated ground water. Therefore, bioremediation of contaminated ground water can be enhanced (accelerated) via the introduction of nitrate and/or sulfate into the contaminated region. However, sulfate reduction, denitrification, and fermentation/methanogenesis can all occur naturally under unaugmented conditions. Therefore, the benefits of accelerated degradation via nitrate/sulfate augmentation must be assessed vis-a-vis natural attenuation (intrinsic bioremediation), which is less costly to implement.
- Ground water contaminated from gasoline contains not only BTEX compounds, but many other gasoline components as well. At the Seal Beach site, much of the injected nitrate and sulfate was utilized by bacteria to degrade non-BTEX hydrocarbons. This makes it difficult to predict the amount of electron acceptor(s) that will be needed for complete BTEX removal.
- At the Seal Beach site, nitrate utilization was fast. Most or all of the injected nitrate was consumed within 30 days after injection ceased for the third augmentation, and even faster for the first and second augmentations. Sulfate utilization was also relatively fast in the region where sulfate was the only electron acceptor injected. In the region where both sulfate and nitrate were injected, the sulfate utilization was much slower.
- Injection of nitrate at very high concentrations (greater than 100 mg/L) might lead to the formation of nitrogen gas bubbles, which could alter the hydraulic character of the aquifer.
- Methanogenesis was observed in all three treatment zones during the demonstration, but was most apparent in the zone where neither nitrate nor sulfate was added. In two of the zones, several mg/L methane were generated (5–6 mg/L in zone 3, and 3–5 mg/L in zone 4), which would correspond to the degradation of several mg/L fuel hydrocarbons. However, it did not appear that benzene was effectively removed via methanogenesis.
- The removal rate and the removal sequence for BTEX compounds depends on a number of factors, including the terminal electron acceptor. Toluene concentrations were very low even at the onset of the demonstration, consistent with previously-observed preferential toluene degradation under sulfate-reducing, denitrifying, and methanogenic conditions. Augmentation with nitrate was effective for the removal of all BTEX compounds except benzene. Augmentation with sulfate accelerated the removal of xylenes, particularly o-xylene, but not the other BTEX compounds.
- Benzene was effectively removed via flushing, in part because it does not sorb strongly to aquifer materials. However, benzene biodegradation was slow if it occurred at all. Some previous studies have shown that benzene biodegradation occurs only when no other BTEX compounds are present, indicating a preferential removal sequence.

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- During periods when the injection and extraction wells were not operated, a rebound in the BTEX concentrations was observed, especially for benzene. This probably indicates the presence of a residual non-aqueous liquid (NAPL) phase, but it might also indicate the desorption of BTEX compounds from aquifer solids, the diffusion of BTEX from highly contaminated areas that are not effectively flushed, and/or slow encroachment of highly contaminated ground water.
- Many of the design parameters for this system depend on site-specific contamination and hydrogeologic conditions. Some of these design parameters include: the number and location of extraction and injection wells; the injection and extraction flow rates; the method of above-ground treatment for extracted water; the choice of electron acceptors injected; the concentrations of electron acceptors injected; and the duration of flushing, augmentation, and no-flow periods.

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Figure 1. Location of the Naval Weapons Station, Seal Beach and the Seal Beach National Wildlife Refuge.

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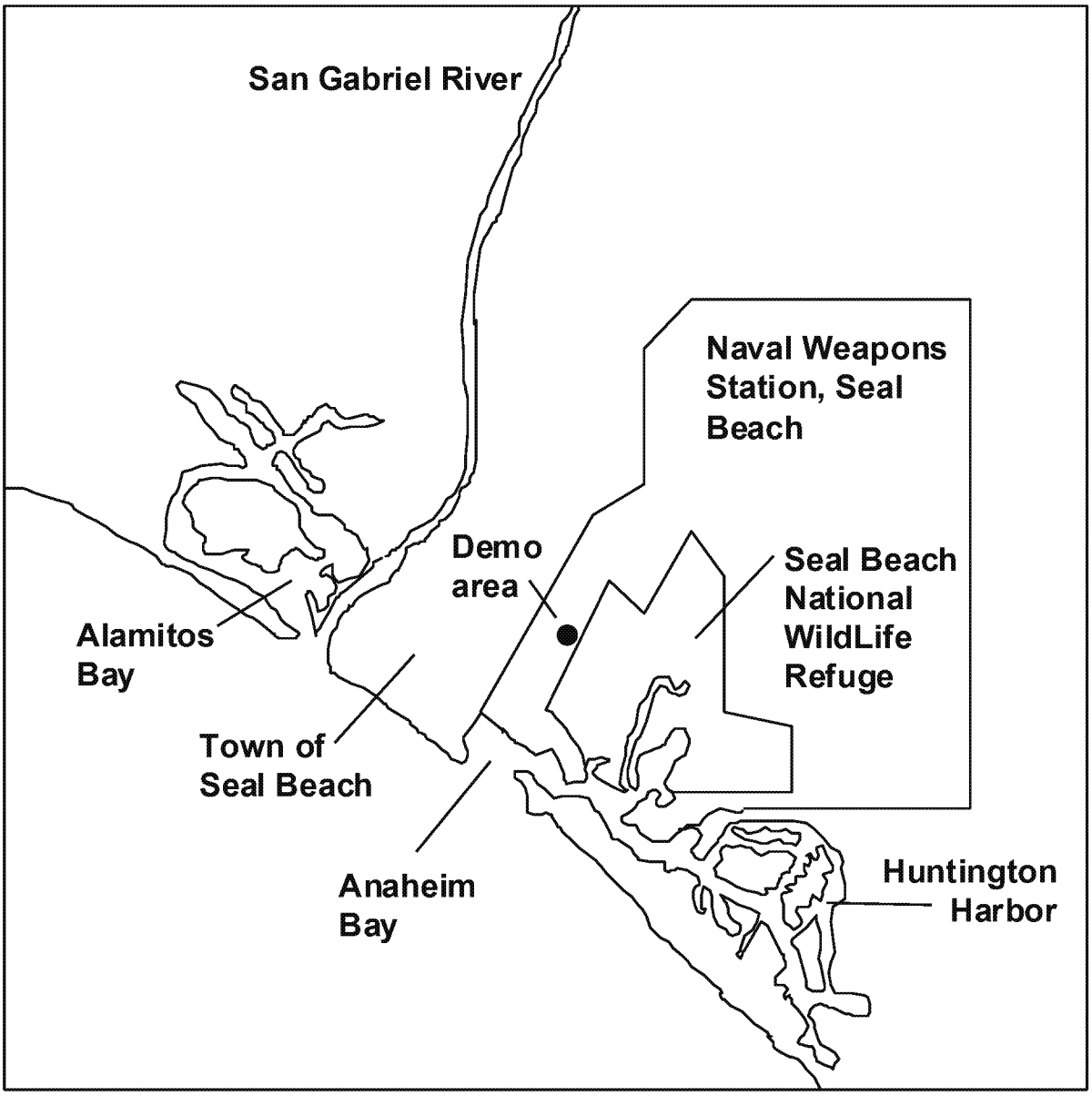

Figure 2. Detailed location of the Naval Weapons Station, Seal Beach and the Seal Beach National Wildlife Refuge.

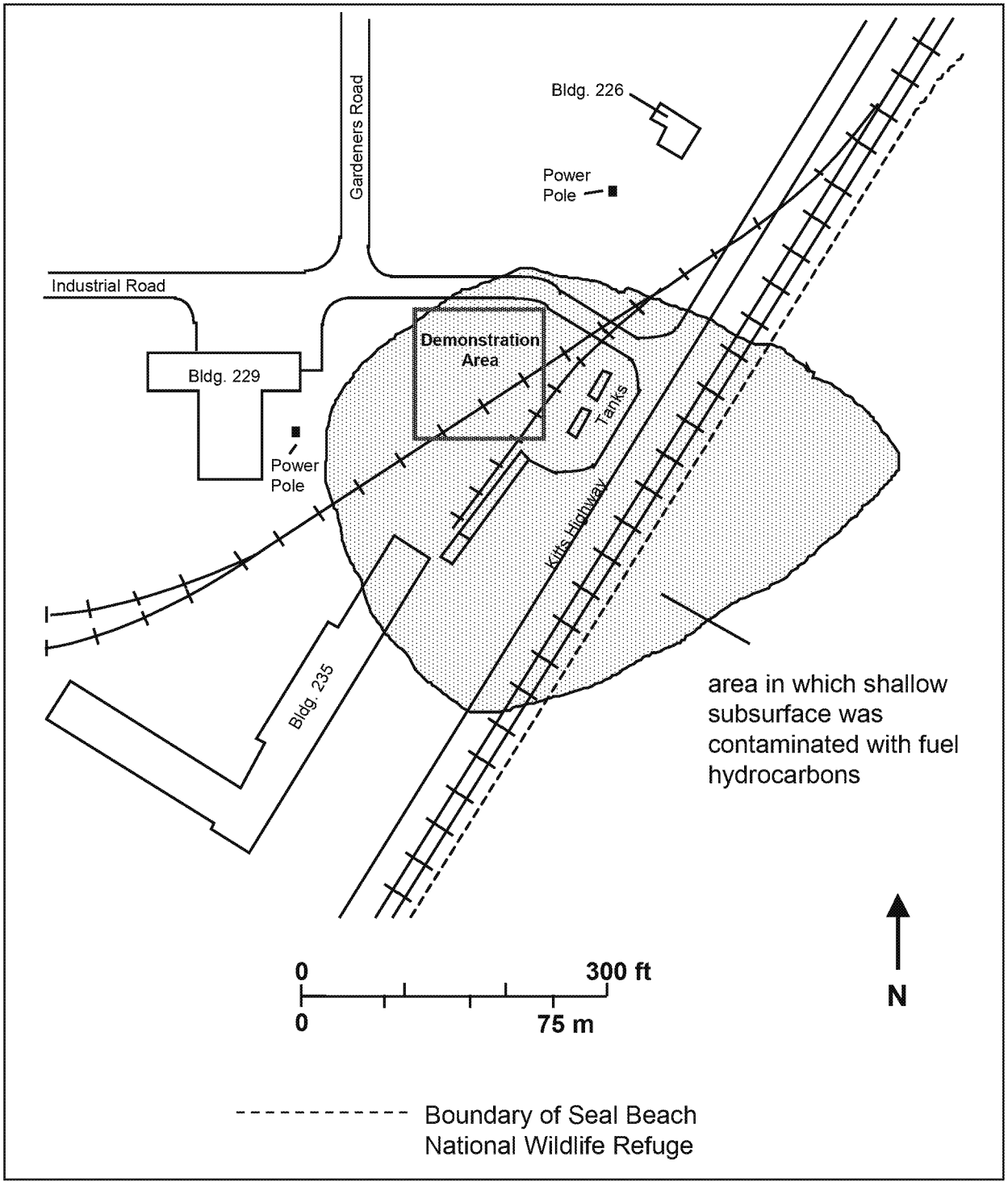

Figure 3. Plan view of the region of ground water contamination and the demonstration area.

