In Situ Measurement and Numerical Simulation of Oxygen Limited Biotransformation

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Abstract

Enhanced subsurface biorestoration is rapidly becoming recognized as a valuable tool for the restoration of hydrocarbon-contaminated aquifers and sediments. Previous field and laboratory studies at a former wood creosoting facility near Conroe, Texas, have indicated that insufficient oxygen is the primary factor limiting the biotransformation of polynuclear aromatics (PNAs) in sediments and ground water at this site. A series of laboratory experiments and field push-pull injection tests were performed as part of this project to: (1) study the effect of low oxygen concentrations on the biotransformation of PNAs; (2) identify the minimum concentration of PNAs that could be achieved through the addition of oxygen alone; (3) confirm that enhanced subsurface biorestoration is feasible at this site; and (4) test an existing numerical model of the biotransformation process (BIOPLUME). The laboratory studies demonstrated that biotransformation of the PNAs was not inhibited at dissolved oxygen concentrations as low as 0.7 mg/L although this work did suggest that there may be a minimum PNA concentration of 30 to 70 μg/L total PNAs below which biotransformation was inhibited. The field push-pull tests confirmed that addition of oxygen was effective in enhancing the subsurface biodegradation of the PNAs. The minimum concentration achieved using oxygen alone was approximately 60 μg/L total PNAs. Minimal biotransformation of these compounds was observed without oxygen addition. The numerical model BIOPLUME was tested against monitoring data from the field experiments and appears to provide a good approximation of the biodegradation process.

Introduction

Microbial activity in subsurface environments contaminated with organic chemicals can be limited by the availability of inorganic nutrients and dissolved oxygen (DO), the absence of an acclimated microbial population, and the presence of toxicants (Thomas et al. 1987). At a site contaminated with wood creosoting wastes near Conroe, Texas, DO appeared to be the major limitation on biodegradation once the microbial population had acclimated to the contaminants (Lee and Ward 1985, Wilson et al. 1985). An active microbial population has been found in the creosote-contaminated aquifer material and ground water that is capable of rapidly degrading many of the contaminants including naphthalene, 2-methylnaphthalene, dibenzofuran, fluorene, acenaphthene, anthracene, and 1-methylnaphthalene in laboratory microcosms (Lee et al. 1984, Wilson et al. 1985). However, in areas that have not been impacted by the creosote wastes, the microorganisms in the subsurface material were not acclimated to the contaminants and were unable to reduce the concentrations of a number of aromatics after incubation for eight weeks (Wilson et al. 1985). Wilson et al. (1985) divided the creosoting site into the following four areas based upon the quantities of oxygen, chloride, and polynuclear aromatics (PNAs) found in the ground water: (1) the pristine zone contained little or no PNAs or chlorides and 2 to 3 mg/L DO; (2) a plume of contamination in which high chlorides and PNAs were found and non-detectable oxygen; (3) a zone of active treatment where the chlorides were elevated, oxygen was depleted, and the PNAs were reduced; and (4) a treated zone where chlorides were higher than the background, PNAs were greatly reduced, and low quantities of oxygen were present. Additional monitoring at the creosoting site demonstrated that the ability to degrade benzene, toluene, and *o*- and *m*- xylene was correlated with elevated levels of ATP (Wilson et al. 1986). This correlation may have been the result of increased microbial growth due to the presence of the organics. The creosote contamination may have also affected the composition of the microbial population causing a reduction in the relative percentage of Gram-positive organisms (Smith et al. 1985). Lee and Ward (1985) demonstrated that the addition of inorganic nutrients (nitrogen, phosphate, and trace metals) to creosote-contaminated ground water did not increase the rate of biotransformation of several aromatic compounds, although the minimum concentration achieved by biotransformation was lower than that without nutrients.

The field work and laboratory experiments described in this paper were conducted at or using samples from the former creosoting site in Conroe, Texas. This facility was operated by the United Creosoting Co. (UCC) from 1946 until 1972. Wastes from the operation were disposed in two unlined pits. This resulted in contamination of the shallow aquifer beneath the site. In 1983, the site was added to the National Priorities List of the Comprehensive Environmental Response, Compensation and Liability Act and is currently under investigation to determine the appropriate remedial action for cleanup. The major organic contaminants present in the ground water are PNAs including naphthalene, dibenzofuran, phenanthrene, 2-methylnaphthalene, and fluorene at concentrations up to 3.5 mg/L. Other aromatic compounds are found, but typically at lower concentrations. The geology of the site is extremely heterogeneous with numerous and rapid changes between relatively clean, well-sorted sand, and sandy clay. Two separate water-bearing zones have been identified: (1) a lower permeability unconfined zone; and (2) a slightly higher permeability semiconfined zone. These two zones are separated by a thin semiconfining layer. The push-pull tests were performed in the semiconfined zone approximately 250 feet (75m) south of the former pits. The semiconfined zone is composed of medium fine clayey sand with a permeability of approximately 3 ft/d (1 m/d). The gradient of the piezometric surface in the area is low, resulting in a ground water velocity of approximately 17 ft/yr (5 m/yr). Borden and Bedient (1987) described a three-well injection-production test at this site in which adsorption of naphthalene and 1,4-dichlorobenzene was found to be negligible.

