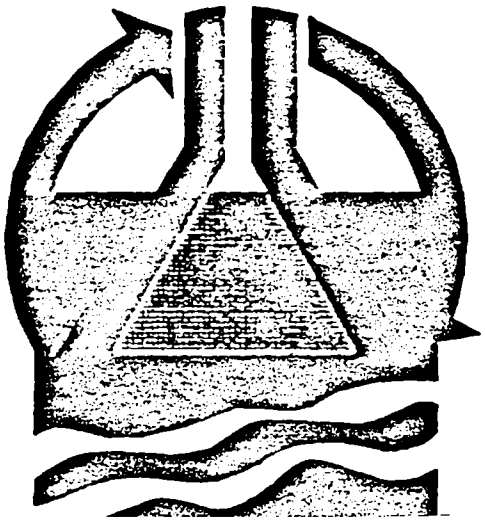
EPA/600/A-92/080

The Gulf Coast Hazardous Substance Research Center

Lamar University
Beaumont, Texas

GROUND WATER: The Problem and Some Solutions



PROCEEDINGS

4th Annual Symposium

April 2-3, 1992

COLUMN STUDIES ON BTEX BIODEGRADATION UNDER MICROAEROPHILIC AND DENITRIFYING CONDITIONS

S.R. HUTCHINS
United States Environmental Protection Agency
Robert S. Kerr Environmental Research Lab, Ada, OK

S.W. MOOLENAAR
D.E. RHODES
Rice University
Houston, TX

ABSTRACT

Two column tests were conducted using aquifer material to simulate the nitrate field demonstration project carried out earlier at Traverse City, Michigan. The objectives were to better define the effect nitrate addition had on biodegradation of benzene, toluene, ethyl benzene, xylenes, and trimethylbenzenes (BTEX) in the field study, and to determine whether BTEX removal can be enhanced by supplying a limited amount of oxygen as a supplemental electron acceptor. Columns were operated using limited oxygen, limited oxygen plus nitrate, and nitrate alone.

In the first column study, benzene was generally recalcitrant compared to the alkylbenzenes (TEX), although some removal did occur. The average benzene break through were 74.3 ± 5.8%, 75.9 ± 12.1%, and 63.1 ± 9.6% in the columns with limited oxygen, limited oxygen plus nitrate, and nitrate alone, respectively, whereas the corresponding average effluent TEX breakthroughs were 22.9 ± 2.3%, 2.9 ± 1.1%, and 4.3 ± 3.3%. In the second column study, nitrate was deleted from the feed to the column originally receiving nitrate alone and added to the feed of the column originally receiving limited oxygen alone. Benzene breakthrough was similar for each column. Breakthrough of TEX decreased by an order of magnitude once nitrate was added to the microaerophilic column, whereas TEX breakthrough increased by 50-fold once nitrate was removed from the denitrifying column. Although the requirement for nitrate for optimum TEX removal was clearly demonstrated in these columns, there were significant contributions by biotic and abiotic processes other than denitrification which could not be quantified.

INTRODUCTION

Leaking underground storage tanks are a major source of ground water contamination by petroleum hydrocarbons. There are approximately two million underground tanks storing gasoline in the U.S., and there have been 90,000 confirmed releases reported in the last two years (OUST, 1990). Gasoline and other fuels contain benzene, toluene, ethylbenzene, and xylenes (collectively known as BTEX) which are hazardous compounds regulated by the U.S. Environmental Protection Agency (EPA, 1977). Aerobic biorestoration, in conjunction with free product recovery, has been shown to be effective for many fuel spills (Thomas et al., 1987; Lee et al., 1988). However, success is often limited by the inability to provide sufficient oxygen to the contaminated zones due to the low water solubility of oxygen (Wilsonet al., 1986; Barker et al., 1987).

Nitrate can also serve as an electron acceptor and results in anaerobic biodegradation of organic compounds via the processes of nitrate reduction and denitrification (Tiedje, 1988). Because nitrate is less expensive and more soluble than oxygen, it may be more economical to restore fuel-contaminated aquifers using nitrate rather than oxygen. Several investigators have observed biodegradation of aromatic fuel hydrocarbons under denitrifying conditions (Kuhn et al., 1988; Major et al., 1988; Mihelcic and Luthy, 1988; Hutchins et al., 1991a). However, these processes are not well understood at field scale where several other processes, including aerobic biodegradation, can proceed concomitantly. Although several field studies have demonstrated partial success in BTEX removal under denitrifying conditions (Battermann, 1986; Lemon et al., 1989; Hutchins et al., 1991b), the complexity of the field sites and the limited monitoring data have precluded a thorough evaluation of the process.

Background

The use of nitrate to promote biological removal of fuel aromatic hydrocarbons was investigated for a JP-4 jet fuel spill at Traverse City, Michigan, through a field demonstration project in cooperation with the U.S. Coast Guard. Laboratory tests had indicated that denitrification would be a suitable alternative for biorestoration of the aquifer, although benzene was not degraded (Hutchins et al.,1991a). The field work showed that BTEX was degraded under denitrifying conditions in conjunction with low oxygen (microaerophilic) levels (Hutchins et al., 1991b). However, a suitable control site was not available to test the effects of treatment without nitrate addition. Therefore, the relative contribution of nitrate to BTEX biodegradation in the field study required further clarification. In addition, although benzene was recalcitrant under strictly denitrifying conditions in the laboratory study, degradation occurred at the field site prior to nitrate addition.

The purpose of this research was to compare BTEX biodegradation by aquifer microorganisms using different electron acceptors and to investigate whether any advantages can be expected under a mixed oxygen/nitrate system. This might prove advantageous in that the demand for oxygen can be supplemented rather than replaced by alternate electron acceptors. This concept, first advanced by Britton (1989), was found to hold true for phenol biodegradation by a mixed culture obtained from activated sludge, a contaminated landfill, and ground water. Theoretically, enough oxygen could be provided to allow the initial oxidation of compounds such as benzene by mono- or dioxygenases, which could then yield oxidized intermediates more susceptible to anaerobic biodegradation using nitrate.

Materials and Methods

Five columns, 1.5 m x 10 cm ID, were constructed of beaded process Pyrex glass with Teflon-lined seals and packed under aerobic conditions at room temperature. Columns were packed with fresh aquifer material representing both the contaminated and the uncontaminated zones from the JP-4 site at Traverse City, outside of the zone of influence from the pilot demonstration project on nitrate bioremediation. Both the site and field project have been described elsewhere (Hutchins et al., 1991b). Uncontaminated material was obtained from depths of 4.9 to 9.1 m below land surface in an area not impacted by the fuel spill. The water table was at 4.6 m below land surface. The contaminated material was obtained at several locations outside of the demonstration project area at depths from 4.0 to 4.6 m below land surface. The average JP-4 content of the contaminated aquifer material was 3750 ± 1600 mg/kg (mean ± standard error), based on the analytical technique of Vandegrift and Kampbell (1988).

The columns were designed to be operated in an up-flow condition. Packing material consisted of glass wool followed by 2.5 cm of porcelain berl saddles at the bottom of the columns. A series of three screens (#40 mesh, #80 mesh, #40 mesh) were placed on top of the column packing followed

by 2.5 cm of clean aquifer material. This was followed by 7.6 cm of contaminated aquifer material to simulate the contaminated region in the field demonstration project (Figure 1). The aquifer material was wet-packed by systematically distributing and mixing 2.5-cm depth aliquots (approximately 200 g) with the lower layers using a 5-cm steel blade attached to a rod. The remainder of each column received 1.4 m uncontaminated aquifer material. The column packing, in combination with a flow rate of 0.5mL/min, was designed to represent the entire treatment zone of the field site on a residence-time basis. That is, the contaminated interval should have had a residence time of 8 hr and the entire column should have had a residence time of approximately 1 wk.

The basic feed solution for the columns consisted of a mixture of ground water obtained from a local artesian well (Byrd's Millspring) mixed 1:1 with deionized water to yield a groundwater whose chemistry approximated that found in Traverse City. The feed solution was delivered to each column using a peristaltic pump with Tygon tubing. Because this could allow gas transfer and sorb organics, degassing and BTEX addition was conducted down-gradient. Degassing was accomplished by passing a gas stream into a chamber containing a gas-permeable feed solution flow line (Figure 1). The chamber was constructed of a plexiglass column (30 cm x 5 cm ID) with rubber stoppers and contained either 1 or 2 solution lines, each 7.6 m in length, of 2.4 mm OD x 0.8 mm ID silicone tubing. All tubing was stainless steel beyond this point to prevent gas transfer and sorption of organics. Sample tees containing stainless steel Luer-Lok valves were placed in-line at several points for BTEX addition and sample collection. The solution BTEX spike was continuously added using a syringe pump to deliver a controlled rate of flow. The column effluent end-piece was also modified to allow removal of accumulated gases during operation (Figure 1).

Columns were operated as illustrated in Figure 2. Each column was designed to represent a unique treatment scheme, or appropriate control, without replicates. The column designations and initial operating parameters were as follows: a) Column A (microaerophilic), receiving BTEX and low oxygen levels without nitrate addition; b) Column B (microaerophilic/denitrifying), receiving BTEX and low oxygen levels with nitrate addition; c) Column C (denitrifying), receiving BTEX and nitrate alone, with the solution flow diverted through a separate degasser to eliminate oxygen; d) Column D (control), receiving BTEX and nitrate in an analogous manner as the previous column, but with biocide added to the feed reservoir to inhibit microbial activity; and e) Column E (BTEX control), similar to the previous control column except that no BTEX was added. This last column was designed to assess the degree of BTEX removal which occurred through leaching only.

