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Fate of Microbial Metabolites of Hydrocarbons in a Coastal Plain Aquifer: The Role of Electron Acceptors

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Geochemical and Microbiological Methods for Evaluating Anaerobic Processes in an Aquifer Contaminated by Landfill Leachate

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A combined geochemical and microbiological approach was needed to delineate the biogeochemical processes occurring in an aquifer contaminated by landfill leachate in Norman, OK, where the important microbially mediated reactions in an anoxic plume were iron reduction, sulfate reduction, and methanogenesis. The highest rates of sulfate reduction (13.2 $\mu\text{M}/\text{day}$) were detected near the water table where sulfate levels were maximal (up to 4.6 mM). The enrichment of ^{34}S in the sulfate pools ($\delta^{34}\text{S}$ of SO_4^{2-} was 67–69‰), and dissolved hydrogen measurements provided additional support for the importance of sulfate reduction near the water table. Methane was detected in the center of the plume where sulfate was depleted. Microbial incubations demonstrated concomitant sulfate reduction and methanogenesis in the anoxic portion of the plume. Although high concentrations of soluble reduced iron were detected throughout the aquifer and H_2 levels were indicative of iron reduction under steady-state conditions, microbiological experiments showed that iron reduction was active only at the edges of the sulfate-depleted portion of the plume. This study demonstrates the benefits of using a combined geochemical and microbiological approach to elucidate the spatial distribution of biogeochemical processes in contaminated aquifers.

Introduction

More than 3000 landfills are active in the United States, and thousands more are closed (1). Despite increases in recycling and incineration efforts, landfills remain the major waste disposal option in the United States. The contamination of the environment by landfill leachate is an inevitable consequence associated with landfills. Natural attenuation is receiving increased attention as a remedial option for organic components of landfill leachate that find their way into aquifers. Due to increased organic loading, oxygen is usually

depleted rapidly, and biodegradation becomes dependent on the utilization of alternate soluble and insoluble electron acceptors. The availability of alternate electron acceptors and the distribution of microbially mediated redox reactions are important factors for evaluating the efficacy of intrinsic bioremediation (2). As hydrologic and geochemical conditions in an anoxic aquifer change, the dominant terminal electron-accepting processes (TEAPs) shift, resulting in different rates of degradation of the organic contaminants. For instance, toluene biodegradation in a contaminated aquifer was shown to be linked to sulfate reduction, but the rate of degradation was also affected by the presence of $\text{Fe}(\text{OH})_3$ (3). The distribution of TEAPs in contaminated aquifers can vary over small spatial and temporal scales. In a shallow Coastal Plain aquifer that is contaminated with gasoline, the depletion of microbially reducible solid iron oxyhydroxides in thin vertical zones (<1 m) allowed sulfate reduction to occur (4). In a study of a crude oil-contaminated aquifer in Bemidji, MN, microbiological profiles indicated that aerobic, iron-reducing, and methanogenic bacteria exhibited spatial zonation on the scale of tens of centimeters (5).

Biodegradation in aquifers is often evaluated by measuring dissolved chemical species that are characteristic of particular microbial processes; these include the concentration of dissolved electron acceptors, mainly O_2 , NO_3^- , and SO_4^{2-} , or the reduced products of electron acceptor utilization, such as NH_4^+ , HS^- , Fe^{2+} , and CH_4 . For example, the reduction of iron(III) and manganese(IV) oxides in sediments by microbial processes can result in the accumulation of high concentrations of dissolved Fe^{2+} and Mn^{2+} in groundwater (6–9). Reduction of NO_3^- and SO_4^{2-} in contaminated groundwater results in the accumulation of reduced nitrogen species and dissolved sulfide (10, 11). Furthermore, the concentration of dissolved hydrogen in groundwater has been shown to be characteristic of TEAPs in some instances (12, 13), although in non-steady-state systems the interpretation of in situ H_2 concentrations is complicated by the simultaneous occurrence of microbially mediated TEAPs (14). Stable isotope analyses of dissolved electron acceptors and the corresponding reduced products can supply additional support for the occurrence of sulfate reduction and methanogenesis (15, 16).

Terminal electron-accepting processes also can be determined by measuring microbial activity in the aquifer. Microbiological evidence for TEAPs may include the enrichment of microorganisms that use particular electron acceptors. For example, in an aquifer that is contaminated with crude oil, the iron-reducing bacteria *Geobacteraceae* were found to be more numerous in iron-reducing zones of the contaminant plume (17), and populations of methanogens increased where iron-reducer populations declined as iron oxides in the sediments became depleted (18). Alternatively, the microbial utilization of electron acceptors and the production of the corresponding reduced products can be measured in sediment incubations performed in the laboratory or in the field using in situ microcosms.

Previous investigators have used the distribution of redox-sensitive constituents to identify the governing redox processes in landfill leachate plumes (10, 19). Because of the potential for transport of the reduced products of electron acceptor utilization away from the redox zone of formation into downgradient redox zones, interpretation of biogeochemical zones based solely on groundwater analyses is limited. The primary purpose of this research was to identify useful measurements for determining TEAPs in a landfill leachate-impacted aquifer. We used several geochemical and

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TABLE 1. Indicators of Active Terminal Electron-Accepting Processes

TEAP	electron acceptor concn decreased	reduced products increased	microbial activity present (microcosms)	H concn (nM)	isotopic enrichment
CO ₂ reduction	CO ₂	CH ₄	methane production	>4.0	$\delta^{13}\text{C}$ (CO ₂)
SO ₄ ²⁻ reduction	SO ₄ ²⁻	H ₂ S	sulfate reduction	1.0–4.0	$\delta^{34}\text{S}$ (SO ₄ ²⁻)
Fe ³⁺ reduction	solid-phase Fe(III)	Fe ²⁺	iron reduction	0.1–0.8	
NO ₃ ⁻ reduction	NO ₃ ⁻	NH ₄ ⁺	nitrate reduction	<0.10	

microbiological methods for evaluating the microbial processes that occur in an aquifer downgradient from the Norman, OK, Landfill. This aquifer represents an excellent example of the challenges faced in identifying redox zones in contaminated aquifers because of a large, complex source of electron donor and the presence of multiple electron acceptors in the aquifer. We found that an integrated geochemical and microbiological approach, as summarized in Table 1, provided a more comprehensive picture of biogeochemical processes in the contaminated aquifer than would a geochemical approach alone. The geochemical indicators of TEAPs that were measured included hydrogen, dissolved and solid-phase electron acceptors, reduced products, and dissolved stable isotopes ($\delta^{34}\text{S}$ –SO₄²⁻, $\delta^{13}\text{C}$ –dissolved inorganic carbon). The microbiological indicators of TEAPs that were measured included sulfate reduction, iron reduction, and methanogenic activity in sediment samples that were obtained from cores at the same locations of collected groundwater samples.

