Progression of methanogenic degradation of crude oil in the subsurface

Barbara A. Bekins, Frances D. Hostettler, William N. Herkelrath, Geoffrey N. Delin, Ean Warren, and Hedeff I. Essaid

ABSTRACT

Our results show that subsurface crude-oil degradation rates at a long-term research site were strongly influenced by small-scale variations in hydrologic conditions. The site is a shallow glacial outwash aquifer located near Bemidji in northern Minnesota that became contaminated when oil spilled from a broken pipeline in August 1979. In the study area, separate-phase oil forms a subsurface oil body extending from land surface to about 1 m (3.3 ft) below the 6–8-m (20–26 ft)-deep water table. Oil saturation in the sediments ranges from 10–20% in the vadose zone to 30–70% near the water table. At depths below 2 m (6.6 ft), degradation of the separate-phase crude oil occurs under methanogenic conditions. The sequence of methanogenic alkane degradation depletes the longer chain n-alkanes before the shorter chain n-alkanes, which is opposite to the better known aerobic sequence. The rates of degradation vary significantly with location in the subsurface. Oil-coated soils within 1.5 m (5 ft) of land surface have experienced little degradation where soil water saturation is less than 20%. Oil located 2–8 m (6.6–26 ft) below land surface in areas of higher recharge has been substantially degraded. The best explanation for the association between recharge and enhanced degradation seems to be increased downward transport of microbial growth nutrients to the oil body. This is supported by observations of greater microbial numbers at higher elevations in the oil body and significant decreases with depth in nutrient concentrations, especially phosphorus. Our results suggest that environmental effects may cause widely diverging degradation rates in the same spill, calling into question dating methods based on degradation state.

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INTRODUCTION

The potential for degradation of crude-oil compounds under methanogenic conditions has wide-ranging implications for petroleum deposits, environmental cleanup, and global methane budgets. Recent laboratory studies demonstrated methanogenic degradation of hexadecane, which is considered a model compound for the range of n-alkanes found in crude oil (Zengler et al., 1999; Anderson and Lovley, 2000). However, when multiple n-alkanes are present, the methanogenic degradation sequence remains unexplored. Other recent studies have described polar metabolites formed by n-alkane degradation under sulfate-, and nitrate-reducing conditions (Kropp et al., 2000; Rabus et al., 2001) and, to a limited extent, under methanogenic conditions (Townsend et al., 2003). Linking these laboratory results to observations of methanogenic degradation of petroleum in the environment remains to be accomplished.

Understanding methanogenic degradation of crude oil in the subsurface is important for assessing the efficacy of natural attenuation at contaminated sites. Methanogenic conditions commonly occur when separate-phase petroleum hydrocarbons are present at the water table (e.g., Weidemeier et al., 1999). Moreover, crude oil in the subsurface can be extremely difficult to remove. Typically, a residual saturation from 8 to 32% of the pore space is immobile (Abdul, 1992), requiring removal by either soil excavation or enhanced recovery techniques. Where the oil is deep or laterally extensive, these removal strategies may be prohibitively expensive. Regulatory decisions on whether existing crude-oil contamination may be left in the subsurface may depend on estimating the rate of natural attenuation under methanogenic conditions.

Many authors have noted that degradation rates vary according to the quality of subsurface environmental conditions for the degrading microbial populations (Stout et al., 2002). Factors that may influence degradation rates include availability of electron acceptors (Fredrickson and Onstott, 2001), moisture content (Kieft and Brockman, 2001), and microbial nutrient supplies (Ghiorse and Wilson, 1988). Commonly, contamination has been chronic through leakage or a series of spills over an extended period, so that the timing and even the composition of the original products may be in doubt. Studies of spills with a known source and date of origin can provide insights into the factors that govern the degradation rates and the compositional changes that occur during methanogenic degradation.

In this paper, we present data on the degradation of the aliphatic fraction of spilled crude oil 25 yr after the oil entered the subsurface. Although the aliphatic fraction is generally not a factor in risk assessment at contaminated sites, the results provide insights into environmental controls on the long-term fate of crude oil in the subsurface. We relate the observed degradation rates to redox conditions, hydrology, and microbial populations. The paper concludes with a discussion of the implications for hydrocarbon fingerprinting, spill dating, and site cleanup time projections.

SITE DESCRIPTION

The study aquifer, located approximately 16 km (10 mi) northwest of Bemidji, Minnesota, became contaminated after a shallowly buried, high-pressure pipeline ruptured on August 20, 1979. An estimated 1.7×10^6 L of crude oil sprayed and leaked from the rupture onto the land surface. Cleanup operations by the pipeline company during the month following the spill removed about 1.3×10^6 L. The remaining 0.4×10^6 L collected in topographic low areas and trenches where it infiltrated into the subsurface (Hult, 1984). The oil is present in three main subsurface oil bodies (Figure 1a). The largest of these, known as the north pool, extends roughly 25 × 80 m (82 × 262 ft) laterally and, in 1999, was estimated to contain 147,000 L of oil (Herkelrath, 1999).

The contaminated, unconfined aquifer is a surficial formation of glacial outwash sediments. The sediments consist of 20 m (66 ft) of moderately calcareous sand with layers of gravel and thin, discontinuous, lacustrine silts (Franzi, 1988). Underlying the sandy, high-permeability material is a clayey till that is not impacted by the contamination. The surface topography at the site varies gently with a relief of less than 10 m (33 ft). Vegetation consists of grassy meadows and wooded areas with pine trees. Average annual precipitation between 1979 and 2000 was 65 ± 12 cm/yr (26 ± 4.7 in./yr). Average annual recharge estimated using water level rises in nearby wells and a specific yield of 0.3 ranged from 10 to 20 cm/yr (4 to 8 in./yr) (Herkelrath and Delin, 2001).

The water-table depth below the north pool ranges from 6 to 8 m (20–26) below ground level and fluctuates about 0.5 m (1.6 ft) annually. The average groundwater velocity is 6 cm/yr (2.4 in./yr) (Essaid et al., 2003). Susceptible hydrocarbons (e.g., benzene, toluene, ethylbenzene and xylenes [BTEX] compounds) from the north pool oil body continue to dissolve in the groundwater, forming a contaminant plume in the saturated zone. The anaerobic contaminant plume is slowly expanding at a rate of 2–3 m/yr (6.6–10 ft/yr)