Operation of the columns began initially without nutrient, nitrate, or biocide addition. Feed solution of 50% Byrd's Mill Spring water (50% BOW) was prepared without filtering or autoclaving, and amended with sodium bromide to provide a tracer concentration of 50 mg/L bromide. The feed solution flow rate was 0.50 mL/min. For Columns A and B, the degassers were purged with a mixed gas stream containing 21 mL/min helium and 3.4 mL/min air. The remaining column degassers were purged with helium only at 43 mL/min. Solution BTEX spikes were prepared aseptically in an anaerobic glovebox by injecting the compounds directly through Teflon Mininert valves into 160-mL serum bottles containing sterile distilled water, without headspace, and stir bars. The spikes were mixed overnight, combined, and dispensed into each of 4 100-mL glass syringes. The syringes were then removed from the glovebox and loaded onto the syringe pumps, and the flowrate was set at 0.005 mL/min. Following breakthrough of the bromide tracer, bromide addition to the feed reservoirs ceased and nutrients, nitrate, and biocides were added to the appropriate feed reservoirs as shown in Figure 2. Stock solutions were prepared and autoclaved prior to use, and the final feed concentrations were 5mg/L ammonia-nitrogen as NH_4Cl and 2 mg/L phosphate-phosphorus as KH_2PO_4 for the nutrients, 10 mg/L nitrate-nitrogen as KNO_3 for the nitrate, and 100 mg/L $HgCl_2$ for the biocide. Feed solutions were replaced once weekly and flow rates and effluent volumes were recorded each week.

The column influents and effluents were sampled 1 to 2 times per week, except during the tracer study when sampling was more frequent. For the tracer study, 2-mL samples were obtained and analyzed for bromide using ion chromatography with a 590 pump (Waters Associates) and conductivity detector (Dionex). The mobile phase consisted of 0.75 mM $NaHCO_3$ and 2.2 mM Na_2CO_3 at a flow rate of 1.6 mL/min through an HPIC AS4A column (Dionex). The quantitation limit was 0.1 mg/L Br. Samples for BTEX, nitrate, nitrite, ammonia, phosphate, sulfate, pH, and alkalinity were obtained without head space using glass 50-mL syringes. The volatile aromatic hydrocarbons were analyzed by purge-and-trap gas chromatography using a Tekmar LSC-2000 liquid sample concentrator and an HP5890 GC with a flame ionization detector. Hydrocarbons were purged onto a Tenax trap for 6 min at 34°C followed by a 2-min dry purge and desorbed for 4 min at 180°C. For the first column test, samples were chromatographed using a 30 m x 0.32 mm megabore DB-5 capillary column with a 1.0 μm film thickness. The injector temperature was 120°C, and the oven temperature was programmed from 32°C (4-min hold) to 110°C (1-min hold) at 8°C/min with a flow rate of 5 mL/min. This method did not result in separation of all three xyleneisomers, and the column was replaced with a 30 m x 0.53 mm ID megabore DB-wax capillary column with a 1.0 μm film thickness for the second column test. The new temperature program was from 50°C (4-min hold) to 120°C at 8°C/min, and then to 180°C (4-min hold) at 30°C/min. The quantitation limit for these compounds was 0.2 μg/L. The remaining sample was analyzed for the other parameters using standard EPA methods (Kopp and McKee, 1979).

Samples for dissolved gases were obtained using plastic 10-mL or 60-mL syringes which had been stored for one week in the anaerobic glovebox. For the dissolved gases, including oxygen initially, 9 mL were injected under water into evacuated 12-mL headspace vials which had been sealed with butyl rubber stoppers and pressurized and evacuated three times with helium. The vials were then shaken at room temperature for 20 min to equilibrate, and headspace samples were analyzed on an HP 5890 GC with a thermal conductivity detector. The injector and detector temperatures were both set at 120°C, and the samples were chromatographed on a CTR I 2-m concentric column set with 3.2-mm OD inner column packed with a Poropak mix and a 6.4-mm OD outer column packed with activated Molecular Sieve (Alltech Associates) with helium carrier gas at 29 mL/min. The quantitation limits were 0.005% (vol/vol), 0.02%, 0.02%, 0.07%, and 0.5% for carbon dioxide, nitrous oxide, methane, oxygen, and nitrogen, respectively. In addition, headspace samples were analyzed for tracenitrous oxide using a Varian 6000 GC with electron capture detector. The injector and detector temperatures were 120°C and 300°C, respectively. Samples were chromatographed at 35°C on a 2 m x 3.2 mm OD stainless steel column containing 100/120 mesh Poropak Q using a mixed carrier gas stream of 95% argon/5% methane at 30 mL/min. The quantitation limit for nitrous oxide was 0.23 ppm (vol/vol) using this method. Aqueous dissolved gas concentrations were calculated for the original solutions using Henry's constants and correcting for total mass in the gas and liquid phases. For the second column test, the analytical procedure for dissolved oxygen was changed to a modified Winkler titration due to problems of inconsistent air contamination of the syringe needle prior to injection of the headspace gas sample into the GC. The standard Winkler titration method (Kopp and McKee, 1979) was modified for 55-mL volumes and there agents were prepared in the anaerobic glovebox. Samples were obtained using 60-mL plastic syringes and reagents were withdrawn directly into the samples and mixed in the glovebox. The fixed samples were then titrated outside the glovebox using 0.0075 $NNa_2S_2O_3$ with starch indicator.

RESULTS AND DISCUSSION

Because of the large number of parameters which were continually monitored, a complete evaluation of all of the column data is beyond the scope of this discussion. Rather, this report focuses on 1) the controlling parameters (electron acceptors), 2) benzene, and 3) the alkylbenzenes, considered as a

single group. Data on individual compounds, nutrients, pH and dissolved gases are published elsewhere (Hutchins et al., 1992).

Column Test I

The first column test was run for 100 days. Initial operation of the five columns commenced using no nutrients or biocides to simulate the initial flooding period required to establish the water table mound in the Traverse City field project. The columns were operated in this manner for approximately 40 days to deplete internal oxygen reserves. Figure 3 shows the bromide tracer data for the first 30 days of operation, and indicates that the average column residence time is 6 days, with some variability among the columns. On Day 38, nutrient and biocide addition were initiated for the appropriate columns. During the following weeks it became evident that the mercuric chloride biocide was not being properly distributed throughout the control Columns D and E, thereby allowing microbial growth and subsequent BTEX biodegradation. Attempts to mobilize the biocide were not successful, and hence these columns cannot be considered as appropriate controls. The following discussion therefore focuses on Columns A, B, and C.

To avoid a layering effect, the columns had been packed in an unsaturated mode and then flooded, leading to the formation of numerous gas pockets. It was thought that, because of these pockets, it would be difficult to induce anaerobic conditions in the columns. However, as shown in Figure 4, effluent dissolved oxygen profiles dropped to 1 mg/L oxygen in about 20 days. This is identical to what was observed in the field study (Hutchins et al., 1991b). However, unlike the field study, dissolved oxygen continued to drop to 0.2 to 0.4 mg/L in the column effluents, even though influent oxygen concentrations were maintained at 0.8 to 1.0 mg/L in Columns A and B (Figure 4). Hence, there was a significant oxygen demand (approximately 0.5 mg/L) in Columns A and B during the study. After Day 63, influent oxygen levels in these two columns appeared to drop, but this was found to be an artifact caused by the column design. The proper levels of oxygen were being supplied by the degassers, but growth of microorganisms in the inlet lines subsequent to BTEX addition resulted in oxygen consumption prior to samples being obtained through the influent monitoring ports. This problem was corrected for Column Test II.

Nitrate and nutrient addition began on Day 38. Nitrate removal was observed in Columns B and C, with losses ranging from 2 to 7 mg/L nitrate-nitrogen once nitrate began to break through in the column effluents (Figure 5). Effluent nitrate concentrations began to stabilize at Day 60 and were not significantly different, despite the fact that Column B was also receiving approximately 1.0 mg/L dissolved oxygen as an additional electron acceptor. From Day 45 to Day 98, the average nitrate-nitrogen loss was 4.1 ± 0.4 mg/L and 3.6 ± 0.3 mg/L in Columns B and C, respectively. As was also observed in the field study, there was a transient production of nitrite in Columns B and C effluents, with concentrations dropping and stabilizing at 0.6 to 0.8 mg/L nitrite-nitrogen by the end of the test (Figure 6). Only very low concentrations of nitrous oxide were produced, and appearance of this intermediate was transient as well (data not shown). The columns did not appear to be nutrient-limited. Complete breakthrough of ammonia-nitrogen occurred on Day 56 for Columns B and C and on Day 63 for Column A, although some phosphate limitation may have occurred since phosphate did not begin to breakthrough in the column effluents until Day 91 of the test (data not shown).

For several reasons, it was difficult to maintain consistent influent BTEX concentrations during the column tests. These problems were never fully corrected, but the effects were mitigated to the point that conclusions could be made regarding BTEX removal in the separate columns. The majority of this discussion focuses on benzene, the compound of primary interest. The other alkylbenzenes, generally labile under denitrifying conditions, are discussed as a single group consisting of the summation of toluene, ethylbenzene, m, p, - and o-xylene, and 1, 2, 4-trimethylbenzene concentrations (TEX).