Study Methods

Field Site. The field site is a closed municipal landfill in Norman, OK, that is located in the alluvium of the Canadian River. The landfill received solid waste beginning in 1922. The waste was dumped into trenches that were about 3 m deep and that contained water to a depth of 1.5–2.5 m because of the shallow water table; the waste was subsequently covered with 15 cm of sand. No restrictions were placed on the type of material dumped at the landfill. In 1985, the landfill was closed, and the mounds, which had reached a maximum height greater than 12 m, were covered with local clay and silty-sand material. In 1995, the U.S. Geological Survey began an intensive investigation of the closed landfill as part of the Toxic Substances Hydrology Program.

The Canadian River alluvium is 10–12 m thick and is predominantly sand and silty sand, with interbedded mud and gravel. Near the landfill, the water table is less than 2 m below land surface. The hydraulic conductivity of subsurface materials near the landfill, which was measured by use of slug tests, ranged from 7.3×10^{-2} to 24 m/day (20). A discontinuous low hydraulic conductivity interval that consisted of silt and clay was detected about 3–4 m below the water table along the transect where most data have been collected (Figure 1). A high hydraulic conductivity layer containing coarse sand and gravel is located near the base of the alluvium at a depth of about 10–12 m (21). Low-permeability shales and siltstones in the Hennessey Group of Permian age, which act as a boundary to vertical groundwater flow, underlie the alluvium. A shallow stream with ponded areas caused by beaver dams (referred to here as a slough), which is about 0.75 m deep, lies roughly parallel to the landfill and about 100 m to the southwest (Figure 1). Groundwater flows from the landfill toward the slough and the Canadian River (20).

Groundwater Sampling. A network of small-diameter temporary wells (Figure 1) that consisted of 2.5 cm diameter, schedule-80 stainless steel line pipe with a screen length of 8.7 cm were installed in 1996. The temporary wells were driven

at sites where sediment cores were collected for microcosm experiments and geological characterization of the sediment. These wells were driven with an electric jackhammer to the desired sampling depths, which were based on field analysis of the cores, from just below the water table (depth about 1 m) to the base of the aquifer (depth about 11 m). Teflon tubing was attached directly to the screened sandpoints, and samples were withdrawn by using a peristaltic pump. Samples were collected from vertical profiles that are located downgradient from the landfill (well sites 35, 38, and 40) and at an upgradient background location (NPD) that is northwest of the landfill and from the slough (Figure 1).

Permanent multi-level samplers (MLS) were installed in 1997. Each well site contained 7 nested well casings that were set at different depths with 0.12 m length screens. The set of 2.5-cm PVC wells were installed together in a 20-cm augered hole. The auger flights were withdrawn, and the alluvial sands collapsed around the wells. In each cluster, the first six well screens were set about 0.5, 1.0, 2.0, 3.0, 4.0, and 7.0 m below the water table at the time of emplacement, although the screen depths were adjusted up or down small distances to avoid low-permeability zones. The seventh screen was set just above the base of the alluvium at 11–12 m.

Samples were collected from the temporary wells in April 1996 and from the MLS network in April 1997. Cross-sectional profiles of groundwater chemistry from well 35 through well 80 along the transect (Figures 2 and 3) were generated by using the 1997 data (shown as diamonds in the figures) and the 1996 data (shown as triangles in the figures). Sediment samples were collected in March 1996 by using a Geoprobe Macro-Core soil sampler. The cores were immediately flushed with nitrogen, capped, and stored at 4 °C under a nitrogen atmosphere.

Field-Collection Procedures and Analyses. Specific conductance, pH, and temperature of groundwater were determined in the field using specific electrodes with thermocouples; dissolved oxygen (DO), Fe²⁺, NH₄⁺, and H₂S concentrations were determined by using colorimetric CHEMets kits (CHEMetrics Inc., Calverton, VA). Alkalinity was determined by an incremental titration method in a mobile field laboratory. Dissolved hydrogen (H₂) was determined (on samples from only the PVC wells) in the field with a reduction gas analyzer (RGA3, Trace Analytical, Menlo Park, CA) by using the gas-stripping method, which is described by Chapelle et al. (22). Water samples for measurement of CH₄ concentrations were collected in Glasspak syringes that were connected directly to the sample-pump outlet (23). The water samples were transferred from the syringe into 25-mL serum bottles containing mercuric chloride (resulting in a concentration of 0.2 mM Hg). Water that was collected for analyses of nonvolatile dissolved organic carbon (NVDOC) was filtered in line through a 0.45- μm silver filter. Samples collected for determination of anions were filtered through a 0.2- μm Nuclepore filter. Cation samples were filtered through 0.1- μm Nuclepore filters and acidified with HNO₃ to a pH of 2. Samples for determination of $\delta^{13}\text{C}$ of total dissolved inorganic carbon (TDIC) were filtered through 0.4- μm Nuclepore filters, collected in glass bottles,