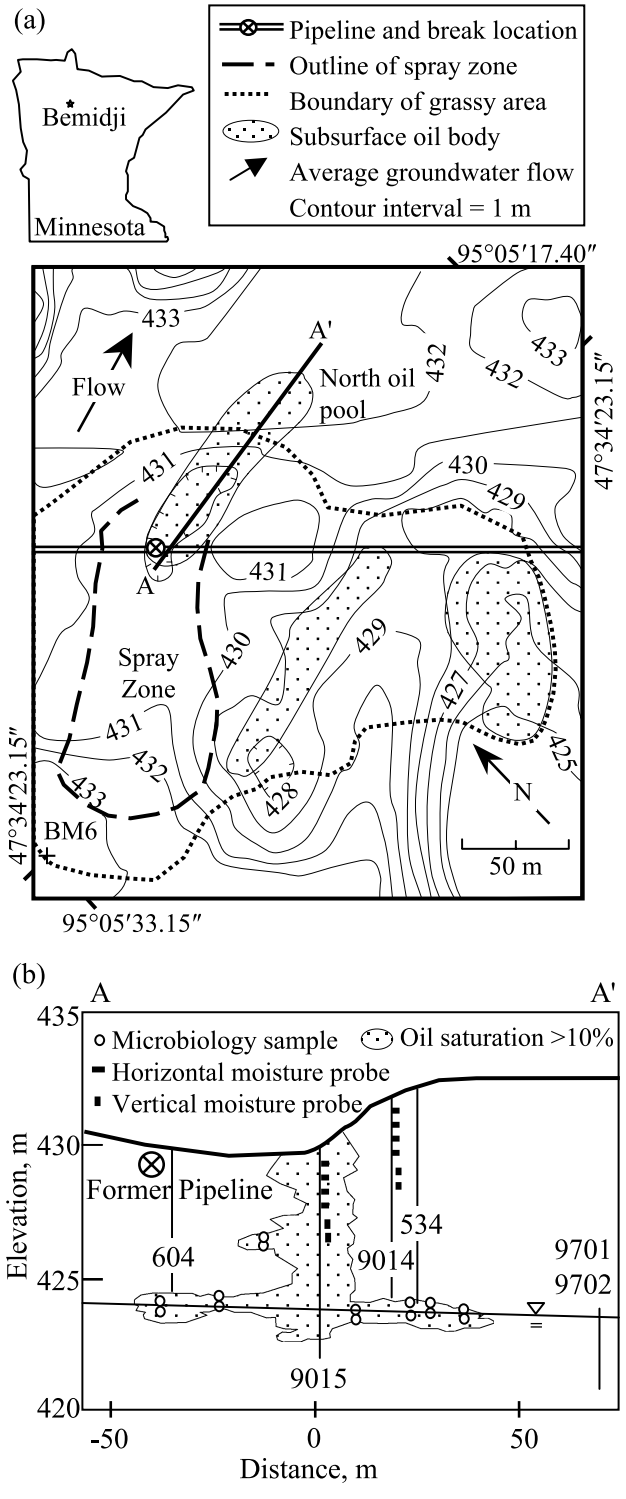


Figure 1. (a) Topographic map of the Bemidji, Minnesota, crude-oil spill site, showing locations of buried oil pipeline and 1979 break, major subsurface oil bodies, area affected by sprayed oil, and demarcation between wooded and grassy area. Line AA′ shows location of cross section. (b) Cross section through north oil pool study area. Gray area contains greater than 10% saturation of separate-phase oil. Locations of moisture probes and lysimeters are 9014 and 9015. At 9015, the top three moisture probes and lysimeters are in oil-free sediments located in front of the line of section. Locations for gas profiles are 604 and 9014. Locations of microbiology samples collected in 2003 are marked with crosses. Profile through iron-reducing zone is at test hole 9701-2.

(Cozzarelli et al., 2001). Numerical modeling of dissolution and degradation indicates that the separate-phase oil is still a significant source of dissolved contaminants (Essaid et al., 2003).

In the area where the oil originally infiltrated, it is present at residual saturations of 20–30% in the vadose zone above the capillary fringe (Figure 1b) (Dillard et al., 1997). Within a 1-m (3.3-ft)-thick zone around the average water-table elevation, the oil reaches saturations of more than 60% and has spread beyond the area of the original infiltration (Dillard et al., 1997).

Analyses of oil samples from several locations in the north pool show substantial variation in the degree of degradation throughout the oil body (Hostettler and Kvenvolden, 2002). Moreover, the pattern of oil degradation differs from that observed under aerobic conditions. Microbial data (Bekins et al., 2001) and vapor data (Chaplin et al., 2002) from the north pool area indicate that methanogenic conditions dominate in the separate-phase oil.

METHODS

Crude-Oil Analyses

In 1998, cores were collected from eight locations along the axis of the oil body and analyzed for soil and water saturation using the methods of Hess et al. (1992). In each core, saturations were determined for 0.075-m (0.25-ft) subsections. The data were contoured to characterize the subsurface oil-saturation distribution.

Procedures for collecting oil-coated sediments for chemical analysis of the oil are described in Eganhouse et al. (1993). New core samples were collected in the summers of 1999–2003, along with some samples from frozen cores originally collected in 1996. All sediment samples were kept frozen until analysis, at which time they were thawed in their glass sample jars. A detailed description of the methods for oil extraction and analyses is provided in Hostettler and Kvenvolden (2002). Briefly, 10 g of sample was extracted with successive volumes of dichloromethane. Anhydrous sodium sulfate and freshly activated copper powder were added, and the mixture was refrigerated overnight. The sample extract was filtered and gently evaporated under a stream of nitrogen to a final volume of 5.0 mL. The approximate concentration of oil was ascertained by weighing the evaporation residue from a 50-mL aliquot. The evaporation residue from another approximately 25 mg of oil was taken up in 5.0 mL of hexane and fractionated on a mixed silica and alumina column into aliphatic and aromatic fractions.

The fractions were analyzed by gas chromatography–mass spectrometry (GC-MS) and, by GC-MS in the selected ion monitoring mode (SIM), monitoring *m/z* 191 (terpanes) and *m/z* 217 (steranes) for aliphatic sterane and hopane biomarkers. Compound identifications were based on comparison with known standards or with previously characterized reference oils. Members of the homologous series were identified by extracted ion chromatograms of characteristic fragment ions. These included n-alkanes (*m/z* 57) and n-alkylated cyclohexanes (*m/z* 83). Ratios of selected components were calculated using peak heights. Only the aliphatic fraction will be discussed here.

Geochemical Analyses

Sediment samples for bioavailable iron analyses were collected from nine locations in the oil body. Samples were immediately wrapped with saran wrap and duct tape and frozen until analysis. The solid-phase iron was extracted with 0.5 M HCl as described by Tuccillo et al. (1999). This method was expected to extract the bioavailable Fe(III) as well as the poorly crystalline Fe(II) from the sediments.

Gas sampling was performed with two vertical arrays of soil gas probes located near each end of the oil body (Figure 1b, sites 604 and 9014). Each vertical array consists of permanently installed gas probes spaced at 1-m (3.3-ft) depth intervals from approximately 1 m (3.3 ft) below ground level to 1 m (3.3 ft) above the water table (Chaplin et al., 2002). Gas samples were obtained using a peristaltic pump in gas-tight glass syringes and analyzed onsite using a Tracor 560 gas chromatograph equipped with flame-ionization and thermal conductivity detectors for hydrocarbon and fixed-gas (O_2, CO_2, CH_4, and N_2) analyses, respectively.