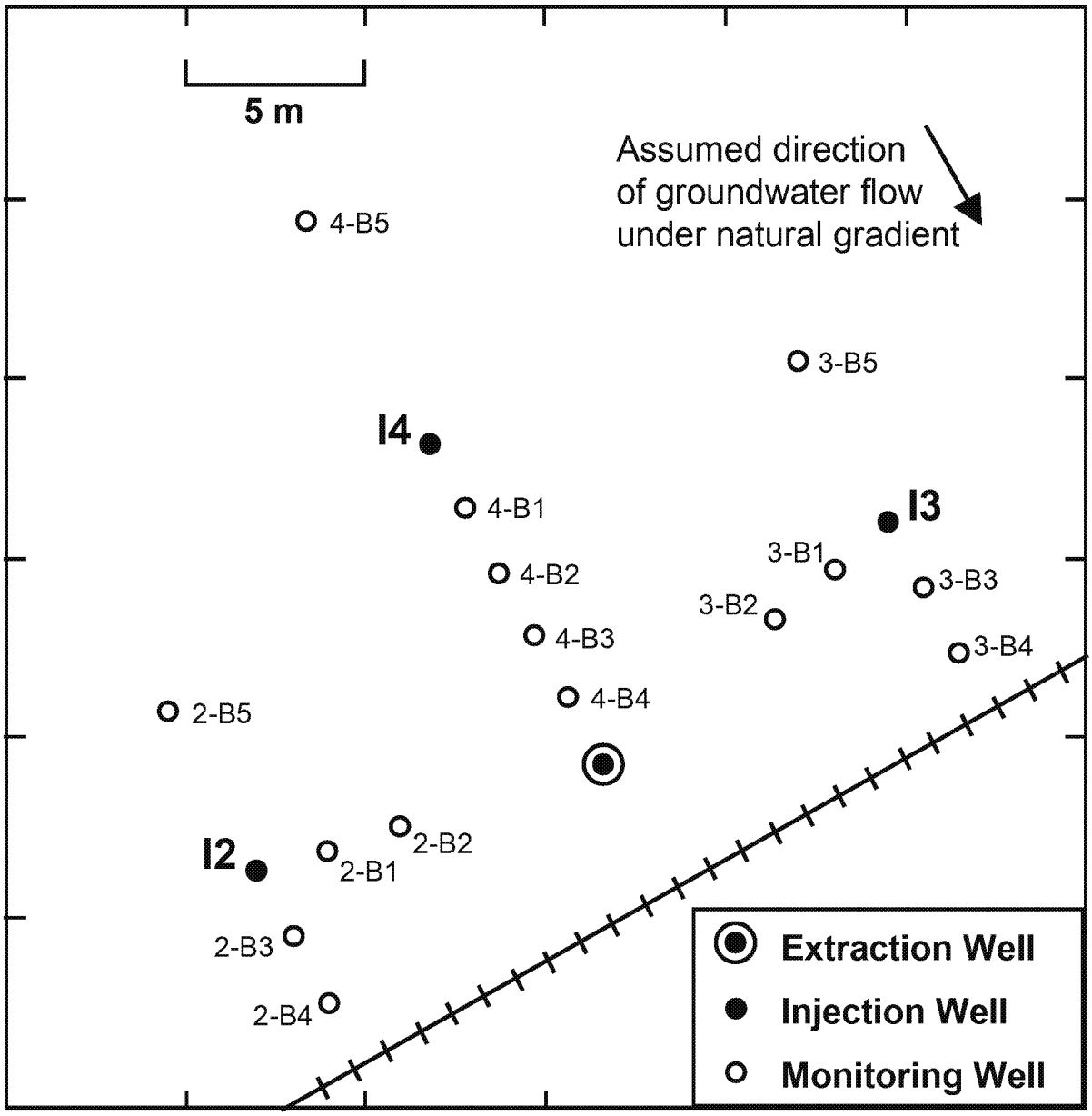

Figure 4. Plan view of the injection wells, extraction wells, and monitoring wells in the demonstration area. Zone 2 is amended with sulfate, Zone 3 is methanogenic (unamended), and Zone 4 is amended with nitrate and sulfate

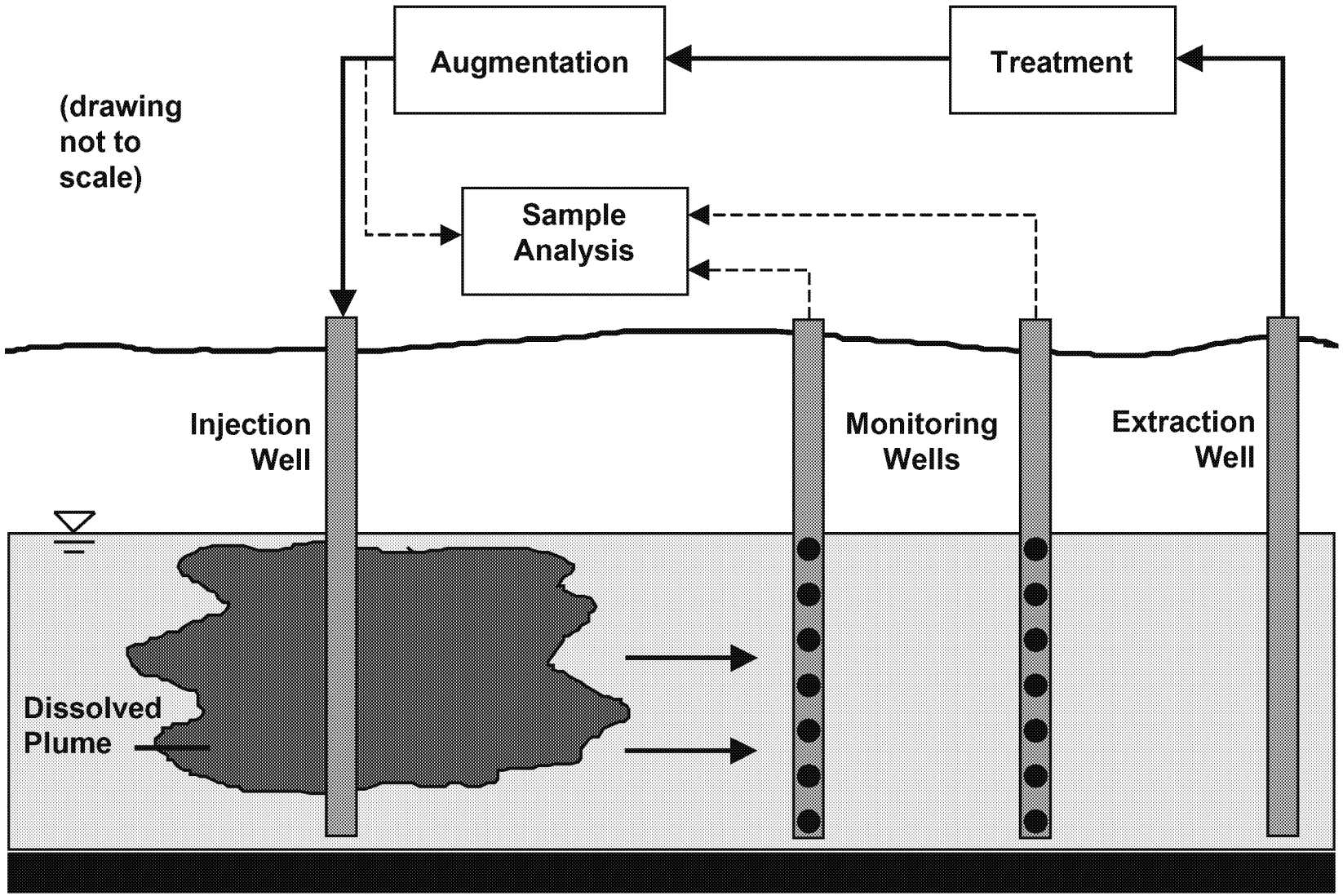

Figure 5. Schematic of injection/extraction well system.

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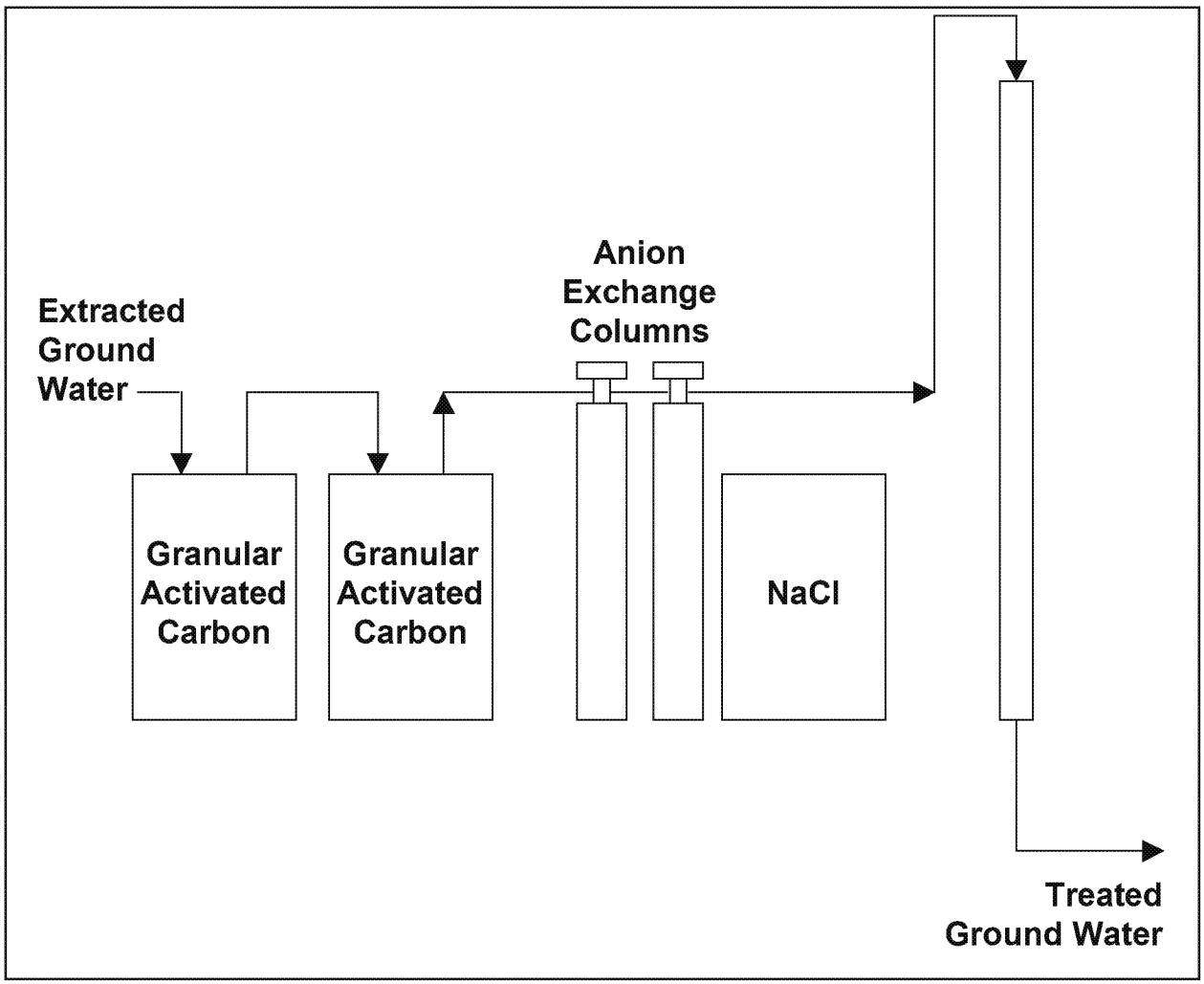

Figure 6. Schematic for the water treatment system that is used to remove hydrocarbons, anions, and gases from extracted water.

