Spatial variability in the naphthalene mineralization response to oxygen, nitrate, and orthophosphate amendments in MGP aquifer sediments

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Abstract

The feasibility of aerobic in situ bioremediation is being investigated for use in a strategy to control subsurface coal tar contamination at the site of a former manufactured gas plant. As part of this investigation, anoxic aquifer sands collected between 11 and 25 m below ground surface were assayed in batch microcosms to measure the singular and combined effects of O_2, NO_3^-, and PO_4^{3-} on ^{14}C-naphthalene mineralization. The influence of these additives varied considerably between sediments. A high initial concentration of O_2 (21 mg/L) promoted the greatest extent of mineralization in the majority of active sediments. NO_3^- (85 mg/L) was observed to enhance, inhibit, or have no effect on the rate of naphthalene mineralization, although significant denitrification was observed in nearly all the active sediments. Data suggest that PO_4^{3-} complexation and/or precipitation with sediment cations limited P bioavailability. The sediments that were incapable of mineralizing naphthalene were characterized by low pH (< 4.1), high SO_4^{2-} (> 500 mg/L), and moderate to high dissolved Fe(II) (30–265 mg/L) when equilibrated aerobically with water. Fe(II) likely exerted a significant O_2 demand that reduced the O_2 available as an electron acceptor for biodegradation. These experiments demonstrate that while aeration/oxygenation can be an effective strategy for enhancing subsurface bioremediation of aromatic hydrocarbons, the biodegradation response to aeration/oxygenation and nutrient addition may vary considerably within an aquifer.

Introduction

Aerobic in situ bioremediation is being considered for treatment of aromatic hydrocarbon contamination at the site of a former manufactured gas plant (MGP). Previous research has determined that bacteria in many of the sediments at the MGP site possess enzymes necessary to aerobically mineralize naphthalene and phenanthrene (Durant et al. 1995). Ongoing studies are aimed at developing a strategy for enhancing the in situ biodegradation of aromatic hydrocarbons at the site.

Intrinsic bioremediation rates for aromatic hydrocarbons are often controlled by the availability of O_2 (Wilson et al. 1985; Barker et al. 1987; Chiang et al. 1989), and aeration techniques such as in situ air-sparging, hydrogen peroxide injection, and membrane oxygen diffusion are commonly used in attempts to enhance the subsurface biodegradation of aromatic hydrocarbons at contaminated sites (Lee et al. 1988; Johnson et al. 1993; Wilson et al. 1994; Gantzer & Cosgriff 1995). Nutrients such as nitrogen (N) and phosphorus (P) are also typically included as in situ bioremediation additives to promote biological growth (Jamison et al. 1975; Lee et al. 1988; Kampfer et al. 1993; Leethem et al. 1995).

Despite the apparent consensus on the necessity of including N and P as in situ bioremediation additives, their effects on biodegradation in soils and aquifer material are not well understood. One reason for this is that when considered collectively, literature studies examining the combined effects of N and P amendments on biodegradation of aromatic hydrocarbons have yielded mixed results. For example, while the combined addition of NO_3^-, NH_4^+, and PO_4^{3-} has been observed to enhance the aerobic biodegradation of gasoline in ground water (Jamison et al. 1975), the combined addition of these species had no effect

or inhibited phenanthrene biodegradation in soil and petroleum-contaminated sand (Morgan & Watkinson 1992; Efroymson & Alexander 1994). When added in the absence of N, PO_4^{3-} amendments (added as K^+ and Na^+ salts) typically stimulate aromatic hydrocarbon biodegradation in soil and water systems (Lewis et al. 1986; Armstrong et al. 1991; Manilal & Alexander 1991). The effects of N in the absence of P amendments are more variable. While Allen-King et al. (1994) found that NH_4^+ and NO_3^- each stimulated aerobic toluene biodegradation in soil, other studies have observed NO_3^- to inhibit phenanthrene biodegradation, and NH_4^+ (added as $(NH_4)_2SO_4$) to inhibit toluene degradation in soil and aquifer material (Swindoll et al. 1988; Manilal & Alexander 1991).

Given the variability in the existing literature, this study was initiated to explore the relative importance of O_2, NO_3^-, and PO_4^{3-} on the biodegradation of aromatic hydrocarbons, and to utilize the results in the formulation of bioremediation additives to be applied at the MGP site. Experiments reported here focused on determining whether O_2, NO_3^-, and PO_4^{3-} can individually, or in combination, stimulate the biodegradation of unsubstituted aromatic hydrocarbons in aquifer sediments, and to investigate the extent to which the effects of these additives varied among aquifer sediments. NO_3^- was selected as the N-source for this work because unlike NH_4^+, NO_3^- doesn't have the tendency to sorb to soil and sediments, and it can also act as an electron acceptor in biodegradation. NO_3^- has been used effectively to enhance biodegradation of aromatic hydrocarbons in several laboratory and field studies (Kuhn et al. 1985; Hutchins et al. 1991; Reinhard et al. 1997), and interest is growing in the use of NO_3^- to complement O_2 in aerobic bioremediation schemes (Hutchins et al. 1992; Anid et al. 1993; Wilson & Bouwer 1997).

Methods

Aquifer sediments were obtained from 3 boreholes at the site of a former manufactured gas plant (Figure 1) using techniques described previously (Durant et al. 1995). The samples were collected from locations exhibiting low to moderate levels of aqueous phase groundwater contamination (naphthalene 100–1000 μg/L), except for the upper aquifer samples of Borehole A, which had been shown to contain active naphthalene degrading populations despite an absence of detectable aromatic hydrocarbon contamination (Durant et al. 1995). The sediments were stored at 4 °C for several months prior to use in these experiments.