Methods — Laboratory Biotransformation Studies

Batch ground water biotransformation studies were carried out to determine the effect of dissolved oxygen on the rate of biotransformation and the minimum achievable PNA concentration. Ground water used in these studies was collected after pumping three or more well volumes from existing monitoring wells. The incubation vessels were replicate 25mL vials capped with Teflon®-lined septa or 1-gallon amber glass bottles. Controls were prepared by adding 1 percent sodium azide, a microbial respiration inhibitor. The DO content of the water was adjusted by purging the water with either air or nitrogen. Dissolved oxygen levels were determined in triplicate using a YSI probe and meter. Organic analyses were performed by extracting a composite water sample using the Sep-pak extraction procedures outlined by Wang et al. (1985). Organic quantification was by gas chromatography using a Hewlett-Packard ultra-performance silica capillary column with cross-lined 5 percent phenyl methyl silicone packing. Peak identifications are based on retention time matching with the quantification based upon relative peak response factors of known standards. The concentrations of the various PNAs were determined over time.

Methods — Field Push-Pull Tests

Two push-pull tests were performed in the semiconfined zone of the shallow aquifer at the UCC site in order to examine the effect of oxygen addition on the in situ biotransformation of PNAs present in the shallow aquifer and to test an existing biotransformation model (BIO-PLUME, Borden and Bedient 1986). In the first test, anoxic ground water from a well in the contaminated zone (RU-2) was pumped into two 55-gallon (7.4 ft^3 or 210 liter) drums, spiked with 280 mg/L chloride, deoxygenated by bubbling with nitrogen for 30 minutes, and injected rapidly into a well in the zone of active treatment (RU-1). Water was withdrawn from this well at a rate of 0.11 ft^3/hr (50 mL/min) and samples were taken periodically for determination of organics, DO, conductivity, and chloride. A second test (aerobic) was conducted in the same manner except that air was used to increase the concentration of DO of the water to about 6.0 mg/L prior to injection. Water samples were collected during both tests by withdrawing water from the well through approximately 30 feet (10m) of 0.25 in (0.6cm) Teflon tubing using a peristaltic pump. Target organics were analyzed by gas chromatography using the methods outlined previously. Chloride was determined by a Hach test kit. Conductivity was measured with a YSI conductivity meter. Oxygen was monitored at the surface with a YSI meter and probe and by a Chemetrics test. During the field tests, the minimum oxygen concentration observed was 0.7 mg/L. This observed minimum is thought to have been due to diffusion of oxygen through the Teflon tubing during sample collection rather than a lower limit of microbial activity. Holm et al. (1986) developed a relationship for estimating the change in oxygen concentration due to diffusion through Teflon tubing. Using this relationship, approximately 0.8 mg/L oxygen would be expected to enter the sample. Based on this analysis, the analytical detection limit for DO has been assumed to be 0.8 mg/L.

Laboratory Results

Laboratory experiments were conducted to determine the effect of low oxygen and organic substrate concentrations on the biotransformation of hazardous organic compounds.

An experiment was designed to examine the effect of oxygen concentration on the removal of the selected aromatics. Water samples from a contaminated well (RU-2) were purged with nitrogen or oxygen to adjust the DO to 1.8 or 8.0 mg/L. Figures 1a and 1b show the decline in the sum of naphthalene, 2-methylnaphthalene, fluorene, phenanthrene, and dibenzofuran (Figure 1a) and oxygen (Figure 1b) concentrations for the samples with high levels of DO, the samples with low levels of DO, and the sodium azide-amended controls. Rapid PNA removal rates were observed for both the high and low DO samples, while less was removed from the sodium azide-amended control. The maximum rate of hydrocarbon removal in the microcosm with high DO levels was 291 μg/L/d (Day 0 to 2, DO = 6.5 to 9.0 mg/L), while the maximum removal rate in the microcosm with low DO levels was 483 μg/L/d (Day 3 to 4, DO = 0.7 mg/L). These data clearly demonstrate that DO is not a major limitation at concentrations greater than 0.7 mg/L. Previous studies have shown that at very low oxygen concen-

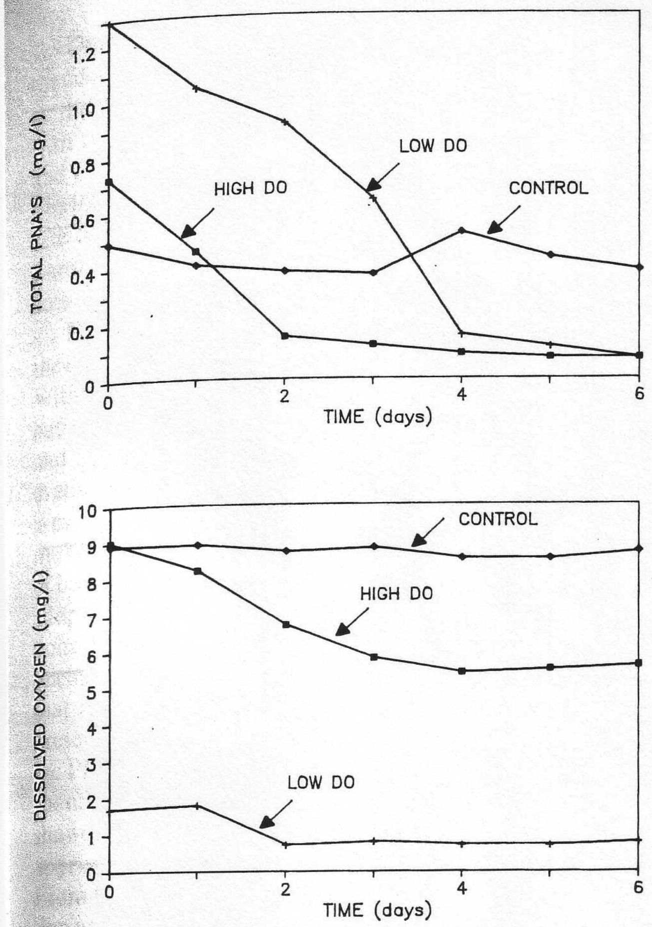

Figure 1. Effect of dissolved oxygen on biotransformation rates. Observed concentrations of: (a) total PNAs (naphthalene, 2-methylnaphthalene, fluorene, dibenzofuran, and phenanthrene); and (b) dissolved oxygen.