Compared to the alkylbenzenes, benzene was generally recalcitrant during treatment in the separate columns, although some removal did occur (Figure 7). Lacking a proper control column, it was not possible to determine whether this removal was biological in nature. Despite the variability in influent benzene concentrations, however, it was possible to compare the extent of benzene breakthrough among the three columns by considering the average percent breakthroughs from Day 45 to Day 98; this represents the time period that nitrate and nutrients were available to the columns. In addition, data from Column E, the control column which did not receive BTEX spike, showed that the total leached BTEX concentration from the contaminated zone of this column was generally less than 10 mg/L after Day 42 (data not shown). Hence, BTEX breakthrough from Day 45 to Day 98 would not represent contributions from leaching of background BTEX from the previously contaminated interval. Based upon this analysis, the average benzene breakthroughs (effluent concentration/influent concentration, expressed as percent) were 74.3 ± 5.8%, 75.9 ± 12.1%, and 63.1 ± 9.6% in Columns A, B, and C, respectively. This indicates that there was little benefit in using nitrate with limited oxygen on benzene removal, compared to either limited oxygen or nitrate alone. Although this does not agree with results from a previous batch microcosm test (Hutchins, 1991), it may more realistically approximate field conditions.

In contrast to benzene, the alkylbenzenes (TEX) were removed more extensively in each column (Figure 8). Again, without an appropriate control, it was not possible to determine to what extent this removal was due to biodegradation. This did, however, correlate well with batch microcosm data (Hutchins et al., 1991a). After nutrient addition on Day 38, effluent TEX concentrations in each column declined, although the rate of decline was more significant in Columns B and C, which received nitrate as well as nutrients (Figure 8). It is of interest to note that effluent dissolved carbon dioxide concentrations, which generally exceeded influent concentrations, exhibited a transient sharp increase in Columns B and C subsequent to the observed rapid decline in effluent TEX concentrations in the respective columns (Figure 9). This may be due to an increase in mineralization of the utilized labile compounds, but it is not clear why the levels continued to drop to below those of the Column A effluent after the initial peak. The cause of the continued decline in effluent TEX concentrations in the microaerophilic Column A is also unclear. No other exogenous electron acceptors were added, and methane was not detected in the column effluent at any time. In addition, there was little sulfate removal from the influent (data not shown). The aquifer solids could conceivably contain exchangeable iron, manganese, and other potential electron acceptors which might augment the role of nitrate and oxygen, but this possibility could not be assessed with the current test design. From Day 45 to Day 98, the average effluent TEX breakthroughs were 22.9 ± 2.3%, 2.9 ± 1.1%, and 4.3 ± 3.3% in Columns A, B, and C, respectively. As observed in a previous batch microcosm study (Hutchins, 1991), these alkylbenzenes were degraded equally well with or without limited oxygen under denitrifying conditions, and final effluent concentrations were generally less than 10 μg/L for total TEX.

An approximation of total mass of hydrocarbon removed and electron acceptor consumed can be made by calculating the average difference between influent and effluent concentrations for any given column, and then multiplying by the total effluent volume collected during that period. This was done for the time during which nitrate was available to the columns. From Day 45 to Day 98, the total effluent volume was 38.8 ± 0.1 liters for Columns A, B, and C. The following theoretical stoichiometric relationships were then used to calculate how much of the observed hydrocarbon removal could be attributed to mineralization under either aerobic or denitrifying conditions:

$$C_{61}H_{87} + 62.2\ H^+ + 62.2\ NO_3^- \rightarrow 61\ CO_2 + 31.1\ N_2 + 64.6\ H_2O$$

$$C_{61}H_{87} + 155.5\ NO_3^- \rightarrow 61\ CO_2 + 155.5\ NO_2^- + 33.5\ H_2O$$

$$C_{61}H_{87} + 75.75\ O_2 \rightarrow 61\ CO_2 + 33.5\ H_2O$$

This assumed that the nitrate which did not account for nitrite production was completely denitrified. In the column study, no significant nitrous oxide accumulation was observed (data not shown). It was also assumed that the nitrogen requirement for cell biomass was satisfied by the ammonium supplement, and that the hydrocarbons were completely mineralized to carbon dioxide and water. These data are summarized in Table 1, and indicate that the actual BTEX removal was approximately twice that of the theoretical removal for Column A, whereas it was only 35% and 80% of the total theoretical removal in Columns B and C, respectively. For the latter two columns, it is quite possible that additional hydrocarbons present in the contaminated interval exerted a significant electron acceptor demand for nitrate; this was also observed in the field study, but to a much greater extent (Hutchins et al., 1991b). Although the higher nitrate consumption observed in Column B is consistent with the hypothesis that preliminary oxidation of the hydrocarbons under microaerophilic conditions could lead to increased utilization of nitrate, the data are not sufficient to formulate definitive conclusions. In addition, the loss of BTEX in Column A, in excess of the electron acceptor supplied, indicates that other electron acceptors may have been present and/or other removal processes were operative. Without an appropriate control column, it was not possible to determine the extent to which abiotic processes contributed to BTEX removal.

Column Test II

The second column test ran from Day 170 to Day 270. Initially, the only test parameters that were changed from Column Test I were that the column operating temperature was raised from 12°C to 20°C, influent oxygen levels to Columns A and B were increased to 1.5mg/L, and the mercuric chloride biocide was replaced with 0.01 N Na OH for the control columns. However, the 50% BMW used for the stock feed to Columns A, B, and C was replaced with deionized water during the test to eliminate microbial growth in feed lines and reservoirs. On Day 216, nitrate was deleted from the feed for Column C so that this column now had no added electron acceptor. In addition, nitrate was added to the feed for Column A so that the operating parameters were now identical for Columns A and B.

Oxygen removals were similar throughout Column Test II for Columns A and B with an average loss of 1.0 ± 0.1 mg/L dissolved oxygen. There was no net consumption of oxygen in either of the other columns. Removal of nitrate was more complex. Initially, effluent nitrate values were much higher for Column B than the other columns; once the 50% BMW feed was replaced with deionized water on Day 184, nitrate levels began to rise in the effluents of both Columns B and C (Figure 10). The reason for this is not clear, since the decrease in background total organic carbon available for denitrification (about 0.3 mg/L) would be insufficient to account for this on a mass basis. Nitrate was removed from the Column C feed solution on Day 216, and its effluent nitrate levels dropped to below detection soon thereafter (Figure 10). Similarly, nitrate was added to the Column A feed solution at the same time, and its effluent nitrate levels increased to those observed for Column B. From Days 231 to 268, the average nitrate-nitrogen removal was 1.6 ± 0.4 mg/L and 1.3 ± 0.2 mg/L for Columns A and B, respectively. In terms of nitrate removal, therefore, these columns were operating similarly with little or no acclimation period observed for Column A. Unlike the first test, Column D appeared to be an adequate control with respect to denitrification in the second column test (Figure 10). The average loss of nitrate was only 0.2 ± 0.1 mg/L nitrate-nitrogen in this column. Nitrite levels continued to remain below 0.5 mg/L nitrite-nitrogen in the column effluents, except for a transient production of nitrite in Column C following the switch to deionized water in the feed (Figure 11). This was accompanied by a transient production in nitrous oxide which peaked at 0.8 mg/L. There was no production of nitrite or nitrous oxide observed in Column D.

Even with the changes incorporated into the operating procedure, there continued to be problems in maintaining consistent BTEX inputs to the columns (Figure 12). Column E received no BTEX input, and benzene concentrations were typically below 1 µg/L in its effluent; similarly, the total concentrations of the other alkylbenzenes (TEX) were consistently less than 5 µg/L (data not shown). Hence,

operation of Column E will not be considered in this discussion. Addition of nitrate to the feed for Column A on Day 216 appeared to have little effect on benzene removal (Figure 12a), as was expected from the Column B results during Column Test I. Surprisingly, benzene concentrations in the effluent of Column C appeared to decrease once nitrate was removed from the column feed (Figure 12c), but this decline did not continue. A similar decline was observed for Column D (Figure 12d), indicating that the drop may have been an artifact, since nitrate was still available in the control column influent. In addition, both of these columns were serviced by the same syringe pump used to deliver BTEX to the column influents and thus failure of the pump may have been responsible. However, a corresponding drop was not observed in effluent TEX concentrations for Column C (Figure 13), as would be expected based on syringe pump failure. The reason for this decline is therefore not clear. As shown in Figure 13, TEX concentrations gradually increased in Column C following nitrate removal from the feed, and decreased in Column A following addition of nitrate to the feed.

Despite the fluctuating BTEX levels in the column influents, an analysis of the effects of the operating parameters on BTEX removal was possible by calculating average percent breakthroughs of the various components during selected time intervals (Table 2). Average percent breakthroughs were considered during the entire test period for Columns B and D, since electron acceptor levels were not changed in the feed solutions. For columns A and C, two time periods were considered, corresponding to the initial part of the test prior to switching the feed solutions (Days 169 to 210), and to the time of nitrate breakthrough in the Column A effluent after switching the feed solutions (Days 231 to 262). Table 2 shows that benzene breakthrough was similar for Columns A, B, and C during each time period, with the exception of a slight decrease in Column A following nitrate addition. While this decrease is in agreement with the results of the previous batch microcosm data using limited oxygen plus nitrate (Hutchins, 1991), it is not statistically significant given the variability in influent benzene concentrations during the test. At least part of the removal of benzene in the first three columns may have been due to biological processes, since breakthrough in the control column was approximately twice that of the others (Table 2). The requirement for nitrate as an electron acceptor became more apparent with the labile alkylbenzenes (TEX). Breakthrough of TEX decreased by an order of magnitude once nitrate was added to the microaerophilic column A, whereas TEX breakthrough increased by 50-fold once nitrate was removed from the denitrifying Column C (Table 2). Even so, TEX breakthrough was still twice that in the control column, indicating that other biotic processes may have been operative. Although nitrate and nitrite concentrations dropped rapidly in the Column C effluent once the feed amendment was stopped, TEX concentrations rose much more gradually (Figure 13c). Therefore, although the requirement for nitrate for optimum TEX removal was clearly demonstrated in these columns, there were significant contributions by biotic and abiotic processes other than denitrification which could not be quantified using the given experimental design.