Ten sediments (medium- to coarse-grained sands) were analyzed to measure the singular and combined effects of O_2, NO_3^-, PO_4^{3-}, and pore water chemistry on biodegradation of naphthalene. Each sediment was mixed thoroughly prior to use in the microcosm assay in order to promote uniform chemistry and biomass densities within sediments. Naphthalene was selected as the model aromatic compound for these experiments because of its widespread occurrence in petroleum-contaminated aquifers, and because the sediments at the MGP site contain bacteria that can readily degrade naphthalene under aerobic conditions (Durant et al. 1995). Sediment-water microcosms were aseptically prepared in 25-mL serum vials, and consisted of 7 g moist sediment, 4 mL filter-sterilized groundwater, 6.7×10^{-2} μCi ^{14}C-naphthalene, 4 mg/L unlabeled naphthalene, and approximately 17 mL of air-filled headspace. The ^{14}C-naphthalene was added using methanol as a carrier solvent (254 mg/L, final concentration). The groundwater was obtained from a relatively pristine location at the site, and contained < 1 mg/L NO_3^-, 10 mg/L SO_4^{2-}, and no detectable organic contaminants. To ensure oxygen availability, the groundwater was equilibrated with the atmosphere before use in the microcosms, attaining 8.5 mg/L dissolved O_2. Two killed-control microcosms were prepared for each sediment under each initial condition by autoclaving the microcosms twice, and by adding NaN_3 (250 mg/L, final concentration).

A sterile 2-mL glass vial containing 0.5 mL 1 N KOH was placed in each microcosm to trap $^{14}CO_2$ generated from mineralization of the ^{14}C-naphthalene. Microcosms were capped with Teflon^{TM}-lined butyl rubber stoppers and aluminum crimp seals. $^{14}CO_2$ evolved in each microcosm was determined periodically by sampling and replacing the KOH with a syringe inserted through the stopper. Radioactivity was counted by liquid scintillation with a Beckman LS 3801 liquid scintillation counter. Biodegradation was determined as the percent ^{14}C-naphthalene mineralized to $^{14}CO_2$, less the percent of radioactivity detected in the KOH in the control microcosms.

The ability of each of the ten sediments to mineralize ^{14}C-naphthalene was measured in triplicate under five separate initial conditions: a) no nutrient amendments; b) 85 mg/L NO_3^-; c) 90 mg/L PO_4^{3-}; d) 25 mg/L NO_3^- and 90 mg/L PO_4^{3-}; and e) 21 mg/L O_2 and no nutrient amendments. These relatively high
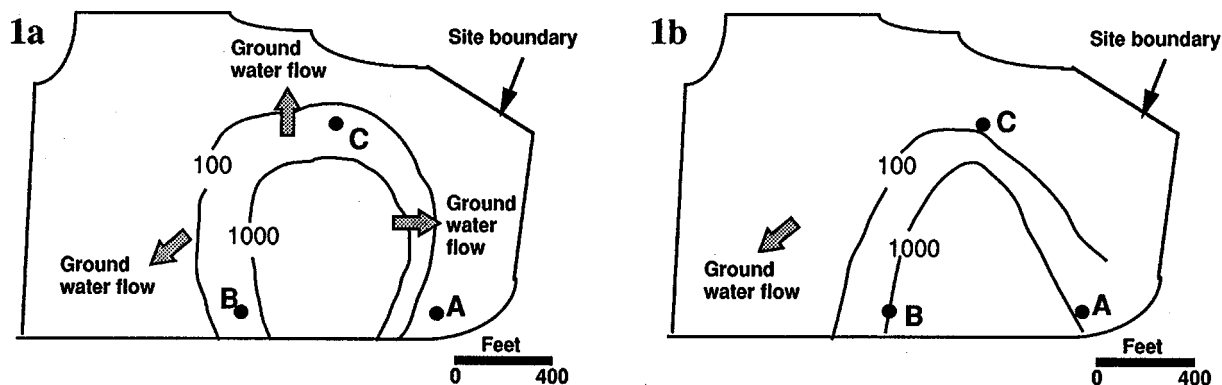
Figure 1. Borehole locations A, B, and C, ground water flow directions, and naphthalene plume configuration (μg/L) in (**1a**) the upper aquifer (approx. 11–16 m) and (**1b**) the lower aquifer (approx. 17–27 m).

concentrations of PO_4^{3-} and NO_3^- were selected with the goal of providing excess PO_4^{3-} and NO_3^- availability for naphthalene biodegradation while recognizing that various other organic and inorganic processes might also compete for these two species. Differences in the ^{14}C-naphthalene mineralization response to the various treatments were evaluated with a t-test. A significant difference was attributed to tests producing a $P < 0.05$.

All microcosms except those assayed under the high O_2 conditions (21 mg/L) had an initial O_2 concentration of approximately 8.5 mg/L. High O_2 conditions were created by purging a portion of the groundwater with pure O_2 before adding it to the microcosms. PO_4^{3-} was added from a sterile stock containing KH_2PO_4 (25.7 mg/L), K_2HPO_4 (66.0 mg/L), and Na_2HPO_4-$7H_2O$ (101.2 mg/L). NO_3^- was added as $NaNO_3$. All microcosms were incubated at 10 °C to simulate aquifer temperatures.

Portions of the ten sediments were analyzed for background N, P, and organic carbon (f_{OC}) content. NH_4^+ extracted into 2 M KCl was reacted with indophenol in the presence of hypochlorite and quantified on a Shimadzu UV-160 spectrophotometer at A_{636} (Keeney & Nelson 1982). Soluble reactive phosphate in filtered $NaHCO_3$ extracts was measured at A_{882} using the molybdenum blue assay with ascorbic acid (Olsen & Sommers 1982). Filtered aqueous extracts were analyzed for NO_3^- by ion chromatography. The organic carbon content was determined using a UIC Coulometric Model 5120 Total Carbon Analyzer.