trations, DO will limit the biotransformation of organics (Lee and Ward 1985, Atlas 1981). In this study, the minimum DO concentration was apparently not low enough to significantly inhibit biotransformation. The one unexpected finding of this study was the apparent persistence of low concentrations of organics in the microcosms. Removal of the organics ceased at concentrations ranging from 5 to 30 μg/L for the individual compounds. From Day 4 until the end of the experiment, the sum of the PNAs remained essentially constant at 70 to 80 μg/L in the high DO microcosm even though 5 mg/L of oxygen remained. The apparent persistence of these organics suggests that there may be a minimum concentration below which biotransformation does not occur. Results from previous studies (Lee and Ward 1985) indicate that the addition of inorganic nutrients may reduce the level of PNAs that can be achieved by microbial biotransformation.

A second experiment was performed to determine the long-term persistence of a more extensive variety of polynuclear aromatics. Ground water samples from a contaminated well (RU-2) were incubated in amber 1-gallon glass bottles for 28 weeks. The total PNA concentration was calculated as the sum of the following organic compounds: o-cresol, borneol, naphthalene, 2-methylnaphthalene, 1-methylnaphthalene, biphenyl, 1,2-dimethylnaphthalene, acenaphthene, dibenzofuran,

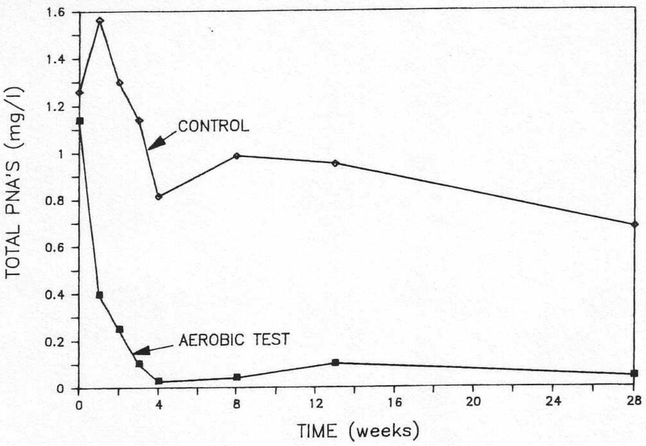

Figure 2. Total PNA concentration in ground water from RU-2 over a 28-week incubation period. Total PNA defined as the sum of o-cresol, borneol, naphthalene, 2-methylnaphthalene, 1-methylnaphthalene, biphenyl, 1,2-dimethylnaphthalene, acenaphthene, dibenzofuran, fluorene, dibenzothiophene, phenanthrene, and carbazole.

fluorene, dibenzothiophene, phenanthrene, and carbazole. The decline in the total PNA concentration during the 28-week incubation is shown in Figure 2 for the unamended ground water and the sodium azide-amended control. The total PNA concentration dropped rapidly in the unamended ground water during the first three weeks of the experiment before reaching a plateau of approximately 30 to 40 μg/L. All of the compounds from o-cresol to fluorene could be detected at Week 28 at concentrations between 3 and 35 μg/L (data not shown), which suggests that the microorganisms cannot completely remove the contaminants or that their activity is significantly reduced. Dissolved oxygen levels in the sample were sufficient; 6.5 mg/L of oxygen was measured in the test sample after 13 weeks incubation. The small increase in total PNA concentration observed during Week 13 is thought to be experimental error. Some disappearance of PNAs was also detected in the sodium azide-treated ground water. This may have been due to volatilization or sorption to the bottle, or the organisms may have adapted to the sodium azide inhibitor. Thomas et al. (1988) provide information of the breakdown of individual PNAs.

Push-Pull Test Results

The chloride, conductivity, oxygen, and PNA concentrations observed during the two push-pull tests are shown in Table 1. Direct comparison of data from the two tests is complicated by differences in the injection and background concentrations of the different compounds. A convenient method of analyzing data under these conditions is to plot the normalized concentration (c′) where

$$c' = \frac{\text{(observed concentration - background concentration)}}{\text{(injection concentration - background concentration)}}$$

For example, the normalized chloride concentration at Day 1 for the anoxic test was (189-70)/(280-70) or c′ = 0.57. Normalized concentration curves for chloride, conductivity and the sum of the four quantified PNAs (naphthalene, 2-methylnaphthalene, dibenzofuran, and

TABLE 1
Push-Pull Test Results

Anoxic Test

Day	Volume (cu. ft.)	Chloride (mg/L)	Conductivity (μmho)	Oxygen (mg/L)	Total PNA (mg/L)
0	0	280	830	0.9	1.175
1	2.76	189	580	<0.8	0.521
2	5.24	129	432	<0.8	0.270
3	7.38	99	370	<0.8	0.208
4	9.50	77	328	<0.8	0.156
6	9.50	83	322	<0.8	0.120
8	14.57	74	262	<0.8	0.170