To assess whether microbial growth nutrients affect the degradation rates, sediment samples were collected from three vadose zone profiles located adjacent to sites 604, 9014, and 534. The samples were analyzed for total inorganic nitrogen and extractable orthophosphate. Inorganic nitrate and ammonia were determined by equilibrium extraction with potassium chloride and subsequent determination by flow-injection analyzer (see http://groups.ucanr.org/danranlab/Soil_Analysis/index.htm#312). Orthophosphate was determined using the method of Olsen-P (see http://groups.ucanr.org/danranlab/Soil_Analysis/index.htm#340).

Soil Moisture

Soil-moisture probes were installed at the 9015 and 9014 sites to estimate recharge (Figure 1b). Each profile consisted of six Campbell Scientific CS615 probes connected to automated data-logging systems. At each site, a 2-m (6.6-ft)-deep pit was dug, and the uppermost four probes were pushed horizontally into the undisturbed pit wall at depths of 50, 100, 150, and 200 cm (19, 39, 59, and 79 in.). The lowermost probes were installed vertically at depths of 250 and 300 cm (98 and 118 in.) in 10-cm (4-in.)-diameter boreholes in the bottom of the pit. The pit and boreholes were then backfilled with native soil. The soil-moisture data were collected every 4 hr from November 2001 to November 2002.

The cumulative annual recharge was estimated by summing the total moisture that propagates below a depth of 1 m (3.3 ft) during each recharge event. Recharge events are identified using precipitation records and the soil moisture data. For each event, the total increase in moisture was computed by summing the product of the moisture increase at each probe over the pre-event value and the spanned vertical interval between probes.

Microbial Enumerations

Microbial concentrations were determined using a five-tube Most Probable Number (MPN) analysis. Details of the method of sediment sampling and MPN determination are described in Bekins et al. (1999). Briefly, approximately 10 g of sediment collected from the center of a core under a flow of oxygen-free nitrogen gas was added to a prereduced and anaerobically sterilized (PRAS) mineral salts solution containing nonionic surfactant to detach microbes from the sediment (Yoon and Rosson, 1990). The tube was sealed, mixed well, allowed to stand for 2 hr, and then sonicated under a flow of sterile oxygen-free nitrogen gas to dislodge the bacteria. Samples were serially diluted by orders of magnitude into PRAS mineral salts solutions.

Microorganisms capable of fermentation were enumerated using PRAS prepared Schaedler's Broth (Difco, Detroit, Michigan). Tubes with turbid growth or clumps of particulates after incubation for 1 week were scored positive. Iron-reducing bacteria were enumerated using PRAS media containing sodium acetate and poorly crystalline iron (modified from Lovley and Phillips, 1986). After inoculation, the serum bottles were aseptically pressurized with a 70:30 mix of H_2–CO_2 to 140 kPa and then incubated at least 6 weeks. A serum bottle was scored positive if greater than 2 mg/L of reduced iron was present as determined with bipyridine. Hydrogen-using methanogens were enumerated by aseptically pressurizing the serum bottles after inoculation with a 70:30 mix of H_2–CO_2 to 140 kPa. The serum bottles were allowed to incubate for a minimum of 10 weeks. The presence of methane in the head space was determined by gas chromatography–flame ionization detection analysis (Godsy, 1980). All incubations were at room temperature.

RESULTS

Patterns of Oil Degradation

Figure 2 shows chromatograms for three samples illustrating the range of oil-degradation states found in the oil body. The degradation state can be assessed on the basis of the C_{17} to pristane (Pr) ratios, which show that sample a is relatively undegraded, whereas in sample c, the straight-chain n-alkanes have completely degraded. The intermediate degradation state in sample b is characterized by greater losses in the C_{18}–C_{25} range of n-alkanes than in the C_{10}–C_{15} n-alkane range. Hostettler and Kvenvolden (2002) noted that the initial loss of mid- and high-range n-alkanes is opposite to that documented for aerobic degradation of oil. Under aerobic conditions, typical of small spills near the land surface, the degradation proceeds from the low-order n-alkanes, to the midrange, and finally, to the high-range n-alkanes (see, e.g., Kaplan and Galperin, 1996).

Figure 3a shows the locations and degradation states (classified into four levels) for all the samples analyzed from the oil body. Several features are apparent in the distribution of degradation states. In most of the samples from the upgradient end of the oil body, the n-alkanes have been completely degraded (white). Only a few samples located at depth have some C_{10}–C_{15} n-alkanes present. Starting at about 20 m (66 ft) downgradient from the center of the oil body, the situation changes. Here, most of the samples show very little degradation (black). Chromatograms of these samples show only slight n-alkane losses, and some straight-chain alkanes still exceed the isoprenoids in concentration (Figure 2a). One sample located closest to the land surface in the center of the oil also has undergone almost no degradation despite its proximity to atmospheric oxygen supply. Intermediate degradation

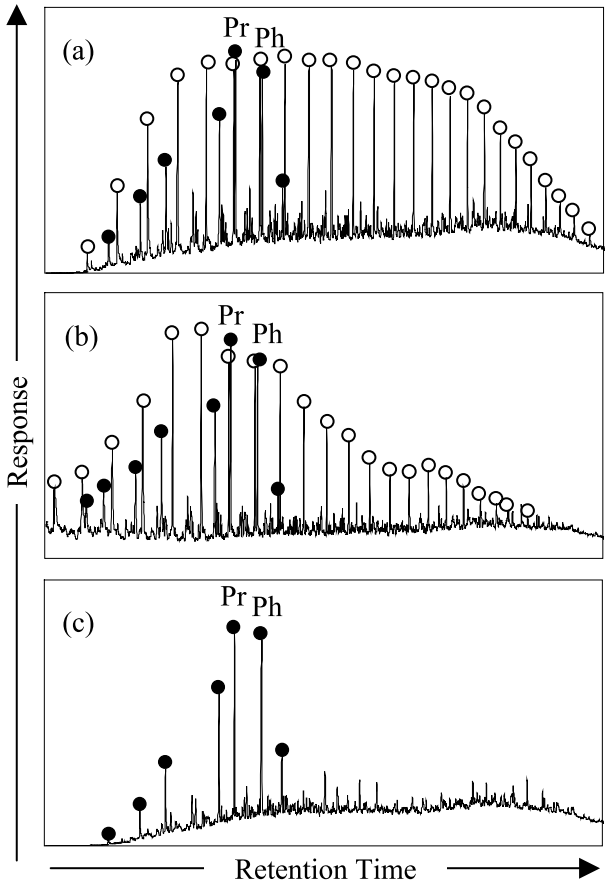


Figure 2. Constituent-extracted ion chromatograms of oil samples from locations shown in Figure 3 with varying degrees of degradation. Peaks marked with open circles are n-alkanes, and black circles are isoprenoids. The isoprenoid peaks for pristane (Pr) and phytane (Ph) are marked. (a) Almost undegraded sample with n-alkane concentrations greater than isoprenoids. (b) Somewhat degraded sample showing that C_{16} to C_{25} n-alkanes are degraded before the lighter n-alkanes (c) Highly degraded sample with n-alkanes completely degraded.

states (gray) are found in a transitional zone between the highly degraded and the undegraded ends of the oil body. The advancing front of the floating oil body also seems to be slightly more degraded.