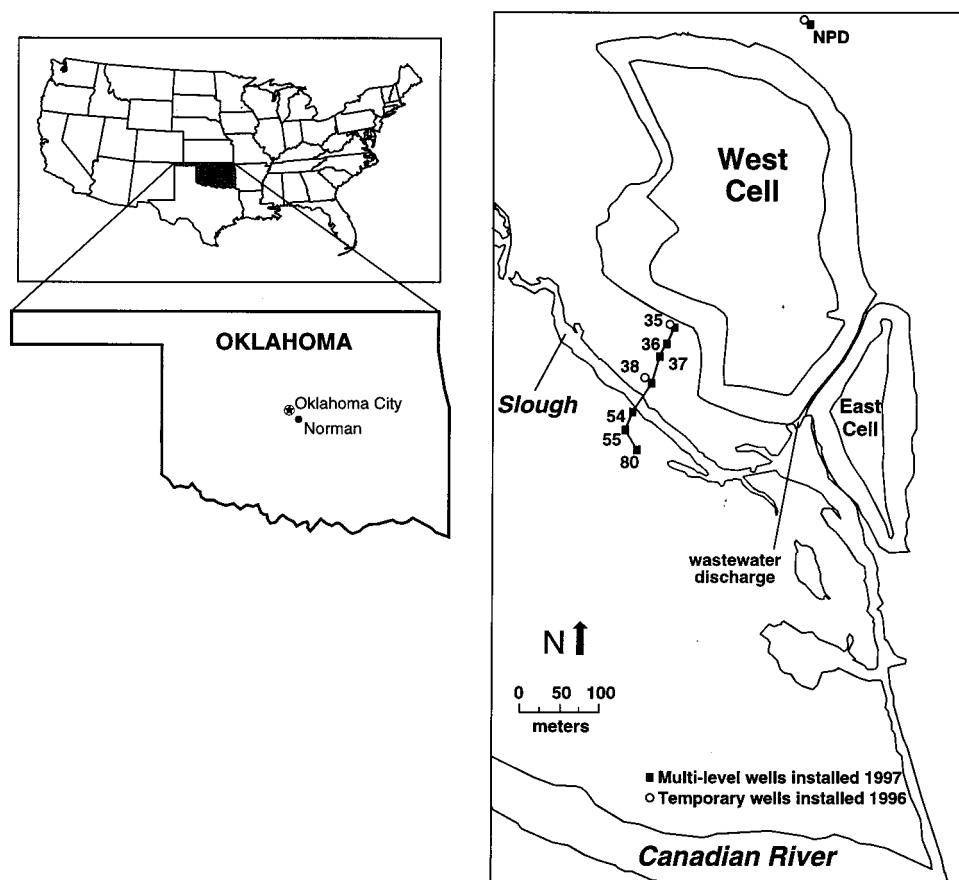


FIGURE 1. Map of the Norman Landfill, showing the location of the multi-level samplers and temporary wells.

and preserved with a ammonium hydroxide/strontium chloride solution as described by Gleason et al. (24). Samples for measurement of $\delta^{34}\text{S}$ of SO_4^{2-} were filtered through 0.2- μm Nuclepore filters, and samples collected for δD and $\delta^{18}\text{O}$ of H_2O were collected without filtration. All samples were stored on ice after collection and during transport to the laboratory.

Laboratory Analytical Procedures. Dissolved methane concentrations in groundwater were measured by headspace analysis and gas chromatography using a molecular sieve capillary column (23). Anion concentrations were measured by ion-exchange chromatography. Concentrations of NVDOC were determined by the persulfate wet-oxidation technique by using a carbon analyzer (23). The determination of $\delta^{13}\text{C}$ values of TDIC was made on SrCO_3 precipitates with an isotope ratio mass spectrometer (23).

Iron Reduction and Methanogenesis Experiments. Microbial activity assays of sediments were performed within 2 days after collecting the samples. The sediments were processed inside an anaerobic glovebag that was filled with 100% N_2 . Anoxic sediment slurries (15 g/15 mL) were prepared in 160-mL serum bottles with sediment from cores and groundwater collected from the adjacent monitoring wells. The slurries were incubated under a N_2/CO_2 (80/20) headspace in the dark at 18 °C (the approximate in-situ temperature of the aquifer at the time of sampling). During the experiments, pH values were monitored and remained near neutral. Autoclaved sediment was incubated to test for abiological activity. Production of soluble Fe^{2+} was monitored by periodic removal of liquid samples for analysis by ferrozine assay as previously described by Lovley and Phillips (25). Measurement of CH_4 concentrations in the microcosms was by gas chromatography analysis according to the procedure of Beeman and Suflita (26).

Sulfate-Reduction Activity and Iron Sulfide Analyses.

For the sulfate-reduction assays, sediment samples were obtained by pushing 5-mL syringe cylinders (with distal end removed) into the cores. An anoxic solution of $^{35}\text{SO}_4^{2-}$ (100 μL) (20 $\mu\text{Ci mL}^{-1}$; carrier free; ICN) was injected into the samples with a syringe and needle. The incubations were sealed with butyl rubber stoppers and placed inside ammunition boxes under an N_2/CO_2 (80:20) atmosphere. The samples were incubated for 17 h at 18 °C. Sulfate-reduction activity was then determined by extracting ^{35}S -labeled total reducible inorganic sulfides ($^{35}\text{TRIS}$) as described by Ulrich et al. (27). Iron monosulfides and iron disulfides were determined as described by Ulrich et al. (27).

Results and Discussion

Geochemical Indicators of Redox Processes. Chloride and NVDOC profiles along the well 35 to well 80 transect confirm that the plume extends through the entire thickness of the alluvium between the landfill and the slough and has migrated beneath the slough (Figure 2A,B). Groundwater downgradient from the landfill has high concentrations of NVDOC (Figure 2B) as compared to groundwater collected upgradient and northeast of the landfill at site NPD (Figure 1), where the average concentration is <0.2 mM. The high NVDOC concentrations result from the dissolution and partial degradation of organic waste in the landfill. The maximum concentration of NVDOC (17 mM) was measured close to the landfill edge at site 40 (Figure 1).

Degradation of organic compounds to inorganic carbon is evident by the increase in alkalinity (up to 7 times the background concentrations) of groundwater downgradient from the landfill, resulting in an alkalinity plume similar in shape to the NVDOC plume (Figure 2C). Background water pH values ranged from 6.9 to 7.3, whereas values throughout