CONCLUSIONS

These studies have shown that alkylbenzenes can be degraded under denitrifying conditions, even when a limited amount of oxygen is present. There is some evidence that the addition of a limited amount of oxygen can facilitate benzene removal under denitrifying conditions, but the controlling parameters have not been defined. However, there were no adverse effects observed with the use of oxygen in addition to nitrate in the column studies, indicating that a mixed oxygen/nitrate system could be used for biorestoration of fuel-contaminated aquifers. The column data show that nitrate is required for optimal BTEX removal, although some removal does occur without nitrate addition. The nature of these processes could not be determined with the given column design, but appeared to be biotic for at least a portion of the removal. If these results are extrapolated to the field, they show that nitrate addition had a significant effect on BTEX removal in the field demonstration project at Traverse City.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. John T. Wilson for technical advice during the project and ManTech Environmental Services for providing analytical support. Although the research described in this paper has been funded wholly or in part by the U.S. EPA and the U.S. Air Force (MIPRs N90-43, N91-22, and N91-31, Air Force Engineering and Services Center (AFCESA/RAVW), Tyndall Air Force Base), it has not been subjected to Agency review and therefore does not necessarily reflect the views of the Agency, and no official endorsement should be inferred.

TABLE 1

MASS BALANCE FOR BTEX REMOVAL AND ELECTRON ACCEPTOR CONSUMPTION FROM DAY 45 TO DAY 98 OF COLUMN TEST I

Parameter	Units	Column A Microaerophilic	Column B Microaerophilic/ Denitrifying	Column C Denitrifying
Oxygen Removed	mg	15.5 ± 3.9	15.5 ± 3.9	0.0 ± 3.9
Nitrate-Nitrogen Removed	mg	15.5 ± 0.0	155 ± 16	140 ± 12
Nitrite-Nitrogen Added	mg	0.0 ± 0.0	50.4 ± 11.6	69.8 ± 11.6
Theoretical BTEX Demand	mg	19.4	120	89.6
BTEX Removed	mg	36.0 ± 3.3	42.3 ± 4.4	70.8 ± 11.7

TABLE 2

BREAKTHROUGH OF BENZENE AND TEX DURING SELECTED TIME INTERVALS OF COLUMN TEST II

Parameter	Time Period Days	Column A Microaerophilic*	Column B Microaerophilic/D enitrifying	Column C Denitrifying*	Column D Control
Benzene Break-through	169-210	29 ± 5%	---	31 ± 7%	---
Benzene Break-through	231-262	21 ± 4%	---	32 ± 5%	---
Benzene Break-through	169-262	---	26 ± 2%	---	65 ± 6%
TEX Break-through	169-210	11 ± 2%	---	0.5 ± 0.2%	---
TEX Break-through	231-262	1.1 ± 0.3%	---	26 ± 3%	---
TEX Break-through	169-262	---	1.7 ± 0.3%	---	59 ± 3%

* Initial conditions. Nitrate added to Column A feed and removed from Column C feed on day 216.

FIGURE 1.
COLUMN DESIGN SCHEMATIC

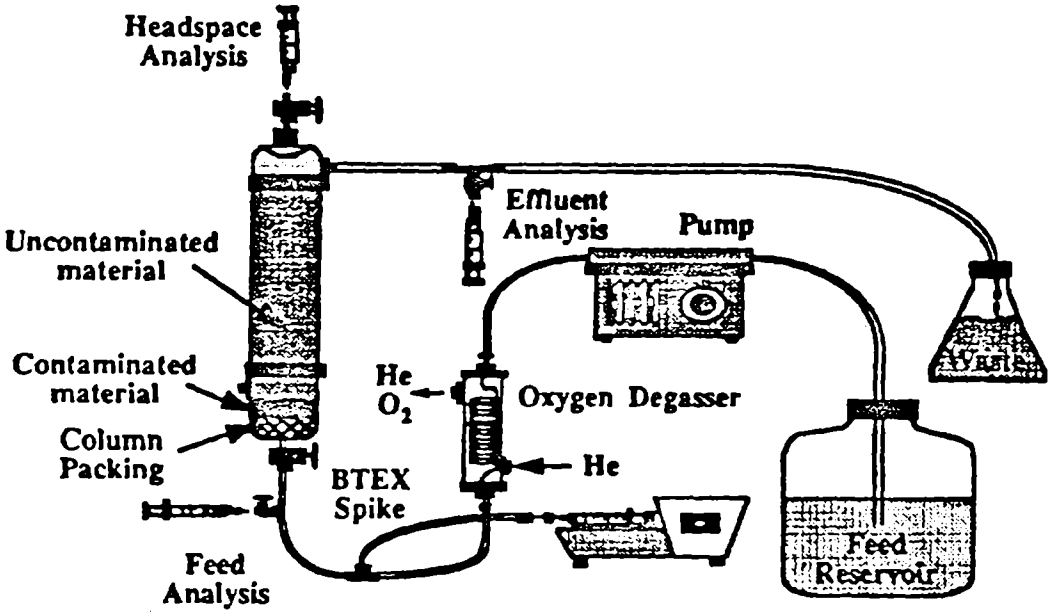


FIGURE 2.
COLUMN DESIGNATION AND SYSTEM OPERATION

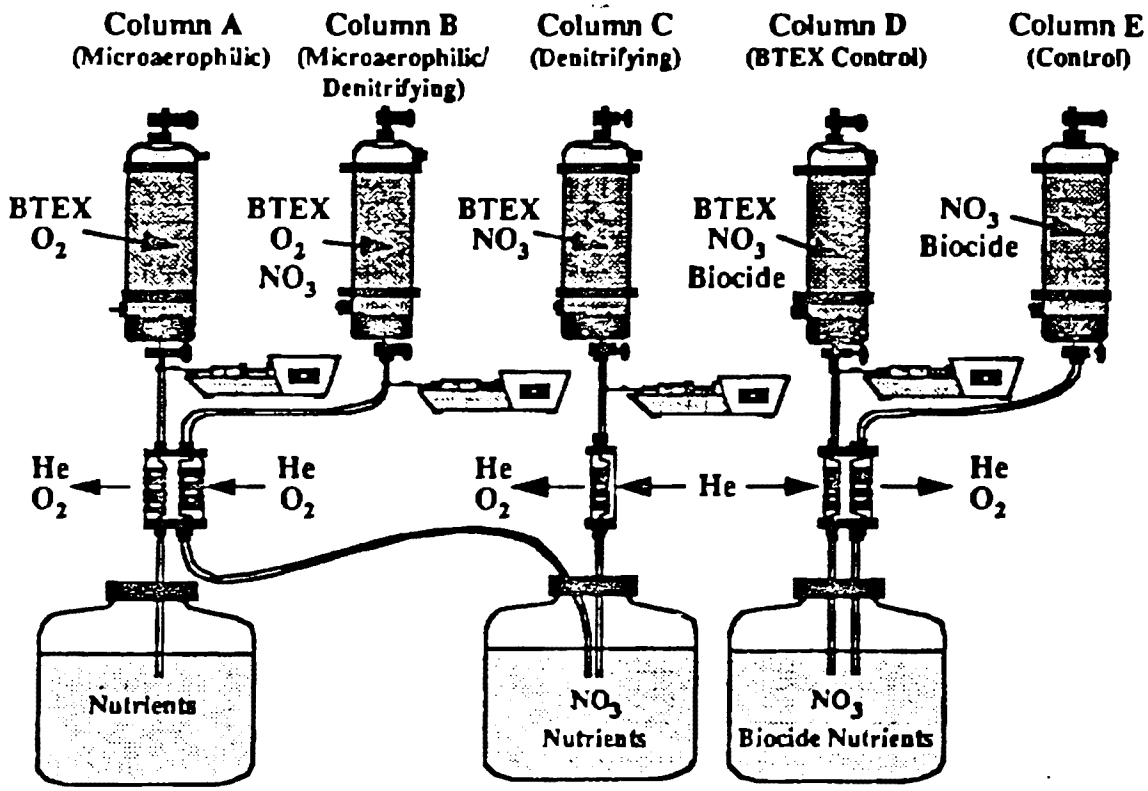


FIGURE 3.
BREAKTHROUGH OF BROMIDE TRACER IN COLUMNS

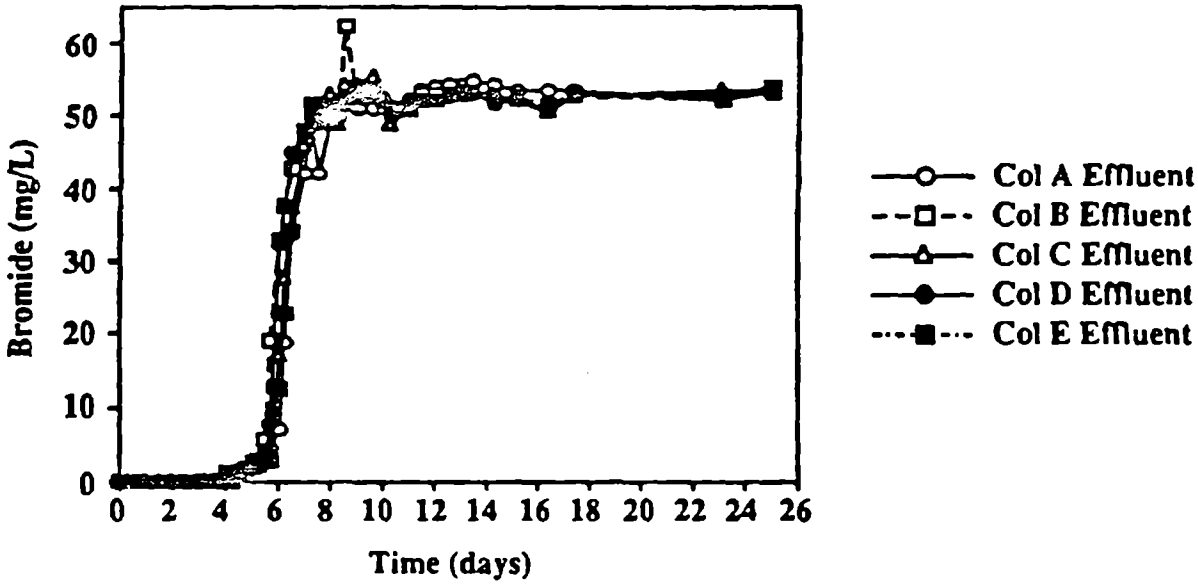


FIGURE 4.
DISSOLVED OXYGEN PROFILES IN COLUMNS A (MICROAEROPHILIC), B (MICROAEROPHILIC/DENITRIFYING), AND C (DENITRIFYING) DURING COLUMN TEST I