Aerobic Test

Day	Volume (cu. ft.)	Chloride (mg/L)	Conductivity (μmho)	Oxygen (mg/L)	Total PNA (mg/L)
0	0	290	905	4.0	0.936
1	2.18	229	700	0.8	0.075
2	4.33	166	540	<0.8	0.060
3	6.59	119	418	<0.8	0.121
5	11.52	89	348	<0.8	0.106

fluorene) vs. total volume of water produced from the well are shown in Figures 3 and 4 for the anoxic and aerobic push-pull tests, respectively. These four organics were selected because they occur in the highest concentrations at the site and generally constitute 70 percent to 80 percent of the total hydrocarbons present. Oxygen concentrations are not plotted here because there was only one observation during the anoxic test and two observations during the aerobic test that were above the analytical detection limit of 0.8 mg/L. Injection and background concentrations used in the calculations are shown in Table 2.

Chloride concentration and conductivity decreased rapidly during both tests, returning to background levels after approximately 12.4 ft^3 (354 L) had been produced. This is somewhat surprising because approximately 14.7 ft^3 (416 L) of water was injected during both tests. Classical advection-dispersion theory would predict that the normalized concentration of chloride and conductivity would be greater than 0.50 until at least one injection volume had been produced. Various hypotheses were examined to try to explain this apparent inconsistency. The only hypothesis found that adequately matched the chloride and conductivity data assumes that a fraction of the injection fluid was somehow lost. During the push-pull tests, the injection rates were very high to avoid loss of the organics during the injection phase (Reynold's number = 10 at injection well). These high injection rates may have forced some of the injection water through the semiconfining layer where it could not be recovered. In all further data analysis, a fraction of the injection volume has been assumed lost from the system.

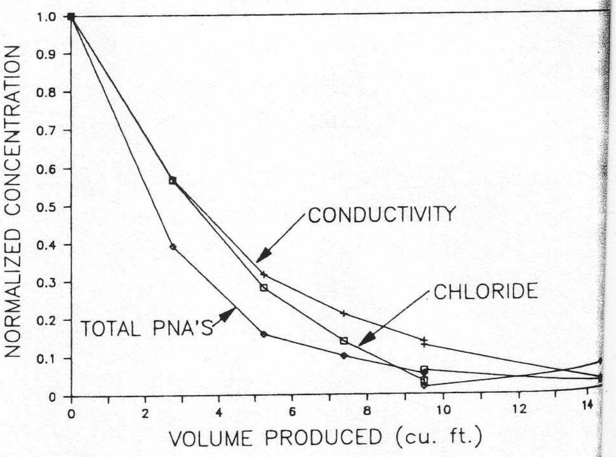

Figure 3. Anoxic push-pull test results. Variation in the normali chloride (□), conductivity (+), and total PNA (◆) concentrations vs. produced volume.

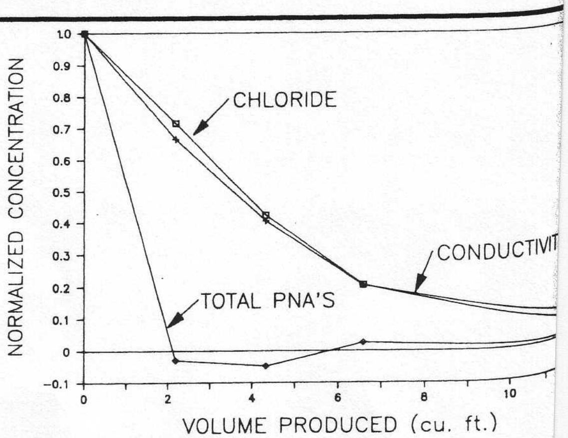

Figure 4. Aerobic push-pull test results. Variation in the norm ized chloride (□), conductivity (+), and total PNA (◆) concen tions vs. produced volume.

During the anoxic test, there was a general decline in the concentrations of the PNAs, conductivity, and chloride as they approached background levels due to dilution with the less contaminated ground water. The chloride and conductivity followed each other well as expected. The total PNA concentration dropped at a slightly greater rate than chloride. This greater rate may be due to biotransformation resulting from the introduction of a small amount of oxygen during the injection procedure.

During the aerobic test, there was a rapid decrease in the concentrations of the organics and oxygen (Figure 4). Within 24 hours (2.18 ft^3) the total PNA concentration had dropped from 936 $\mu g/L$ to 75 $\mu g/L$ while the chloride and conductivity decreased by only 15 percent. This dramatic difference between the anoxic and aerobic tests is evidence of: (1) the importance of oxygen addition in the biotransformation of the PNAs; and (2) the high rates of biotransformation possible when both oxygen and an adapted microbial population are present. After an additional 24 hours, the total PNA concentration was relatively unchanged at 60 $\mu g/L$. Mass balance calculations indicate that at this time there should still have been oxygen present in the formation. The minimal transformation of the PNAs below a concentration of 60 to 75 $\mu g/L$ corresponds with the previous laboratory experiments, which showed little biotransformation below a concentration of approximately 30 to 40 $\mu g/L$ for the sum of 13 PNAs. From Day 2 to 5, a slight increase in the PNA concentration was observed as the injection water was diluted with formation water containing elevated levels of organics and no oxygen.