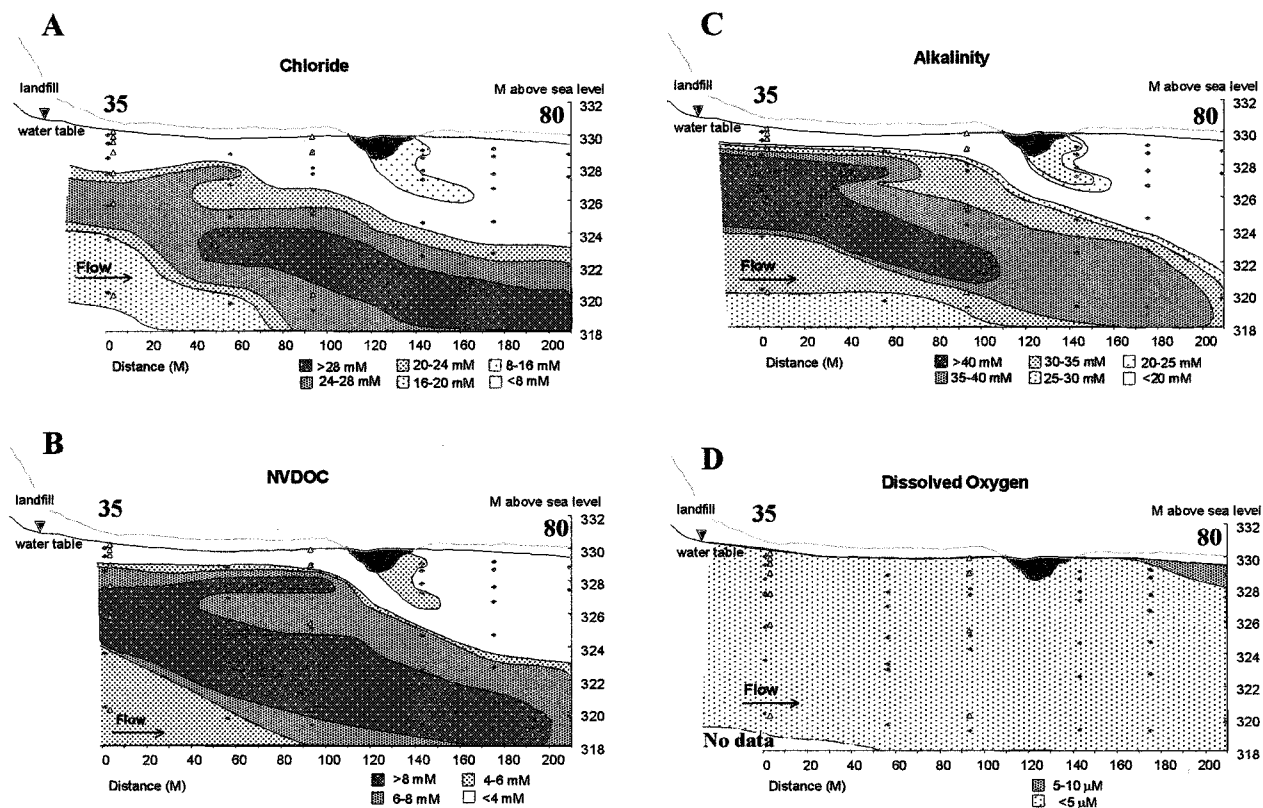


FIGURE 2. Distribution of (A) chloride (Cl^-) concentrations (in mM), (B) nonvolatile dissolved organic carbon (NVDOC) concentrations (in mM), (C) alkalinity values (in mM HCO_3^-), and (D) dissolved oxygen (DO) concentrations (in μM) in groundwater downgradient from the Norman Landfill along a transect from site 35 to site 80.

the high chloride plume along transect 35–80 ranged from 6.7 to 7.3. Degradation processes in the leachate-contaminated aquifer have resulted in the depletion of oxidized chemical species (such as O_2 and SO_4^{2-}) and the accumulation of reduced products (such as Fe^{2+}) in groundwater (Figures 2 and 3). The entire area that was sampled along the transect (wells 35–80) between the landfill and the slough is anoxic ($<5 \mu\text{M}$ DO, Figure 2D). Consequently, aerobic respiration is probably a degradation process of limited importance in the majority of the contaminated aquifer. High levels of ammonium in the groundwater immediately downgradient from the landfill ($>5 \text{ mM}$, Figure 3B) are consistent with nitrate reduction, yet nitrate levels in the contaminated aquifer and in the background water are very low ($<0.1 \text{ mM}$). The large NH_4^+ plume downgradient from the landfill most likely results from the transport of NH_4^+ produced during the fermentation of organic matter within the refuse mounds. At the boundaries of the plume there is a sharp decrease in the concentrations of NH_4^+ . Ammonium sorption onto sediment or oxidation along the plume boundary may control the transport of ammonia. The oxidation of ammonia may create local zones, such as near the water table, where NO_3^- may be produced and is available as an electron acceptor. Nitrate concentrations as high as 2.7 mM were periodically detected at the water table in 1999 during monthly sampling of the MLS well at site 35.

High concentrations of Fe^{2+} in the groundwater ($>0.25 \text{ mM}$, Figure 3A) are consistent with microbial dissolution of iron oxides in the aquifer. However, reactive iron oxides were depleted from sediments within the contaminated region of the aquifer (28). Hematite grain coatings remained on all samples collected within the anoxic plume (29) and may be available as a source of electron acceptor to iron-reducing bacteria. Nevertheless, the persistence of hematite relative to iron oxyhydroxides in the Norman Landfill plume suggests

that the microbial reduction of hematite, if it does occur, proceeds at very slow rates.

Sulfate reduction in the plume has resulted in the depletion of SO_4^{2-} from the main body of the plume (Figure 3C). Dissolved sulfate at the edge of the landfill (site 35) was enriched in ^{34}S . At the water table (1.0 m below land surface (bls)), $\delta^{34}\text{S}$ of SO_4^{2-} was 33‰ , and at 1.6 m bls the $\delta^{34}\text{S}$ of SO_4^{2-} was 67‰ . In contrast, sulfur isotope analyses of SO_4^{2-} in groundwater from uncontaminated alluvium showed that the $\delta^{34}\text{S}$ of SO_4^{2-} was -5‰ at 1.0 m bls. Although dissolved sulfide concentrations in the plume were low ($0.3\text{--}5.0 \mu\text{M}$), analysis of the sediment cores indicated that iron sulfides have accumulated in the aquifer. The highest concentrations of iron sulfides were detected just beneath the water table where increased sulfate reduction rates were measured (Figure 4). The high concentrations of dissolved SO_4^{2-} at the water table may result from the reoxidation of these solid sulfides or may be supplied during recharge events from the dissolution of sulfate-containing construction wastes or sulfate deposited in the unsaturated zone during dry periods. The oxidation of sedimentary sulfides from the contaminated regions of the aquifer (mainly FeS_2 and FeS) was evaluated in the laboratory. The sulfides were stable under anoxic conditions but were oxidized rapidly when exposed to oxygen, indicating that this reaction may occur on a relatively short time scale. Another possible source of sulfate within the anoxic plume may be barite dissolution. Barite was identified in the alluvium (29), and high concentrations of barium (up to $100 \mu\text{M}$) were detected in the contaminant plume, where sulfate is depleted relative to background wells. In the sulfate-depleted zone, the groundwater is undersaturated with respect to barite. Barite in the alluvial sediments dissolves, resulting in a small source of SO_4^{2-} as an electron acceptor and the relatively high concentrations of barium in solution.