Modeling indicates that all of the oil infiltrated the subsurface within a few months of the August 1979 spill (Dillard et al., 1997). Thus, the current variation in degradation states reflects widely varying biodegradation rates during the 25 yr since the spill. Figure 3b shows a temporal progression of degradation from 1996 to 2001 for the vertical profile marked in Figure 3a. The shallowest location shows almost no degradation from the time of the spill through 1999. Two locations in the center of the profile underwent substantial degradation between 1996 and 1999. In the deepest locations, the degradation remains at an intermediate state from 1996 to 2001.

The large-scale pattern of degradation along the transect seems to reflect the topography. More degraded locations lie beneath a local topographic low (Figure 1a, 430 m [1410 ft] contour). In addition, with the exception of sample a, the locations at higher elevations in the oil body are generally more degraded than lower elevations (Figure 3a, b). Both these observations suggest that there may be a relation between recharge infiltrating from the land surface and degradation rates.

Hydrology

In this section we explore how the hydrologic data from the site can provide insights into the spatial pattern of oil degradation. Two issues are addressed. The first is the anomalously low degradation state of the oil sample located closest to land surface. The second is the large-scale pattern of highly degraded oil beneath the topographic low and comparatively undegraded oil located 5–10 m (16–33 ft) downgradient beneath an area where the land surface elevation is higher.

To understand why the sample closest to land surface remains undegraded, it is instructive to look at the distribution of subsurface moisture content. Figure 4 is a plot of oil and water saturations for a vertical profile through the center of the oil body from the land surface to below the water table. The residual oil saturation in the vadose zone is relatively constant at about 20% and increases to about 60% near the capillary fringe. Water saturations are only approximately 10% near land surface and increase to about 60% below approximately 1.5 m (5 ft) depth. The two undegraded oil samples were located within 1 m (3.3 ft) of land surface, where water saturations are extremely low. The oil-coated sediments sampled from this location are extremely dry and friable. The low degree of oil degradation is most likely caused by the lack of moisture at this location.

To explore the influence of hydrology in determining the large-scale variation in degradation rates, we compared recharge estimates between two ends of the oil body in 2002. The soil moisture probe arrays used to estimate recharge are located at sites 9015 and 9014 in Figure 1b. Table 1 shows the estimated values obtained by summing the moisture increase from each rainfall event. The results show that the recharge over the less-degraded end of the oil body was about 20% of the precipitation, which is typical for this area (Delin

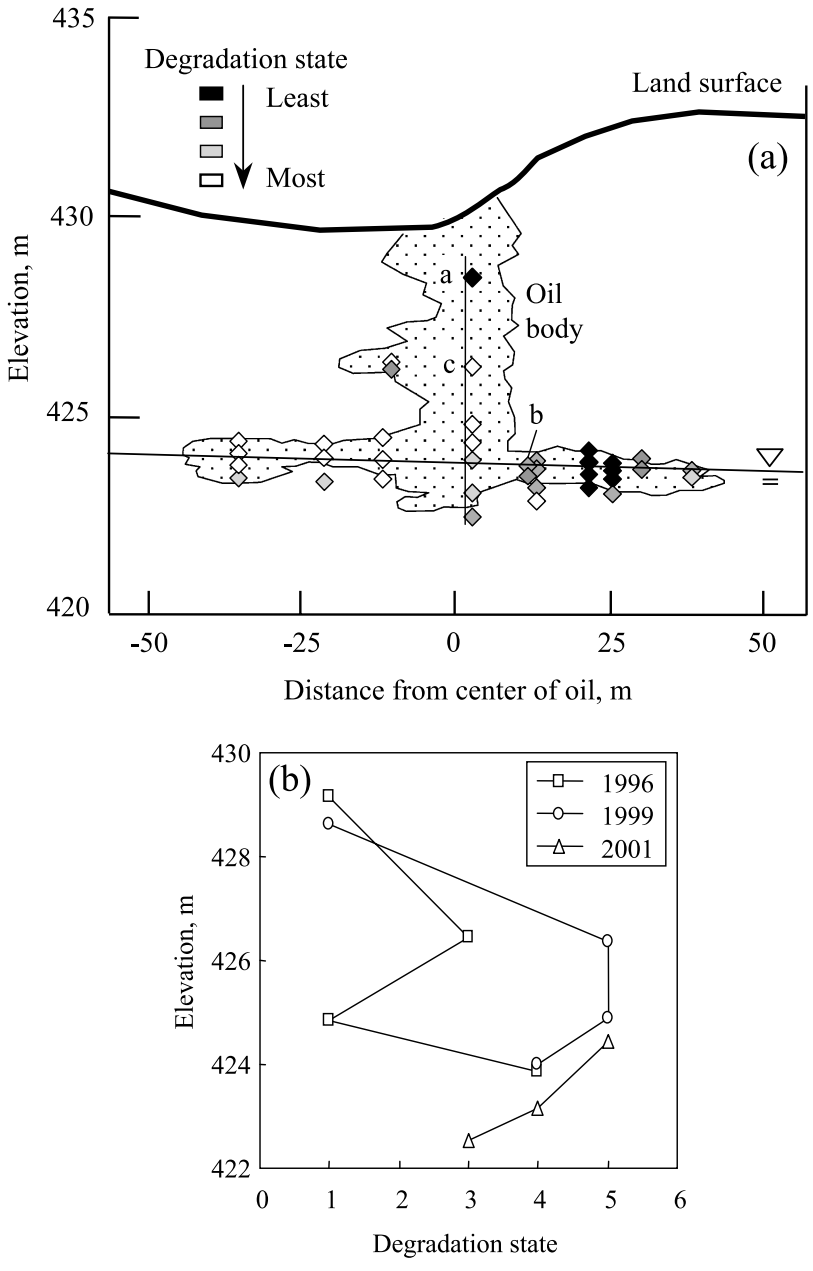


Figure 3. (a) Cross section of oil body along the line AA′ (Figure 1a) showing oil sample locations and degree of degradation for samples collected during 1999–2003. The samples marked a (black), b (dark gray), and c (white) correspond those shown in Figure 2a–c, respectively. The direction of groundwater flow is from left to right. (b) Degradation state of samples collected in 1996, 1999, and 2001 for the profile marked in (a). 1 = least degraded; 5 = most degraded.

et al., 2000). In contrast, the recharge over the more degraded end of the oil body was over twice this rate.

The observed difference in recharge rates is a consequence of the local topography and the distribution of surface vegetation. The higher rate occurs in a local topographic depression (Figure 1a), which is consistent with topographic focusing of recharge in sandy aquifers noted by Delin and Landon (2002). In addition, pine trees overlie the lower recharge area, whereas grass overlies higher recharge area (Figure 1a).