Hydrophobic adsorption cannot explain the large loss of organics during the aerobic test because previous field studies at this location have shown adsorption to be minimal. Borden and Bedient (1987) found no retardation of naphthalene or para-dichlorobenzene relative to a chloride tracer (retardation factor = 1.0 ± 0.08) using a three well injection-production test. This small amount of hydrophobic adsorption is not unexpected because the organic carbon concentration in this aquifer is very low (less than 0.01 percent by weight).

Numerical Modeling of Push-Pull Tests

The transport and aerobic biodegradation of polynuclear aromatic hydrocarbons during the push-pull tests was simulated using a general mathematical model (BIOPLUME) developed by Borden and Bedient (1986). This model was modified to: (1) account for oxygen demand during both cell growth and cell decay; (2) simulate the apparent minimum degradable hydrocarbon concentration (H_{min}); and (3) simulate one-dimensional radial flow away from the injection well. Transport of microorganisms within the aquifer was assumed to be negligible. The final equations used in the simulations are:

$$\frac{\partial H}{\partial t} = \frac{\partial}{\partial r}\left(D_l \frac{\partial H}{\partial r} - vH\right) - M k \left(\frac{H}{K_H + H}\right)\left(\frac{O}{K_O + O}\right)\left(\frac{H - H_{min}}{H}\right)$$

$$\frac{\partial O}{\partial t} = \frac{\partial}{\partial r}\left(D_l \frac{\partial O}{\partial r} - vO\right) - M k F_1 \left(\frac{H}{K_H + H}\right)\left(\frac{O}{K_O + O}\right)\left(\frac{H - H_{min}}{H}\right) - F_2 k_d M \left(\frac{O}{K_O + O}\right)$$

$$\frac{\partial M}{\partial t} = M k Y \left(\frac{H}{K_H + H}\right)\left(\frac{O}{K_O + O}\right)\left(\frac{H - H_{min}}{H}\right) - k_d M$$

where

D_l = a_l*v
a_l = longitudinal dispersivity
v = $q/2\pi rbn$
q = injection/pumping rate
r = radial distance from well
b = saturated thickness of aquifer
n = aquifer porosity
H = total hydrocarbon concentration
O = oxygen concentration
M = microorganism concentration
k = maximum hydrocarbon utilization rate per unit mass microorganisms
Y = cell yield coefficient
F_1 = ratio of oxygen to hydrocarbon consumed during cell synthesis
F_2 = ratio of oxygen to cell material consumed during cell decay
K_H = hydrocarbon half saturation constant
K_O = oxygen half saturation constant
H_{min} = minimum degradable hydrocarbon concentration
k_d = microbial decay rate.

The spatial derivatives were approximated using a fully upwinded finite difference approximation. This resulted in a series of ordinary differential equations that were solved using a fourth-order Runga-Kutta solution. Numerical dispersion was accounted for using the correction factor suggested by Lantz (1971). This procedure was compared with the analytical solution of Gelhar and Collins (1971) and closely agreed. Additional details on the solution procedure can be found in Borden and Bedient (1986).

The transport portion of the biodegradation model was first calibrated by matching the chloride recovery curves. The effective injection volume was estimated as the produced volume where the normalized chloride concentration equaled 0.50. An effective injection volume was used because of the apparent loss of a portion of the injection fluid to the overlying unit. The longitudinal dispersivity (a_l) was then adjusted to match the observed recovery curves. The best estimate of a_l is 0.1 foot for both the anoxic and aerobic tests. Simulated and observed

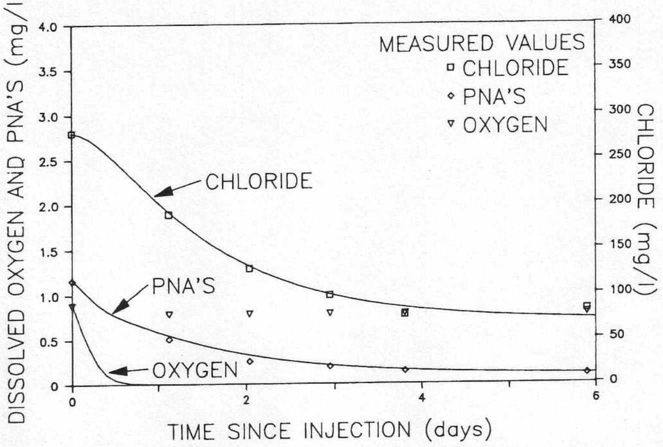

Figure 5. Comparison of numerical model and field monitoring data: anoxic push-pull test (solid lines are simulation results).

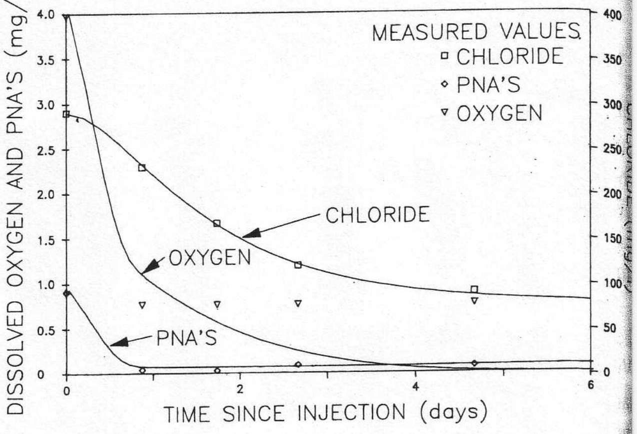

Figure 6. Comparison of numerical model and field monitoring data: aerobic push-pull test (solid lines are simulation results).

chloride recovery curves are shown in Figures 5 and 6.