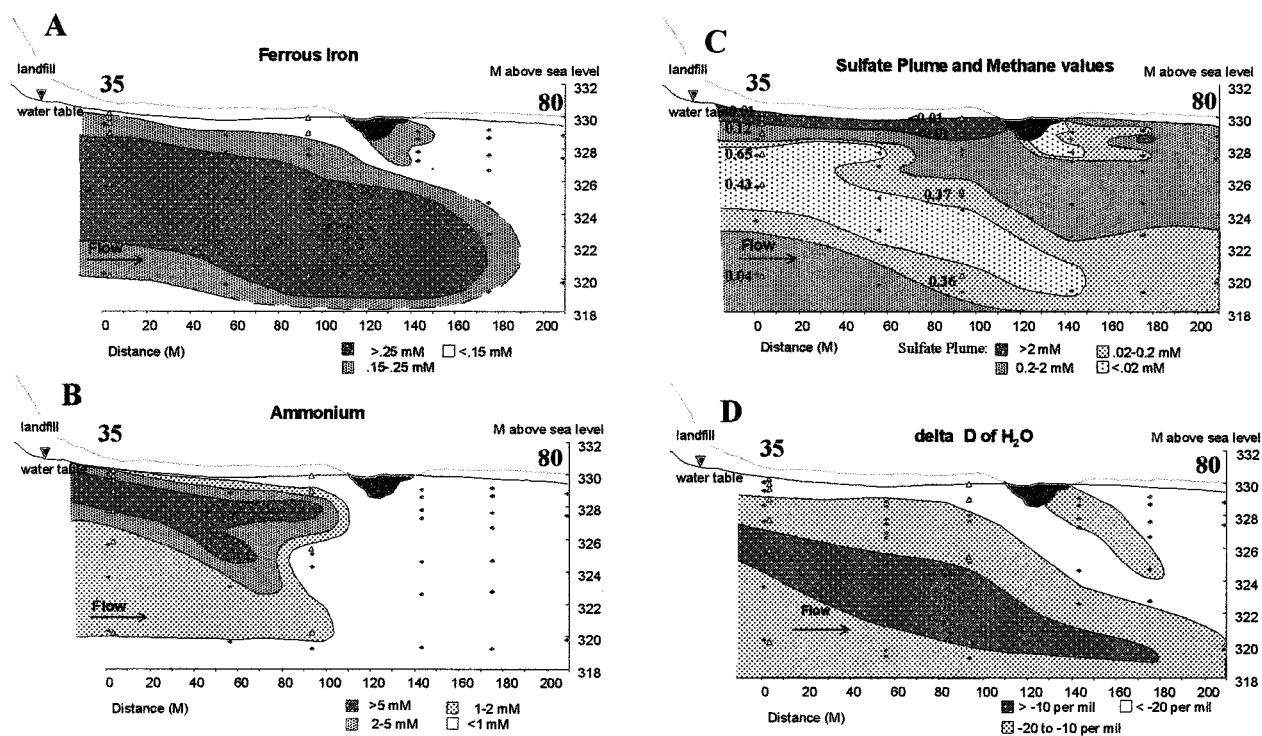


FIGURE 3. Distribution of (A) ferrous iron concentrations (in mM), (B) ammonium concentrations (in mM), (C) sulfate concentrations (in mM) and methane concentrations (in mM, shown on the plot as individual values at sites 35 and 80), and (D) δD of H_2O values (‰ relative to VSMOW) in groundwater downgradient from the Norman Landfill along a transect from site 35 to site 80.

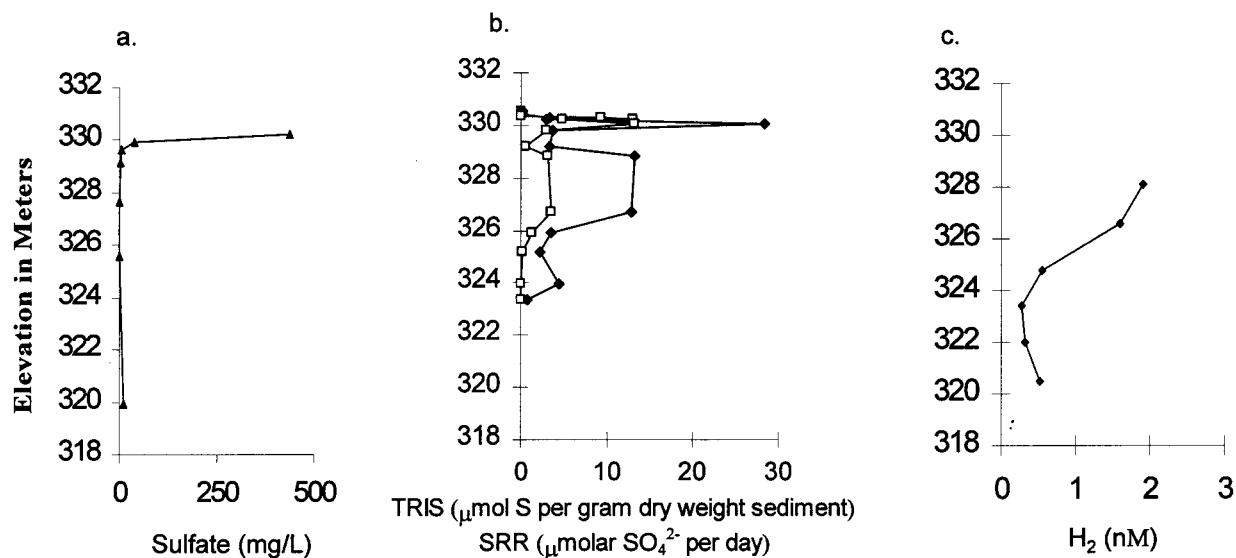


FIGURE 4. (A) Sulfate concentrations (mg/L). (B) Sulfate reduction rates (SRR, open squares on the plot) in $\mu\text{mol } SO_4^{2-} \text{ /day}$ and total reduced inorganic sulfur (TRIS, solid diamonds on the plot) in $\mu\text{mol S/g}$ dry weight of sediment from site 35. (C) Hydrogen concentrations (nM) in groundwater at site 35.

At the edge of the landfill, concentrations of CH_4 are highest in the center of the anoxic plume. The highest CH_4 concentrations occurred slightly beneath the top of the sulfate-depleted zone (Figure 3C). The δD values for groundwater downgradient from the landfill indicate that the groundwater is enriched in deuterium (Figure 3D). Groundwater that is contaminated by leachate discharging from other landfills is enriched in deuterium (10, 30, 31). Hackley et al. (30) reported 30–60‰ deuterium enrichment in leachate from three landfills in Illinois and speculated that most of the enrichment was a result of methanogenesis, with some enrichment resulting from isotopic exchange with hydrogen sulfide. The greatest enrichment in deuterium at our study

site was measured in the center of the plume where δD of H_2O values ranged from -10.6 to -3.4 ‰, as compared to background values of -45.8 to -27.9 ‰. The samples that contained the greatest enrichment in deuterium also had enriched ^{13}C of TDIC values. The $\delta^{13}C$ of TDIC values in the most contaminated groundwater downgradient from the landfill were as heavy as 11.9‰, which indicated significant enrichment in ^{13}C as compared to typical $\delta^{13}C$ values for shallow groundwater from the Central Oklahoma aquifer, which ranged from -17.8 to -12.5 ‰ (32). This large shift in isotopes to enriched values in the groundwater most likely results from biogenic CH_4 production, a process that results in significant fractionation of carbon isotopes (31). The high

concentrations of CH_4 combined with the heavy δD of H_2O values at the edge of the landfill at Norman, OK, indicate that methanogenesis likely occurs within and underneath the landfill and that the products of this process are transported in groundwater downgradient from the landfill (Figure 3C,D).

