Problem and Research Objectives: An environmental necessity, municipal landfills are a threat to local water quality as well as a contributor to global warming. In 1997 there were about 2500 landfills operating in the U.S., down from nearly 8000 in 1988 (Glenn, 1998). While the number of operating landfills is decreasing, the number of closed landfills is increasing, posing an increased risk of leachate contamination into underlying aquifers. Several studies have characterized the anaerobic environments of landfill leachate plumes (e.g., Baedecker and Back, 1979; Lyngkilde and Christensen, 1992; Ludvigsen et al., 1998; Cozzarelli et al., 2000). These plumes typically exhibit a succession of redox conditions with methanogenesis dominating near the source, followed downgradient by sulfate reduction, iron reduction, nitrate reduction, and oxygen reduction. The distribution of these redox states has a profound effect on the degradation of dissolved organic compounds. As a result, the accurate prediction of pollutant destruction in these environments requires precise observation of a number of ecological parameters including the activity of the resident microorganisms.

The work performed by implementation of this proposal has been to determine the presence and distribution of microbial processes that are occurring in the aquifer downgradient from the Norman landfill and to assess the impact of these processes on the quality of the groundwater. This project has been addressed in a variety of ways that include on-site field measurements and observations as well as laboratory experimentation and analyses. The findings are certainly not restricted to the Norman landfill. Many of our results will be useful to other investigators who are working in related areas at other field sites.

Methodology: This project involves the measurement of dissolved hydrogen gas in the groundwater downgradient from the landfill. We are investigating the usefulness of hydrogen as an indicator of the dominant microbial processes that could be occurring at the site. Information concerning the three-dimensional distribution of microbial processes is required in order to accurately describe a subsurface ecosystem and predict the rate of biodegradation of pollutant chemicals. Moreover, microbial activity often changes with time. Dissolved hydrogen measurements made over time may be a useful indicator of the dynamics of microbial communities in soil. If hydrogen can be used to make this description, then the task of characterizing the environment is greatly simplified. Traditionally, this characterization involves obtaining sediment cores that are used in laboratory experiments that can incur a significant expense while often requiring long incubation times and tedious sampling. Groundwater sampling for hydrogen is much simpler and cost effective in that no sediment is required. Water can be pumped from wells that have been installed at the site of interest and can then be removed after hydrogen monitoring is complete, resulting in minimal disturbance to the environment.

A thorough understanding of an aquifer environment requires information concerning the rates of the various microbial processes that are occurring. Often these rates are estimated from laboratory-based measures and extrapolated to the field. While estimating rates from laboratory incubations can be an accurate method, we have applied a more direct means for measuring metabolic activity at the landfill site. We have employed the push-pull test procedure in an effort to quantitate the microbial consumption rates of several compounds of environmental significance. Briefly, a push-pull test involves the removal of groundwater, which is stored in a carboy while chemicals of interest, including a conservative tracer are added after which the solution is reinjected into the formation. As samples are removed over time, the concentration of tracer and analyte will decrease due to microbial consumption and/or dilution. The degree of tracer loss due to dilution is applied to the concentration curve of the analyte. In this way, the decrease in analyte concentration that is due to dilution can be estimated. Any time-dependent decrease of the chemical of interest over and above that which can be attributed to dilution is then recognized as being due to microbial consumption and a rate is estimated based upon that measure. Some of the push-pull tests conducted thus far have examined the rate of sulfate and hydrogen consumption. Sulfate was examined because it is an important electron acceptor at the landfill site and is common to other aquifers as well. Moreover, the degradation of many pollutant chemicals is linked to the use of sulfate reduction as a terminal electron accepting process. Hydrogen is a critical intermediate in organic matter degradation affecting the nature of end products formed as well as the rate of organic carbon oxidation. Thus, describing the capacity of a given environment to process hydrogen is a key component to understanding overall carbon and energy fluxes in the area. In addition, we have used push-pull tests to measure uranium reduction rates in the aquifer. Although this aquifer is not contaminated with uranium, we are using the landfill site as a surrogate from which we can make predictions regarding the capacity of other locations to reduce and thereby immobilize soluble uranium.

A feature of natural systems that often complicates the estimation of rate processes at any site is the non-uniform distribution of microbial activity. We have recently removed cores of sediment from several locations downgradient from the landfill and are currently studying the two-dimensional distribution of sulfate reducing activity in incubations of this material. The spatial distribution of sulfate reduction activity can be visualized based on a direct imaging procedure that we have developed. Radioactive sulfate is applied to the face of a core sample; the sulfate is reduced by the resident microflora yielding radioactive sulfide that precipitates on the soil particles. The unreacted sulfate is then washed away leaving only sulfide, which can be examined qualitatively, and quantitatively using a specialized autoradiography instrument. This procedure allows for direct viewing of the distribution of microbial activity in a minimally disturbed sample giving the researcher an accurate representation of how the processes occur in the field.

Once the distribution of sulfate reduction activity has been observed, we can investigate the factors that limit sulfate reduction rates. Consequently, in addition to describing the distribution of activity at the site, this work will enhance our understanding of the underlying factors responsible for driving sulfate reduction at the landfill and other locations.

Molecular techniques are valuable tools for the characterization of microbial populations in soil. The various methods however must prove to be consistent and critically examined to assure their reproducibility. To that end, we have employed two popular methods of nucleic acid extraction for the examination of DNA present in sediment samples at the Norman landfill. Two locations at the landfill, designated 35 and 40 have been thoroughly examined previously and were thus chosen for this study. The first extraction method involved several freeze-thaw-grind cycles followed by an alcohol extraction and precipitation. The second method consisted of beat-beating, using the Bio101 FastDNA Soil Extraction kit (Qbiogene, Inc.). The DNA present in the sediment was purified from other components by binding to silica particles and washing by centrifugation with a series of buffers. Examination of the results from these different procedures will better define any limitations and inconsistencies that may exist when nucleic acid analysis of soil is necessary.

Stable isotopes have been widely used as tracers for methane oxidation (Grossman, 2001). Microorganisms preferentially consume $^{12}CH_4$ resulting in an enrichment of $^{13}CH_4$ in residual methane. Numerous studies have used stable carbon isotopes as a tracer for *aerobic* methane oxidation in landfill soils, but none have investigated anaerobic methane oxidation at a landfill site. We have used carbon isotopic measurements to investigate *anaerobic* methane oxidation in leachate-contaminated groundwaters at the Norman Landfill. We combined these results with hydrogeologic information to estimate the rate of natural attenuation of methane as it travels within the leachate plume. Isotope analysis was conducted on several water samples along the MLS35 to MLS80 transect using permanent multi-level samplers installed in 1997. We sampled for carbon isotopic analyses of dissolved inorganic carbon and methane in June 1998, April 1999, July-August 1999, and December 1999. The waters collected in April, 1999 were subject to a comprehensive array of chemical analyses. Sampling and analytical methods are described in Cozzarelli et al. (2000).