Although the 2002 recharge estimates provide compelling evidence that faster rates of degradation occur in areas of enhanced recharge, the mechanism for the faster rates is not clear. One possibility is that the higher water flux through the oil enhances dissolution of the soluble fraction. Although the mid- and high-range n-alkanes have low solubility, degradation rates of these compounds might be enhanced once the soluble fraction is removed by dissolution. The dissolution rate is equilibrium controlled (Eganhouse et al., 1996) and thus depends on the rate that water contacts the oil. Contact between oil and water is increased both by greater recharge and by lower oil saturations. For example, Essaid et al. (2003) showed with model simulations that locations with lower oil saturations experience greater simulated benzene losses because of enhanced

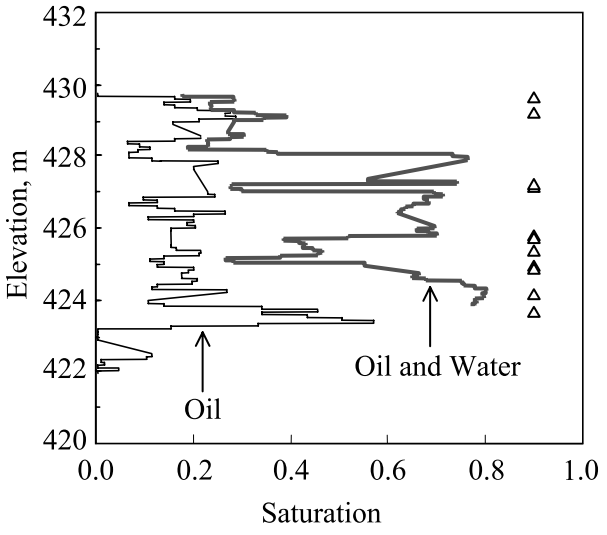


Figure 4. Oil and total fluid (oil plus water) contents for the vertical profile shown in Figure 3b (Dillard et al., 1997). The locations of the oil samples in Figure 3b are marked with triangles on the right-hand side of the profile. The samples that were relatively undegraded in 1996 and 1999 are located at the top of the profile in sediments with very low water contents.

dissolution. Thus, if the dissolution rate is important in explaining the degree of degradation, there should be a direct correlation between oil saturation and degradation rate. Figure 5a shows a contour plot of oil saturation in the area of the oil body lying within 4 m (13 ft) of the water table. The saturations at the less degraded end of the oil body reach peak values of more than 60% compared to less than 30% at the more degraded end. To evaluate the possibility that locations containing higher concentrations of oil might be less degraded, we plotted degradation state vs. weight percent of oil in Figure 5b. The data show no significant relation between oil content and degradation state. Thus, the association between recharge and degradation state is not simply caused by enhanced dissolution of the soluble oil fraction by higher recharge rates.

Geochemical Environment of the Oil Body

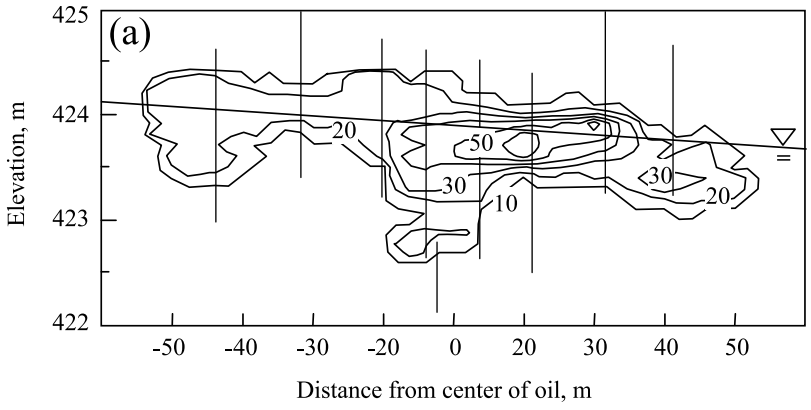
Another hypothesis to explain the contrast in degradation states in the oil body is differing redox conditions. Bekins et al. (1999) examined microbial populations in the contaminated portion of the aquifer and concluded that fermentation and methanogenesis are the dominant degradation processes in the oil body. Their survey of the aquifer MPN values for methanogens, fermenters, and iron reducers showed that the microbial numbers corresponded very well with other geochemical indicators of the microbial processes at the site. Moreover, the fine-scale distribution of methanogens and iron reducers was consistent with the theoretical expectation that there is more rapid transition from iron reduction to methanogenesis in areas of high hydrocarbon flux. They documented the dominance of methanogens in two high-flux environments: the first in the separate-phase oil body and the second in high-permeability zones in the contaminant plume (Bekins et al., 2001). The consistency of the MPN data with other geochemical data and the expected pattern of progression from iron reduction to methanogenesis indicate that MPN data provide an accurate picture of in-situ microbial activities. To illustrate the validity of the MPN data as a measure of in-situ activities, Figure 6 compares microbial numbers from a vertical profile in the iron-reducing zone of the contaminant plume to those from the oil body. The results show that in the oil body iron reducer numbers are 100–1000 times lower, whereas methanogens and fermenters are 100–1000 higher compared to the iron-reducing zone. This observation is consistent with the high concentrations of 15–20% methane found by Chaplin et al. (2002) in the vadose zone above the oil body (Figure 7a, b).

To examine whether enhanced recharge facilitates transport of dissolved oxygen to the oil, Figure 7a and b shows vertical profiles of soil gas methane and oxygen contents above two locations in the oil body. Near land surface, oxygen is present at atmospheric concentrations. At the base of the profiles, very high concentrations of methane are maintained by the ongoing methanogenic degradation of the underlying oil (Chaplin et al., 2002). In both profiles, aerobic degradation of methane completely depletes the oxygen below a depth of 2–3 m (6.6–10 ft) (Amos et al., 2005). Thus, the recharge does not appear to transport dissolved oxygen through the vadose zone to the depth of the oil floating at the water table.

Differences in rates of other anaerobic degradation processes caused by variation in the supply of electron acceptors do not appear to be important between the two ends of the oil body. Table 2 shows the concentrations of sulfate and nitrate from lysimeter samplers

Table 1. Recharge Estimates

Site	Recharge (cm)	Percent of Precipitation
9014	12.1	20
9015	38.9	65



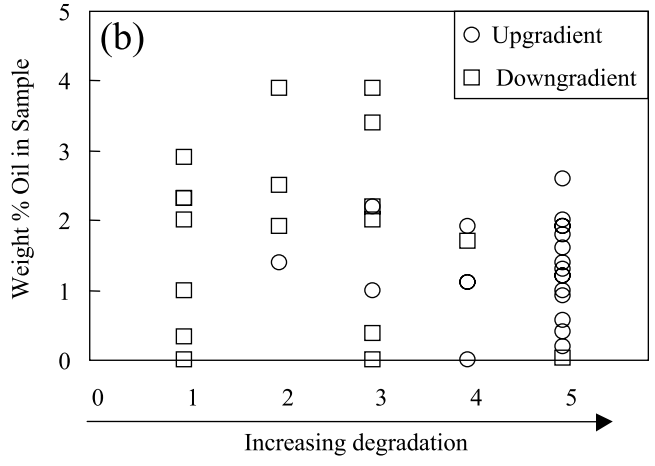


Figure 5. (a) Contour plot of oil saturation at the water table. The oil saturations approach 60% at the less degraded end and are closer to 20% at the highly degraded end of the oil body. The vertical lines show the locations of soil cores used for oil determinations. (b) Degree of oil degradation compared to weight percent oil in sample, showing that there is a slight dependence on oil quantity, but mainly the degree of degradation is related to position in the oil body.