At the end of the 6-week mineralization assay, the pore water and sediment in each microcosm were ana-

lyzed to measure the fate of supplements (PO_4^{3-} and NO_3^-), and to investigate whether other parameters (i.e. pH, SO_4^{2-}, and iron content) correlated with observed trends in naphthalene biodegradation. Anions in the pore water were separated on a Dionex IonPac AS4A-SC 4 mm column and measured on a Dionex 2010i conductivity detector. Fe^{2+} in the filtered pore water and in sediment extracts was reacted with ferrozine and measured at A_{562} (Stookey 1970). Standards were prepared from anaerobic stock solutions of ferrous ethylenediammonium sulfate tetrahydrate dissolved in 0.5 HCl. Fe^{2+} in the moist sediments was extracted in 1 N HCl for 7 days, and total iron (Fe^{2+} and Fe^{3+}) was extracted for the same duration in a solution of 0.5 N HCl and 0.5 N NH_2OH-HCl (hydroxylamine reduces Fe^{3+} to Fe^{2+} under acidic conditions (Lovley & Phillips 1987)). While extraction in 1 N HCl does not provide an absolute measure of iron content, it has been demonstrated to yield a reasonable approximation of amorphous Fe^{3+} and reduced species such as FeS(s) and $FeCO_3$(s) (Heron et al. 1994).

The approach used in these experiments provides a model for how nutrient and O_2 addition affect biodegradation of aqueous-phase (bioavailable) naphthalene by sediment-bound bacteria. It does not attempt to investigate how nutrient and O_2 addition might affect the desorption and biodegradation of sorbed naphthalene in contaminant-aged sediments. It should be recognized that the observed biodegradation responses were likely more pronounced than they would have been had these experiments examined the biodegradation of sorbed ^{14}C-naphthalene in aged sediments.
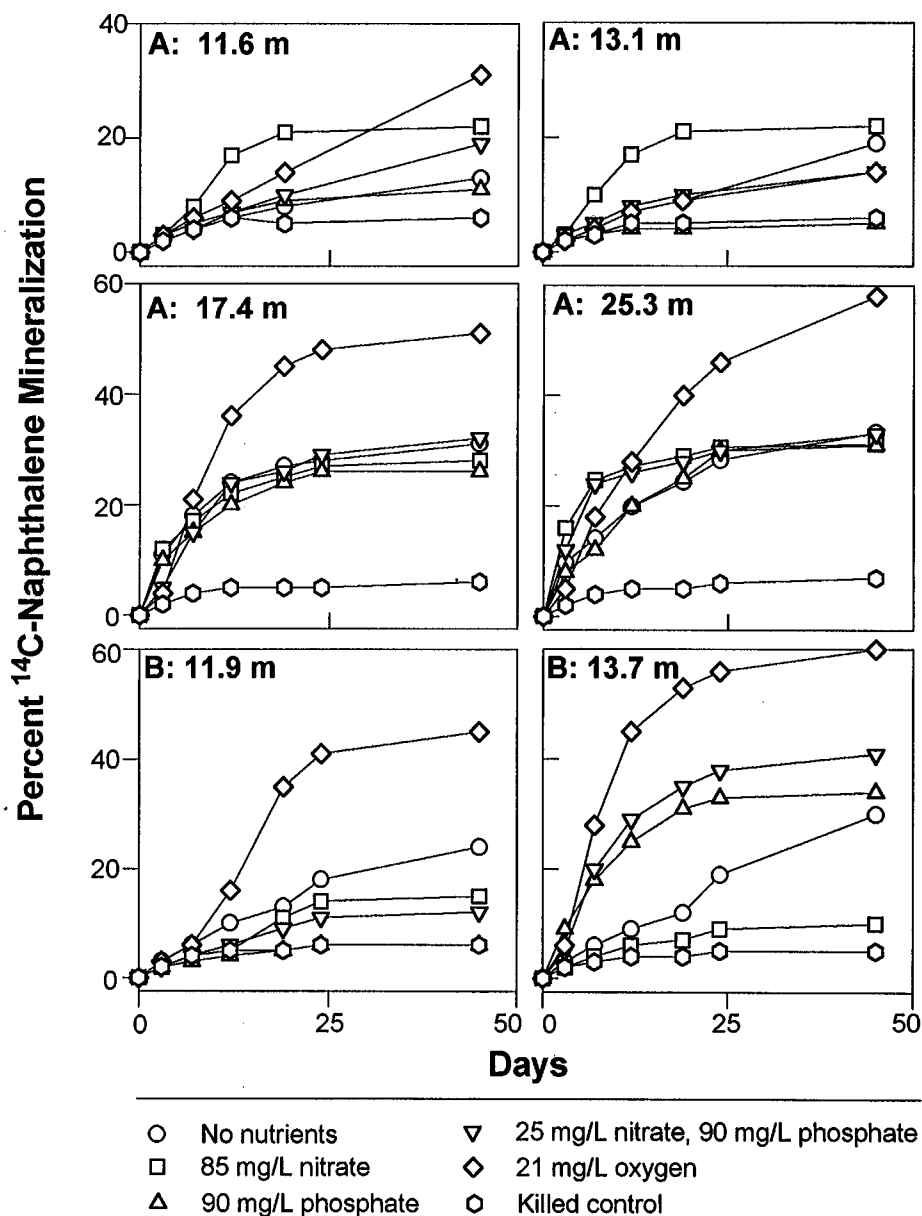
Figure 2. Mineralization of ^{14}C-naphthalene in aerobic sediment-water microcosms incubated at 10 °C. Except for the killed control curves, each curve represents the average of triplicate microcosms sampled over time. The killed control curves represent the average of 10 microcosms.