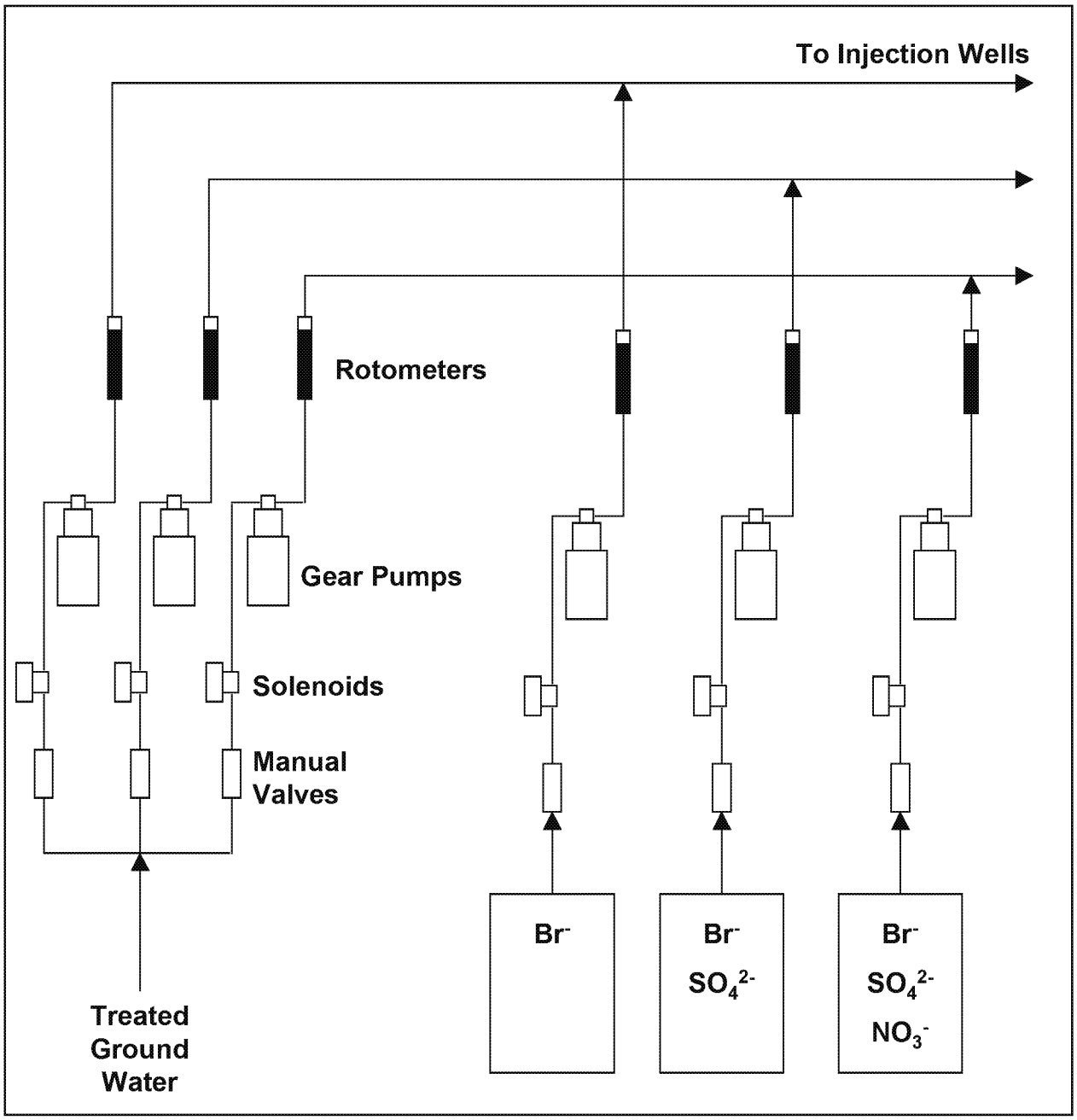

Figure 7. Schematic for the chemical delivery system that is used to augment water with sulfate and/or nitrate prior to re-injection.

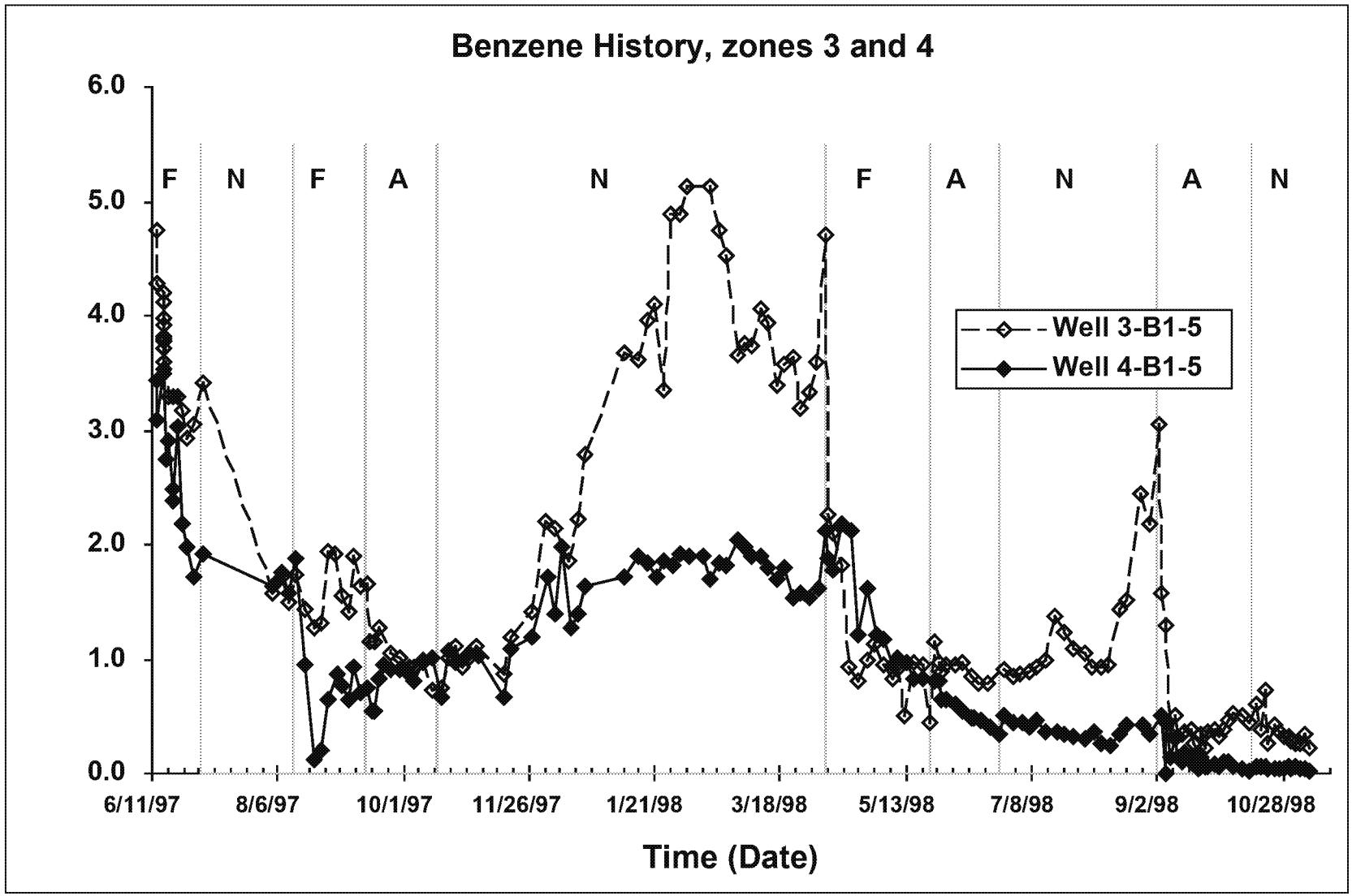

Figure 8. Benzene concentration histories at monitoring Points 3-B1-5 and 4-B1-5.

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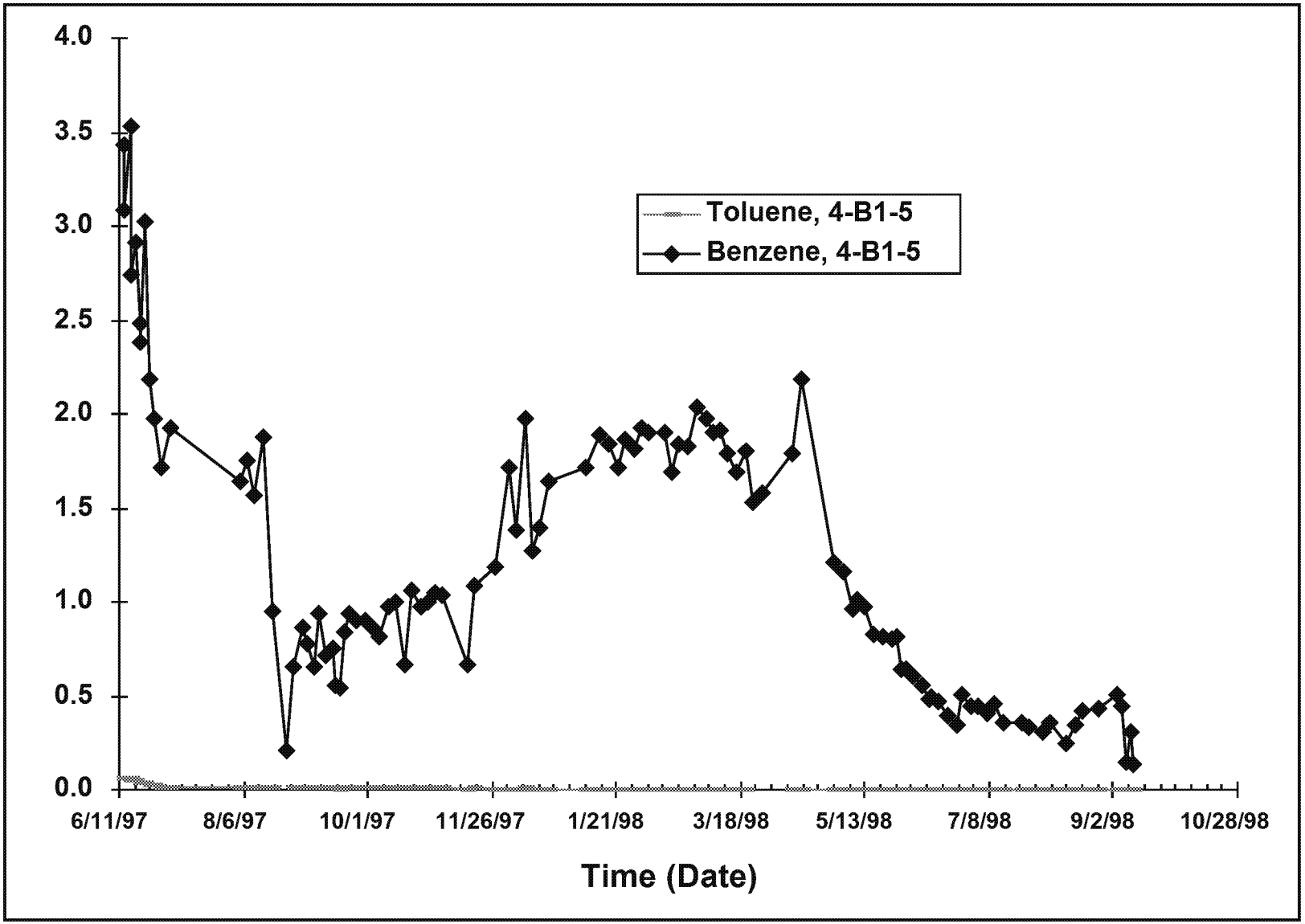

Figure 9.Toluene and benzene concentration histories at Point 4-B1-5.
Toluene concentrations are near zero even at the start of the demonstration.