located at either end. Both sulfate and nitrate concentrations are higher at the less degraded end of the oil body. A small population of active iron reducers is also present in the oil body. It seems plausible that recharge could transport humic acids through the vadose zone that could stimulate oil degradation by iron-reducing bacteria (e.g., Lovley et al., 1994). Figure 7c shows extracted sediment Fe(III) vs. distance along the length of the oil body. The values of Fe(III) are only 13–30% of the background value of 24 μM/g (Tuccillo et al., 1999). However, no significant differences in Fe(III) exist between the two ends, indicating that differing degrees of degradation under iron-reducing conditions cannot explain the oil data.

Microbial Populations

The final hypothesis to consider is that enhanced recharge transports nutrients required for microbial growth to the oil body. If this explanation is correct, then microbial numbers should be higher at the more degraded end of the oil body. In addition, greater numbers should be found at higher elevations, where there is better access to nutrients transported from land surface. Figure 8a and b shows the numbers of

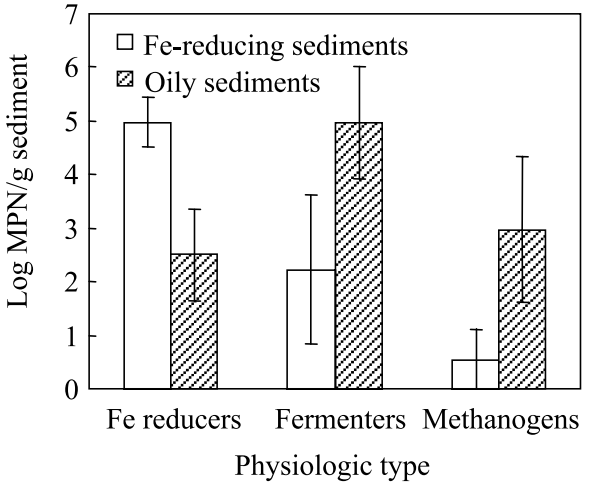


Figure 6. Bar charts comparing average populations of three microbial physiologic types from 26 locations in the oil body to a profile at the edge of the anoxic plume (9701-2, Figure 1b), which is dominated by iron reducers (Bekins et al., 2001).

methanogens and fermenters vs. distance along the oil body. Each plot has data collected from different elevations in the same borehole. Shallower populations of methanogens in the center of the oil body are 100–1000 times greater than deeper ones (Figure 8a). Observing just the methanogen profile for the deeper samples shows that the average number of methanogens in the more degraded end of the oil body is about 1000/g compared to about 100/g at the less degraded extremity. The fermenter numbers seem to show less sensitivity to position and elevation. However, their numbers are 10 times greater at the higher elevations in the less-degraded extremity of the oil body (Figure 8b). Overall, the drop in microbial numbers with depth suggests that an essential growth nutrient supplied from land surface is present in greater concentrations at shallower depths. The fact that the fermenter numbers drop with depth only at the less degraded extremity of the oil body may indicate that the vertical decrease in nutrients is more pronounced there.

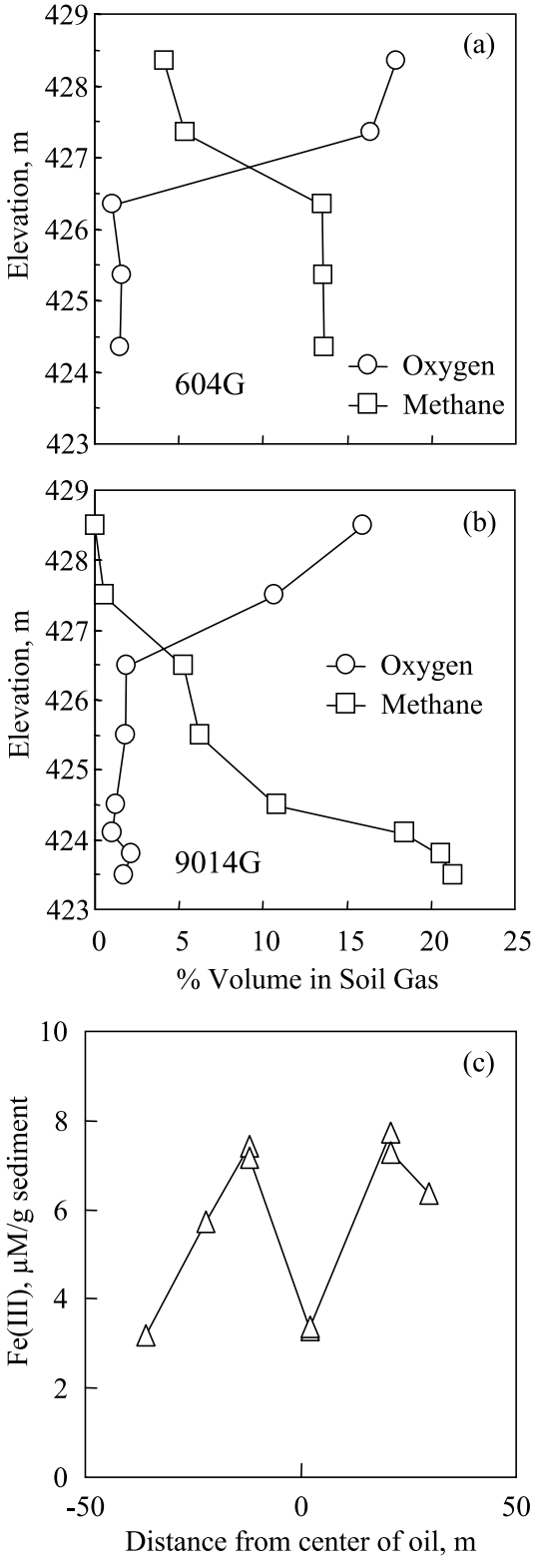


Figure 8a and b shows concentrations of sediment-extracted total inorganic nitrogen and orthophosphate for three vertical profiles spanning the vadose zone. None of the three nitrogen profiles show a significant decrease with depth. At the base of the profile for site 534, an increase in nitrogen concentration is even present. This observation is consistent with results from Amos et al. (2005), who compared argon and nitrogen gas concentrations to infer that nitrogen fixation may be occurring in the oil body. In contrast to nitrogen, phosphate concentrations drop steeply with depth. Most notably, at site 9014, the deepest sample has an orthophosphate concentration below the detection limit. Moreover, the lowest numbers of methanogens in the entire oil body are found at site 9014. Evidence that phosphorus limits microbial growth matches results of Rogers et al. (1998), who showed that the microbial populations in the contaminated aquifer scavenge phosphorus from feldspars.