Results

Six of the ten sediments analyzed exhibited significant mineralization of ^{14}C-naphthalene ($< 52\%$) relative to killed controls over 6 weeks of incubation (Figure 2). In the four most active sediments (Borehole A 17.4 and 25.3 m, and Borehole B 11.9 and 13.7 m), substantial

(20 to 30%) naphthalene mineralization occurred in the absence of nutrient amendments. Nutrient amendments were not a prerequisite for microbial activity, as background concentrations of N and P were detected in all the sediments. $NO_3{}^-$-N ranged from 0.3 to 0.7 mg/kg, while NH_4^+-N (4.4–7.1 mg/kg) was only
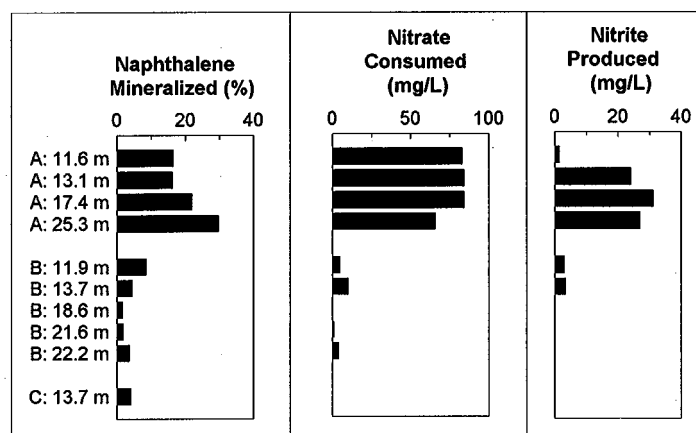
Figure 3. ^{14}C-Naphthalene mineralization, nitrate consumed, and nitrite produced in microcosms initially amended with 85 mg/L nitrate. The vertical axis indicates the Borehole (A, B, or C) and sample depth.
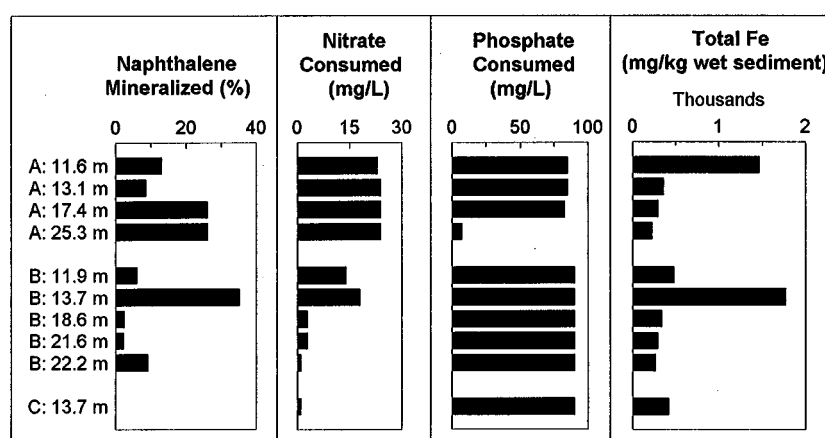

Figure 4. ^{14}C-Naphthalene mineralization, nitrate consumed, phosphate consumed, and total iron concentrations in microcosms initially amended with 25 mg/L nitrate and 90 mg/L phosphate. The vertical axis indicates the Borehole (A, B, or C) and sample depth.

detected in the Borehole B and C sediments. Phosphorus concentrations ranged from 0.3 to 2.1 mg/kg.

A high initial O_2 concentration (21 mg/L) maximized the extent of ^{14}C-naphthalene mineralization in the active sediments (Figure 2). O_2 conditions also typically stimulated mineralization rates, but for three of the sediments, initial mineralization rates were significantly ($P < 0.05$) faster in microcosms amended with 85 mg/L NO_3^- than in microcosms amended with high O_2 (Borehole A: 11.6, 13.1, and 25.3 m). In the Borehole A 13.1 m sediment, O_2 addition failed to stimulate mineralization relative to unamended conditions, and naphthalene mineralization favored nitrate-amended conditions. Denitrification (indicated by NO_3^- consumption and NO_2^- production) was detected in all sediments exhibiting microbial activity, with the most denitrification occurring in samples from Borehole A (Figures 3 and 4). NO_3^- did not consistently stimulate mineralization among all the active sediments. In the Borehole A 17.4 m and Borehole B 11.9 m samples, naphthalene mineralization under NO_3^--amended conditions was not significantly different from unamended conditions, and mineralization in the Borehole B 13.7 m sediments was inhibited by NO_3^- amendments.

The effect of PO_4^{3-} amendments on naphthalene mineralization also varied between sediments (Figure 2). In three of the six active sediments (Borehole A

11.6, 17.4, and 25.3 m), PO_4^{3-} did not significantly effect mineralization relative to unamended microcosms. PO_4^{3-} was also observed, however, to significantly enhance (Borehole B 13.7 m) and inhibit (Borehole A 13.1 m and Borehole B 11.9 m) mineralization. Analysis of the microcosm pore water indicates that, with the exception of one sample (Borehole A 25.3 m), greater than 90% of the added PO_4^{3-} was removed from solution by the end of the 6 week incubation period (Figure 4), presumably by abiotic reactions.

In the majority of cases, the mineralization response in microcosms receiving 25 mg/L NO_3^- and 90 mg/L PO_4^{3-} appeared slightly greater than the response in microcosms receiving PO_4^{3-} alone, but the difference between these two treatments was not significant. For the Borehole A 13.1 m and 25.3 m samples, however, the combined addition of NO_3^- and PO_4^{3-} significantly stimulated mineralization relative to microcosms receiving only PO_4^{3-}, reflecting the same positive effect NO_3^- alone had on these two sediments.

Four of the ten sediments (Borehole B 18.6, 21.6, and 22.2 m, and Borehole C 13.7 m) were not able to mineralize naphthalene during the six week incubation, nor were they able to consume NO_3^-. The aqueous chemistry in the microcosms for these sediments possessed some common characteristics, including a presence of detectable dissolved Fe^{2+} (30–265 mg/L), low pH (< 4.1), and high SO_4^{2-} concentrations ranging from 600 to 1300 mg/L (Figure 5).