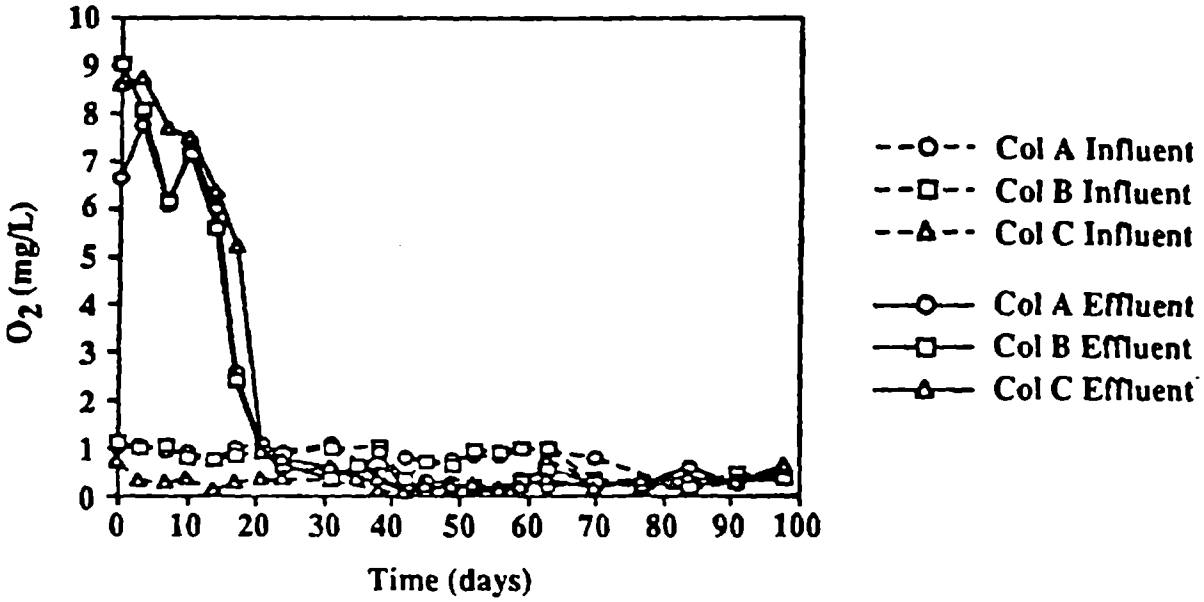


FIGURE 5.
NITRATE-NITROGEN PROFILES IN COLUMNS A (MICROAEROPHILIC), B (MICROAEROPHILIC/DENITRIFYING), AND C (DENITRIFYING) DURING COLUMN TEST I

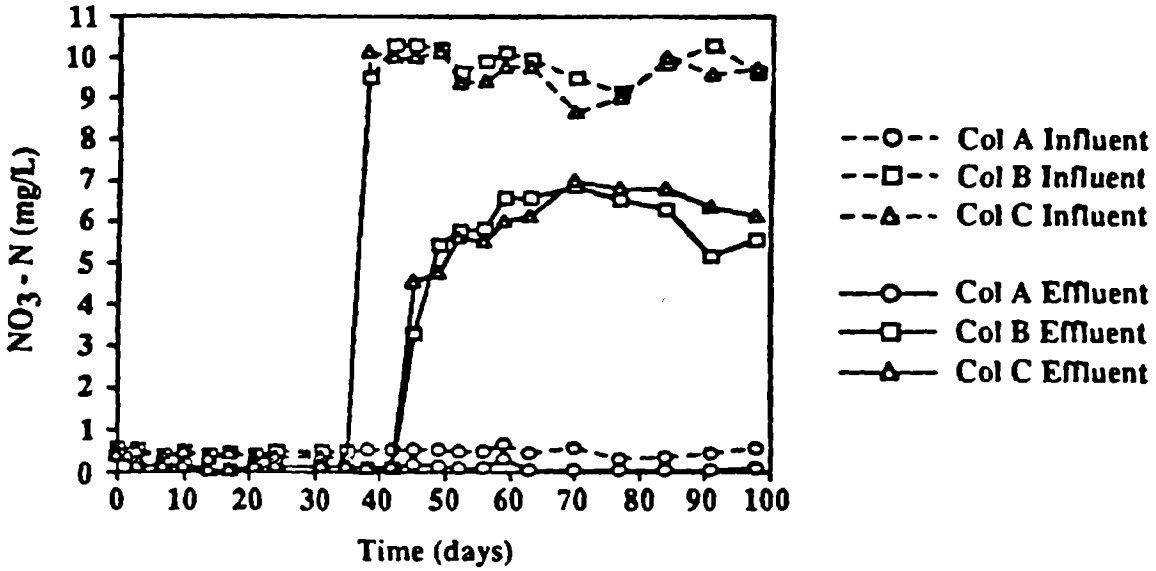


FIGURE 6.
NITRATE-NITROGEN PROFILES IN COLUMNS A (MICROAEROPHILIC), B (MICROAEROPHILIC/DENITRIFYING), AND C (DENITRIFYING) DURING COLUMN TEST I

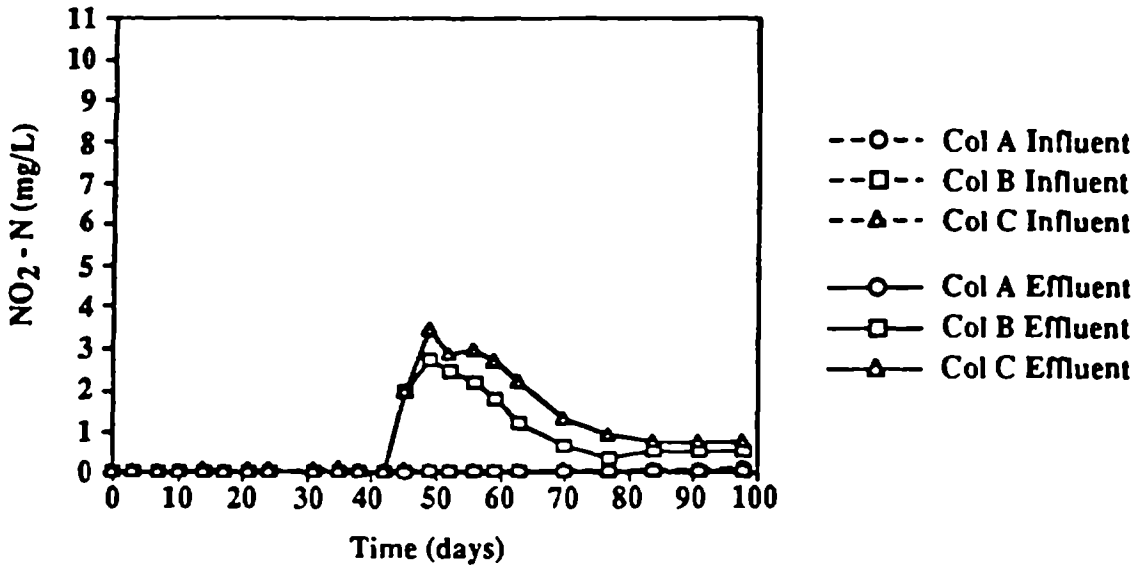


FIGURE 7.
INFLUENT AND EFFLUENT BENZENE CONCENTRATIONS IN (A) COLUMNS A (MICROAEROPHILIC), AND (B) B (MICROAEROPHILIC/DENITRIFYING), AND (C) C (DENITRIFYING) DURING COLUMN TEST I