Concentration gradients near the landfill are steepest near the water table. Sulfate concentrations in the leachate plume vary by more than 3 orders of magnitude downgradient from the landfill (from 0.001 to 5.27 mM (Figure 3C)), whereas concentrations in the upgradient water vary by a factor of 2 (0.72–1.62 mM). Ferrous iron and NH_4^+ concentrations vary by more than 2 orders of magnitude (Figure 3A,B) in the contaminated water. The depletion of SO_4^{2-} and increase in Fe^{2+} and CH_4 concentrations in the deep water zones indicate that the most reduced part of the plume is within a zone 3–7 m below the water table. This zone contains the highest concentrations of CH_4 , about 0.65 mM (Figure 3C).

Microbial Measurements of Redox Processes. The chemical concentration gradients observed in the groundwater reflect the different microbial processes that occur in the contaminated aquifer. The laboratory microcosm experiments conducted with sediment collected at site 35 are consistent with the water composition data. Dissolved hydrogen measurements indicated that sulfate reduction was the dominant TEAP in a zone just below the water table where increased levels of sulfate were detected (Figure 3). Sulfate reduction rates measured in sediment from site 35 were greatest a few centimeters below the water table where dissolved SO_4^{2-} concentrations were highest (Figure 4A,B). Dissolved H_2 concentrations measured in groundwater at elevations above 325 m at site 35 were in the range of 1.6–1.9 nM (Figure 4C); this supports the assumption that sulfate reduction was a dominant microbial process in this region of the aquifer (22). High iron sulfide content of the sediment (TRIS, Figure 4B) and an enrichment of isotopically heavy SO_4^{2-} in the groundwater ($\delta^{34}\text{S}$ of SO_4^{2-} were as heavy as 69‰) provide additional evidence that sulfate reduction is likely the dominant terminal electron-accepting process just below the water table.

The rates of sulfate reduction and the concentrations of reduced sulfur in the sediment in the upper 1 m of the saturated zone vary widely over short vertical distances. This is likely due to the transient nature of the cycling of sulfur in this zone as oxygenated recharge water infiltrates after rain events. A rate of sulfate reduction of about $3.5 \mu\text{M SO}_4^{2-}/\text{day}$ in the center of the anoxic plume (Figure 4B, at an elevation of 327 m) would result in accumulation of S in the sediment at a rate of about $0.15 \mu\text{mol/g dw}$ of sediment in 1 year. At this rate, it would take about 80 years to accumulate the sulfur measured in the sediment at this location, consistent with the age of the landfill.

Under the sulfate-depleted zone, at elevations below 324 m near the landfill edge, the groundwater still contains high concentrations of dissolved iron and has more sulfate than the water in the center of the plume. This is likely attributed to mixing of the plume with background water that contains higher levels of sulfate. At the edge of the landfill (site 35), hydrogen concentrations are low at this depth (Figure 4C), which is consistent with iron reduction as the dominant degradation reaction (28). A small amount of iron reduction, $2 \text{ nmol of Fe}^{2+} \text{ g}^{-1} \text{ day}^{-1}$ calculated for the first 25 days of the experiment, was detected in sediments collected at this location (at 324 m, Figure 5), which supports the hypothesis that iron reduction may be occurring at the boundaries of the highly reduced, sulfate-depleted zone of the plume. Iron reduction also was detected in samples collected near the water table at a rate of $6.5 \text{ nmol of Fe}^{2+} \text{ g}^{-1} \text{ day}^{-1}$ (calculated for the first 21 days for the sediment incubations at 329.4 m, Figure 5). Iron reduction was not detected in autoclaved

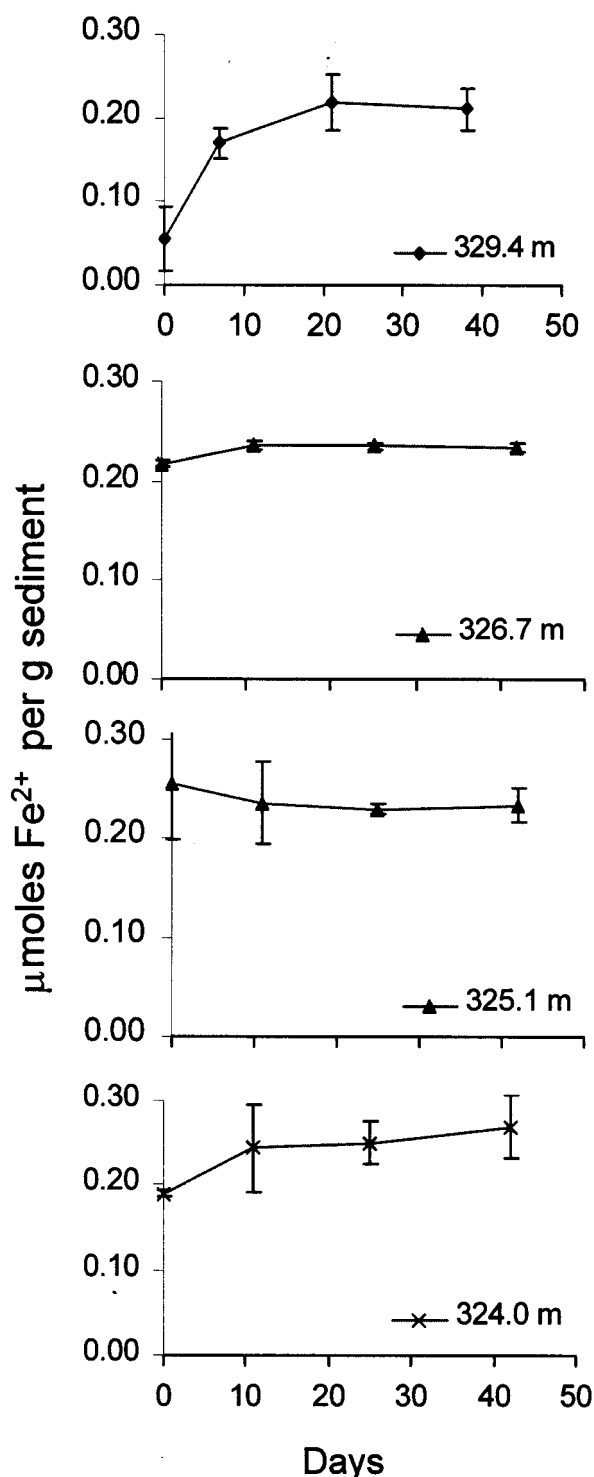


FIGURE 5. Iron production over time in biologically active microcosm experiments using sediments collected at four elevations from site 35.

incubations, indicating that the iron-reducing activity was microbially catalyzed. A source of microbially reducible oxidized iron near the water table may be produced when oxygenated recharge water mixes with the shallow anoxic plume and iron oxyhydroxides precipitate. The iron oxyhydroxides are subsequently available for iron reduction as the water table rises. However, sediments from only 30 cm higher (329.7 m) in the same core showed much less iron reduction, indicating that this process may vary over short distances near the water table.