Discussion

Oxygen limitations

For the majority of active sediments, O_2 amendments substantially improved the extent of naphthalene removal, highlighting the crucial role O_2-availability plays in governing the fate of unsubstituted aromatic compounds in contaminated aquifers. Since methanol was introduced into the microcosms as the carrier solvent for naphthalene, it was not possible to quantify the amount of O_2 consumed during ^{14}C-naphthalene degradation. Stoichiometric relationships indicate, however, that while the initial dissolved O_2 was sufficient to mineralize 4 mg/L naphthalene, the methanol exerted a large additional oxygen demand that could only be satisfied by O_2 mass transfer from the microcosm headspace (Table 1). Considering these stoichiometric requirements along with the observed

effect of O_2 on naphthalene mineralization, it is likely that the rate and extent of naphthalene mineralization in these sediments was limited primarily by O_2 availability.

Abiotic reactions may have also reduced O_2 availability. At near neutral pH, O_2 is rapidly consumed in the oxidation of Fe^{2+} by the reaction: $Fe^{2+} + 1/4O_2 + 5/2H_2O => Fe(OH)_3(s) + 2H^+$. Since the concentrations of HCl-extractable Fe^{2+} in all the sediments were substantial (33–697 mg/kg) even at the end of the 6 week incubation (Figure 5), it is likely that Fe^{2+} exerted a significant abiotic O_2 demand in some cases. Moreover, dissolved Fe^{2+} (30–265 mg/L) was only detected in microcosms for those sediments in which no naphthalene mineralization was observed (Figure 5). In these sediments the presence of dissolved Fe^{2+} provided an immediate abiotic O_2 demand that may have reduced the concentration of O_2 available for biodegradation.

These data do not prove that Fe^{2+} inhibited aerobic naphthalene mineralization, but they are useful to the extent that they underscore the importance of recognizing abiotic oxygen demands in the design of aerobic bioremediation strategies and the prediction of aerobic bioremediation rates. In addition, the oxidation of ferrous iron Fe^{2+} is undesirable because the resulting formation of $Fe(OH)_3(s)$ can lead to plugging of aeration wells, and the pH drop caused by this reaction may inhibit microbial proliferation (Applin & Zhao 1989; Bajpai et al. 1994). To minimize the adverse effects of dissolved Fe^{2+} oxidation, aerobic bioremediation strategies in reduced aquifers should employ ground water extraction and reinjection systems that include Fe-removal (as $Fe(OH)_3(s)$) and pH neutralization as components in the surface pretreatment train, prior to reinjection. In cases where significant concentrations of FeS(s) and $FeCO_3(s)$ are present in the target formation, it may be appropriate to spike the injectate with a carbonate buffer to neutralize the pH and/or an iron-sequestering agent to inhibit crystal nucleation of $Fe(OH)_3(s)$ (Bajpai et al. 1994; Bowlen & Kossen 1995).

Effect of NO_3^-

Although the effect of NO_3^- varied considerably, denitrification was evident in all six of the active sediments (Figures 3 and 4). These data indirectly support the conclusion that oxygen concentrations were low in the sediment-water microcosms because denitrification does not typically occur at O_2 concentrations
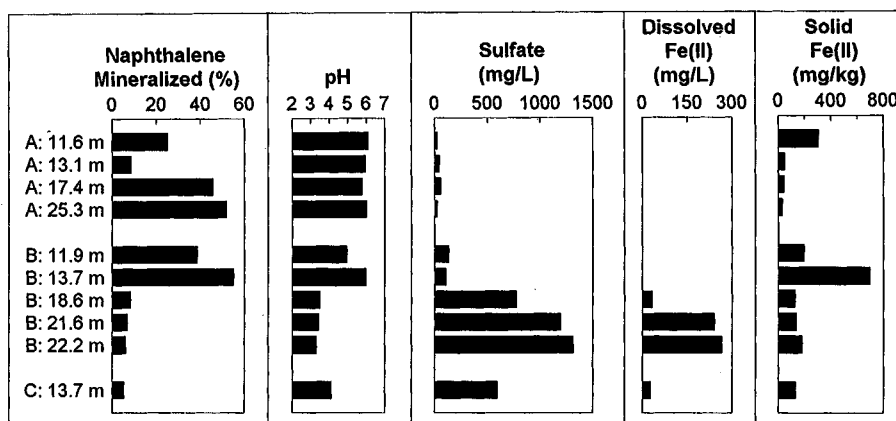
Figure 5. ^{14}C-Naphthalene mineralization, pH, and sulfate and Fe(II) concentrations measured in high-oxygen (21 mg/L at start of experiment) microcosms after 6 weeks of incubation. The vertical axis indicates the Borehole (A, B, or C) and sample depth.

Table 1. Stoichiometry of naphthalene ($C_{10}H_8$) and methanol (CH_3OH) biodegradation under aerobic and denitrifying conditions (after McCarty, 1975)

$$C_{10}H_8 + 5.83O_2 + 0.88NO_3^- + 0.88H^+ \Rightarrow 0.88C_5H_7O_2N + 5.6CO_2 + 1.34H_2O$$
$$C_{10}H_8 + 5.66NO_3^- + 5.66H^+ \Rightarrow 0.86C_5H_7O_2N + 5.71CO_2 + 3.81H_2O + 2.40N_2$$
$$CH_3OH + 0.49O_2 + 0.14NO_3^- + 0.14H^+ \Rightarrow 0.14C_5H_7O_2N + 0.29CO_2 + 1.57H_2O$$
$$CH_3OH + 0.62NO_3^- + 0.62H^+ \Rightarrow 0.12C_5H_7O_2N + 0.38CO_2 + 1.87H_2O + 0.25N_2$$

above 2 mg/L (Wilson & Bouwer 1997). In the Borehole A 11.6, 13.1, and 25.3 m sediments, the initial rate of naphthalene mineralization was fastest in microcosms amended with 85 mg/L NO_3^- (Figure 2), and this mineralization was accompanied by substantial denitrification (Figure 3). Stoichiometric relationships (Table 1) indicate that for those active sediments in which naphthalene mineralization was either stimulated or unaffected by the addition of 85 mg/L NO_3^-, the amount of NO_3^- consumed exceeded the amount necessary for mineralizing the 4 mg/L naphthalene. Since the fraction of natural organic carbon in the sediment is relatively low ($f_{OC} < 0.001$), it is likely that methanol served as an electron donor in denitrification (Metcalf and Eddy 1991). Naphthalene oxidation coupled to denitrification has been reported previously (Mihelcic & Luthy 1988), but this possibility could not be evaluated in this work since the microcosms were prepared and incubated under aerobic conditions.