Overall, the microbial and nutrient data, together with previous results from the site, provide the best explanation for the difference in degradation rates in the lower part of the oil body. Higher recharge and associated transport of phosphorus facilitates growth

Figure 7. Profiles of methane and oxygen in the vadose zone at sites 604 and 9014 (locations shown in Figure 1b) above the (a) highly degraded and (b) less degraded ends of the oil, respectively, show no difference in the availability of oxygen at the two ends of the oil body. Methane is produced at both ends, consistent with methanogenic degradation conditions. (c) Extractable iron vs. distance along the axis of the oil body shows that Fe(III) values are similar at both ends and highly depleted compared to the background value of 24 μM/g.

Table 2. Lysimeter Data

Site	Depth (m)	Nitrate (mg/L)	Sulfate (mg/L)
More degraded			
9015	1.5	nondetected	0.2
9015	4.5	0.1	1.3
Less degraded			
9014	3.0	0.3	52.3
9014	4.5	0.8	30.2

of the methanogens at the more degraded end of the oil body. Although enhanced growth of the fermenters is not apparent, it is well established that methanogenesis is the rate-limiting step during methanogenic degradation of complex carbon substrates (e.g., Schink, 1997). Downward transport of dissolved phosphorus could conceivably double with the observed difference in recharge at the more degraded end of the oil. The dissolved phosphorus could come either from land surface or from the overlying vadose zone where methane oxidation is occurring (Amos et al., 2005).

DISCUSSION

It has only recently been established in the laboratory that n-alkanes biodegrade under methanogenic conditions (Zengler et al., 1999; Anderson and Lovley, 2000). We have documented that this process can occur rapidly in the subsurface. The potential for methanogenic crude-oil degradation indicates that substantial production of biogenic methane may be possible at low temperatures in petroleum hydrocarbon reservoirs. One approach to assessing whether this process is important is to look for the characteristic degradation fingerprint that we have documented at the Bemidji site.

The subsurface methanogenic degradation rates seem to be strongly affected by moisture content and the transport of nutrients from land surface. In remediation by natural attenuation, the site hydrology is not manipulated, as it would be in an engineered cleanup scheme. Our results show that subtle, small-scale variations in the hydrologic conditions at a site may lead to rapid degradation rates in some locations and minimal degradation in nearby locations. Moreover, when soils become hydrophobic as a result of a hydrocarbon fuel spill, more extreme variations in recharge and associated degradation rates may result. Thus, when assessing the progress of natural attenuation for a separate-phase product in the subsurface, it is important to routinely analyze samples from several locations.

The study also has some implications for engineered remediation schemes. First, because nitrogen fixation occurs under a range of anaerobic conditions, it may be that phosphorus limitation is more typical in large subsurface petroleum spills. Second, where the oil is present near land surface, the soils have become very dry, suggesting that addition of water may accelerate the degradation. However, water does not easily penetrate into the pore space, suggesting that such soils may require more aggressive treatment than natural attenuation can provide. Finally, in some cases

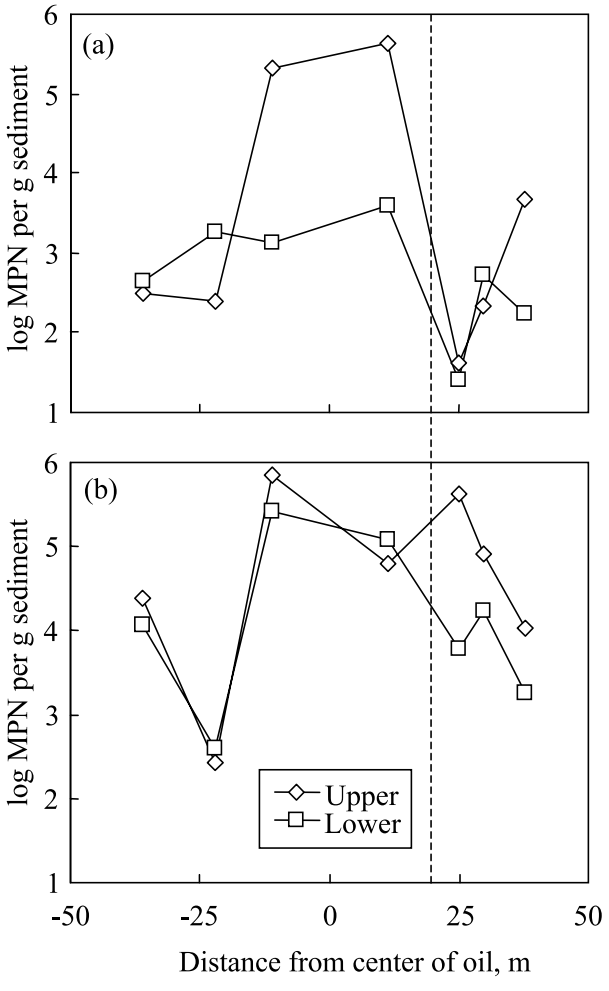


Figure 8. Most Probable Numbers vs. distance along the axis of the oil body of (a) hydrogen-using methanogens and (b) heterotrophic fermenters. Sample locations are shown in Figure 1b. "Upper" and "Lower" refer to relative elevation in the borehole at each sampling site. Dashed line marks the boundary between the highly degraded and less degraded ends.

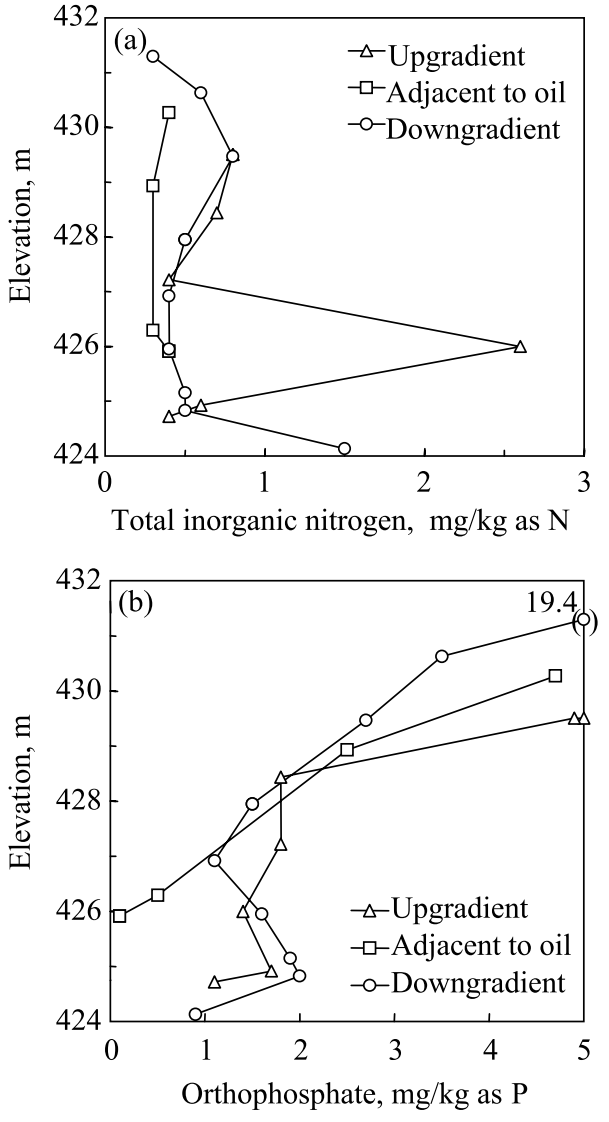


Figure 9. Nutrient profiles from three locations upgradient (site 604), adjacent to the oil (site 9014), and downgradient (site 534). Locations are plotted in Figure 1b. (a) Total inorganic nitrogen. (b) Orthophosphate.