The high concentrations of Fe^{2+} measured in the groundwater within the sulfate-depleted zone in the center of the anoxic plume (Figure 3A), coupled with the very low SO_4^{2-} concentrations (Figure 3C) indicated that iron reduction was potentially an important process. Dissolved hydrogen measurements made in groundwater at an elevation of 325 m and below are less than 1 nM, suggesting that iron reduction is the dominant terminal electron-accepting process in this region of the aquifer (12, 13). Iron reduction, however, was not detected in microcosm experiments that were conducted with sediments from within this zone (326.7 and 325.1 m, Figure 5). It should be noted that slow rates of iron reduction would not have been detected with this assay because of the high background concentrations of Fe^{2+} in the water. Nevertheless, the microcosm results demonstrate that it is likely that the source of the dissolved Fe^{2+} at this location may be from reduction of iron oxyhydroxides upgradient from the landfill or the dissolution of iron-containing wastes in the landfill. The increased levels of dissolved methane (Figure 3C) and the detection of methane production in sediment slurry incubations (Figure 6) indicate that methanogenesis was an important process in the center of this plume, where sulfate was depleted. These results illustrate the importance of coupling microbiological evidence with geochemical measurements when assessing active biogeochemical processes within a contaminant plume. Similarly, Albrechtsen et al. (33) found that high dissolved iron concentrations in groundwater downgradient from the Vejen Landfill in Denmark did not correlate with an iron-reducing zone in the aquifer. Jakobsen et al. (14) also observed low H_2 concentrations (indicative of iron-reducing conditions using the scale suggested in ref 13) in areas of the Grindsted Landfill leachate plume that supported sulfate reduction and methanogenesis. They postulate that this apparent discrepancy is the result of non-steady-state conditions and may be typical for anaerobic plumes.

The rates of the microbial processes that were measured in the anoxic plume at the Norman, OK, Landfill are comparable to measurements made in sandy aquifers contaminated with landfill leachate in Denmark. The rate of Fe^{2+} production in the aquifer sediments collected at an elevation of 329.4 m at Norman Landfill was $6.5 \text{ nmol of Fe}^{2+} (\text{g of sediment})^{-1} \text{ day}^{-1}$, whereas at an elevation of 324.0 m the rate was $1.9 \text{ nmol of Fe}^{2+} (\text{g of sediment})^{-1} \text{ day}^{-1}$. These rates are in the same range as those measured by Ludvigsen et al. (34) in the sands of the leachate-polluted aquifer at the Vejen Landfill ($7\text{--}12 \text{ nmol of Fe(II)} (\text{g dw of sediment})^{-1} \text{ day}^{-1}$). The sulfate-reduction rate in the center of the anoxic plume at the edge of the Norman Landfill, which was $0.67 \text{ nmol of SO}_4^{2-} (\text{g of sediment})^{-1} \text{ day}^{-1}$ at the 327 m elevation (Figure 4B), is within the range of the rates of sulfate reduction ($0.01\text{--}1.3 \text{ nmol of SO}_4^{2-} (\text{g dw of sediment})^{-1} \text{ day}^{-1}$) measured by Ludvigsen et al. (34) in the leachate plume of the Grindsted Landfill, Denmark.

The methane-production rate detected in the center of the Norman Landfill leachate plume at an elevation of 326.7 m (Figure 6), $0.20 \text{ nmol of CH}_4 (\text{g of sediment})^{-1} \text{ day}^{-1}$ for the first 75 days, is slightly higher than the maximum methane production rate measured in the Grindsted Landfill. In the microcosm experiments conducted with Norman Landfill sediment, other degradation processes were not inhibited, and the higher rate of methane production observed after 75 days of incubation (Figure 6) might have been caused by the depletion of electron acceptors or donors used by competing microbial populations in these sediments. Therefore, these higher rates may be less representative of the rates occurring in situ. Regardless of the actual rates, however, these microcosm experiments indicate that the Norman Landfill sediments in the center of the leachate plume (at an elevation of 326.7 m) support both methane production and sulfate

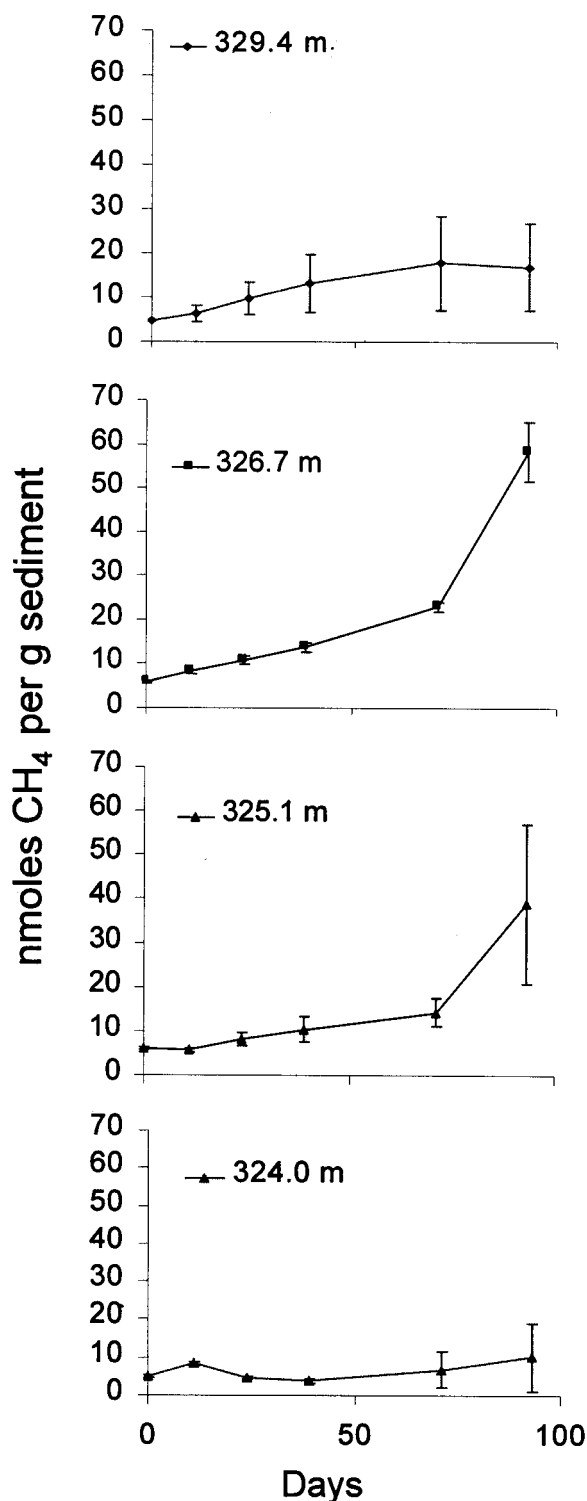


FIGURE 6. Methane production over time in biologically active microcosm experiments using sediments collected at four elevations from site 35. Twice autoclaved controls showed no increase in methane over the duration of the experiment.

reduction, which indicates that both of these processes may be occurring in this region of the plume. The low concentrations of sulfate in the center of the leachate plume probably are too low to inhibit methane production (26), which allows methanogenesis and sulfate reduction to occur simultaneously, similar to the observations of Ludvigsen et al. (34) and Vroblesky et al. (35). Measurement of H_2 concentrations in this area of the plume did not prove to be a useful tool

in sorting out these processes; at 325 m the concentrations (0.6 nM) were in the range indicative of iron reduction (13), yet incubations of sediment from this zone showed sulfate reduction at a slow rate (0.2 $\mu\text{M SO}_4^{2-}/\text{day}$), methane production, and no iron reduction. A possible explanation for the apparent inconsistency in process indicators is that the methanogens in this system may rely on substrates other than H_2 to produce CH_4 .

Within the center of the plume, the stability of the remaining iron oxides, including hematite, likely limits iron reduction and may allow sulfate reduction to become energetically favorable at the expense of iron reduction, as described by Postma and Jakobsen (36). In this same region of the plume, the iron reduction rate was not measurable. Iron reduction, however, does appear to occur above the most anoxic part of the plume and near the bottom of the plume (Figure 5). Thus, the zones of iron reduction appear to overlap with the zones of sulfate reduction but not the area that supports methanogenesis.

Significance of Microbial Degradation Rates. The degradation of organic contaminants in the Norman Landfill aquifer by the processes of microbially mediated iron reduction, sulfate reduction and methanogenesis is dependent on the balance between the rates at which these processes occur and the rate at which contaminants are transported downgradient in the flowing groundwater. The amount of total dissolved carbon that could be consumed as water moves through the center of the anoxic plume from site 35 to site 38 was calculated by using an average groundwater flow velocity of 0.041 m/day (calculated from slug test data of Scholl and Christenson (20)), an average porosity of 39%, and a bulk dry density for the aquifer sediment of 1.62 g/cm³. Assuming the rates of sulfate reduction and methanogenesis that were determined from the experiments on sediment from site 35 were sustained throughout the anoxic, low-sulfate plume (from 0 to 140 m, Figure 3C), it was determined that 14.9 mM C could be consumed by sulfate reduction along this flow path and that 3.1 mM C could be consumed by methanogenesis. Examination of the geochemical data from the field, however, indicate that the maximum concentration of C in this region of the aquifer is 13.2 mM, and the concentrations of NVDOC, when corrected for dilution and dispersion by Cl^- concentrations, does not change significantly along this distance (Figure 2B). The persistence of the NVDOC through the anoxic core of the plume may be due to the age of the organic matter in the landfill. In the microbial assays, the microorganisms may be using the small amount of more labile or volatile carbon that is present in the aquifer at site 35 near the edge of the landfill (37). As these compounds are degraded along the flow path between 35 and 80, the rates of microbial processes may decline. Thus, the use of laboratory-based rates may overestimate the actual in-situ rates of microbially catalyzed redox reactions when extrapolated to the entire flow path because of changes in the availability of electron donor in situ.

Conceptual Model of Active Microbial Processes. The combination of geochemical and microbiological measurements used in this study can be used to generate conceptual models of the microbially mediated terminal electron-accepting processes in shallow aquifers. Within the section of the leachate plume examined in this study, the cycling of sulfate and iron and the formation of methane are important biogeochemical processes that affect aquifer geochemistry downgradient from the Norman Landfill. Although the core of the plume is strictly anaerobic and supports both sulfate reduction and methanogenesis, the edges of the plume appear to support iron reduction and, to a greater extent, sulfate reduction, possibly due to the increased availability of readily reactive electron acceptors at these boundaries.

The nonuniform availability of electron acceptors and the mixing of the contaminant plume with oxygenated water at the plume boundaries appear to have a significant impact on biogeochemical processes and result in sharp concentration gradients in the groundwater. High rates of sulfate reduction near the water table appear to be controlled by the rate of sulfate supply from sulfide oxidation and/or dissolution of sulfate during recharge events. Although sulfate reduction and methane production occur within the anoxic core of the plume, there are high concentrations of NVDOC transported through this region of the aquifer. The rates of sulfate reduction and methanogenesis measured in the laboratory microcosm experiments on anoxic sediment near the edge of the landfill do not appear to be sustained through the anoxic plume.

Since the availability of electron acceptors for degradation reactions is an important control on the fate of organic compounds, the elucidation of the TEAPs in contaminated hydrogeologic environments, such as the Norman Landfill, is critical to understanding the natural attenuation of organic contaminants. Measurements of dissolved chemical species, such as ferrous iron and sulfide, at specific locations in this leachate-contaminated aquifer were found to be poor indicators by themselves of active microbial processes. In addition, measurements of H_2 concentrations alone were inconclusive and likely reflect the fact that the redox processes in this system are not mutually exclusive but rather occur simultaneously. The microbial incubations combined with geochemical measurements provided a more robust identification of areas of in-situ sulfate reduction activity and, to a lesser extent, methanogenesis. In contrast, the presence of iron reduction was less evident because of the limitations inherent in the iron-reduction assays. The results of this study underscore the benefits of conducting microbiological analyses along with measuring chemical concentrations. This creates a stronger basis for evaluating the biogeochemical processes that occur in contaminated subsurface environments, such as landfill leachate-contaminated aquifers, that are unlikely to be in a steady state.

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