Relative to unamended microcosms, NO_3^- amendments inhibited naphthalene mineralization in the Borehole B 13.7 m sample. This same phenomena has been observed by others, but the mechanism of inhibition is not understood (Manilal & Alexander 1991).

Morgan and Watkinson (1992) proposed that inhibition by NO_3^- can be caused by the oligotrophic nature of aquifer bacteria and/or shifts in the predominating populations and metabolic pathways of the consortia.

Effect of PO_4^{3-}

For microcosms amended with PO_4^{3-}, the most consistent observation among all the sediments was the removal of PO_4^{3-} from solution after 6 weeks of incubation (Figure 4). In six of the ten sediments, the phosphate was removed to non-detectable levels, and in three out of the four remaining sediments > 90% of the phosphate was removed from the porewater. Assuming an average cellular composition of $C_5H_7O_2N_1P_{0.0833}$ (Metcalf and Eddy 1991), only a fraction of the added 90 mg/L PO_4^{3-} could have been consumed for biomass growth during the degradation of naphthalene and methanol. Since dissolved and solid iron phases were abundant in the microcosms (Figures 4 and 5), the bioavailability of PO_4^{3-} was probably limited by sorption onto ferric-(hydr)oxide surfaces (McLaughlin et al. 1981) and/or by precipitation of iron phosphates such as strengite (Fe(III)PO$_4$-

$2H_2O(s))$ and vivianite $(Fe(II)_3(PO_4)_2\text{-}8H_2O(s))$. The sorption hypothesis is supported in that the only sample in which significant concentrations of PO_4^{3-} remained in solution (Borehole A 25.3 m) also had the lowest concentrations of HCl-extractable iron (Figure 4). Calculations also indicate that the phosphate-amended microcosms for the four sediments with dissolved Fe^{2+} were highly oversaturated with respect to vivianite (assumes $K_{SO} = 10^{-36}$ at 25 °C (Niragu 1972)). It should be recognized that phosphate availability is also significantly controlled by Ca^{2+}, a parameter which was not measured in this study.

In the four active sediments (Borehole A 11.6, 13.1, 17.4, and 25.3 m) in which detectable amounts of PO_4^{3-} remained in solution at the end of six weeks, PO_4^{3-} was not observed to stimulate naphthalene mineralization relative to unamended microcosms. This result suggests that the background supply of P was sufficient to support biodegradation in these four sediments. In the Borehole B 13.7 m sample, however, PO_4^{3-} clearly enhanced mineralization, indicating P-limited biodegradation.

Given the work of Swindoll et al. (1988), the finding that PO_4^{3-} inhibited mineralization in some sediments was not unexpected, but the causes of such inhibition remain unclear. Steffensen & Alexander (1995) proposed that in systems containing a bacterial consortia and multiple substrates, bacterial competition for added inorganic nutrients may promote the biodegradation of certain substrates over others. The addition of PO_4^{3-} may have stimulated the growth of methanol-degrading bacteria, out-competing the naphthalene-degrading bacteria for electron acceptors and other growth factors.

Because PO_4^{3-} only stimulated degradation in one of the sediments studied, and substantial concentrations of PO_4^{3-} appeared to sorb and/or precipitate consistently, it will no longer be considered as a bioremediation additive at the MGP site. Solid and dissolved phase iron concentrations at the site are too high to permit effective use of PO_4^{3-} for in situ bioremediation. Future efforts will investigate the biodegradation response to using alternative P-sources such as metaphosphates. These P-sources are attractive for in situ bioremediation because they hydrolyze slowly, and are thus less reactive than introducing pure orthophosphate (Aggarwal et al. 1991; Stieof & Dott 1995).

Spatial heterogeneity in biodegradation response to stimuli

Investigations on subsurface microbiology typically observe significant heterogeneity in microbial distribution and biodegradation activity within and between aquifers (Hickman & Novak 1989). In most studies, these two parameters correlate positively with sediment texture and formation permeability (Fredrickson et al. 1989; Sinclair et al. 1990). A variety of other factors may also contribute to heterogeneity in microbial activity between aquifer sediments, including the type and concentration of available electron acceptors, pH, the adaptation to the presence of organic contaminants, and the f_{OC}. Sediments with a high f_{OC} tend to reduce the bioavailability of hydrophobic substrates, thus reducing biodegradation and microbial growth rates.

The present study attempted to control the variable impacts of texture, adaptation, and bioavailability by working with samples that were 1) of relatively uniform texture (medium-to-coarse grained sands), 2) collected from areas that had been exposed to low to moderate levels of naphthalene contamination for decades prior to this study, and 3) of low organic carbon content ($f_{OC} < 0.001$). From the results it is apparent that the distribution of aerobic naphthalene-degrading bacteria is non-uniform within the contaminated, sandy layers of the aquifer. Among the active sediments, O_2 stimulated naphthalene mineralization in most cases, but the effects of NO_3^-, and PO_4^{3-} varied considerably. These data likely reflect some combination of heterogeneity in the bioavailability of nutrients, in the distribution of abiotic species likely to compete with bacteria for PO_4^{3-}, and in the occurrence of bacteria capable of using NO_3^- as an electron acceptor in the degradation of naphthalene and/or naphthalene biotransformation intermediates. In MGP aquifers where NO_3^- and PO_4^{3-} (or other N and P forms) are used to augment aerobic in situ bioremediation, practitioners should expect that N and P amendments will have the potential to enhance bioremediation in certain zones of the target formation and have a negligible effect on bioremediation in other zones. In addition, these nutrients may stimulate the biodegradation of certain pollutants while failing to effect the biodegradation of others.