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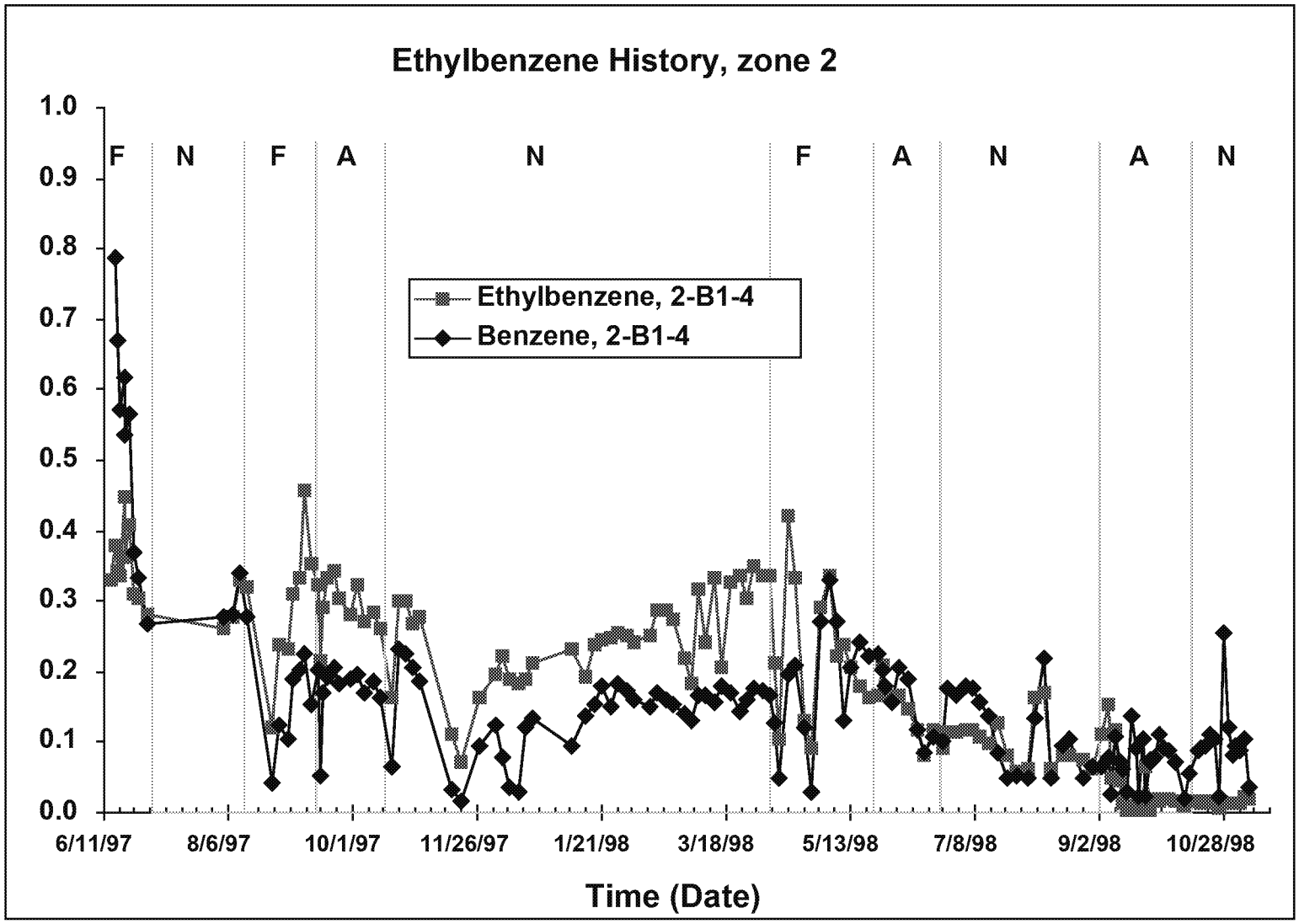

Figure 10. Ethylbenzene and benzene concentration histories at monitoring Point 2-B1-4.

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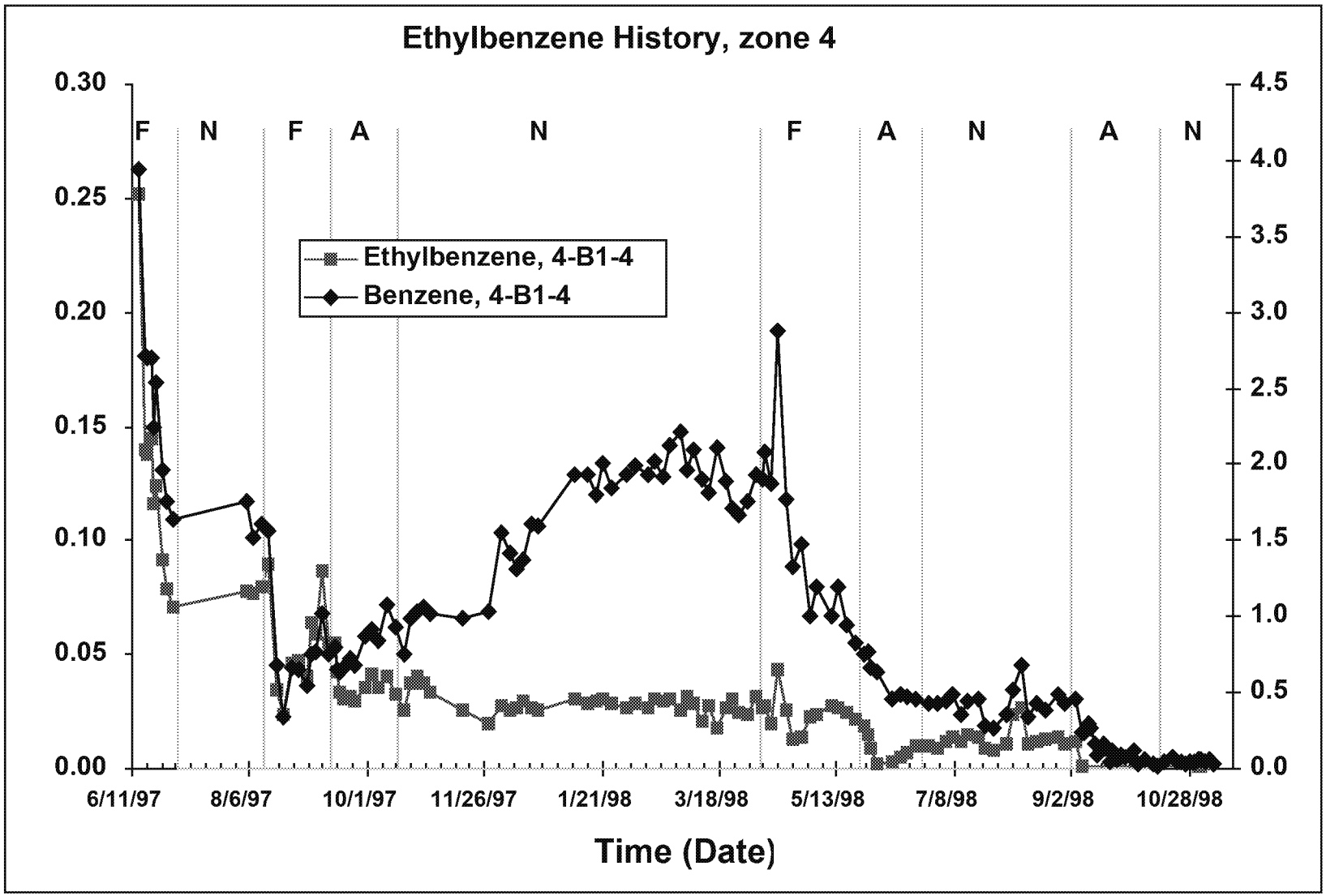

Figure 11. Ethylbenzene and benzene concentration histories at monitoring. Point 4-B1-4.

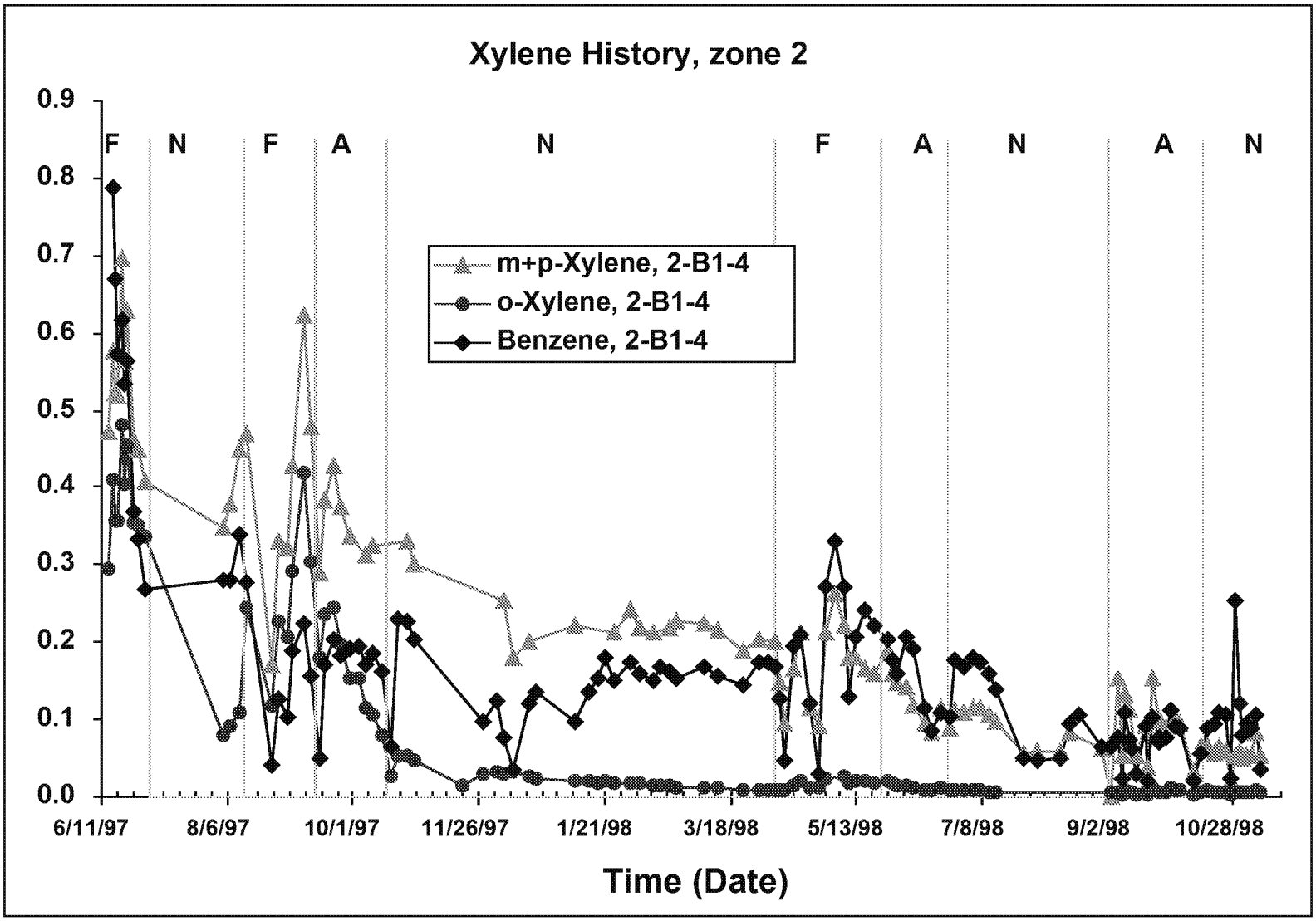

Figure 12. Concentration histories of xylene isomers and benzene at monitoring. Point 2-B1-4.