where source removal is prohibitively expensive, subsurface hydrocarbon source areas are sometimes capped to limit further growth of a groundwater plume. The results presented here indicate that capping probably slows down the degradation rate of the separate-phase source.

This site provides an excellent control for forensic fingerprinting of hydrocarbon contaminants because the spill occurred at a single time, and the composition of the original spilled product is known. Moreover, redox conditions at the site have been monitored since 1983. Methanogenic degradation began in the oil by 1987 (Baedecker et al., 1993), and by at least 1996, a methanogenic consortium dominated the microbial population in the oil (Bekins et al., 1999). Under methanogenic conditions, the n-alkane fingerprint of the partially degraded oil forms a distinct pattern that has only recently been recognized (Hostettler and Kvenvolden, 2002). The methanogenic fingerprint, shown in Figure 2b, consists of initial loss of the high-end n-alkanes with possible enhancement in the low-end n-alkanes. When not recognized as a product of methanogenic biodegradation, this pattern could be mistaken for a mixture of a light fuel and a heavily degraded crude oil (Hostettler and Kvenvolden, 2002).

The large observed variation in the degree of degradation states has implications for forensic dating of hydrocarbon spills. The dating method of Christensen and Larsen (1993) is based on measuring the current n-C_{17}/Pr ratio. If we apply this method to the Bemidji study site, the inferred dates for the original spill range from 2 to 25 yr ago (Figure 10). Christensen and Larsen (1993) recognized the need to minimize variability in

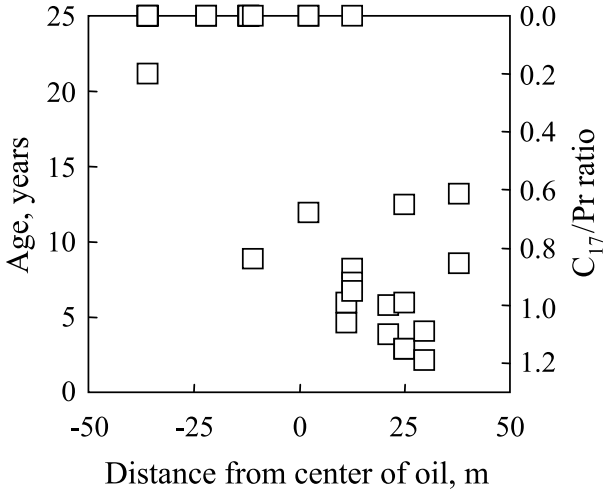


Figure 10. Estimated time since the oil spill vs. distance from the center of the oil body. The 30 samples shown were collected 22–24 yr after the August 1979 spill. The mean time estimate is 23 yr for the highly degraded area and 7.5 yr for the less degraded area. To compute the time estimates, we used the method of Christensen and Larsen (1993), which assumes constant degradation rates; complete loss of n-C_{17} occurs at T = 25 yr, and the C_{17}:Pr of the original spilled product corresponds to T = 0 yr. The method yields the equation $T = 25[1 - (\text{n-}C_{17}/\text{Pr})/1.3]$, where T is the time in years and n-C_{17}/Pr is the ratio of normal C_{17} alkane to pristane concentrations. Our equation differs slightly from that of Christensen and Larsen because our C_{17}/Pr = 1.3 for the original spilled crude oil, whereas their estimated mean value for diesel fuel was C_{17}/Pr = 1.2.

subsurface conditions when collecting samples for dating of subsurface diesel spills and mention several restrictions on the soil samples to be used in their method. They required diesel concentrations greater than 100 ppm; and samples located at least 1 m (3.3 ft) deep in the vadose zone below a paved area. Our samples meet all of these criteria except for being from below a paved area. Ironically, the oil sample locations that best match the correct date for the spill are from the enhanced recharge area. Stout et al. (2002) discussed the use of this dating method and concluded that there is a need for more studies that examine environmental effects on subsurface degradation rates. Our results show that environmental effects may cause such widely diverging degradation rates in the same spill that dating methods based on degradation state are invalid.

SUMMARY AND CONCLUSIONS

A comprehensive study of degradation of separate-phase crude oil in the subsurface was conducted at a crude-oil spill site in northern Minnesota. The oil has been in the subsurface for more than 25 yr and has undergone substantial degradation. In the study area, an oil body extends from land surface to below the water table. Oil saturations range from 20% in the vadose zone to more than 60% near the water table, and methanogenic conditions prevail over all but the top 2 m (6.6 ft) of this area. Substantial degradation of the n-alkane fraction has occurred under methanogenic conditions. The methanogenic degradation of n-alkanes first depletes the C_{18} and higher fraction, which is the reverse of the aerobic degradation progression.

Systematic variation exists in the degradation rate with position in the oil body. Very low degradation rates occur 1–2 m (3.3–6.6 ft) below land surface because of extremely low moisture conditions in the oil. In contrast, profoundly enhanced degradation rates occur 6 m (20 ft) below the land surface where there is high groundwater recharge. Recharge rates over twice the normal value expected for the region occur at one end of the oil body in a topographic low. Possible explanations for an association between recharge and degradation rates include increased transport of favorable anaerobic electron acceptors and microbial growth nutrients or enhanced dissolution of the soluble oil fraction. We present site data to examine each of these possibilities and conclude that the most likely explanation for the variation in degradation rates is recharge-facilitated transport of microbial growth nutrients from the land surface. Dissolved phosphate and nitrate data and previous work at the site suggest that the limiting microbial growth nutrient is phosphate.

The results have several general implications for sites with subsurface oil contamination. The natural hydrologic conditions at a site will control the degradation rates through both moisture content and transport of essential nutrients. Thus, the long-term natural attenuation rates of oil in the subsurface may vary dramatically in space. As a consequence, techniques for dating the time of a spill on the basis of the degree of degradation may yield very different results, depending on where the sample was collected. Techniques to identify the spilled product based on fingerprinting may provide misleading results when methanogenic conditions are present. The fingerprint of the degraded product in such cases differs from the expected pattern under aerobic conditions.

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