Principle findings and significance: Hydrogen measurements indicate that various bacterial processes are dominant at different locations within the field site. In fact, significant differences in hydrogen values were measured at different depths within the same well (Figure 1). In addition, we have found that dissolved hydrogen values change over time. We have monitored hydrogen levels at 35 different locations, taking a measurement approximately every six weeks for almost one year. Results suggest that the dominant microbial process is not constant over time, but changes with seasonal patterns, possibly as a function of groundwater temperature. Our work has shown that the concentration of hydrogen in groundwater can indeed be a useful indicator. However, under some environmental conditions its usefulness is limited and needs to be considered in concert with other groundwater geochemical measurements. Future work in this area will further define the usefulness of dissolved hydrogen measurements and provide an additional tool for site characterization to workers in several disciplines including microbiology, geochemistry and various engineering sciences.

Direct rate measurements made in the laboratory suggest that sulfate reduction and methanogenesis are the most important microbial reactions in the aquifer. At one location, the hydrogen values measured in the field agree with the laboratory experiments in predicting sulfate reduction as a dominant process (Figure 2). In another area of the aquifer, the hydrogen values implicate iron reduction as the dominant process but laboratory rate measurements could not confirm this prediction. This

finding implies that caution is necessary in the interpretation of dissolved hydrogen data and that other methods should be employed, in addition to hydrogen measurements, in efforts to accurately map the distribution of microbially catalyzed redox reactions.

Sulfate reduction rates based on the push-pull test method were found to be approximately 5 ?M/day (Figure 3). This value is in good agreement with laboratory incubations prepared using material from the same location. Our sulfate reduction rate estimates are similar to those measured at other sites. This finding confirms the integrity of the laboratory-based analyses and provides yet another method to estimate sulfate reduction activity. The advantages of the push-pull test are similar to that of dissolved hydrogen in that soil and expensive laboratory incubations are not necessary.

The degradation rate of higher molecular weight molecules in aquifers impinges on the rate and extent of hydrogen removal from the system. Therefore, accurate measurements of hydrogen consumption rates in soil are required for the precise definition of fate processes in groundwater environments. We used the push-pull test method discussed above to measure the hydrogen consumption rate at a particularly well-studied location at the Norman landfill. Our calculations included kinetic estimates of the K_m as well as measures of the maximum rate (Figure 4). We compared our values with those determined from other sites and found similar kinetic properties existed at different locations, suggesting that the hydrogen consuming capacity at the Norman landfill may be representative of other field sites.

In addition to determining the dominant microbial processes that are occurring at a site, the factors that limit the activity of the resident microorganisms also need to be investigated in order to maximize their biodegradative capacity. Sulfate-reducing bacteria are widely distributed in many environments and have the capacity to degrade compounds that are metabolized slowly or not at all under other electron-accepting conditions. We have found that sulfate reduction is an important process at the landfill and our experimental evidence has identified several factors that limit the rate of sulfate reduction at various locations in the aquifer. One of these factors is the concentration of sulfate. In some cases, simply adding exogenous sulfate to incubations of aquifer slurries increased the rate of sulfate reduction. At a different location, sulfate was available in excess but a push-pull test indicated sulfate reduction was very slow. When sediment cores were incubated in the laboratory, the addition of readily degradable organic matter in the form of lactate, resulted in increased sulfate reduction activity (Figure 5). Often overlooked is the ability of material from dead cells to supply nutrients and energy for live cells in soil environments. When a heat-killed suspension of sulfate reducing bacteria stimulated sulfate reduction, however, the addition of a live culture of sulfate reducers had a much smaller effect. These results indicate that at this location, the organisms capable of catalyzing the reduction of sulfate are present, but their activity is limited by the quality of electron donor. This limitation can be relieved upon the introduction of labile organic matter that may be in the form of lactate or even neighboring, dead cells.

Clay is a noteworthy component of sediments in many areas and microbial activity is believed to be relatively low in zones containing highly consolidated soils of low pore volume. Several locations within the aquifer at the landfill site have clay layers that extend horizontally for tens of meters and can have a vertical thickness of several centimeters or more. Because of the common incidence of clay at the landfill site and other locations, we have investigated the effect of this material on sulfate reduction rates in aquifer slurries supplemented with native clay as well as commercially available clay minerals. Incubations containing clay from the aquifer showed a high level of inhibition relative to incubations devoid of clay. Some of the purchased clays also affected a decrease in sulfate reduction rates with the level of inhibition dependent on the type of clay used. A standard treatment in the study of clay minerals involves washing the material with water prior to incubation. Washed clay exhibited an inhibitory effect that was statistically lower than that seen when unwashed clay was used. Moreover, the addition of the wash supernatant was found to inhibit sulfate reduction in pure cultures of sulfate reducing bacteria. The clay minerals found to exert the greatest inhibition were kaolin, barasym (a synthetic clay), and bentonite (Figure 6). Chemical analysis of these clays reveals relatively high amounts of aluminum oxide, a mineral common to clays but existing in various amounts depending on the source. We have identified aluminum oxide as a component that could potentially inhibit sulfate reduction. Indeed, sulfate reduction rates were lower when pure aluminum oxide (alumina) was added to incubations of soil, further suggesting that at least part of the inhibitory effect of clay material is due to the aluminum oxide content (Figudre 6). Inhibition was relieved by addition of a number of salts to mixtures of landfill sediment and bentonite clay suggesting a possible charge-related mechanism for inhibition. To investigate this

possibility, we are currently examining clay samples by scanning electron microscopy (SEM) in an effort to delineate the various mineral forms that may play a role in the distribution of charged species in the different clays.

Two rounds of push-pull tests were performed at the Norman Landfill to assess the extent of uranium reduction/immobilization under *in situ* conditions. The tests were designed to assess the impact of electron donors and alternate terminal electron acceptors (nitrate and sulfate) on the reduction of uranium. Addition of the electron donors formate, acetate, and lactate, did not appear to have any impact on uranium immobilization relative to the unamended well, suggesting that sufficient electron donor was present in the native groundwater to drive complete reduction of the added uranium (1.5? M). Slight inhibition of uranium immobilization by sulfate was observed, while nitrate was able to cause a more extensive inhibition of uranium immobilization. In a second round of tests, uranium and nitrate were injected at two nitrate concentrations (Figure 7). These tests were performed in wells that had been treated with uranium in the previous round of experiments. As nitrate reduction proceeded, previously immobilized uranium was apparently remobilized (Figure 7). Recent laboratory experiments suggest that intermediates in denitrification can oxidize uranium and are likely responsible for the remobilization observed in this experiment. Other work in this area has taken advantage of the ability of sulfate reducing bacteria to immobilize cobalt via sulfide precipitation. In soil cores amended with cobalt and radioactive sulfate, the areas where sulfide production occurred were visualized and soil samples from discrete locations within the cores were then removed for cobalt analysis (Figure 8). The areas that had high levels of sulfate reduction activity also showed a greater extent of cobalt immobilization indicating that the activity of sulfate reducing bacteria can play a role in detoxification of soil contaminated with cobalt.

The two DNA extraction procedures we performed indeed revealed inconsistencies that will be of interest to individuals working in this area of research. Using 5g of landfill sediment, the freeze-thaw method produced DNA pellets from both sites even upon five successive extractions suggesting that a single treatment may be insufficient for collection of a representative nucleic acid sample. We were not able to amplify the 16S rDNA gene using the first extraction from site 40. However, DNA from the well-characterized bacterium *Pseudomonas putida* was successfully purified by this method suggesting that the DNA from the landfill sample was not of PCR quality due to PCR inhibitors that were likely present in the site 40 soil sample. In contrast, we were able to PCR-amplify and eliminate the inhibition in the site 35 sample after further purification of the DNA using the silica-binding of the Bio101 Extraction kit. These preliminary results outline some of the current limitations associated with extracting and amplifying DNA from environmental samples and future work will further clarify measures necessary for success in this burgeoning field of research.