Conclusions

Contaminant-acclimated sediments from a MGP aquifer have the capacity to aerobically mineralize significant amounts of naphthalene without the addition of N and P. In certain portions of the aquifer, the rate at which aqueous phase aromatic hydrocarbons biodegrade appears to be controlled primarily by oxygen availability, and the addition of O_2 can accelerate this biodegradation substantially. O_2 addition failed to stimulate mineralization in samples from other portions of the aquifer, indicating that an efficient aerobic bioremediation strategy will require a site-specific design within the MGP aquifer. It is encouraging that NO_3^- was observed to stimulate the initial rate of naphthalene mineralization in some sediments, but conclusive evidence of naphthalene mineralization under denitrifying conditions was not obtained. Nonetheless, use of NO_3^- as an electron acceptor for bioremediation of naphthalene-contaminated aquifers offers promise because it can promote biodegradation of co-occurring alkylated and hydroxylated aromatic compounds, as well as naphthalene biotransformation intermediates.

Geochemistry plays a fundamental role in governing the spatial trends observed in studies examining the biodegradation potential of aquifer sediments, both in the presence and absence of nutrient amendments. Some of the aquifer sediments examined in this work exhibited a strong tendency to sorb and/or precipitate PO_4^{3-}, and some were characterized by inhibitory pH values (< 4.1). In addition, the presence of dissolved and solid phase Fe^{2+} in some sediments created a significant abiotic O_2 demand that likely affected the availability of O_2 for biodegradation. Accordingly, studies investigating biodegradation potential and the influence of various parameters on biodegradation in aquifer sediments need to adopt routine analyses of the inorganic chemistry in the sediment and sediment pore water. In addition, if engineers continue to use PO_4^{3-} for in situ bioremediation applications, research is needed to investigate the bioavailability of sorbed or complexed forms of PO_4^{3-} in aquifer sediments.

Acknowledgments

References

Aggarwal PK, Means JL & Hinchee RE (1991) Formulation of nutrient solutions for in situ bioremediation. In: Hinchee RE & Olfenbuttel RF (Eds) In Situ Bioreclamation: Applications and Investigations for Hydrocarbon and Contaminated Site Remediation (pp. 51–66). Butterworth-Heinemann, Stoneham, MA

Allen-King RM, Barker JF, Gillham RW & Jensen BK (1994) Substrate- and nutrient-limited toluene biotransformation in sandy soil. Environ. Tox. Chem. 13: 693–705

Anid PJ, Alvarez PJJ & Vogel TM (1993) Biodegradation of monoaromatic hydrocarbons in aquifer columns amended with hydrogen peroxide and nitrate. Water Res. 27: 685–691

Applin KR & Zhao N (1989) The kinetics of Fe(II) oxidation and well screen encrustation. Ground Water 27: 168–174

Armstrong AQ, Hodson RE, Hwang H-M & Lewis DL (1991) Environmental factors affecting toluene degradation in ground water at a hazardous waste site. Environ. Tox. Chem. 10: 147–158

Bajpai RK, Zappi ME & Gunnison D (1994) Additives for establishment of biologically active zones during in situ bioremediation. Annals of the New York Academy of Sciences 450–465

Barker JF, Patrick GC & Major D (1987) Natural attenuation of aromatic hydrocarbons in a shallow sand aquifer. Ground Water Monit. Rev. 7: 64–71

Bowlen GF & Kosson DS (1995) In situ processes for bioremediation of BTEX and petroleum fuel products. In: Microbial Transformation and Degradation of Toxic Organic Chemicals (pp. 515–542). Wiley & Sons, New York

Chiang CY, Salanitro JP, Chai EY, Colthart JD & Klein CL (1989) Aerobic biodegradation of benzene, toluene, and xylene in a sandy aquifer – data analyses and computer modeling. Ground Water 27: 823–834

Durant ND, Wilson LP & Bouwer EJ (1995) Microcosm studies of subsurface PAH-degrading bacteria from a former manufactured gas plant. J. Cont. Hydrol. 17: 213–237

Efroymson RA & Alexander M (1994) Biodegradation in soil of hydrophobic pollutants in nonaqueous-phase liquids (NAPLs). Environ. Tox. Chem. 13: 405–411

Fredrickson JK, Garland TR, Hicks RJ, Thomas JM, Li SW & McFadden KM (1989) Lithotrophic and heterotrophic bacteria in deep subsurface sediments and their relation to sediment properties. Geomicrobiol. J. 7: 53–66

Gantzer CJ & Cosgriff D (1995) Membrane dissolution at the Libby, Montana, Superfund site. In: Hinchee RE, Miller RN & Johnson PC (Eds) In Situ Aeration: Air Sparging, Bioventing, and Related Remediation Processes (pp. 543–549). Battelle Press, Columbus, OH

Heron G, Crouzet C, Bourg ACM & Christensen TH (1994). Speciation of Fe(II) and Fe(III) in contaminated aquifer sediments using chemical extraction techniques. Environ. Sci. Tech. 28: 1698–1705

Hickman GT & Novak JT (1989) Relationship between subsurface biodegradation rates and microbial density. Environ. Sci. Tech. 23: 525–532

Hutchins SR, Downs WC, Wilson JT, Smith GB, Kovacs DA, Fine DD, Douglass RH & Hendrix DJ (1991) Effect of nitrate addition

on biorestoration of a fuel-contaminated aquifer: field demonstration. Ground Water 29: 571–580