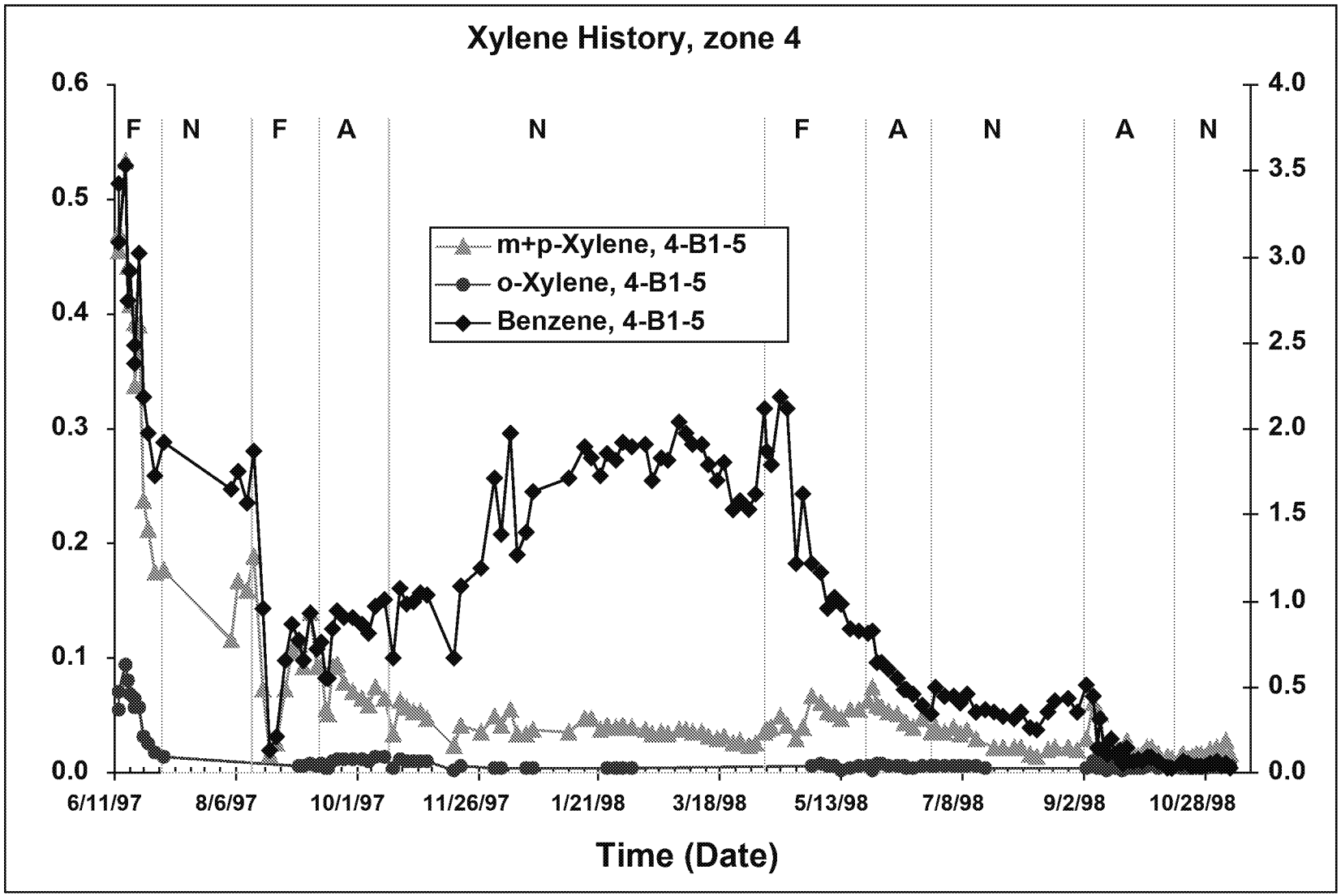

Figure 13. Concentration histories of xylene isomers and benzene at monitoring. Point 4-B1-5.

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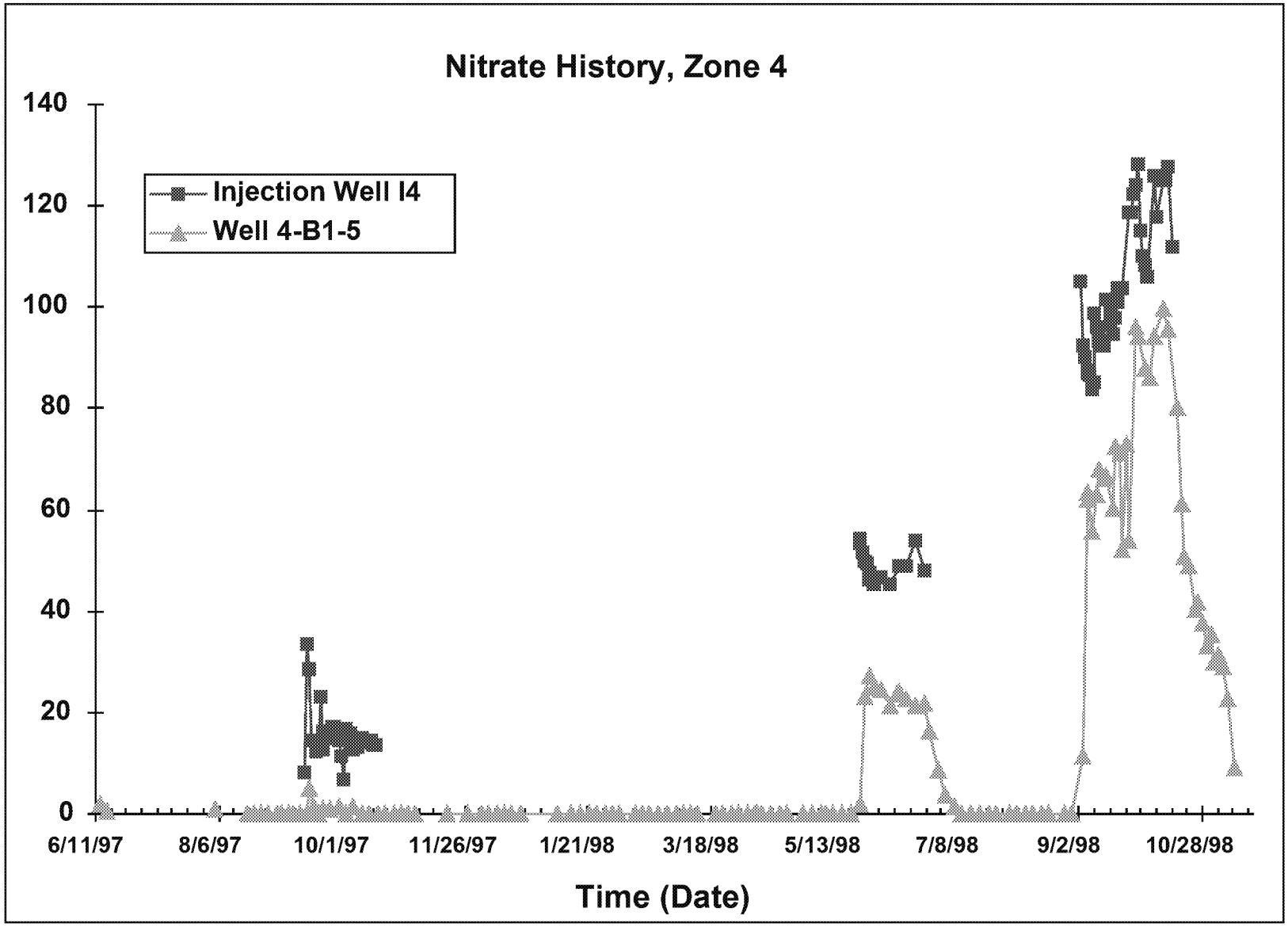

Figure 14. Nitrate concentration histories at injection well I4 and at monitoring Point 4-B1-5.

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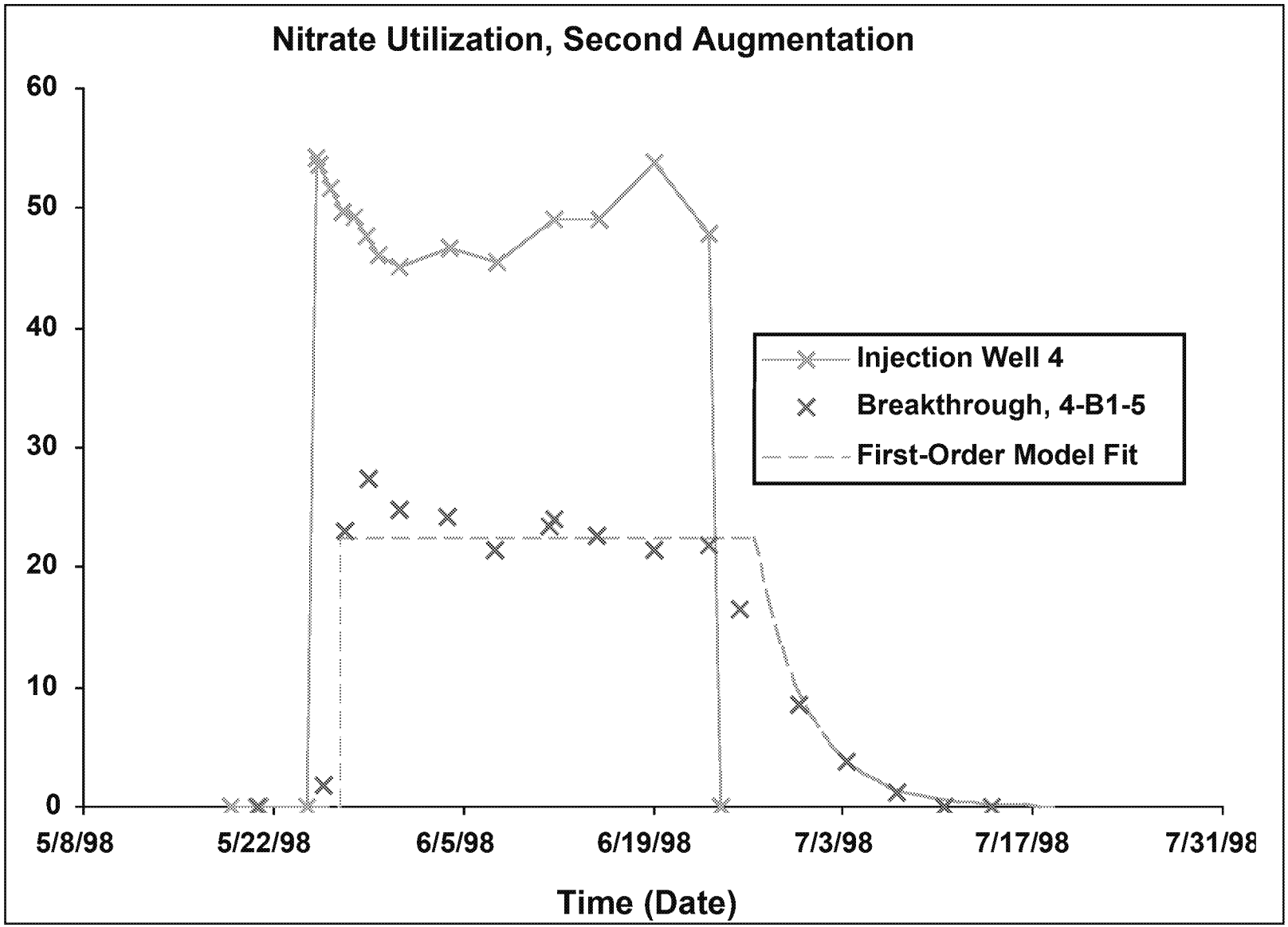

Figure 15. Nitrate utilization in zone 4 after second augmentation.