In addition to sulfate reduction, methanogenesis plays an important role in organic matter degradation at the landfill site. A thorough understanding of the role of methanogenesis often requires quantitation of the organisms. One of the methods used for quantitation of methanogen biomass takes advantage of the fact that an enzymmatic cofactor, coenzyme M (CoM) is found almost exclusively in methanogens. We collected landfill sediments from a thoroughly studied location at the site to use in efforts to standardize and simplify an existing procedure for CoM quantitation. The sediments showed an active methanogen population with 1.1 x 105 cells/g sediment and a CoM content per cell of 0.18 fmol CoM/cell which was lower than the average value of 0.41 fmol CoM/cell from all sites tested. In contrast to the previous procedure, our modification allows for quantitation of methanogen biomass within hours of sample collection and can be done aerobically, greatly simplifying the method.

It is noteworthy that we have successfully developed a sediment-free microbial enrichment capable of degrading cyclohexane carboxylate and cyclohex-1-ene carboxylate. These molecules are likely intermediates in the microbial degradation of several aromatic compounds. Therefore, the demonstration and characterization of this activity in the laboratory is fundamental to the description of aromatic compound destruction in contaminated environments. Current work with these enrichments involves the isolation of pure cultures whose degradative ability is affiliated with the reduction of sulfate, a common electron acceptor at the landfill as well as other sites including marine ecosystems where contamination by aromatic-containing fuels is a current dilemma.

Despite the prevalence of biologically produced methane, few studies have investigated anaerobic methane oxidation in aquifers. The alluvial aquifer adjacent to the Norman landfill provides an excellent opportunity for the study of anaerobic methane oxidation in a landfill-leachate plume. The redox indicators within the plume mimic the succession of energy yields for the various microbial reactions.

Within the plume core near the landfill, methane is present at high concentrations (up to 1135 µM), nearing saturation values (Table 1). Methane concentrations decrease above and below the plume, as well as downgradient within the plume (Figure 9). We have used analyses of ^{13}C, a stable isotope of carbon to investigate the evidence for methane oxidation in the leachate plume.

Carbon isotopic compositions of DIC (?$^{13}C_{DIC}$) within the 4/99 transect vary from –8.8 to +10.3‰ (Table 2; Figure 10). High ?$^{13}C_{DIC}$ values are typical for plume waters and almost always indicate methanogenesis (Grossman, 2001). These waters are also high in DIC concentration and alkalinity. The lowest ?$^{13}C_{DIC}$ values (= –5‰) are found in shallow wells at the downgradient end of the transect (MLS-80) and are representative of pristine groundwaters. The ?$^{13}C_{DIC}$ strongly correlate with DIC content, following a linear trend that cannot be explained solely by conservative mixing (Figure 11). Conservative mixing of the two end-members would generate a curved trend on a ?$^{13}C_{DIC}$ vs. DIC plot because plume waters are rich in DIC compared with native waters (Figure 11), and dominate the DIC reservoir. Methane oxidation may explain why the data fall below the mixing curve. Near the landfill, the low ?^{13}C of the methane (about –54‰), along with the fractionation associated with methane oxidation (about –14‰), will add ^{13}C-depleted DIC (roughly –68‰) to the groundwater. However, the effect of methane oxidation on ?$^{13}C_{DIC}$ is minor because of the low solubility of methane and large concentration of DIC in plume waters. On the other hand, oxidation of other dissolved organic carbon compounds undoubtedly contributes ^{13}C-depleted DIC. Another factor that may contribute to the deviation from the mixing curve is dissolution and precipitation of carbonate minerals. Carbonate minerals occur in low concentration in the sediments (~1%; G. Breit, pers. comm.). Mixing with waters from the slough plume and a secondary plume at MLS35-7 adds additional complexity to ?$^{13}C_{DIC}$ - DIC relationships.

Carbon isotopic compositions of methane (?$^{13}C_{CH4}$) range from –67 to +28‰ and show a progressive enrichment downgradient in all four sampling surveys (Figure 9, Table 1). Furthermore, ?$^{13}C_{CH4}$ increases at the upper and lower plume margins. Near the landfill (MLS35), methane within the plume has ?^{13}C values ranging from –51 to –56‰. The ?$^{13}C_{CH4}$ values within the plume axis increase from about –54‰ to –30‰ (MLS80-7). Over the same interval, methane concentrations decrease from 700 ± 200 mM to 85 ± 5 mM.

Declining methane concentrations can be explained by methane oxidation and by dilution with methane-poor native waters. However, dilution alone would not affect ?^{13}C values. Thus, the increasing ?$^{13}C_{CH4}$ values can only be explained by methane oxidation. On a ?$^{13}C_{CH4}$ versus log CH_4 concentration diagram, the trend for methane oxidation will approximate a straight line. Figure 12 shows the data for the April 1999 transect. Note that the plume and plume margins define two linear trends. The data for slough plume samples, near-surface samples, and background samples do not define a distinct trend because of mixing and variability in the initial concentration and initial ?^{13}C.

To determine the isotopic fractionation associated with methane oxidation, concentration data must be corrected for mixing. Hydrogen isotopic analyses were used to estimate the fraction of native water and thus calculate the original methane concentration. These data are only available for the April 1999 transect. Table 1 shows the methane concentrations corrected for mixing. After this correction, the plume and plume-margin waters continue to define two parallel linear trends (Figure 13). The aberrant plume-margin datum (in parentheses) is one of two plume margin waters with ?D and ?^{18}O values that do not fall on either the meteoric water line or the plume-native water mixing trend. Thus, the estimate of mixing based on ?D is likely compromised. The slopes of the regression for plume and margin water data define fractionation factors of 0.9864 ± 0.0010 and 0.9870 ± 0.0017, respectively. These equate to CO_2-CH_4 enrichment factors of –13.6 ± 1.0‰ and –13.0 ± 1.7‰, respectively. These two values are statistically indistinguishable and are within the range of values observed for aerobic methane oxidation (e.g., Barker and Fritz, 1981). For later calculations we will use the enrichment factor for the plume, –13.6‰, which is better constrained by the data. To our knowledge this is the first determination of the carbon isotopic fractionation associated with anaerobic methane oxidation in an aquifer system.

The fraction of methane remaining can be calculated based only on the carbon isotopic composition of the methane. No assumptions beyond those used to define the fractionation factors are required to calculate the fraction of methane oxidized. Figure 14 shows the percent methane oxidized along the MLS35-80 transect. At least 84% of the methane is oxidized in the 14 years it takes the plume to travel along the 210-m transect.