The majority of the microbiological parameters were determined in laboratory studies at Rice University. Oxygen was assumed to be the only electron acceptor. The four monitored PNAs were assumed to constitute 70 percent of the total hydrocarbon. This percentage is based on more detailed quantification of the contaminated ground water performed by Bedient et al. (1984). The ratio of oxygen to hydrocarbon consumed in cell synthesis was estimated from the following equation:

$$7\,C_{10}H_8 + 56\,O_2 + 4\,NO_3^- + 4\,H^+ \rightarrow 4\,C_5H_7O_2N + 16\,H_2O + 50\,CO_2.$$

The oxygen demand due to cell decay was calculated assuming complete mineralization to carbon dioxide and water. The initial microbial population was estimated by matching the PNA recovery curve. The maximum hydrocarbon utilization rate (k) and hydrocarbon half-saturation constant (K_H) were estimated from a series of batch degradation studies in which the initial hydrocarbon concentration was varied from 4.9 to 798 μg/L (naphthalene used as model hydrocarbon, Thomas et al. 1985). The oxygen half saturation constant (K_O) was estimated from oxygen limitation studies described by Lee (1986). The minimum degradable hydrocarbon concentration was estimated to be 0.1 mg/L (70 μg/L of the four PNAs) based on the previously described laboratory studies. No data could be obtained from the UCC site on the microbial cell yield (Y), or decay coefficient (b). Values for these were obtained from literature sources (Wodzinski and Johnson 1968, Weston 1971). The physical and microbial parameters used in all simulations are listed in Table 2.

The simulated total PNA concentrations closely matched the observed values for both the anoxic and aerobic push-pull tests (Figures 5 and 6). The H_{min} function incorporated into the biodegradation model was able to closely match the presistent low PNA concentrations observed during the aerobic field test. The simulated oxygen concentrations could not be adequately tested because of the high detection limit for oxygen. The model as calibrated did not simulate the observed increase in total PNAs during the end of the aerobic test. This observed increase may have been due to incomplete re-

TABLE 2
Best Estimates of Physical and Microbial Parameters at the UCC Site

	Anoxic Test	Aerobic Test
Production rate (cu. ft./d)	2.45	2.47
Longitudinal dispersivity (ft)	0.1	0.1
Porosity	0.3	0.3
Saturated thickness (ft)	5.	5.
Background oxygen (mg/L)	0.01	0.01
Injection oxygen (mg/L)	0.9	4.0
Background microorganism (mg/L)	1.5	1.5
Background hydrocarbon (mg/L)	0.143	0.143
Injection hydrocarbon (mg/L)	1.679	1.337
Background chloride (mg/L)	70.	75.
Injection chloride (mg/L)	280.	290.
Background conductivity (μmho)	248.	293.
Injection conductivity (μmho)	830.	905.
Maximum hydrocarbon utilization rate (1/d)	2.0	2.0
Ratio monitored PNAs to total hydrocarbon	0.7	0.7
Cell yield coefficient	0.5	0.5
Ratio oxygen to hydrocarbon consumed in cell synthesis	2.0	2.0
Ratio oxygen to cell material consumed in cell decay	2.0	2.0
Hydrocarbon half saturation constant (mg/L)	0.1	0.1
Oxygen half saturation constant (mg/L)	0.6	0.6
Minimum degradable hydrocarbon (mg/L)	0.1	0.1
Microorganism decay coef. (1/d)	0.01	0.01

moval of the organics added to the formation during the anoxic test, which resulted in a higher effective background concentration during the aerobic test.

Discussion

The results from the field push-pull tests clearly demonstrate that oxygen is the primary factor limiting biotransformation of hydrocarbons at the United Creosoting Co. site. This is not surprising because: (1) the rate of oxygen transfer in the subsurface is low; and (2) oxygen is commonly assumed to be necessary for the biotransformation of most hydrocarbons.

A number of investigators have shown oxygen to be the primary factor limiting biotransformation in other aquifers. Ehrlich et al. (1982 and 1983) demonstrated that anaerobic metabolism of phenolics was occurring at a wood creosoting site in Minnesota. However, naphthalene and other polynuclear aromatics persisted in the anaerobic aquifer. They attributed this persistence to the absence of molecular oxygen.

Barker et al. (1987) performed a detailed field experiment to study the biotransformation of benzene, toluene, and xylene (BTX) in a shallow aerobic aquifer. Their monitoring data indicated a rapid loss of BTX during the early portion of their test followed by a period of much slower hydrocarbon loss. The period of slower hydrocarbon loss was attributed to the persistence of benzene in anoxic layers within the aquifer.

Previous studies have also suggested the possibility of a minimum substrate concentration below which biotransformation is inhibited. This concentration appears to be highly dependent on environmental conditions. Boethling and Alexander (1979) observed a sharp drop in the rate of microbial mineralization of 2,4-D dissolved in stream water at concentrations between 22 μg/L and 220 μg/L 2,4-D. Other studies (Wang et al., 1984) have shown that the minimum concentration may be dependent on the concentration of secondary organic substrates and inorganic nutrients. The mineralization of isopropyl N-phenylcarbamate (IPC) at 400 μg/L was increased by the addition of inorganic nutrients or a mixture of nonchlorinated pollutants. In contrast to this, addition of yeast extract, excretions from primary producers, or mixtures of aromatic compounds had little effect on the apparent threshold concentration (Wang et. al., 1984).