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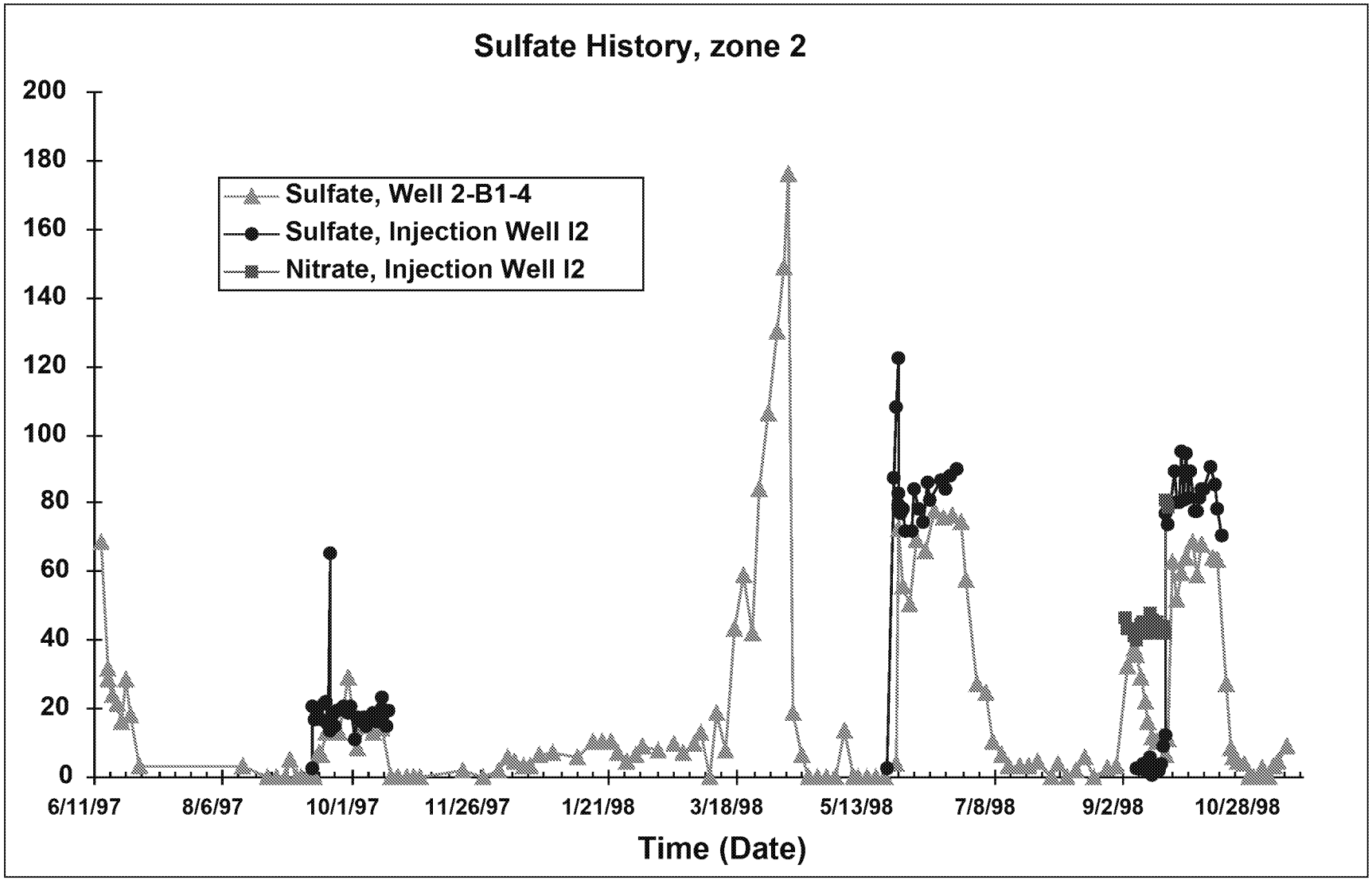

Figure 16. Sulfate and nitrate concentration histories at injection well I2, and sulfate concentration history at monitoring Point 2-B1-4.

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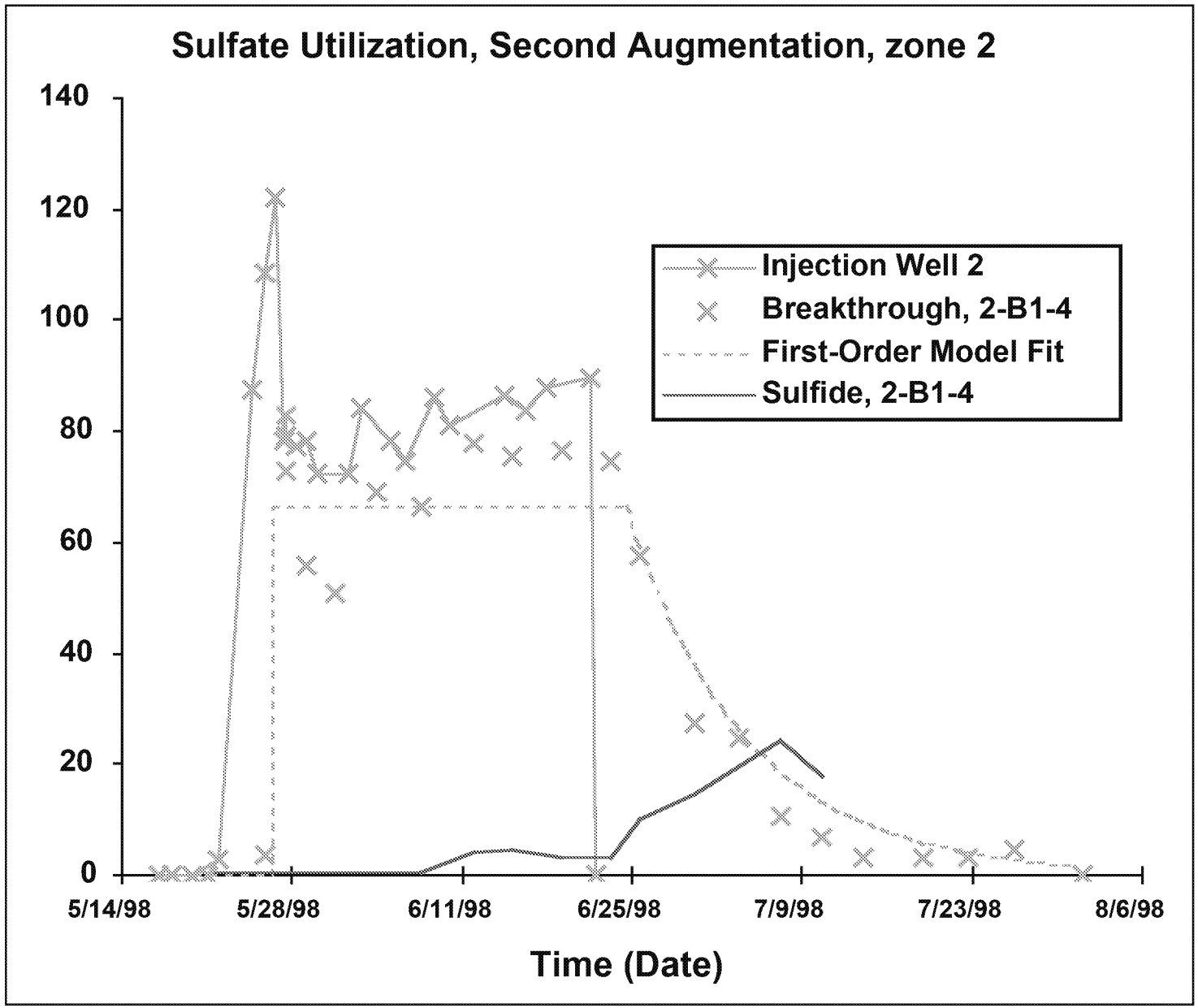

Figure 17. Sulfate utilization and sulfide generation in zone 2 after second augmentation.

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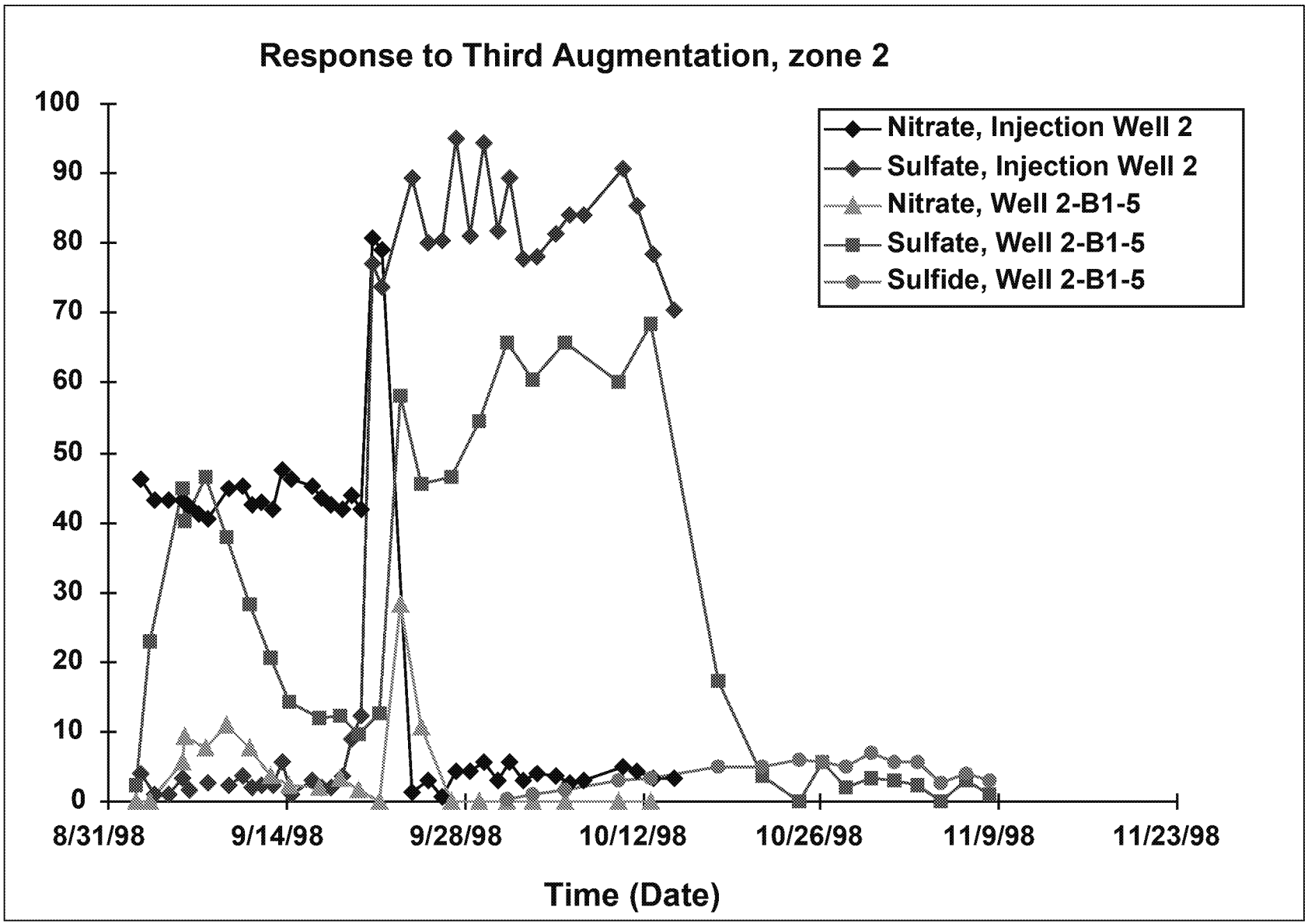

Figure 18. Nitrate and sulfate utilization and sulfide generation in zone 2 after third augmentation.