The rate of methane oxidation along the plume can be calculated using a first-order rate equation

$$d(CH_4)/dt = -k\,(CH_4) \qquad (4)$$

where k = the first-order rate constant. The rate constant equals the $-\ln [(CH_4)/(CH_4)_{0}]/t = -(\ln f)/t$, where t = time. To calculate f (fraction of methane remaining) we used the $?^{13}C$ of the methane for three segments of the transect. We used the flow rate (15 $m \cdot y^{-1}$; Scholl et al., 1999) to calculate t (Figure 14). The rate constants for the reaction vary from 0.06 to 0.23 y^{-1}, which yield half-lives for methane of 3 to 12 y. The 0.23 y^{-1} value is almost identical to that obtained in anoxic culture experiments with Big Soda Lake water (0.20 y^{-1}; Iverson et al.,1987). Rates were calculated from the rate constant x concentration. They vary from 18 $\mu M \cdot y^{-1}$ in the far reaches of the transect (MLS80) to 230 $\mu M \cdot y^{-1}$ in the mid-section of the plume where methane content is high and electron acceptors (SO_4^{2-}?) become available through mixing.

The methane oxidation rates calculated for the Norman Landfill site are three orders of magnitude lower than aerobic methane oxidation rates in water-saturated landfill sediments (Whelan et al., 1990; Figure 15). In terms of natural attenuation, aerobic oxidation is a far more efficient sink for landfill methane, than anaerobic oxidation. However, oxygen is relatively insoluble and is rapidly consumed in contaminated aquifers compelling the use of alternative electron acceptors for the oxidation of reduced compounds such as methane. The anaerobic methane oxidation rates observed at Norman Landfill are of the same order of magnitude as those in two other freshwater systems (Figure 15). In a Cape Cod aquifer, natural gradient tracer tests with nitrate as an electron acceptor yielded anaerobic oxidation rates of 150 $\mu M \cdot y^{-1}$ (Smith et al. , 1991). Anaerobic methane oxidation rates in the sulfate reducing zone of Big Soda Lake, Nevada were found to be of 18 to 31 $\mu M \cdot y^{-1}$ (Iverson et al. , 1987). In contrast, anaerobic methane oxidation associated with sulfate reduction in marine sediments (Hansen et al., 1998) and sediments within a marine-groundwater mixing zone (Bussmann et al., 1999) progresses at rates that are more than one to two orders of magnitude greater (6200 - 72,000 $\mu M \cdot y^{-1}$) than those of the freshwater systems. The greater rates noted for marine environments may be sustained by the increased availability of sulfate.

These data suggest methane is naturally attenuated as the landfill-leachate plume travels from the landfill to the Canadian River. Methane oxidation occurs by a novel anaerobic process that has not been previously characterized in an aquifer system. It is likely that other contaminants, such as chlorinated hydrocarbons, are cometabolized with this methane. Methane is anaerobically oxidized at rates of 18 to 230 $\mu M \cdot y^{-1}$, yielding methane half-lives of 3 to 11 years along the 210-m plume. These results demonstrate that anaerobic methane oxidation is viable sink for methane in contaminated aquifers. The mechanism for anaerobic methane oxidation in sulfate-reducing zones is hotly debated. Numerous studies have suggested possible mechanisms for methane oxidation on the absence of oxygen (Hoehler et al., 1994; Zehnder and Brock, 1980;Valentine and Reeburgh 2001). Allthough our data suggests this activity occurs in the aquifer, detailed studies of Norman Landfill microorganisms are required to investigate the mechanism for anaerobic methane oxidation. Further research is also required to investigate the role of anaerobic methane oxidation in the natural attenuation of toxic compounds such as chlorinated hydrocarbons.

Table 1. Concentration and carbon isotopic composition of methane in Norman Landfill groundwaters, along with hydrogen isotopic composition of water.

	April, 99	June, 98	April, 99		July-August, 99		December, 99		Mean			Mean		
Sample	?D in H2O	CH4 ?13C	CH4	CH4 ?13C	CH4	CH4 ?13C	CH4	CH4 ?13C	CH4 ? or MD^		N	CH4 ?13C	? or MD	N
	(‰)	(‰)	(uM)	(‰)	(uM)	(‰)	(uM)	(‰)	(uM)	(uM)		(‰)	(‰)	
MLS 35-2	-27.8	-43.8	56	-52.7			123	-31.2	89	34	2	-42.6	10.8	3
MLS 35-3	-15.4	-52.5	571	-53.6	1113	-54.7	936	-50.6	873	276	3	-52.8	1.7	4
MLS 35-4	-14.0	-54.1		-55.2	1138	-53.9			1138		1	-54.0		2
MLS 35-5	-9.8	-54.9	827	-55.3	1250	-54.8	349	-50.7	809	451	3	-54.0	2.3	4
MLS 35-6	-16.7	-21.9	28	-15.8	25	-8.6	34	-6.2	29	5	3	-13.1	7.1	4
MLS 35-7	-16.4	-31.9	70	-42.0	117	-41.3	2	-17.6	63	58	3	-33.2	11.4	4
MLS36-1	-41.5		13	-54.1					13		1	-54.1		1
MLS36-2	-26.8		23	-30.8					23		1	-30.8		1
MLS36-3	-25.6		142	-30.4	77	-13.1	400	-28.9	206	171	3	-24.1	9.6	3
MLS36-4	-15.4		532	-56.2	1131	-53.8	646	-47.2	770	318	3	-52.4	4.6	3
MLS36-5	-17.0		137	-51.6	205	-52.2	228	-43.4	190	47	3	-49.1	4.9	3
MLS36-6	-18.3		13	-7.8	13	7.2	14	-2.6	13	1	3	-1.1	7.6	3
MLS36-7	-18.0		13	-12.9	9	22.6	13	11.7	12	2	3	7.1	18.2	3
MLS37-1	-30.6													
MLS37-2	-21.1		23	-31.2			6	-8.0	15	9	2	-19.6	11.6	2
MLS37-3	-15.9		101	-43.8	481	-52.9	238	-49.0	273	192	3	-48.6	4.5	3
MLS37-4	-15.4		57	-40.1	57	-31.1	266	-44.4	127	121	3	-38.5	6.8	3
MLS37-5	-12.3		567	-53.8	1166	-54.7	621	-52.0	785	331	3	-53.5	1.4	3
MLS 37-6	-11.0		478	-52.8	1097	-52.5	899	-52.0	825	316	3	-52.4	0.4	3
MLS 37-7	-17.6		26	-12.7	32	-1.5	33	-13.0	30	4	3	-9.1	6.5	3
MLS 38-1	-44.1													
MLS 38-2	-25.2		30	-51.7					30		1	-51.7		1
MLS 38-3	-18.0		70	-47.3	374	-54.7	250	-46.0	231	153	3	-49.3	4.7	3
MLS 38-4	-15.2		185	-54.1	480	-56.5	70	-34.5	245	211	3	-48.4	12.1	3
MLS 38-5	-7.7	-48.9	569	-50.1	1147	-50.3	1005	-44.8	907	301	3	-48.6	2.6	4
MLS 38-6	-4.6	-48.8	724	-49.8	1383	-49.7	1244	-49.1	1117	347	3	-49.3	0.5	4
MLS 38-7	-10.8		202	-40.2	502	-43.0	282	-43.0	329	155	3	-42.1	1.6	3
MLS 40-3					1218	-52.1			1218		1	-52.1		1
MLS 40-4					113	-27.7			113		1	-27.7		1
MLS 54-1	-20.7													
MLS 54-2	-18.5		333	-54.1					333		1	-54.1		1
MLS 54-3			66	-50.0			6	5.8	36	30	2	-22.1	27.9	2
MLS 54-4	-17.1		19	-20.4			25	-1.4	22	3	2	-10.9	9.5	2
MLS 54-5	-14.2		41	-19.6			40	-13.9	41	1	2	-16.7	2.9	2
MLS 54-6	-12.3		128	-33.6			96	-27.6	112	16	2	-30.6	3.0	2
MLS 54-7	-11.5		224	-39.5			241	-38.4	233	9	2	-38.9	0.6	2
MLS 55-1	-46.2													
MLS 55-2	-29.5		7	-64.5					7		1	-64.5		1
MLS 55-3	-21.0		4	-35.3			370	-63.7	187	183	2	-49.5	14.2	2
MLS 55-4	-24.1		12	-48.0			6	-35.9	9	3	2	-42.0	6.1	2
MLS 55-5	-29.0		15	-41.4			16	-2.6	16	1	2	-22.0	19.4	2
MLS 55-6	-16.7	-25.4	105	-25.1			76	-24.7	91	15	2	-25.1	0.4	3
MLS 55-7	-11.9	-39.8	181	-36.8			168	-35.8	175	7	2	-37.5	2.1	3
MLS 80-1	-21.0													
MLS 80-2														
MLS 80-3	-21.2		TS*	TS					3		1	-34.3		1
MLS 80-4	-27.7		TS	TS					5		1	-53.9		1
MLS 80-5	-29.5		TS	TS					6		1	-52.6		1
MLS 80-6	-24.9		13	8.8			7	28.3	10	3	2	18.6	9.8	2
MLS 80-7	-12.6		89	-27.8			140	-32.4	115	26	2	-30.1	2.3	2
MLS NDP2	-33.6		TS	TS					1					
MLS NPD-5	-30.7		TS	TS					0					
MLS NPD-6	-32.0		TS	TS					0					
WLMLF	-3.5		1031	-52.9					1031		1	-52.9		1