Hutchins SR, Moolenaar SW & Rhodes DE (1992) Column studies on BTEX degradation under microaerophilic and denitrifying conditions. J. Haz. Materials 32: 195–214

Jamison VW, Raymond RL & Hudson Jr. JO (1975) Biodegradation of high-octane gasoline in groundwater. Develop. Ind. Microbiol. 16: 305–312

Johnson RL, Johnson PC, McWhorter DB, Hinchee RE & Goodman I (1993) An overview of in situ air sparging. Ground Water Monit. and Remediation 13: 127–135

Kampfer P, Stieof M, Becker PM & Dott W (1993) Characterization of chemoheterotrophic bacteria associated with the in situ bioremediation of a waste-oil contaminated site. Microb. Ecol. 26: 161–188

Keeney DR & Nelson DW (1982) Nitrogen – Inorganic Forms. In: Page AL, Miller RH & Keeney DR (Eds.) Methods of Soil Analyses Part 2: Chemical and Microbiological Properties, 2nd Edition (pp. 643–698). American Society of Agronomy, Madison, WI

Kuhn EP, Colberg PJ, Schnoor JL, Wanner O, Zehnder AJB & Schwarzenbach RP (1985) Microbial transformations of substituted benzenes during infiltration of river water to groundwater: laboratory column studies. Environ. Sci. Tech. 19: 961–968

Lee MD, Thomas JM, Borden RC, Bedient PB, Ward CH & Wilson JT (1988) Biorestoration of aquifers contaminated with organic compounds. CRC Crit. Rev. in Environ. Cont. 18: 29–89

Leethem JT, Beeman RE, Lee MD, Biehle AA, Ellis DE & Shoemaker SH (1995) Anaerobic in situ bioremediation: Injected nutrient and substrate fate and transport. In: Hinchee RE, Kittel JA & Reisinger HJ (Eds) Applied Bioremediation of Petroleum Hydrocarbons (pp. 271–280). Battelle Press, Columbus, OH

Lewis DL, Kollig HP & Hodson RE (1986) Nutrient limitation and adaptation of microbial populations to chemical transformation. Appl. Environ. Microbiol. 51: 598–603

Lovley DR & Phillips EJP (1987) Rapid assay for microbially reducible ferric iron in aquatic sediments. Appl. Environ. Microbiol. 53: 1536–1540

Manilal VB & Alexander M (1991) Factors affecting the microbial degradation of phenanthrene in soil. Appl. Microbiol. Biotech. 35: 401–405

McCarty PL (1975) Stoichiometry of biological reactions. Prog. Water Technol. 7: 157–172

McLaughlin JR, Ryden JC & Syers JK (1981) Sorption of inorganic phosphate by iron and aluminum-containing components. J. Soil Sci. 32: 365–377

Metcalf and Eddy, Inc. (1991) Wastewater Engineering Treatment, Disposal, and Reuse, 3rd Edition (pp. 359–444). McGraw-Hill, New York

Mihelcic JR & Luthy RG (1988) Microbial degradation of acenaphthene and naphthalene under denitrification conditions in soil-water systems. Appl. Environ. Microbiol. 54: 1188–1198

Morgan P & Watkinson RJ (1992) Factors limiting the supply and efficiency of nutrient and oxygen supplements for the in situ biotreatment of contaminated soil and groundwater. Water. Res. 26: 73–78

Niragu JO (1972) Stability of vivianite and ion-pair formation in the system $Fe_3(PO_4)_2$-H_3PO_4-H_2O. Geochim. Cosmochim. Acta 36: 459–470

Olsen SR & Sommers LE (1982) Phosphorus. In: Page AL, Miller RH & Keeney DR (Eds) Methods of Soil Analyses Part 2: Chemical and Microbiological Properties, 2nd Edition (pp. 403–430). American Society of Agronomy, Madison, WI

Reinhard M, Shang S, Kitanidis PK, Orwin E, Hopkins GD & Lebron CA (1997) In situ BTEX biotransformation under enhanced nitrate and sulfate reducing conditions. Environ. Sci. Tech. 31: 28–36

Sinclair JL, Randtke SJ, Denne JE, Hathaway LR & Ghiorse (1990). Survey of microbial populations in buried-valley aquifer sediments from Northeastern Kansas. Ground Water 28: 369–377

Steffensen WS & Alexander M (1995) Role of competition for inorganic nutrients in the biodegradation of mixtures of substrates. Appl. Environ. Microbiol. 61: 2859–2862

Stieof M & Dott W (1995) Application of hexametaphosphate as a nutrient for in situ bioreclamation. In: Hinchee RE, Kittel JA & Reisinger HJ (Eds) Applied Bioremediation of Petroleum Hydrocarbons (pp. 301–310). Battelle Press, Columbus, OH

Stookey LL (1970) Ferrozine – a new spectrophotometric reagent for iron. Anal. Chem. 42: 779–781

Swindoll MC, Aelion CM & Pfaender FK (1988) Influence of inorganic and organic nutrients on aerobic biodegradation and on the adaptation response of subsurface microbial communities. Appl. Environ. Microbiol. 54: 212–217

Wilson JT, Armstrong JM & Rafai HS (1994) A full-scale field demonstration on the use of hydrogen peroxide for in situ bioremediation of an aviation gasoline-contaminated aquifer. In: Flathman PE, Jerger DE & Exner JH (Eds) Bioremediation: Field Experience (pp. 333–359). Lewis Publishers, Boca Raton, FL

Wilson JT, McNabb JF, Cochran JW, Wang TH, Tomson MB & Bedient PB (1985) Influence of microbial adaptation on the fate of organic pollutants in ground water. Environ. Tox. Chem. 4: 721–726

Wilson LP & Bouwer EJ (1997) Biodegradation of aromatic compounds under mixed oxygen/nitrate electron acceptor conditions. J. Ind. Microbiol. Biotech. 18: 116–130