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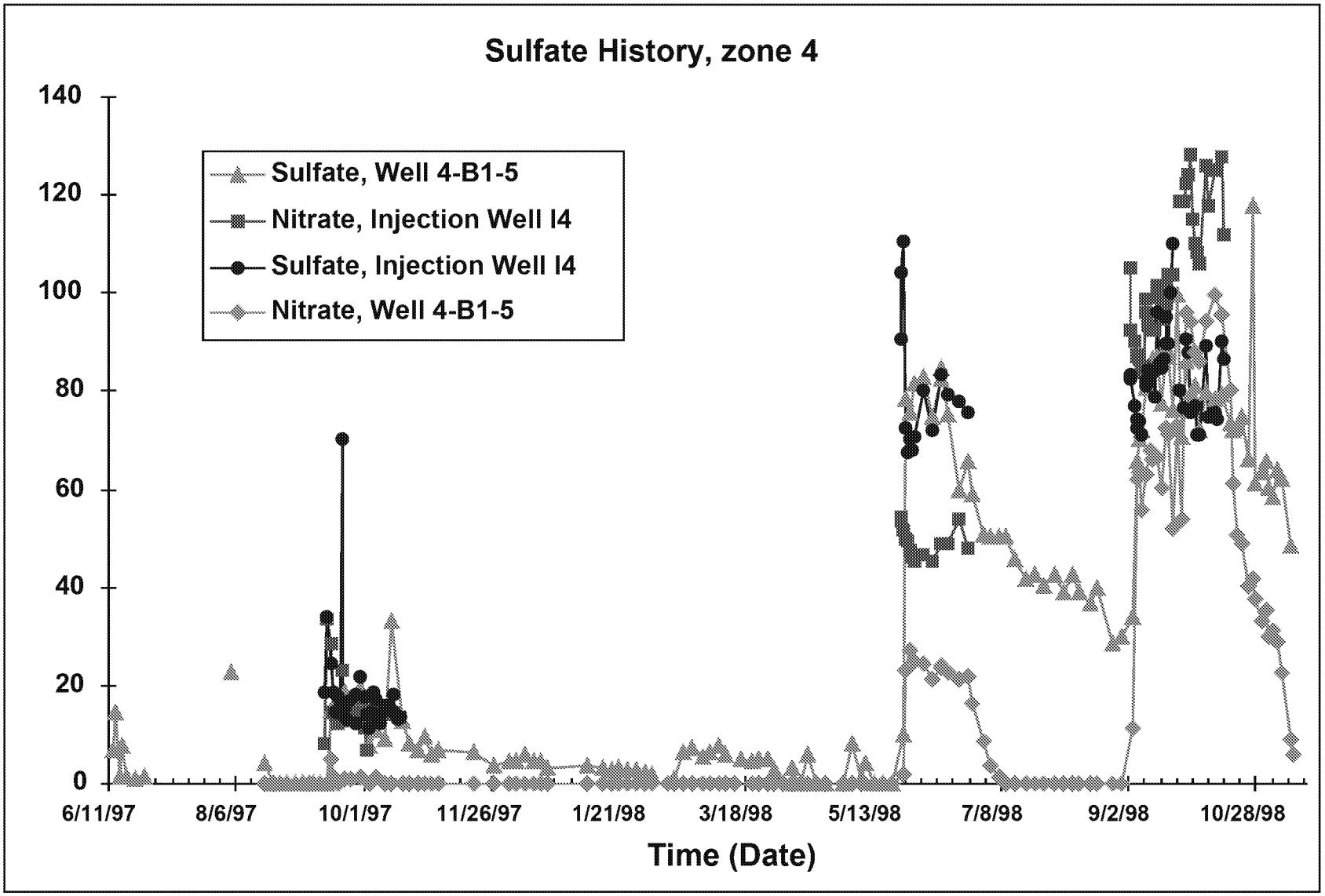

Figure 19. Sulfate and nitrate concentration histories at injection Well I4 and at monitoring Point 4-B1-5.

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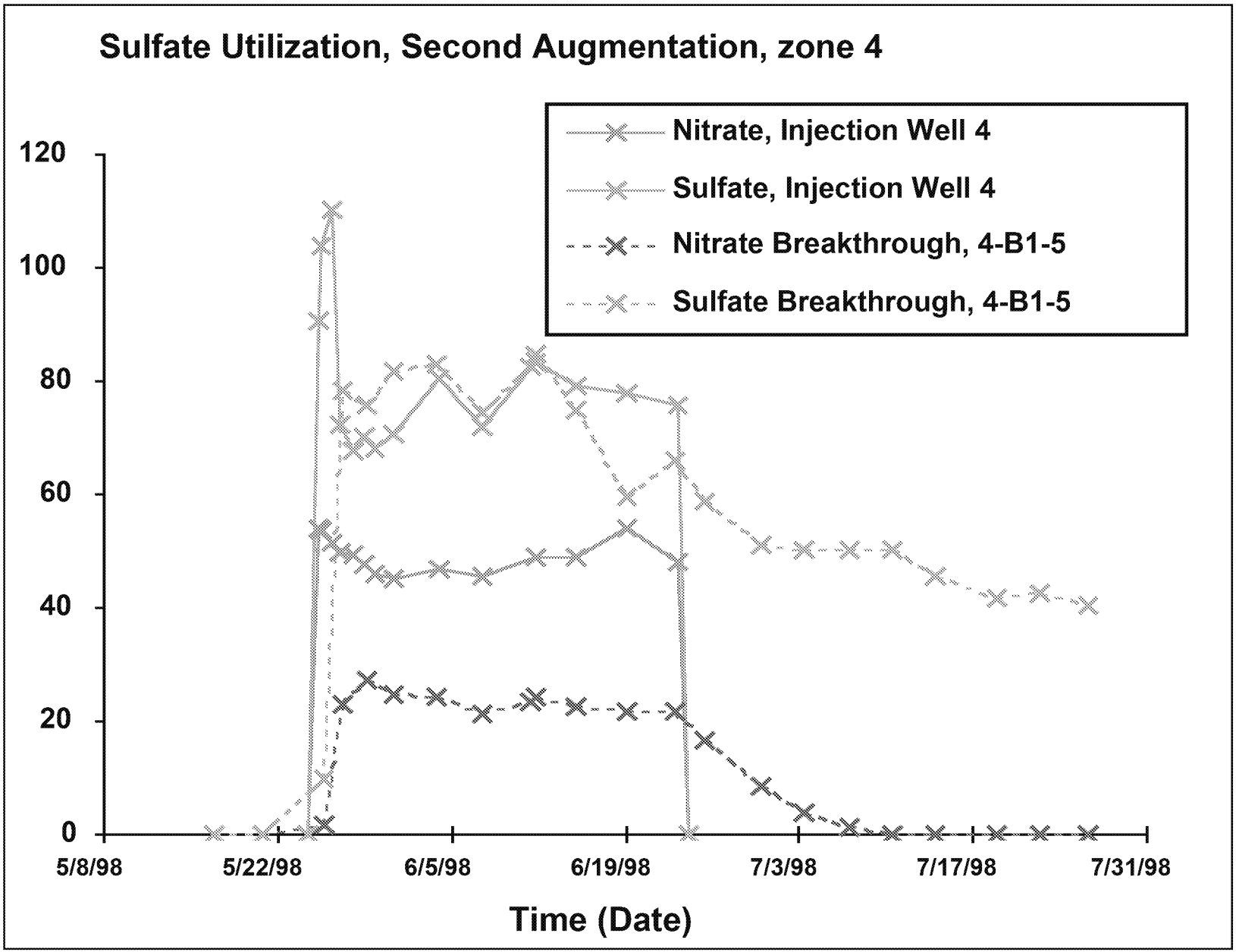

Figure 20. Simultaneous sulfate and nitrate utilization in zone 4 after second augmentation.

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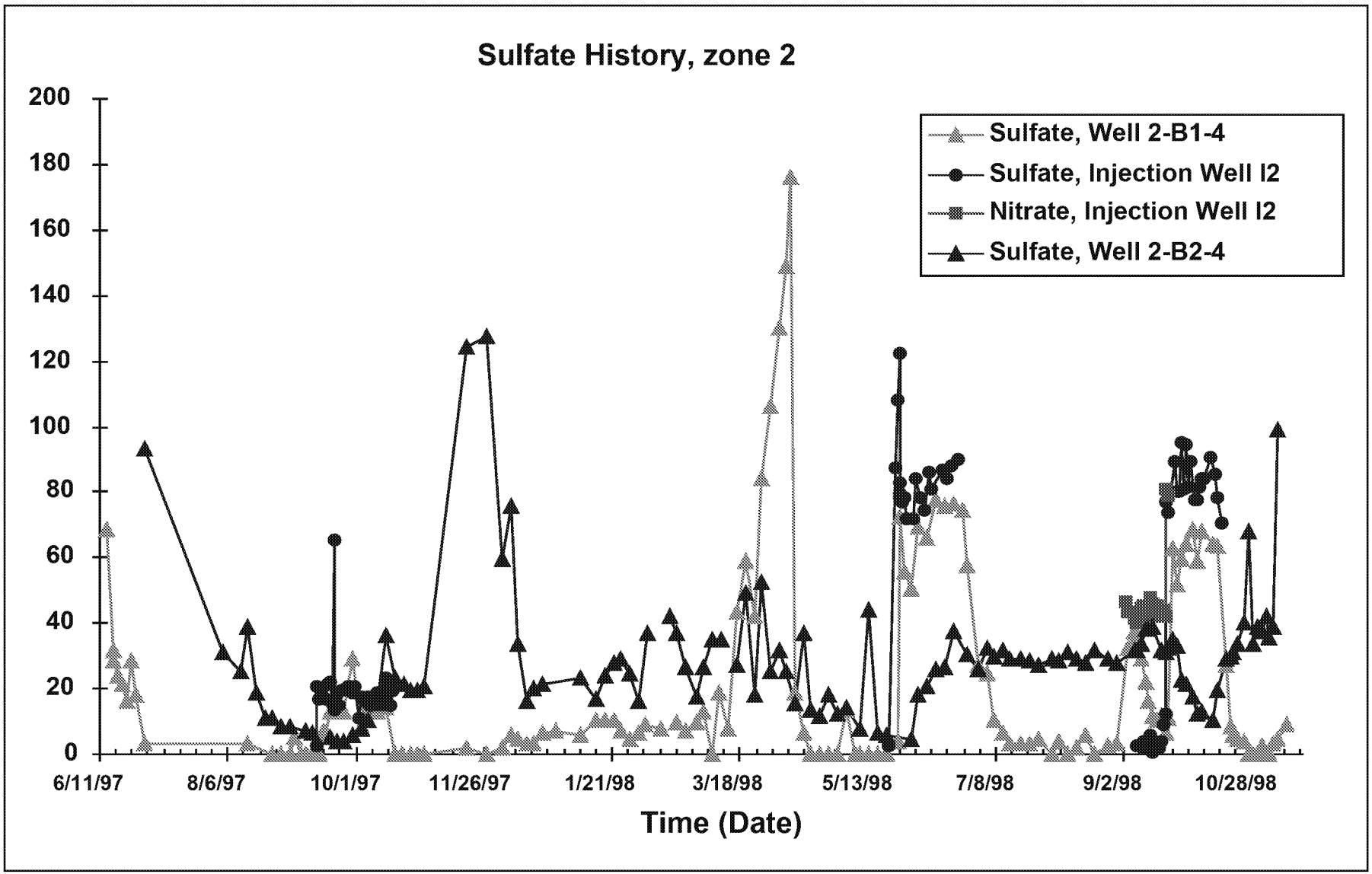

Figure 21. Sulfate and nitrate concentration histories at injection Well I2 and sulfate concentration history at Wells 2-B1-4 and 2-B2-4.