*TS = too small to analyze. Blanks represent intervals not sampled. Many shallow wells either were above the water table or did not contain significant methane.

Table 2. Concentration and stable isotopic composition of Norman Landfill dissolved inorganic carbon (DIC).

Sample	June 1998		December 1999	
	DIC (mM)	?^{13}C (‰)	DIC (mM)	?^{13}C (‰)
MLS 35-2	46.8	11.6	62.3	6.2
MLS 35-3	51.8	13.3	48.1	9.0
MLS 35-4	43.3	10.0		
MLS 35-5	51.2	10.0	45.8	7.9
MLS 35-6	>60	7.1	34.6	6.0
MLS 35-7	>60	8.3	39.1	6.2
MLS37-2			32.1	7.6
MLS37-3			50.7	7.8
MLS37-4			51.2	8.4
MLS37-5			52.4	9.6
MLS 37-6			47.5	8.9
MLS 37-7			44.3	7.4
MLS 38-2	15.1	-5.5		
MLS 38-3	>60		45.8	7.5
MLS 38-4	11.9	9.6	49.7	7.4
MLS 38-5	53.2	10.3	47.5	10.4
MLS 38-6	6.4	11.0	58.5	10.3
MLS 38-7	>60		50.2	8.9
MLS 54-3			37.6	-0.2
MLS 54-4			37.4	1.9
MLS 54-5			48.3	7.4
MLS 54-6			51.8	7.8
MLS 54-7			47.3	9.1
MLS 55-1	6.4	-11.6		
MLS 55-2	>60	-9.3		
MLS 55-3	>60	-0.4	23.2	-3.0
MLS 55-4	>60	-1.5	28.2	1.9
MLS 55-5	11.4	-10.3	15.8	-5.9
MLS 55-6	47.0	7.3	53.9	7.5
MLS 55-7	46.9	9.4	50.0	8.4
MLS 80-4			18.2	-4.6
MLS 80-5			12.6	-8.8
MLS 80-6			31.6	2.3
MLS 80-7			50.1	9.2

Figure1. Hydrogen values change over time and depth in well # 35

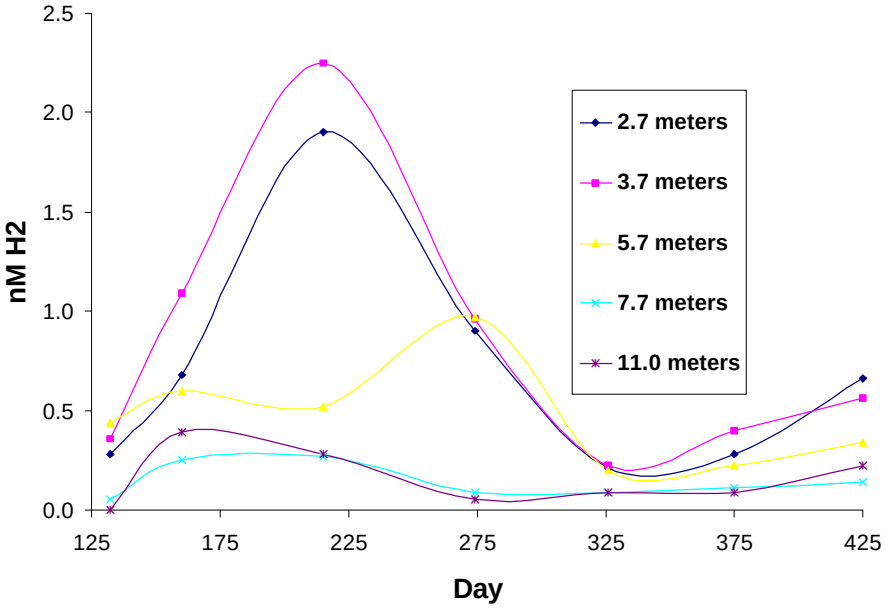


Figure2. Relationship between sulfate reduction and dissolved hydrogen in well # 35

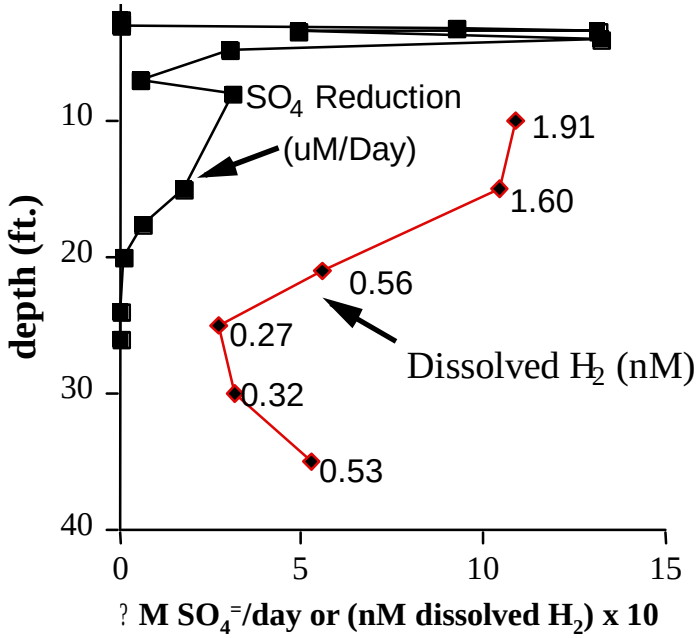


Figure 3. Sulfate consumption rate from push-pull test conducted near well #35.