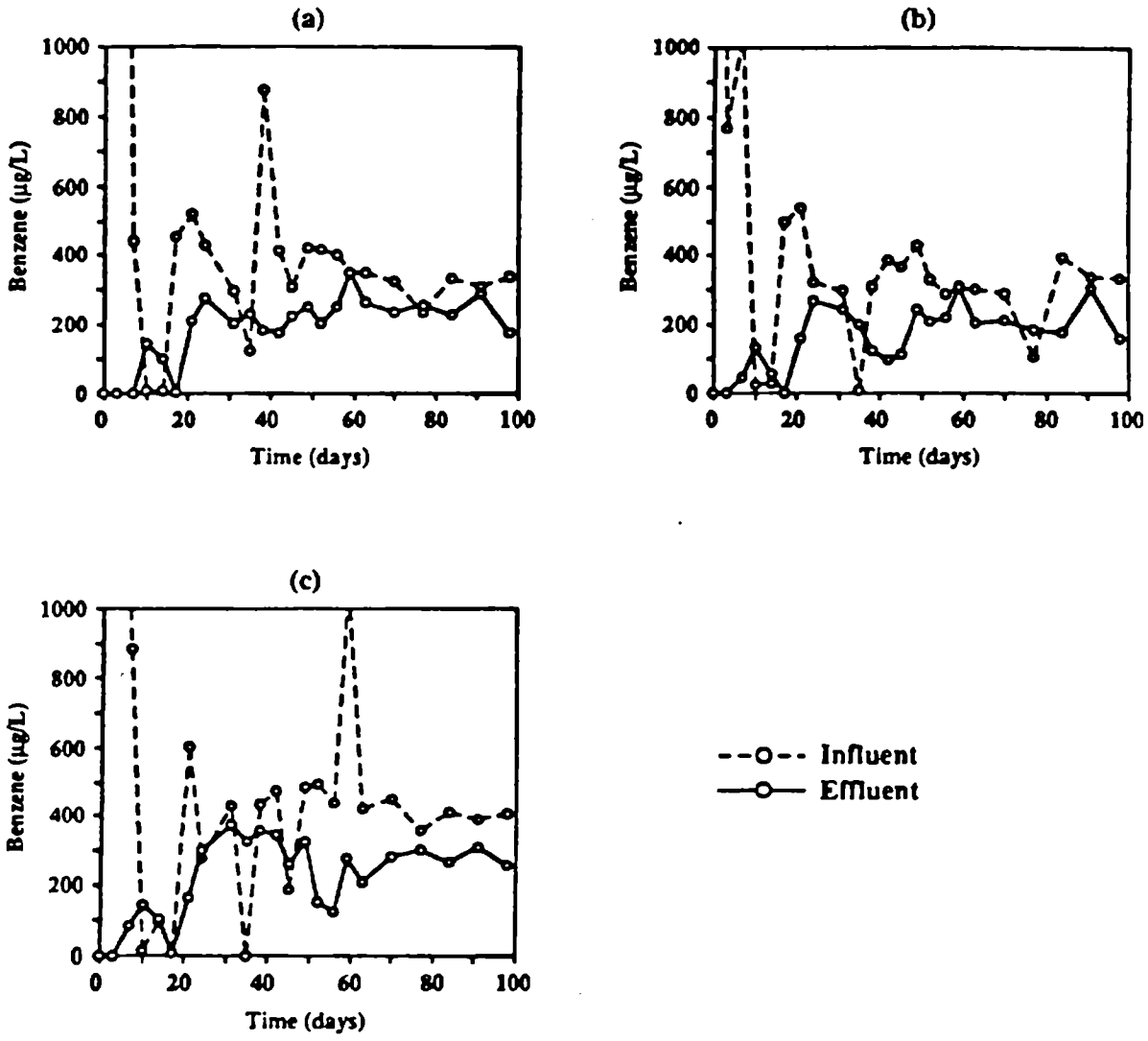


FIGURE 8.
(A) INFLUENT AND (B) EFFLUENT TEX CONCENTRATIONS IN COLUMNS A (MICROAEROPHILIC), B (MICROAEROPHILIC/DENITRIFYING), AND C (DENITRIFYING) DURING COLUMN TEST I

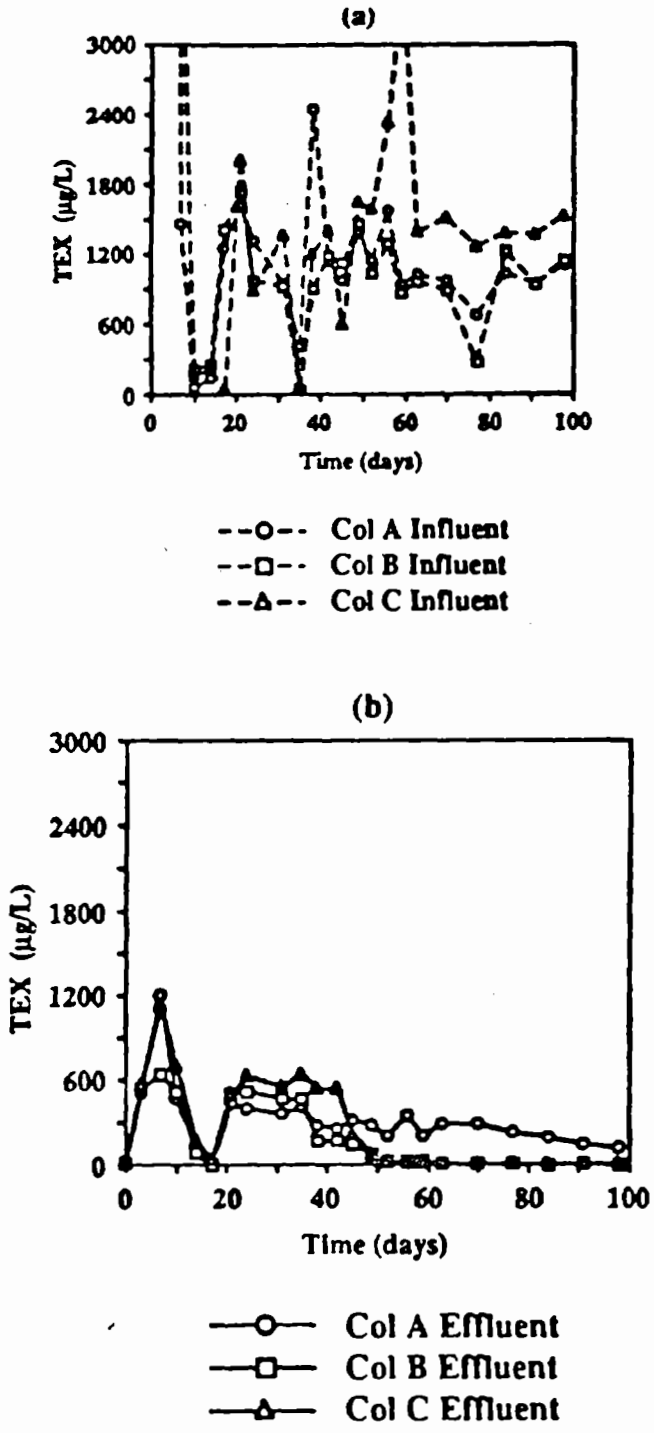


FIGURE 9.
INFLUENT AND EFFLUENT DISSOLVED CARBON DIOXIDE CONCENTRATIONS IN COLUMNS A (MICROAEROPHILIC), B (MICROAEROPHILIC/DENITRIFYING), AND C (DENITRIFYING) DURING COLUMN TEST I

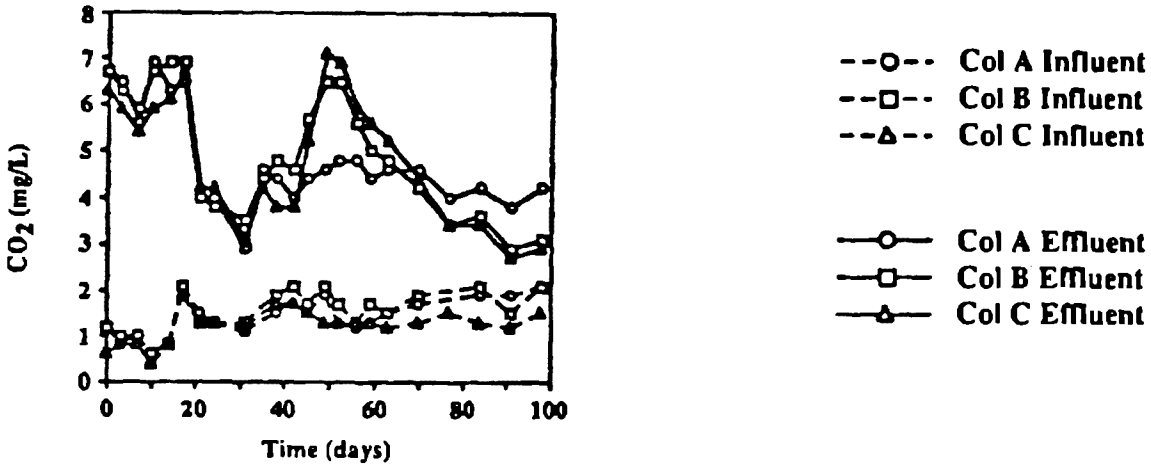


FIGURE 10.
NITRATE-NITROGEN PROFILES IN COLUMNS A (MICROAEROPHILIC), B (MICROAEROPHILIC/DENITRIFYING, C (DENITRIFYING), AND D (CONTROL) DURING COLUMN TEST II

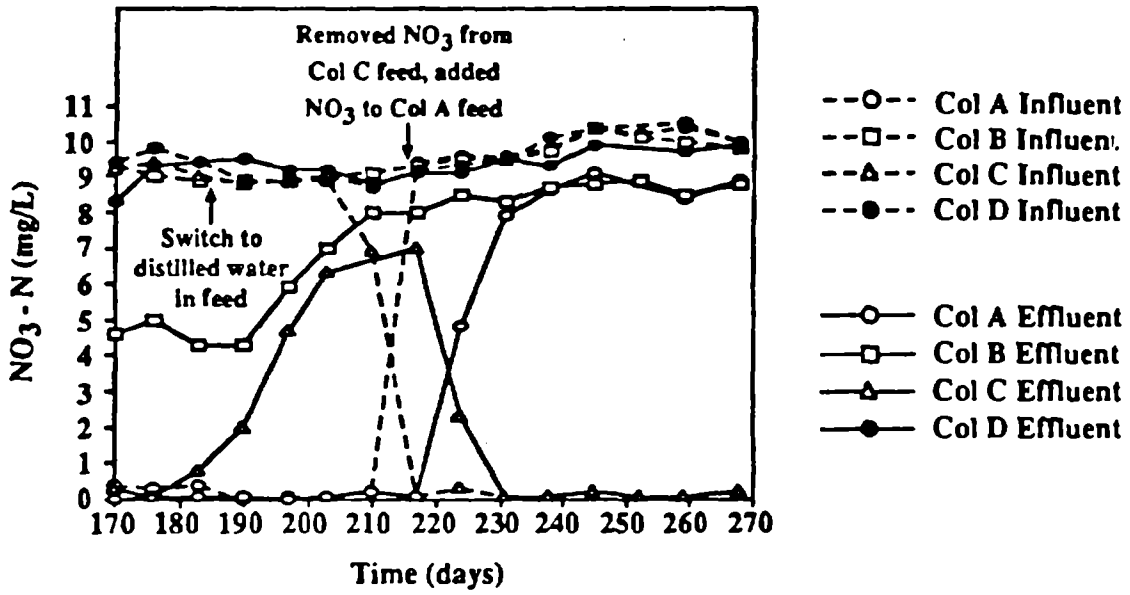


FIGURE 11.
NITRATE-NITROGEN PROFILES IN COLUMNS A (MICROAEROPHILIC), B (MICROAEROPHILIC/DENITRIFYING), C (DENITRIFYING), AND D (CONTROL) DURING COLUMN TEST II