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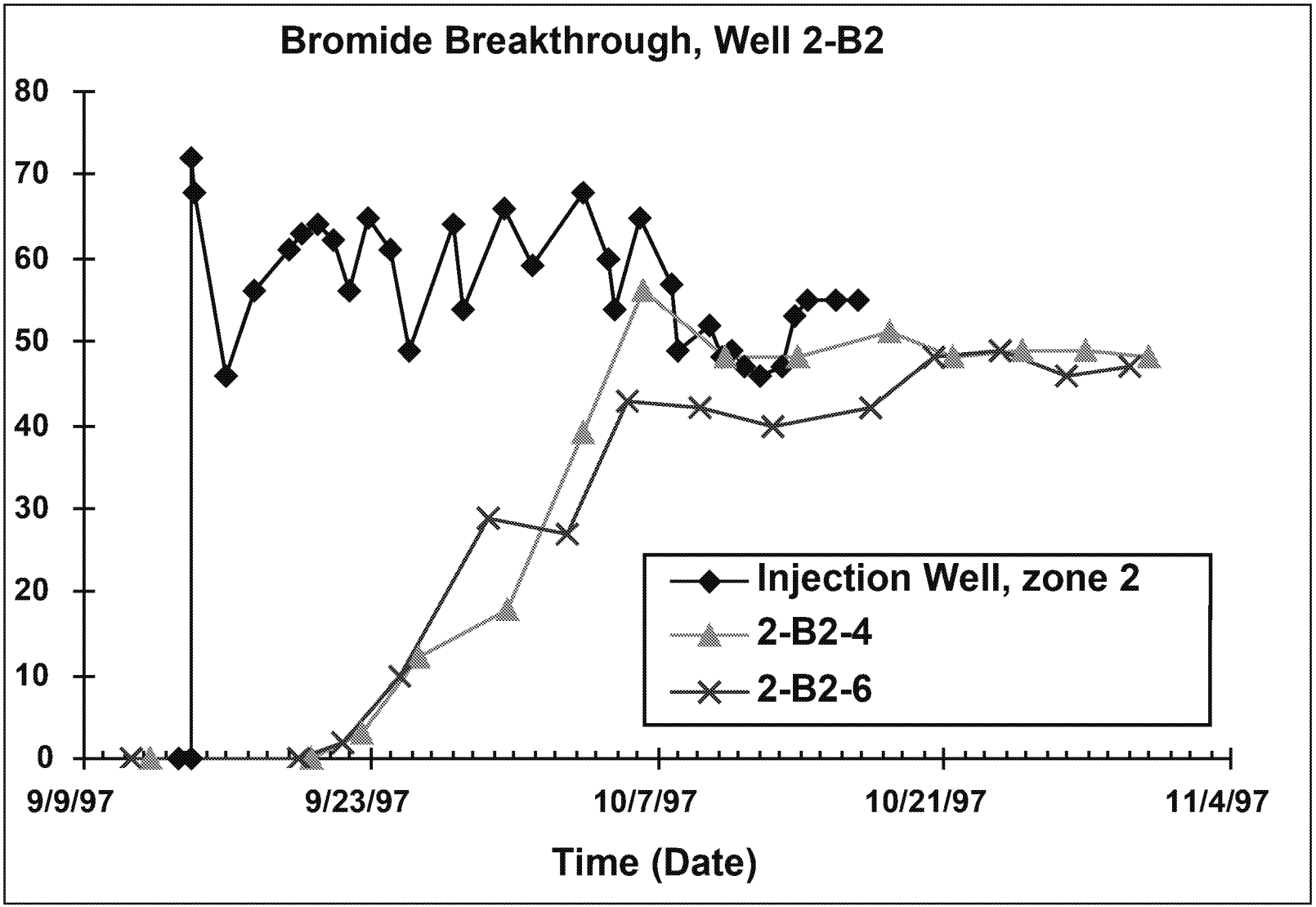

Figure 22: Bromide breakthrough curves at monitoring Wells 2-B2-4 and 2-B2-6.

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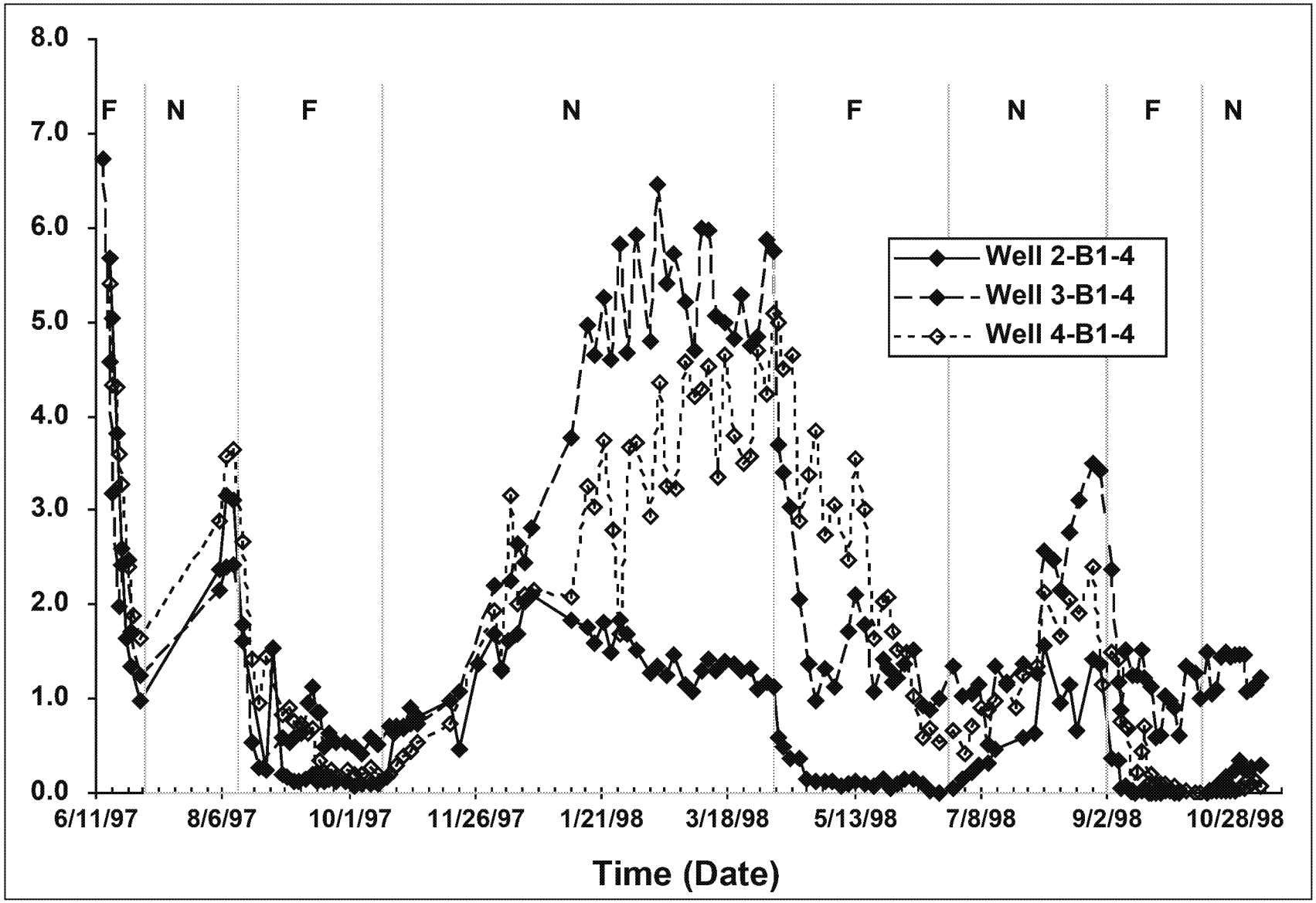

Figure 23. Methanogenesis in the three different treatment zones.

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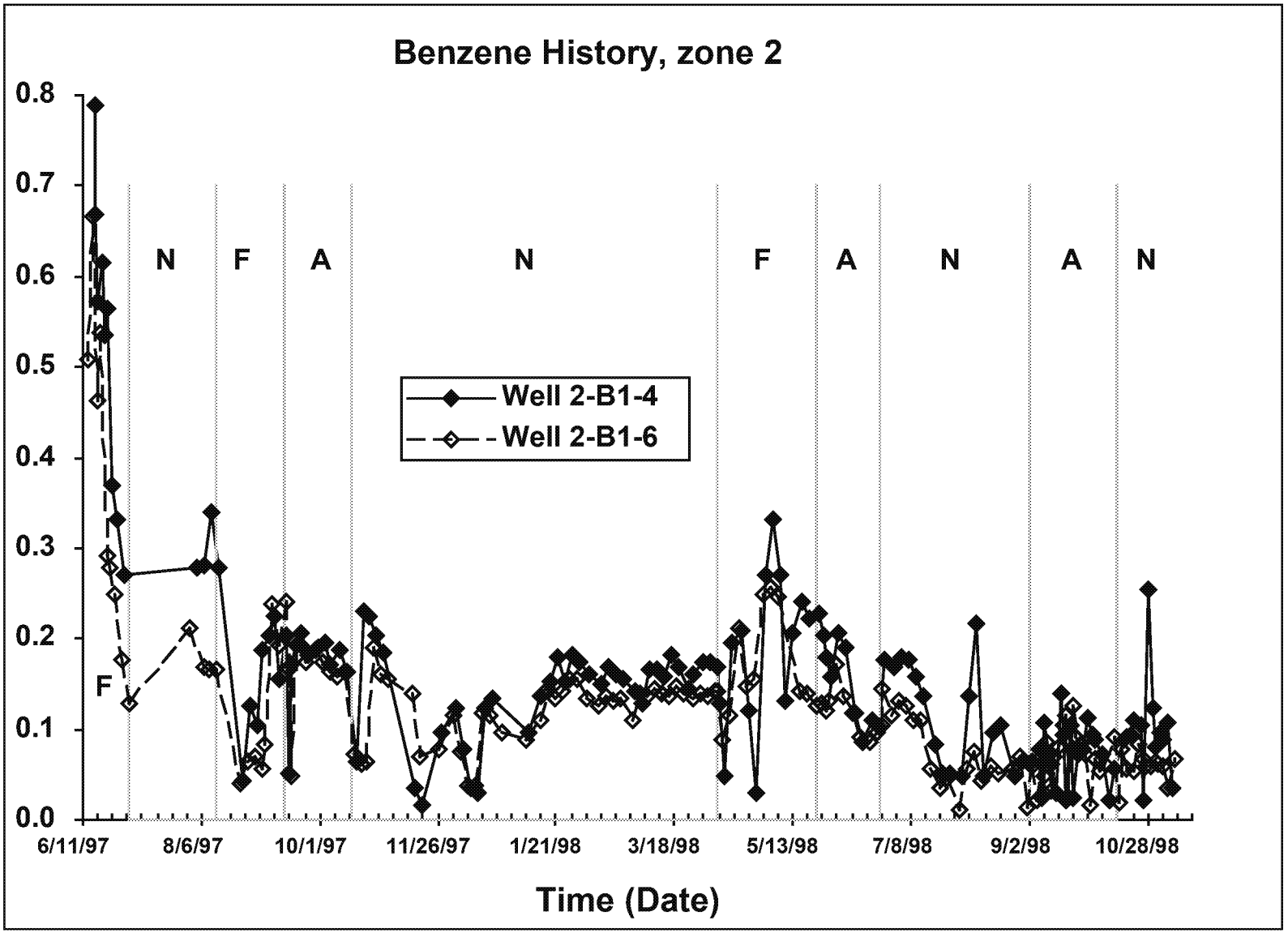

Figure 24. Comparison of measured benzene concentrations at two different veritcal locations - Wells 2-B1-4 and 2-B1-6.

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