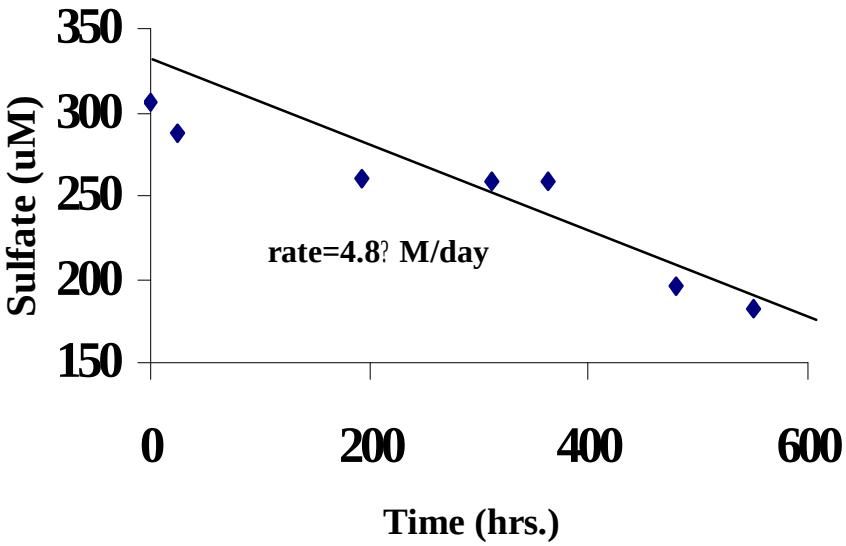


Figure 4. Hydrogen consumption curve from push-pull test conducted near well #35

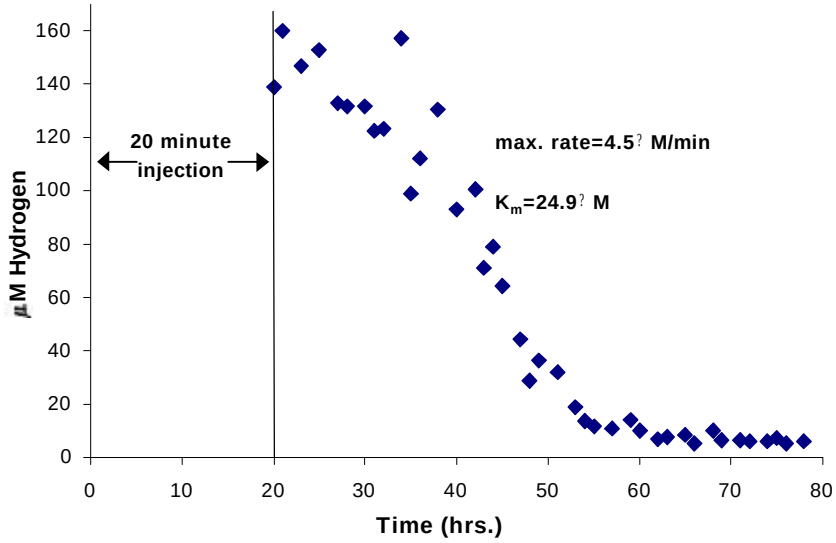
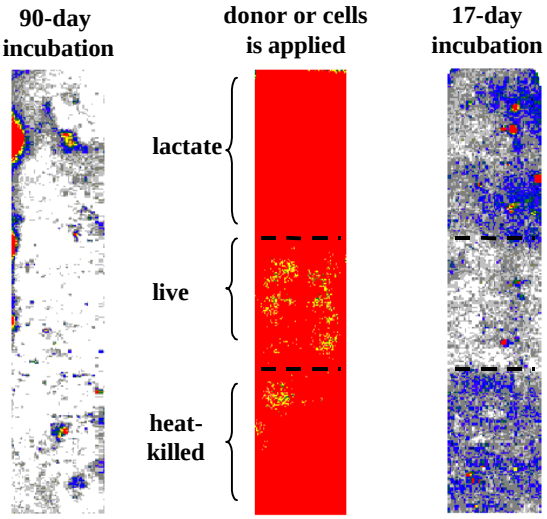


Figure 5. Sulfate reduction in an unamended core and in response to addition of electron donor and *Desulfovibrio* preparations.



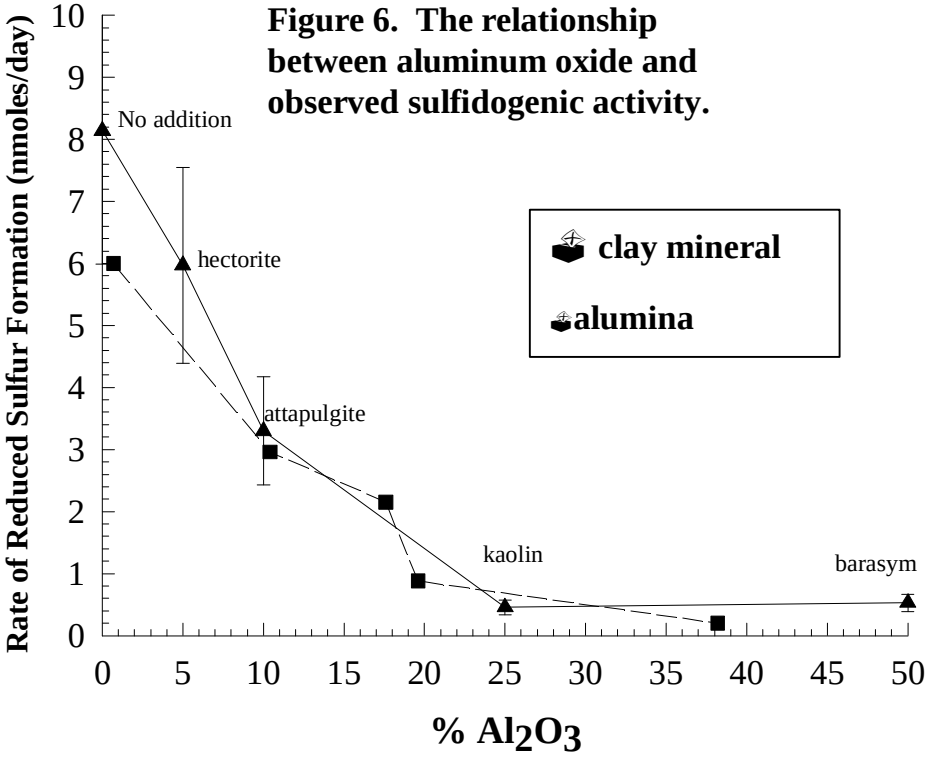


Figure 6. The relationship between aluminum oxide and observed sulfidogenic activity.

Figure 7. Effect of nitrate amendment on remobilization of uranium.