Laboratory studies performed as part of this work on ground water from the UCC site with no added nutrients indicated a threshold of 30 to 70 μg/L total PNAs below which biotransformation was significantly reduced. The field data from the push-pull tests was best fit to the biodegradation model using a minimum degradable concentration of 70 μg/L PNAs. These results roughly fall within the range of 22 μg/L to 220 μg/L where Boethling and Alexander (1979) observed a marked decrease in the rate of 2,4-D biotransformation. Previous laboratory studies by Lee and Ward (1985) suggest that the minimum concentration of contaminants achievable at the UCC site may be reduced through the addition of excess inorganic nutrients and/or DO.

From an ecological standpoint, it may be useful to consider what factors cause this apparent minimum degradable substrate concentration and how the subsurface microflora can survive once the concentration of organic substrate drops below this level. If a few adapted microorganisms and suitable electron acceptors are present, a significant population can be expected to develop and rapidly reduce the concentration of organic substrate. As the substrate concentration drops, the flux of substrate into the cell will also decrease. At some point, the energy yield from substrate metabolism will not meet maintenance requirements. At this point, the cell can continue to operate and exhaust remaining energy reserves or become dormant. Roszak and Colwell (1987) discuss various strategies for bacterial survival under nutrient deprivation. The classical response appears to be the initiation of a dormant stage, which may include formation of a spore or the formation of a non-vegetative stage in which metabolic processes are greatly reduced.

Previous studies at the UCC site and in other aquifers have shown large differences between the number of cells actively respiring and the total number of cells in the subsurface. Webster et al. (1985) observed that between 0 and 5 percent of the total bacterial population at the UCC site was metabolically active. Total bacterial counts were estimated by acridine orange staining while respiring bacteria were estimated as those organisms, which reduced INT [2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazolium chloride] forming granules within the cell. A second method based on ATP content indicated similar metabolic activity. The presence of dormant microbial cells due to nutrient deprivation could explain the observation of large differences between the total number of cells and those actively respiring.

The impact of non-respiring or very slowly respiring cells on the biotransformation of low concentrations of organics in the subsurface is not yet known. Monitoring data collected as part of a Superfund cleanup at the UCC site (Weston 1986) and analyzed by Borden et al. (1986) indicated that biotransformation had limited the downgradient movement of naphthalene and other PNAs. There was no indication from the field monitoring data that a minimum degradable concentration might exist. This suggests that the dormant population could potentially be capable of using dissolved PNAs at low concentrations given sufficient time. Much more research is needed on the long-term impact of the subsurface microflora on the biotransformation of low concentrations of organics and the effect of electron acceptor and inorganic nutrient concentrations on this process.

Summary

A series of experiments was performed to determine the potential for enhanced biotransformation of the PNAs present in the shallow aquifer at the United Creosoting Co. Inc. Superfund site near Conroe, Texas. Previous laboratory studies had indicated that DO was required for biotransformation of the organics. Laboratory experiments performed as part of this project indicate that maintenance of a DO concentration greater than 0.7 mg/L will be adequate for biotransformation down to some minimum degradable concentration. Biotransformation of the polynuclear aromatic hydrocarbons present in the formation appears to be inhibited below a concentration of 30 to 70 total PNAs $\mu g/L$ under ambient nutrient conditions.

Two single-well push-pull tests were performed by injecting ground water containing PNAs and chloride into a portion of the shallow aquifer at the UCC site where an active microbial population was expected. During the second test, oxygen was added to the injection water. These tests have demonstrated the importance of oxygen in hydrocarbon biotransformation. When oxygen was added to the injection water, the PNAs were rapidly degraded. When no oxygen was added, there was no significant loss of the PNAs.

Experimental results from the field test were also compared with mathematical model (BIOPLUME) simulations of the biotransformation process. The mathematical model assumes that oxygen is the primary factor limiting biotransformation of the PNAs. The model provided a very good match to the field monitoring data when modified to account for a minimum degradable hydrocarbon concentration.

Acknowledgments

This work was supported in part by the National Center for Ground Water Research. Although the research described in this article has been funded in part by the United States Environmental Protection Agency, it has not been subject to the Agency's required peer and policy review and does not necessarily reflect the views of the Agency and no official endorsement should be inferred. Special thanks to Jack Clark for allowing access to the field site.

References

Atlas, R.M. 1981. Microbial degradation of petroleum hydrocarbons: an environmental perspective. *Microbiol. Reviews,* v. 45, pp. 180-209.

Barker, J.R., G.C. Patrick, and D. Major. 1987. Natural attenuation of aromatic hydrocarbons in a shallow sand aquifer. *Ground Water Monitoring Review,* v. 7, no. 1, pp. 64-71.

Bedient, P.B., A.C. Rodgers, T.C. Bouvette, M.B. Tomson, and T.H. Wang. 1984. Ground water quality at a creosote waste site. *Ground Water,* v. 22, pp. 318-329.

Boethling, R.S. and M. Alexander. 1979. Effect of concentration of organic chemicals on their biodegradation by natural microbial communities. *Appl. Environ. Microbiol.,* v. 37, pp. 1211-1216.

Borden, R.C. and P.B. Bedient. 1986. Transport of dissolved hydrocarbons influenced by oxygen limited biodegradation: 1. theoretical development. *Wat. Resources Res.,* v. 22, pp. 1973-1982.