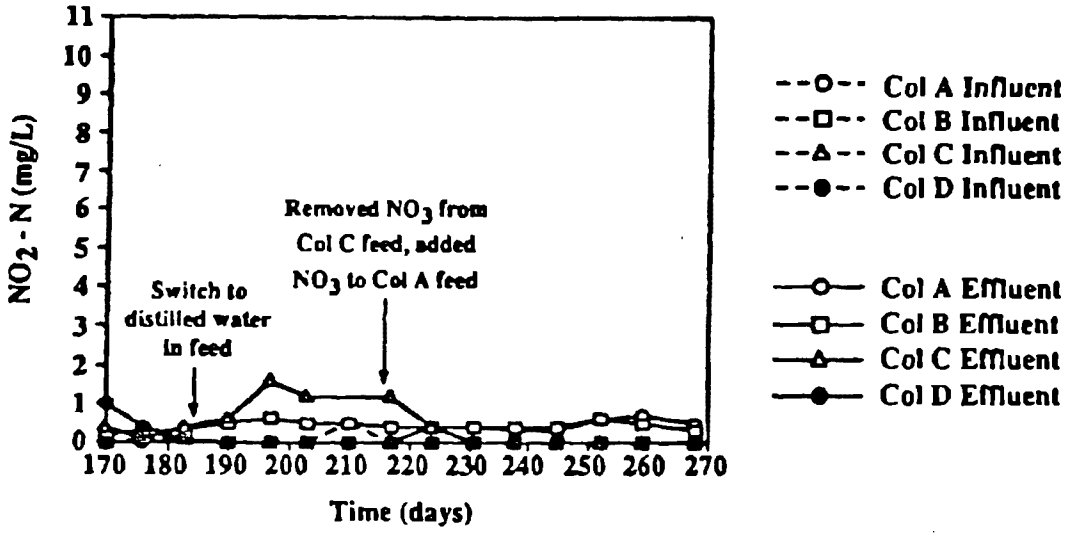


FIGURE 12.
INFLUENT AND EFFLUENT BENZENE CONCENTRATIONS IN COLUMNS (A) A (MICROAEROPHILIC), (B) B (MICROAEROPHILIC/DENITRIFYING), (C) C (DENITRIFYING), AND (D) D (CONTROL) DURING COLUMN TEST II

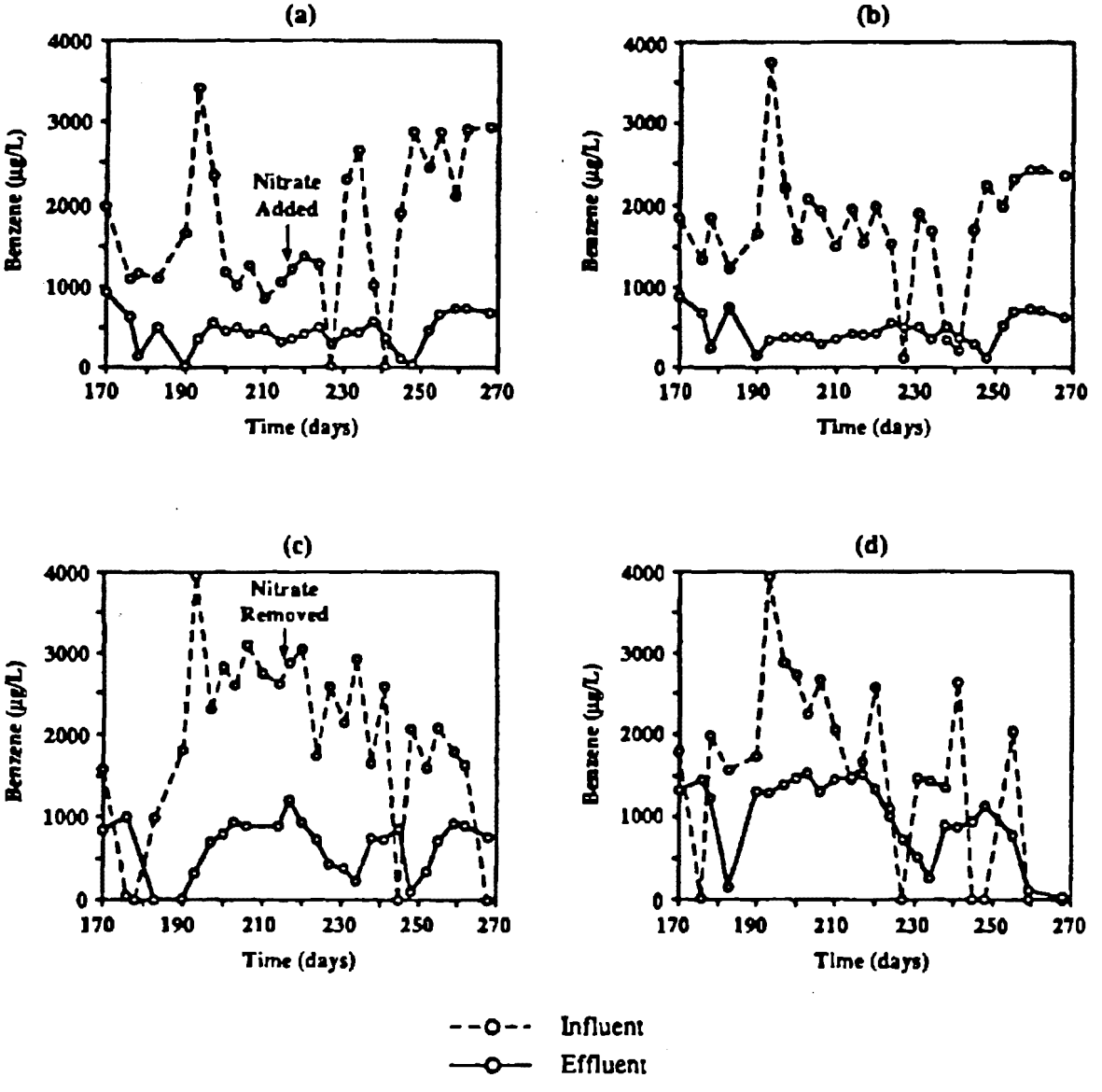
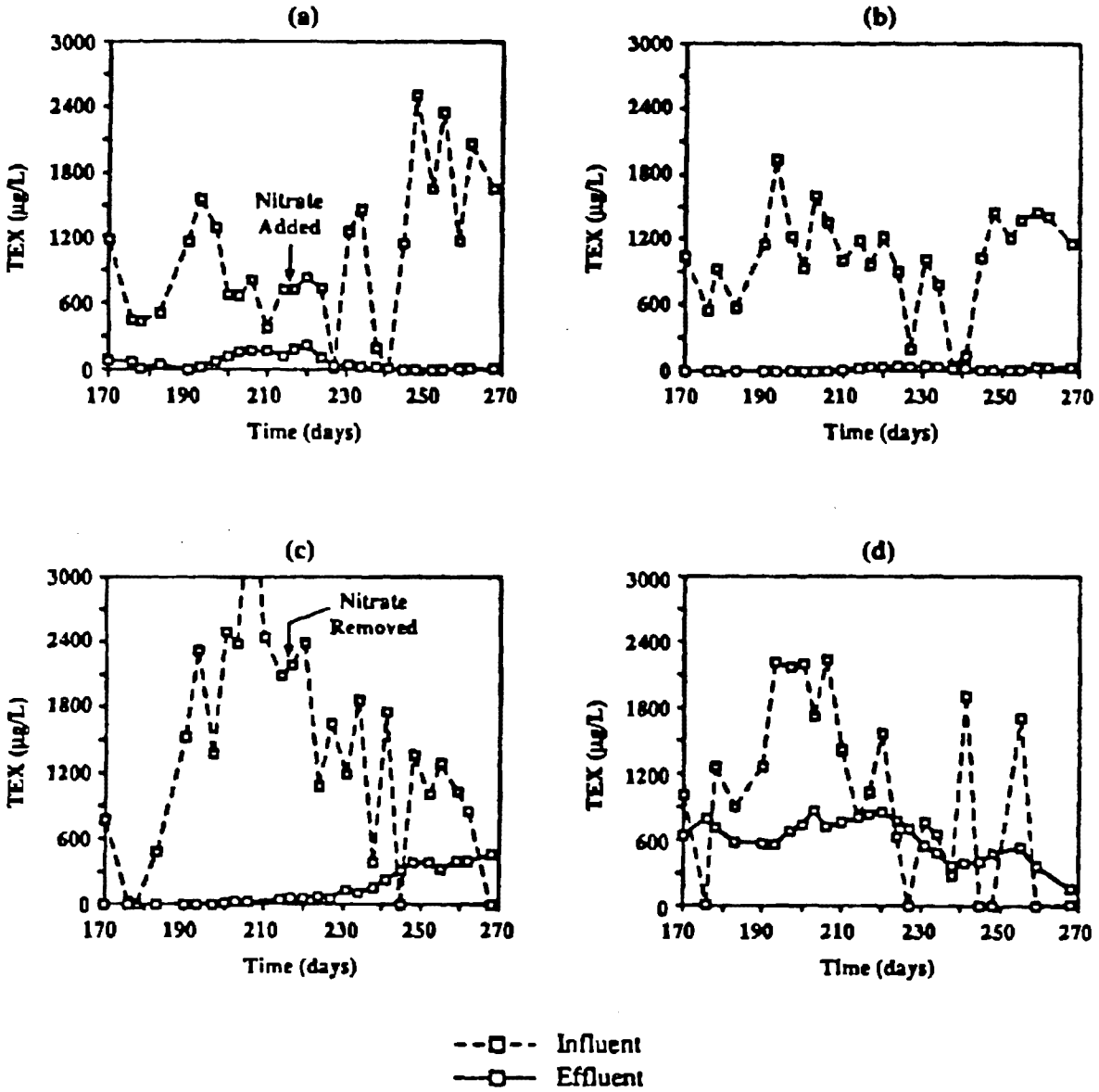