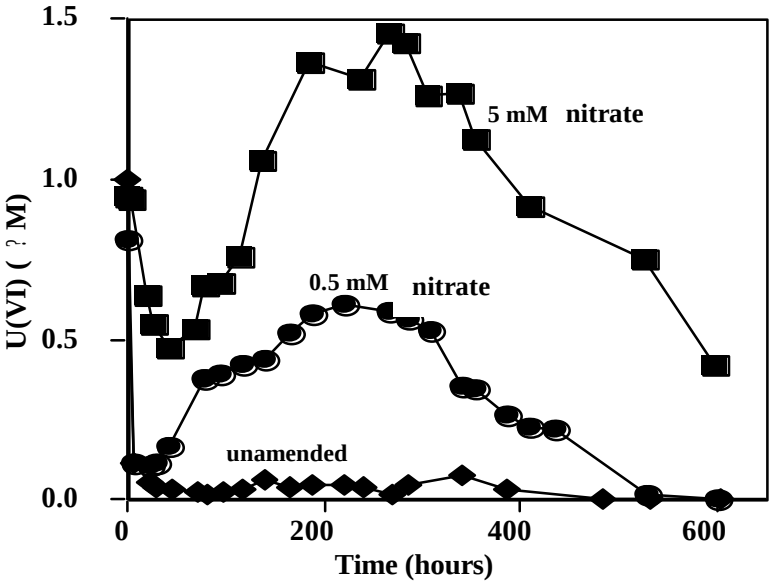


Figure 8. Sulfide production and corresponding degree of cobalt immobilization in cores from the landfill site

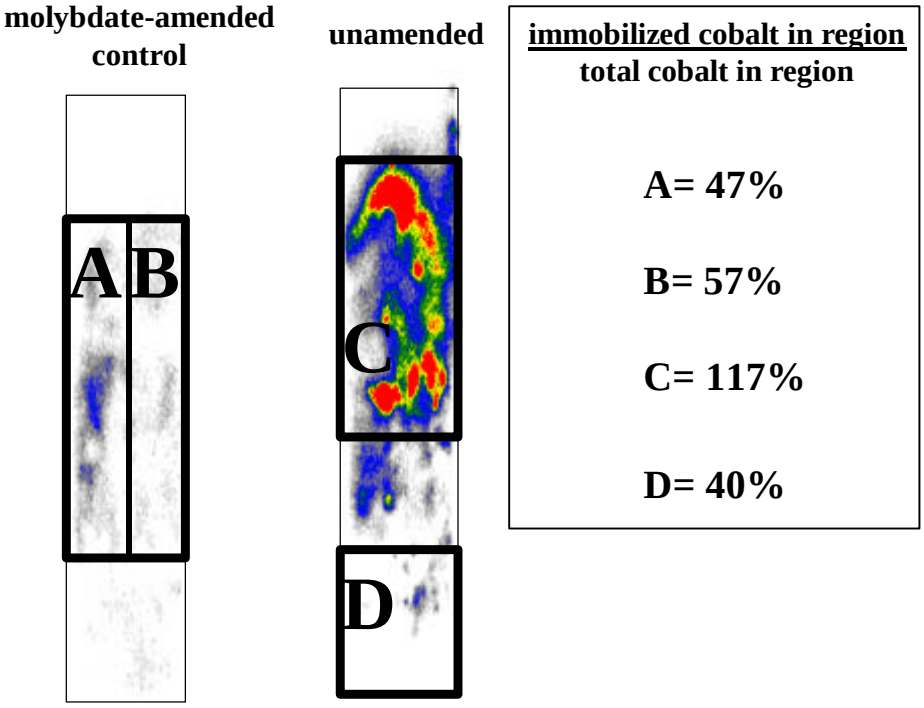


Figure 9. Distribution of methane and methane ? ^{13}C values across the MLS 35 to MLS 80 transect. Values are averages of all samples (Table 1).

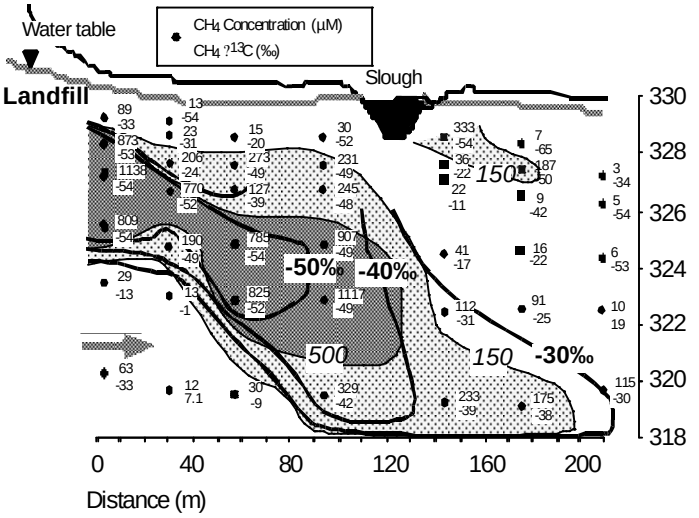


Figure 10. Distribution of dissolved inorganic carbon (DIC) and DIC? ^{13}C values across the MLS 35 to MLS 80 transect. Values are for the December 1999 transect (Table 2).

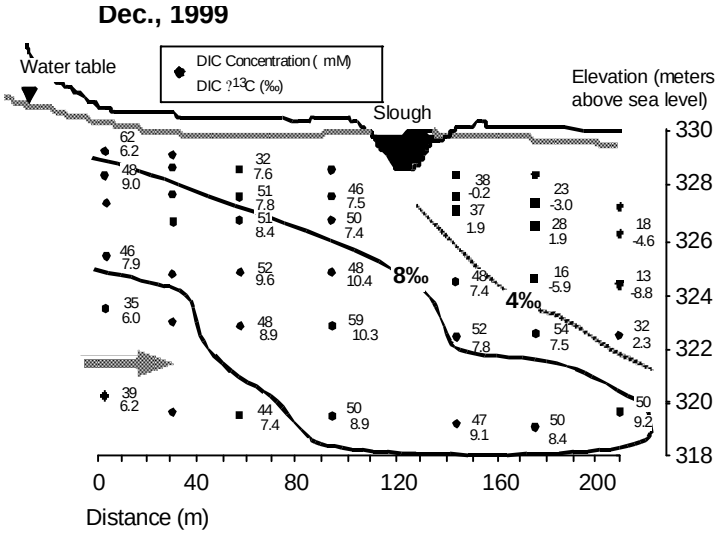


Figure 11. ? ^{13}C of DIC versus DIC content. Thick curve represents mixing between idealized landfill water (50mM DIC, 10‰ ? $^{13}C_{DIC}$) and native water (6.4mM DIC,-11.6 ‰ ? $^{13}C_{DIC}$). Arrow shows trend associated with methane oxidation in the vicinity of MLS 80-6.

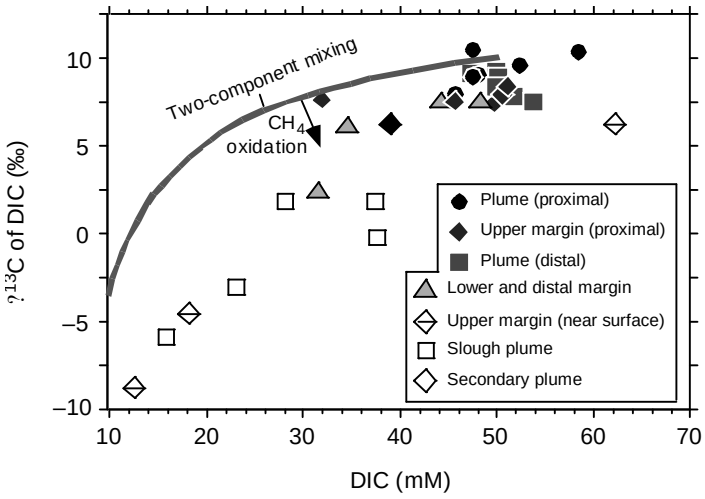


Figure 12. ? ^{13}C of dissolved methane versus methane concentration for April 1999 transect. Oxidation of methane in a closed system will yield a straight line on this plot. Symbols keyed to different parts of the transect.

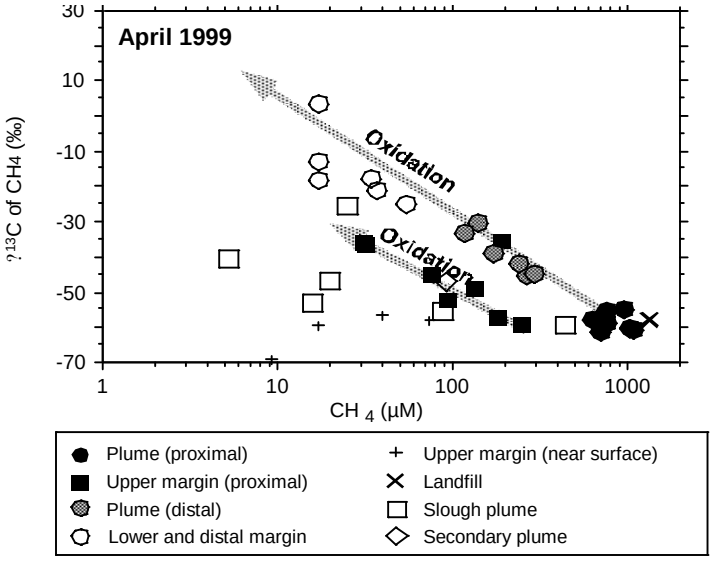
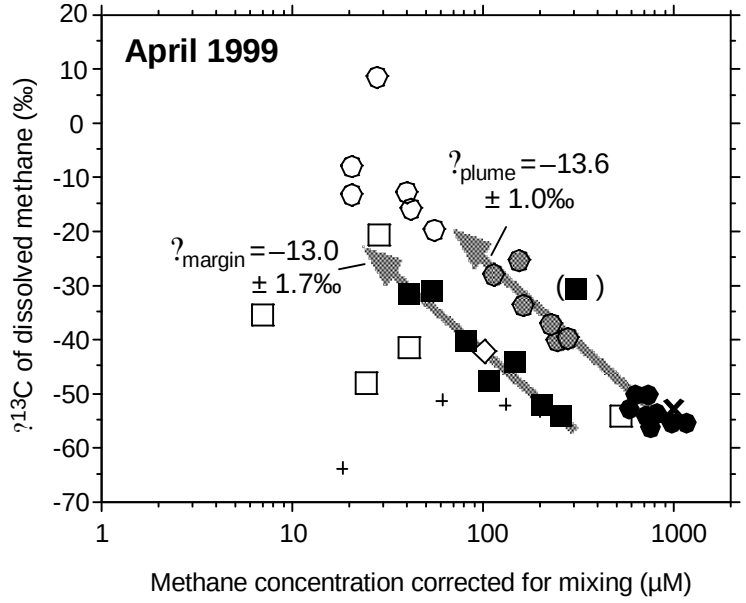
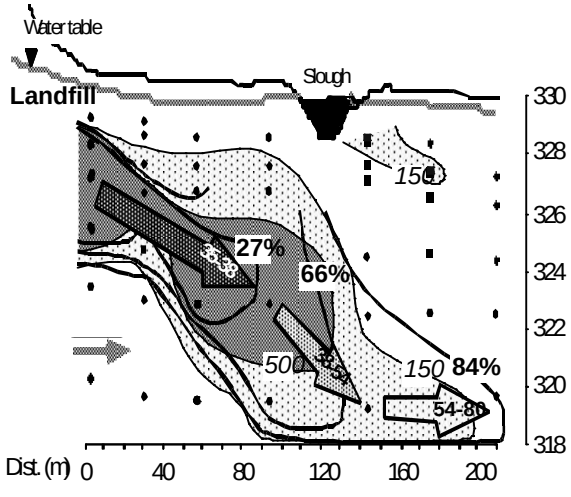


Figure 13. $\delta^{13}C$ **of dissolved methane versus methane concentration for April 1999 transect. Data are corrected for mixing using hydrogen isotopic composition of water as a conservative tracer.** CO_2**-**CH_4 **enrichment factor (s) for methane oxidation (with standard errors) calculated from regressions of plume and upper margin (proximal) data. Symbols defined in figure 5.**





Wells	MLS35-38	MLS38-54	MLS54-80
k(1°) (y^{-1})	0.06	0.23	0.16
Half-life (y)	10.9	3.0	4.5
R(1°) (µM y^{-1})	~56	47 - 202	16 - 32

Figure 14. MLS 35-80 transect with countours for methane content (italics) and percent methane oxidized (bold; from $\delta^{13}C$ **contours). Also shown are the three segments modeled for anaerobic methane oxidation rates, and the rate constants, half-lives, and rates.**

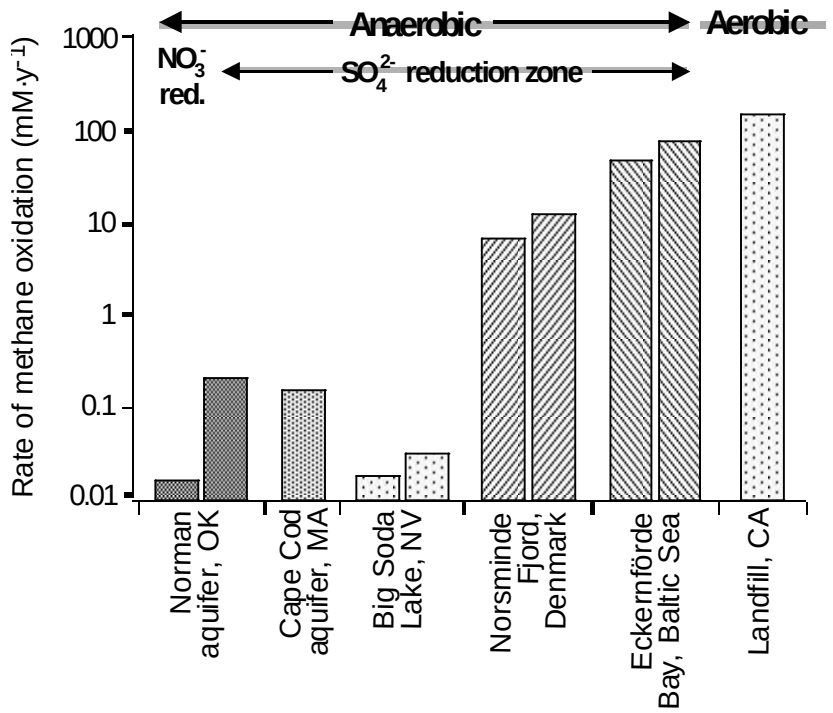


Figure 15. Comparison of rates of methane oxidation in the Norman aquifer leachate plume, Cape Cod aquifer (Smith et al., 1991), Big Soda Lake (Iverson et al., 1987), Norsminde Fjord (Hansen et al., 1998), Eckernförde Bay (Bussmennet et al., 1999). Where two bars are shown, data are minima and maxima.

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