Borden, R.C., P.B. Bedient, M.D. Lee, C.H. Ward, and J.T. Wilson. 1986. Transport of dissolved hydrocarbons influenced by oxygen limited biodegradation: 1. field application. *Wat. Resources Res.,* v. 22, pp. 1983-1990.

Borden, R.C. and P.B. Bedient. 1987. In situ measurement of adsorption and biotransformation at a hazardous waste site. *Wat. Resources Bull.,* v. 23, pp. 629-636.

Ehrlich, G.G., D.F. Goerlitz, E.M. Godsy, and M.F. Hult. 1982. Degradation of phenolic contaminants in ground water by anaerobic bacteria: St. Louis Park, Minnesota. *Ground Water,* v. 20, pp. 702-710.

Ehrlich, G.G., E.M. Godsy, D.F. Goerlitz, and M.F. Hult. 1983. Microbial ecology of a creosote-contaminated aquifer at St. Louis Park, Minnesota. *Dev. Ind. Microbiol.* v. 24, pp. 235-245.

Gelhar, L.W. and M.A. Collins. 1971. General analysis of longitudinal dispersion in nonuniform flow. *Wat. Resour. Res.,* v. 19, pp. 1511-1521.

Holm, T.R., G.K. George, and M.J. Barcelona. 1986. *Dissolved oxygen and oxidation-reduction potentials in ground water.* U.S. Environmental Protection Agency, Wash., D.C., EPA/600/2-86/042.

Lantz, R.B. 1971. Quantitative evaluation of numerical diffusion truncation error, *Soc. Petrol. Eng. J.,* v. 25 pp. 315-320.

Lee, M.D., J.T. Wilson, and C.H. Ward. 1984. Microbial degradation of selected aromatics at a hazardous waste site. *Dev. Ind. Microbiol.,* v. 25, pp. 557-575.

Lee, M.D. and C.H. Ward. 1985. Microbial ecology of hazardous waste disposal site: enhancement of biodegradation. In Proceedings, *Second International Conference on Groundwater Quality Research,* ed N.N. Durham and A.E. Redelfs, Oklahoma Stat Univ., Stillwater, Oklahoma, pp. 25-27

Lee, M.D. 1986. Biodegradation of organic contaminant in the subsurface of hazardous waste sites. Ph.D. Thesi Rice University, Houston, TX.

Roszak, D.B. and R.R. Colwell. 1987. Survival strategies of bacteria in the natural environment. *Microbiol. Reviews,* v. 51, pp. 365-379.

Smith, G.A., J.S. Nickels, J.D. Davis, R.H. Findlay, P.S. Vashio, J.T. Wilson and D.C. White. 1985. Indices identifying subsurface microbial communities that adapted to organic pollution. In Proccedings, *Second International Conference on Groundwater Quality Research,* ed. N.N. Durham and A. E. Redelfs, Oklahoma State Univ., Stillwater, Oklahoma, pp. 210-213.

Thomas, J.M., M.D. Lee, and C.H. Ward. 1985. Microbial numbers and activity in the subsurface at a creosote waste site. (abstract), *Amer. Soc. Microbiol.,* Las Vegas, Nevada.

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.,* U.S. Environmental Protection Agency, Washington, D.C., EPA/600/2-87/008.

Thomas, J.M., M.D. Lee, M.J. Scott, and C.H. Ward. 1988. Microbial ecology of the subsurface at an abandoned creosote waste site. Accepted for publication, *J. Indus. Microbiol.*

Wang, H.T., C.M. Curran, P.B. Bedient, and M.B. Tomson. 1985. Ground water contamination at Conro waste disposal site. In Proceedings, *Second International Conference on Groundwater Quality Research* ed. N.N. Durham and A.E. Redelfs, Oklahoma Stat Univ., Stillwater, Oklahoma, pp. 50-53.

Wang, Y., R.V. Subba-Rao, and M. Alexander. 198 Effect of substrate concentration and organic an inorganic compounds on the occurrence and rate mineralization and cometabolism. *Appl. Envi*

Microbiol., v. 47, pp. 1195-1200.

Webster, J.J., G.J. Hampton, J.T. Wilson, W.C. Ghiorse, and F.R. Leach. 1985. Determination of microbial cell numbers in subsurface samples. *Ground Water*, v. 23, pp. 17-25.

Weston, R.F., Inc. 1971. *Process design manual for upgrading existing wastewater treatment plants.* U.S. Environmental Protection Agency, Washington, D.C.

Weston, R.F. Inc. 1985. *Final site investigation technical report*, United Creosoting Co., Houston, Texas.

Wilson, J.R., J.F. McNabb, J.W. Cochran, T.H. Wang, M.B. Tomson, and P. B. Bedient. 1985. Influence of microbial adaptation on the fate of organic pollutants in ground water. *Environ. Toxicol. Chem.*, v. 4, pp. 721-726.

Wilson, J.T., G. Miller, W.C. Ghiorse, and F.R. Leach. 1986. Predicting biotransformation in subsurface: relationships between ATP content of subsurface material and the rate of biodegradation of alkylbenzenes and chlorobenzene. *J. Contaminant Hydrology*, v. 1, pp. 163-170.

Wodzinski, R.S. and M.J. Johnson. 1968. Yields of bacterial cells from hydrocarbons, *Appl. Envir. Microbiol.*, v. 16, pp. 1886-1891.

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