FIGURE 13.
INFLUENT AND EFFLUENT TEX CONCENTRATIONS IN COLUMNS (A) A (MICROAEROPHILIC), (B) B (MICROAEROPHILIC/DENITRIFYING), (C) C (DENITRIFYING), AND (D) D (CONTROL) DURING COLUMN TEST II



REFERENCE

1 Barker, J.F., G.C. Patrick, and D. Major. 1987. Natural attenuation of aromatic hydrocarbons in a shallow sand aquifer. Ground Water Monit. Rev. 7:64-71.

2 Battermann, G. 1986. Decontamination of polluted aquifers by biodegradation. In J.W. Assink and W.J. van den Brink (eds.), 1985 International TNO Conference on Contaminated Soil, Nijhoff, Dordrecht. pp 711-722.

3 Britton, L.E. 1989. Aerobic denitrification as an innovative method for in-situ biological remediation of contaminated subsurface sites. Air Force Engineering Services Center, Tyndall Air Force Base. Report ESL-TR-88-40.

4 EPA. 1977. Serial No. 95-12, U.S. Govt. Printing Office, Washington, D.C.

5 Hutchins, S.R., G.W. Sewell, D.A. Kovacs, and G.A. Smith. 1991a. Biodegradation of aromatic hydrocarbons by aquifer microorganisms under denitrifying conditions. Environ. Sci. Technol. 25:68-76.

6 Hutchins, S.R., W.C. Downs, J.T. Wilson, G.B. Smith, D.A. Kovacs, D.D. Fine, R.H. Douglass, and D.J. Hendrix. 1991b. Effect of nitrate addition on biorestoration of fuel-contaminated aquifer: field demonstration. Ground Water 29:571-580.

7 Hutchins, S.R. 1991. Biodegradation of monoaromatic hydrocarbons by aquifer microorganisms using oxygen, nitrate, or nitrous oxide as the terminal electron acceptor. Appl. Environ. Microbiol. 57:2403-2407.

8 Hutchins, S.R., D.E. Rhodes, and S.W. Moolenaar. 1992. Batch and column studies on BTEX biodegradation by aquifer microorganisms under denitrifying conditions. Air Force Engineering Services Center, Tyndall Air Force Base. Report (in press).

9 Kopp, J.F., and G.D. McKee. 1979. Manual - Methods for Chemical Analysis of water and Wastes. EPA-600/4-79-020.

10 Kuhn, E.P., J. Zeyer, P. Eicher, and R.P. Schwarzenbach. 1988. Anaerobic degredation of alkylated benzenes in denitrifying laboratory columns. Appl. Environ. Microbiol. 54:490-496.

11 Lee, M.D., J.M. Thomas, R.C. Borden, P.B. Bedient, J.T. Wilson, and C.H. Ward. 1988. Biorestoration of aquifers contaminated with organic compounds. Crit. Rev. Environ. Control 18:29-89.

12 Lemon, L.A., J.R. Barbaro, and J.F. Barker. 1989. Biotransformation of BTEX under anaerobic denitrifying conditions: evaluation of field observations. In: Proceedings, FOCUS Conference on Eastern Regional Ground Water Issues, NWWA, Dublin, pp 213-227.

13 Major, D.W., C.I. Mayfield, and J.F. Barker. 1988. Biotransformation of benzene by denitrification in aquifer sand. Ground Water 26:8-14.

14 Mihelcic, J.R., and R.G. Luthy. 1988. Microbial degredation of acenaphthene and naphthalene under denitrification conditions in soil-water systems. Appl. Environ. Microbiol. 54:1188-1198.

15 OUST, 1990. LUST Trust Fund Monthly Progress Report, Sept 1990, Office of Underground Storage Tanks, U.S. EPA, Washington, D.C.

16 Sheehan, P.J., R.W. Schneiter, T.K.G. Mohr, and R.M. Gersberg. 1988. Bioreclamation of gasoline contaminated groundwater without oxygen addition. In: Proceedings, Second National Outdoor Action Conference on aquifer Restoration, Ground Water Monitoring, and Geophysical Methods, NWWA, Dublin, pp 183-199.

17 Thomas, J.M., M.D. Lee, P.B. Bedient, R.C. Borden, L.W. Canter, and C.H. Ward. 1987. Leaking underground storage tanks: remediation with emphasis on in situ biorestoration. RSKERL Publication, EPA 600/2-87/008.

18 Tiedje, J.M. 1988. Ecology of denitrification and dissimilatory nitrate reduction to ammonium. In: Biology of Anaerobic Microorganisms, (A.J.B. Zehnder, ed.) John Wiley and Sons, New York. pp. 179-244.

19 Vandegrift, S.A. and D.H. Kampbell. 1988. Gas chromatographic determination of aviation gasoline and JP-4 jet fuel in subsurface core samples. J. Chromatogr. Sci. 26: 566-569.

20 Wilson, J.T., L.E. Leach, M. Henson, and J.N. Jones. 1986. In situ biorestoration as a ground water remediation technique. Ground Water Monitor. Rev. 6:56-64.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before comple

1. REPORT NO.	2.	3.
EPA/600/A-92/080		

4. TITLE AND SUBTITLE
COLUMN STUDIES ON BTEX BIODEGRADATION UNDER MICROAEROPHILIC AND DENITRIFYING CONDITIONS

5. REPORT DATE

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
S.R. HUTCHINS[1]
S.W. MOOLENAAR[2]
D.E. RHODES[2]

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
[1]U.S. EPA, RSKERL, P.O. BOX 1198, ADA, OK 74820

[2]RICE UNIVERSITY, HOUSTON, TEXAS

10. PROGRAM ELEMENT NO.
TEKY1A

11. CONTRACT/GRANT NO.
DW-14935081

12. SPONSORING AGENCY NAME AND ADDRESS
ROBERT S. KERR ENVIRONMENTAL RESEARCH LABORATORY - ADA, OK
U.S. ENVIRONMENTAL PROTECTION AGENCY
P.O. BOX 1198
ADA, OKLAHOMA 74820

13. TYPE OF REPORT AND PERIOD COVERED
PROCEEDINGS MAY 1988 - JULY 1993

14. SPONSORING AGENCY CODE
EPA/600/15

15. SUPPLEMENTARY NOTES PUBLISHED IN:
GROUND WATER: The Problem and Some Solutions - Proceedings, 4th Annual Symposium
The Gulf Coast Hazardous Substance Research Center, Beaumont, TX, April 2-3, 1992 pp 67-90

16. ABSTRACT

Two column tests were conducted using aquifer material to simulate the nitrate field demonstration project carried out earlier at Traverse City, Michigan. The objectives were to better define the effect nitrate addition had on biodegradation of benzene, toluene, ethylbenzene, xylenes, and trimethylbenzenes (BTEX) in the field study, and to determine whether BTEX removal can be enhanced by supplying a limited amount of oxygen as a supplemental electron acceptor. Columns were operated using limited oxygen, limited oxygen plus nitrate, and nitrate alone.

In the first column study, benzene was generally recalcitrant compared to the alkylbenzenes (TEX), although some removal did occur. The average benzene breakthroughs were 74.3 ± 5.8%, 75.9 ± 12.1%, and 63.1 ± 9.6% in the columns with limited oxygen, limited oxygen plus nitrate, and nitrate alone, respectively, whereas the corresponding average effluent TEX breakthroughs were 22.9 ± 2.3%, 2.9 ± 1.1%, and 4.3 ± 3.3%. In the second column study, nitrate was deleted from the feed to the column originally receiving nitrate alone and added to the feed of the column originally receiving limited oxygen alone. Benzene breakthrough was similar for each column. Breakthrough of TEX decreased by an order of magnitude once nitrate was added to the microaerophilic column, whereas TEX breakthrough increased by 50-fold once nitrate was removed from the denitrifying column. Although the requirement for nitrate for optimum TEX removal was clearly demonstrated in these columns, there were significant contributions by biotic and abiotic processes other than denitrification which could not be quantified.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field Group
GROUND WATER AQUIFER BIODEGRADATION ANAEROBIC BIOREMEDIATION DENITRIFICATION COLUMN	BTEX NITRATE BENZENE TOLUENE ETHYLBENZENE XYLENE	

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	
	20. SECURITY CLASS (This page)	22. PRICE
	UNCLASSIFIED	

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE