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Environmental factors influencing methanogenesis in a shallow anoxic aquifer: a field and laboratory study

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SUMMARY

The environmental factors influencing methanogenesis in a shallow anoxic aquifer were probed in a combined field and laboratory study. Field data collected over a year revealed that 'in situ' rates of methane production were depressed in winter and elevated in summer. Over the same period, ground water pH values ranged from 6.0 to 7.8 while temperatures varied from 7–22°C. 'In situ' methanogenesis was severely inhibited at temperatures < 13°C or by pH values < 7. The influence of these factors on microbial methane formation from both endogenous and exogenous substrates were tested in aquifer slurries adjusted to pH 5–9 and incubated at temperatures ranging from 5–45°C. Temperature optima for methane production from endogenous substrates varied as a function of pH, but the pH optimum was 8 at all temperatures. Optimal conditions for acetoclastic methanogenesis were found at pH 8 and 35°C. An analysis of variance revealed that pH, temperature, and a pH-temperature interaction are all significant variables influencing aquifer methanogenesis. In addition transient sulfate accumulations were also found to limit methane production in some areas. A comparison of field and laboratory methane production patterns suggest that pH, temperature, and sulfate accumultations are important, but not the only environmental variables influencing the mineralization of organic matter in shallow aquifers.

INTRODUCTION

Leachates from municipal and industrial landfills are known to pollute ground water resources with a variety of organic contaminants [13,17,18,32,35,38]. Biodegradation of leachate components often progresses to a point where the rate of aerobic heterotrophic respiration exceeds the rate of oxygen supply in the aquifer. As oxygen becomes limiting, alternate electron acceptors like organic acids, nitrate or sulfate, are used to support further anaero-

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bic microbial metabolism. However, as these electron acceptors become depleted, methanogenic conditions develop in leachate-impacted aquifers.

Risk evaluation of landfill leachate pollution in aquifers requires reliable information on the nature of the contamination, the susceptibility of the contaminant to biodegradation and the environmental factors which influence the rate of organic matter decomposition in the subsurface. In a previous study of a shallow anoxic aquifer polluted by a municipal landfill leachate, we noted that sulfate limited areas of active methanogenesis [5]. At that time, routine field observations indicated a greater than expected seasonal variation in aquifer temperature and pH values. We therefore designed a field and laboratory study to assess the influence of these variables on anaerobic biodegradation processes as evidenced by the production of methane.

Our findings indicate that low pH and temperature values, in addition to high sulfate levels can inhibit the methanogenic fermentation of organic matter in shallow aquifers. However, a combination of these effects does not entirely explain the depressed field rates of methanogenesis observed at various times of the year.

MATERIALS AND METHODS

Sampling locations

The history of and chemicals leaching from the Norman, OK municipal landfill has been described elsewhere [33,34]. The landfill is located on the banks of the South Canadian River and Fig. 1 shows the location of the sampling sites used in this investigation. Sites A and B are located adjacent to a refuse mound and have been previously described [5]. Site C is located approximately 4 meters (m) from the southwestern edge of the same refuse mound (Fig. 1). Aseptically obtained aquifer sediment samples from all three sites and grab sediment samples from site B were collected as previously described [5].

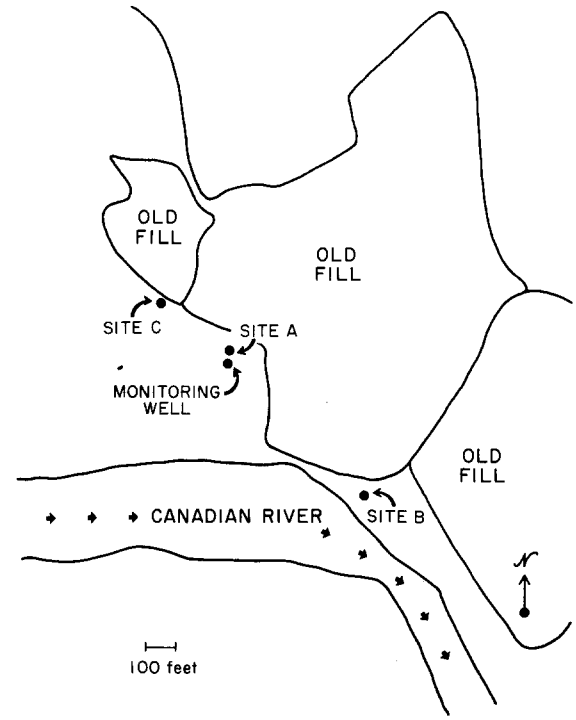

Fig. 1. Map of the municipal landfill in Norman, OK showing the locations of the various aquifer sites and their proximity to a monitoring well.

Field measurements of Aquifer pH, temperature, and methane ebullition

Following the sampling of aquifer sediments, a gas collection device (GCD) was installed below the water table at each site (Fig. 2). The GCD consisted of a 2.1 m long and 6.0 cm diameter PVC plastic pipe connected to a 9.0 cm opening. The GCD was placed approximately 0.3 m into the aquifer and filled to capacity with site ground water. The GCD was closed at the top with a #12 rubber stopper that was pierced with a severed aluminum seal anaerobic culture tube (Bellco Glass Inc., Vineland, NJ. cat #2048-00150). The anaerobic culture tube insert was in turn sealed with a 1 cm thick butyl rubber septum (Bellco Glass Inc. cat #2048-11800). The cap was secured to the PVC cylinder with tape to help maintain a gas tight seal. The remainder of the GCD volume was filled with site ground water.

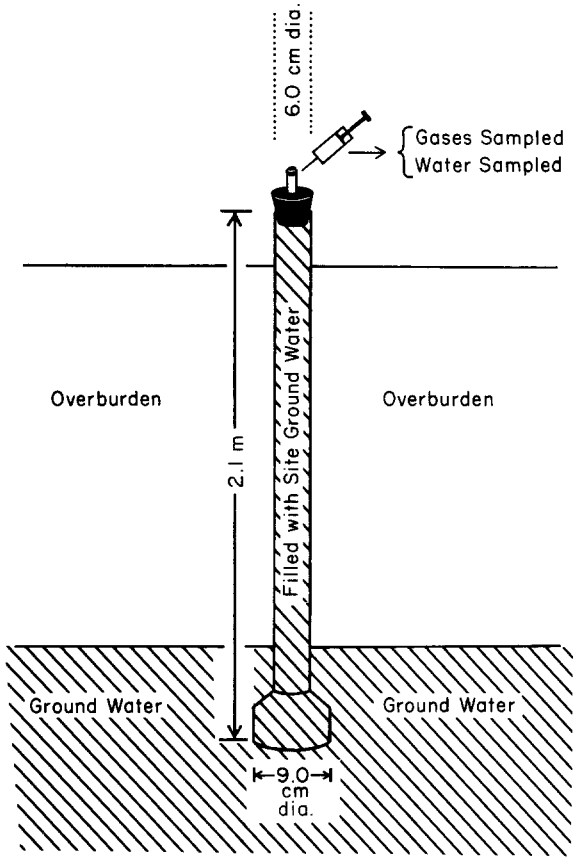

Fig. 2. Field device used to collect gases emanating from the aquifer (Gas Collection Device).

To test for the exchange of ground water between the aquifer and the GCDs, enough KBr was added to the estimated volume of GCD (5.1 l) to reach a final concentration of 7.9 mM. We attempted to mix the KBr solution throughout the volume of the GCDs by repeatedly reinjecting portions of withdrawn ground water through the GCD septum for one minute by needle and syringe. Samples of ground water in the GCD before and after injection of the KBr were monitored for the amount of bromide ion using anion exchange high pressure liquid chromatography (HPLC) and comparing peak retention times of the collected ground water samples against authentic bromide standards.

Gases emanating from the aquifer collected at the top of the GCD. The gases trapped in this fashion were removed through the GCD septum using a 10 ml syringe and a 22 gauge needle. As the aquifer gases were removed, the headspace of the GCD was replaced with ground water that was drawn up by the syringe vacuum. Since the gas samples were withdrawn from the GCDs under a slight vacuum, the syringe was sealed from the atmosphere and the plunger allowed to equilibrate against atmospheric pressure prior to the total gas measurement. Samples of the collected gases were then placed into nitrogen flushed anaerobic culture tubes for transport to the laboratory at 1 atmosphere for subsequent methane analysis.

Ground water from the GCDs was also periodically collected by syringe. The ground water pH was first recorded in the field, then the sample was transported to the laboratory and stored frozen prior to analysis for sulfate or fatty acid composition. A monitoring well close to site A (Fig. 1) was used to measure ground water temperatures. For comparison of pH and sulfate content with ground water, water samples from the South Canadian River were collected at a site approximately 50 m upstream of the landfill and similarly analyzed.

Experiments with aquifer slurries

Site B aquifer slurries were constructed in serum bottles as previously described [5]. Briefly, 50 g of aquifer solids were placed inside a 160 ml serum bottle and mixed with 72 ml of reduced sulfate-free mineral salts media [45]. The bottles were capped with 1 cm butyl rubber septa which were held in place by aluminum crimp seals. The 60 ml headspace was initially adjusted to N_2–CO_2(80:20) using modified Hungate technique [7]. The serum bottles were incubated at temperatures and pH values ranging from 5–85°C and 5–9, respectively. The slurries were adjusted to the desired pH with 6 N HCl or 2 N NaOH and corrected at weekly intervals if necessary. Methane production from endogenous substrates or from 1 mM acetate amendments was measured at the various pH and temperature regimes. All treatments were performed in duplicate.

Statistical analysis

A 2-way analysis of variance (ANOVA) was performed on the methane production data for acetate

amended and unamended aquifer slurries. Temperature and pH served as main effects. Tukey's unplanned comparison [41] was performed by holding the pH constant and letting temperature vary or by holding temperature constant and varying pH.

Microbiological and chemical analytical procedures

Total bacterial counts were measured on aseptically obtained aquifer samples using the acridine orange counting procedures of Ghiorse and Balkwill [16]. Three tube most probable number (MPN) determinations of methanogenic and sulfate reducing bacterial populations were performed on the same samples as previously described [5]. Methane was measured by gas chromatography (GC) while sulfate and bromide analysis was made by anion exchange HPLC [5]. Analysis for volatile fatty acids in selected ground water samples was performed on a Beckman Model 332 HPLC (Beckman Instruments, Berkeley, CA) equipped with a Bio-Rad (Richmond, CA) aminex ion exclusion HPX-87H column (300 mm × 7.8 mm). The mobile phase consisted of 0.016 N sulfuric acid at a flow rate of 0.9 ml/min. Peaks were detected at 210 nm on a variable wavelength spectrophotometer (Beckman, Model 155) and identified by comparing peak retention times of unknowns with authentic compounds. Ground water samples were prepared for HPLC analysis as previously described [5].

RESULTS

Microbiological profile of sampling sites

A microbiological profile of the sampling sites was conducted on aquifer sediments collected immediately before the installation of the GCDs. Total microorganisms as well as specific populations of methanogens and sulfate-reducing bacteria (SRBs) utilizing various electron donors were enumerated for this purpose (Fig. 3). Total organisms were similar at all sites and ranged from 2–4 × 10^7 microorganisms/gram dry weight (gdw). These values are not significantly different from previously reported values measured at either site A or B [5].

As in previous assays [5], all sites contained methanogenic and sulfate reducing bacteria capable of utilizing acetate, formate, and hydrogen as electron donors. Generally, these populations ranged between 10^2 and 10^3 bacterial cells/gdw which also agreed with earlier findings [5]. Greater numbers of SRBs able to use these substrates were found at site A than at either site B or C. In contrast, more methanogens than SRBs were detected at site B when acetate or formate were used as assay substrates, but the same trend is not as obvious at site C. Interestingly, this particular sampling revealed that hydrogen utilizing SRBs were numerically more dominant than methanogens using this electron donor at all three sites. Acetate utilizing methanogens were the most numerically dominant population at site B. Methanogens utilizing methanol as an electron donor could be found at all three sites, while

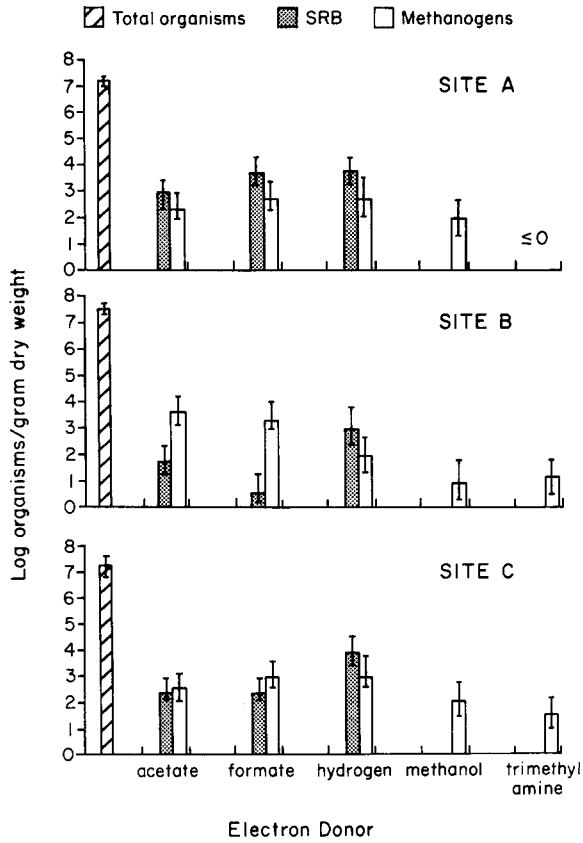

Fig. 3. Total numbers of microorganisms and specific physiological groups of sulfate-reducing (SRB) and methanogenic bacteria present at the aquifer sampling sites when the GCDs were installed.

trimethylamine utilizers were limited to sites B and C. Based on these findings, we concluded that the microbiological profile of the aquifer at the time of GCD installation was not atypical.

Factors influencing aquifer methanogenesis

Adequate hydrological communication between the ground water in the aquifer and the GCD was necessary in order for the field devices to act as semi-quantitative indicators of aquifer methanogenesis. To test the hydrological communication between the ground water in the GCDs and the aquifer we placed KBr into each GCD and collected samples for bromide analysis at various times. The test was conducted in mid-summer 1987 after the GCDs had been in place for approximately 1.5 yrs. Mid-summer was chosen since it had not rained in nearly a month, the amount of water in the South Canadian River was low, and the river meander was away from the landfill sites. These boundary conditions were chosen so that diffusion vectors would be maximized and mixing due to the ground water flow would be minimized. Bromide was not detected in the ground water samples before KBr was placed in the GCDs. All three sites showed approximately first order decay for bromide loss from the GCDs (data not shown). The concentrations of bromide at the first sampling closely matched the predicted value, suggesting that the tracer was adequately mixed throughout the volume of the GCDs. The loss of bromide from the GCDs was rapid with calculated half lives ranging from 6 to 13 min for all three sites. These findings indicate there was good hydrological communication between the ground water in the GCDs and that in the unconfined alluvial sand aquifer. Since temperature differences along the GCDs could influence methane solubility we compared air and ground temperatures (data not shown) to aquifer temperatures and found a maximum differential of + 7°C (air) which occurred during the summer months. Such a difference could account for about a 10% change in methane solubility. Therefore, degassing effects due to temperature differentials in the GCDs were considered minor and not a significant contribution to the methane content in these devices.

Periodically, gas samples from the GCDs were analyzed for the presence of oxygen, but none was ever found. Further, when the GCDs closures were intentionally breached, the water in the devices would immediately fall to the water table level. Since the fall in water levels was not observed during the experiment, the GCDs were adequately protected against atmospheric oxygen intrusion.

Previous results with site A aquifer slurries revealed the presence of methanogens, but only very low rates of methane formation were found 'in vitro' [5]. Consistent with the earlier results, no 'in situ' methane formation could be detected in a GCD placed at site A, yet methanogens were easily enumerated from the underlying aquifer.

Unlike site A, 'in situ' methanogenesis could be detected at sites B and C. The highest methane production rates for these sites were observed in the spring and summer, but neither site exhibited methane ebullition in winter (Fig. 4). Methanogenesis

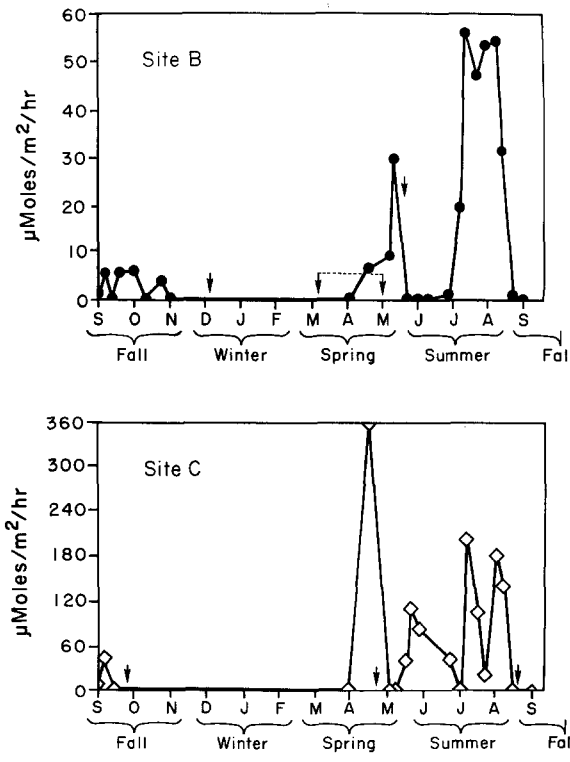

Fig. 4. The seasonal rate of methane formation in the GCDs at sites B and C. Arrows refer to times of the year when sulfate exceeded 1 mM.

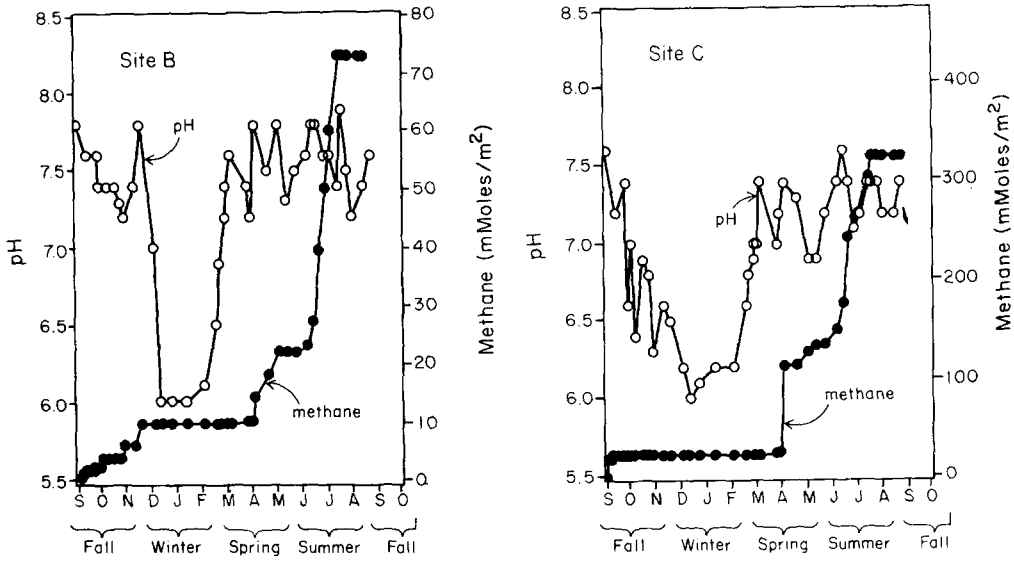

Fig. 5. Cumulative methane formation in GCDs as a function of ground water pH at site B and C.

rates varied from 0–57 μMol/m^2/h at site B and 0–354 μMol/m^2/h at site C. Total methane accumulation in the GCDs during a year of sampling was 71 and 328 mMol/m^2 for sites B and C, respectively.

Figs. 5 and 6 compare the yearly temperature and pH flux in the aquifer with the cumulative amount of methane measured at site B and C. 'In situ' ground water temperature and pH values ranged from 7–22°C and from 6.0–7.8 respectively. The methane production pattern in the GCDs placed at either site B or C were similar during winter, showing no detectable methane formation at either site. Similarly, both environmental parameters reached their minimum values in winter and maximum values in summer. This result suggested that either pH or temperature or both variables exerted an influence on aquifer methanogenesis.

To experimentally evaluate the effect of pH and

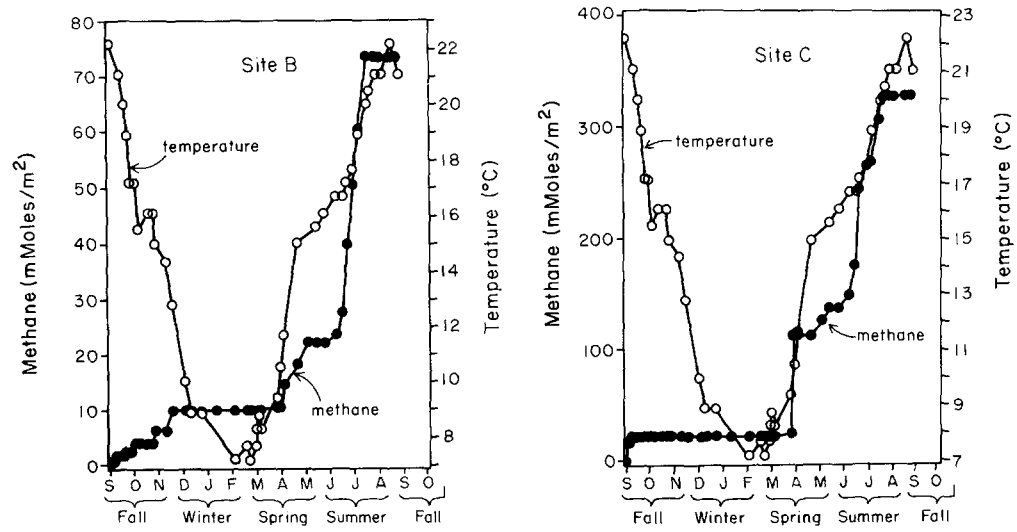

Fig. 6. Cumulative methane formation in the GCDs as a function of ground water temperature at sites B and C.

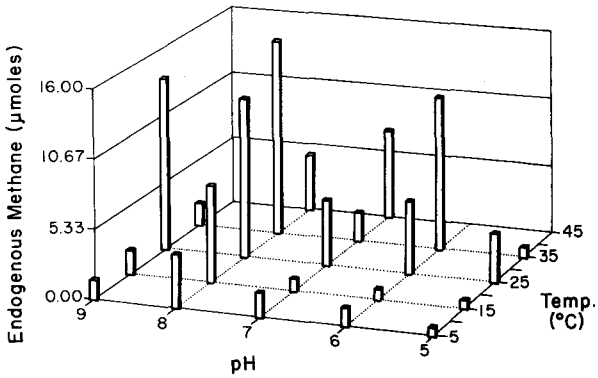

Fig. 7. Headspace methane concentration from endogenous substrates in site B aquifer slurries incubated in the laboratory for 4 weeks under various temperature and pH regimes.

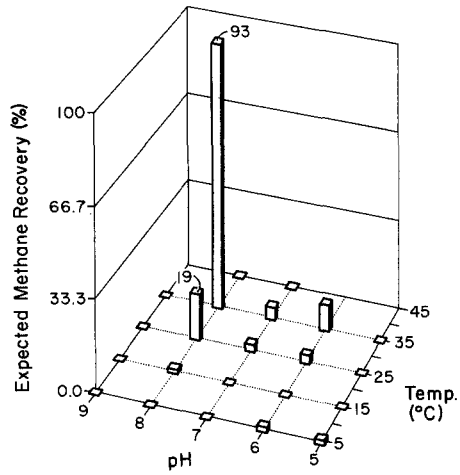

Fig. 8. Methane recovery from site B aquifer slurries amended with 1 mM sodium acetate as a function of temperature and pH. The percent recovery is based on established stiochiometry for acetoclastic methanogenesis [14].

temperature on methanogenesis, we incubated site B aquifer slurries in serum bottles under various pH and temperature regimes and monitored the headspace of the vessels for methane accumulation. The rate and total amount of methanogenesis from endogenous substrates generally increased at all pH values as a function of temperature (Fig. 7). As the optimum temperature was exceeded, methanogenesis decreased. Curiously, this trend was not as obvious at pH 7. The temperature optima for methane formation varied with pH and were 25°C at pH 5 and 9, 35°C at pH6 and 8, and 45°C at pH 7. In contrast, the pH optimum for methanogenesis was 8 at all incubation temperatures. However, high rates of methane production were also noted at pH 9 and 25°C and at pH 6 and 35°C. No methane production was found in aquifer slurries incubated at a pH of 7 and temperatures ranging from 55–85°C.

A much simpler picture emerged when the influence of the same two variables was evaluated on the mineralization of an exogenous substrate. Methane resulting from the metabolism of 1 mM acetate amendments to aquifer slurries was monitored with time. The results of a 3 week incubation are summarized in Fig. 8 and are corrected for endogenous methane production. The figure shows a clear pH and temperature optimum for acetoclastic methanogenesis at 8 and 35°C, respectively. Based on well established stoichiometry [14], 93% of the expected amount of methane was produced under these conditions. Much less acetoclastic methanogenic activity was detected under other pH and temperature incubation conditions. Similar to the unamended aquifer slurries, methane formation from acetate was completely inhibited at temperatures greater than 45°C. This more straightforward response to pH and temperature is likely a simple reflection of the acetoclastic methanogenic population.

A two way analysis of variance (ANOVA) revealed that both pH and temperature had significant effects on the amount of methane produced in both unamended and acetate amended aquifer slurries (Table 1). However, this analysis also showed that the interaction of the two variables was also significant at the 0.0001 level in both cases. Therefore, pH and temperature cannot be considered as completely independent variables influencing aquifer methanogenesis. Due to the significant interaction term in the ANOVA, we used Tukey's unplanned test to statistically evaluate the effects of pH or temperature as individual variables. Results of this statistical analysis showed that pH was a significant variable when temperature was held constant, or that temperature was a significant variable when pH was held constant (data not shown). Thus, temperature, or pH, or temperature–pH interaction

Table 1

Two way analysis of variance (ANOVA) on the unamended and acetate amended aquifer slurries using pH and temperature as main effects

Variable	DF*	SS*	F-value	Significance
Unamended aquifer slurries				
Temperature	3	576	31.8	0.0001
pH	4	598	24.3	0.0001
Temperature–pH interaction	12	512	6.9	0.0001
Error	60	370	–	–
Acetate amended aquifer slurries				
Temperature	3	911	9.7	0.0001
pH	4	1482	11.9	0.0001
Temperature–pH interaction	12	2566	6.9	0.0001
Error	60	1971	–	–

* DF = Degrees of freedom; SS = Sum of squares.

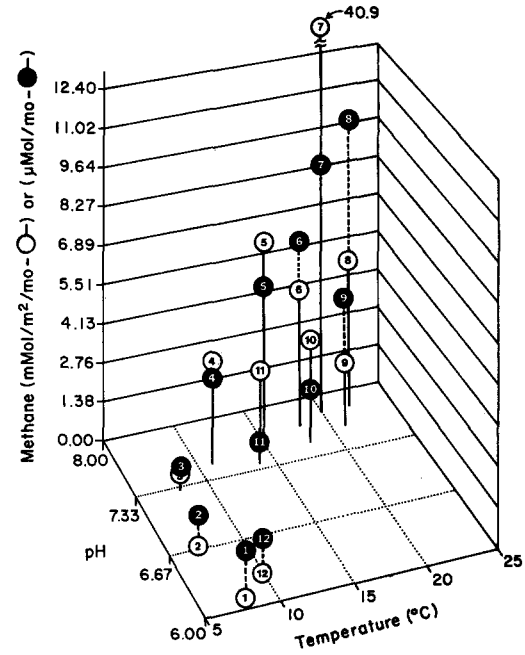

Fig. 9. Comparison of the pattern of methane production observed in the field (○) and in the laboratory (●) as a function of pH and temperature. Numbers refer to months of the calendar year; January (#1) through December (#12). Lines connecting months highlight field accumulation of methane that deviated from the expected pattern based on laboratory findings. The greater the deviation, the longer the connecting line. Dashed and solid lines indicate a negative or positive deviations respectively.

are all statistically significant environmental variables influencing aquifer methanogenesis.

In an effort to compare the relative pattern of methane formation between the laboratory and field, we plotted the monthly amount of methane collected from the GCD at site B as a function of aquifer temperature and pH. On the same graph, we also calculated the amount of methane that would be expected in a four week laboratory incubation of site B aquifer slurries held between the ranges of pH and temperature values measured in the field. To do this calculation, it was necessary to perform a linear interpolation between the data points taken under the experimentally imposed pH and temperature conditions (as in Fig. 7), to the field pH and temperature values. The results are shown in Fig. 9.

There is good agreement between the general pattern of methane production observed in the field and in the laboratory. This finding suggests that a large portion of the variability in field methanogenesis can be attributed to fluctuations in aquifer pH and temperature. However, Fig. 9 also highlights several months during which the differences in the relative pattern of methane formation cannot be attributed solely to these variables. For example, the field methane value for the month of July is much higher than expected based on the laboratory derived pattern. In contrast, the opposite relationship is detected for the month of August. Both of these discrepancies point to some other factor(s) beside pH and temperature which serve to stimulate or depress field rates of methanogenesis.

The effect of sulfate on aquifer methanogenesis

Even though temperature and pH influence both 'in situ' and laboratory rates of methane production, collectively these factors do not explain all the differences between laboratory and field methane data from sites B and C. There are periods of the year where pH and temperature values should not have limited aquifer methanogenesis, yet no methane could be detected (Figs. 5 and 6). We previ-

ously showed that sulfate concentrations could influence carbon and electron flow in this aquifer and limit areas of active methanogenesis [5]. We therefore examined archived frozen ground water samples for the presence of this anion at sites A, B, C, and the South Canadian River.

Fig. 10 shows that sulfate was always present in the ground water at site A. We had previously speculated that the sulfate content at site A might be coming from the river [5]. During the latter portion of the year, the sulfate level of site A and the river exhibited a temporal relationship (data not shown) suggesting that some hydrologic communication between site A and the river was at least possible. However, the concentrations of sulfate in the river and site A did not correlate at all during the early portions of the study. Site A exhibited up to 19.9 mM sulfate, whereas the river only contained up to 6 mM sulfate. Therefore, site A must have other sources of sulfate besides (or in addition to) the South Canadian River. We further suggested that the absence of 'in situ' methane formation at site A is at least in part due to the high levels of sulfate present at this site [5].

An analysis of sulfate at sites B and C revealed that it was only transiently present at both sites during the course of our study (Fig. 10). During most times of the year, sulfate levels were below detectable limits (0.1 mM). However, when site B or C contained sulfate concentrations greater than 1 mM there was a concomitant decrease in the 'in situ' rate of methane ebullition (Fig. 4).

An analysis of those times of the year when 'in situ' methane production was not observed shows that the ground water had: (a) a pH value less than 7.0, (b) a temperature value less than 13°C, (c) sulfate concentration greater than 1 mM, or (d) some combination of these factors. Sites B and C showed times when the absence of methanogenesis could not be explained by low pH, low temperature, or the presence of sulfate. Undoubtedly, other variables besides these also inhibit the methanogenic fermentation of organic matter. However, when the 'in situ' data from sites B or C were examined on a weekly basis, one or more of the above factors

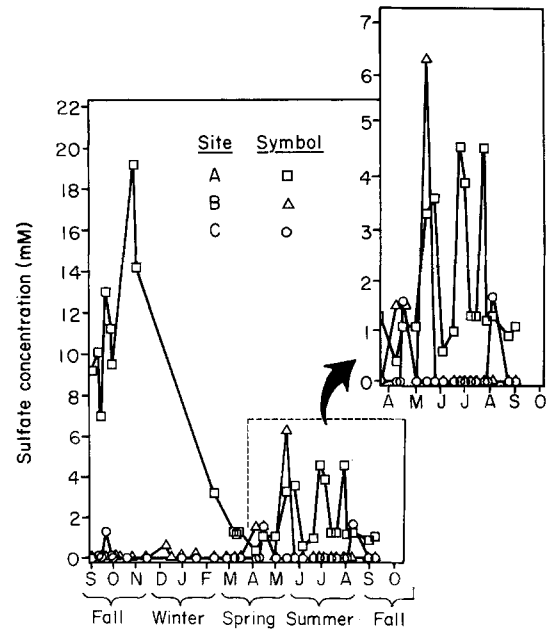

Fig. 10. Sulfate concentrations in ground water sampled over a year (1985–86) from sites A, B, and C.

were noted on 80% of those occasions when aquifer methanogenesis was inhibited.

The presence of low molecular weight volatile fatty acids

The inhibition of methane production in a variety of anaerobic habitats is often associated with a build up of volatile fatty acids (VFAs;27). We questioned whether the lowered aquifer temperature and pH values which depressed aquifer methanogenesis in the field would result in the accumulation of VFAs in the ground water. Unconcentrated ground water samples taken from both site A or B during an active period of methanogenesis (June 1985) did not exhibit detectable concentrations of C1-C5 VFAs (data not shown). Presumably, the VFAs were being consumed about as fast as they are produced. When the same analysis was repeated at the various sites in late March 1986, temperature and pH values were 15°C and 7.2, respectively, thus aquifer methanogenesis was slowed. Under these conditions, VFAs were detected at all three sites. Formate was the only volatile fatty acid common to all sites. In site A, trace concentrations (<1 μM) of

formate were detected, while sites B and C contained 2 and 1 μM respectively. No other VFAs were identified at site A. However, C3-C5 VFAs were detected in the unconcentrated ground water from sites B and C. Site C had relatively high concentrations of butyrate (15 μM) and valerate (7 μM), and also contained 1 μM concentrations of propionate, butyrate, and iso-valerate. In contrast, site B contained 2 μM proprionate and butyrate, but no detectable concentration of valerate. Site B also contained an 8 μM concentration of iso-valerate. The latter VFA probably indicating the decomposition of proteinaceous material in the leachate contaminated ground water [26].

DISCUSSION

Many of the nearly 100 000 municipal and industrial landfills in the United States are located in close proximity to aquifers [42]. Leachate plumes from such sources can occupy areas up to 3000 m long and be greater than 50 m in depth [21]. Leachates can pollute ground waters and render these resources unsuitable for many intended purposes. It is also conceivable that such plumes can migrate under buildings where methane, resulting from the anaerobic fermentation of leachate components, can accumulate to toxic and/or explosive levels. Therefore, risk evaluation of this type of ground water pollution requires reliable information on the susceptibility of leachates to biodegradation and on the environmental factors which influence the rates of substrate mineralization. Yet, relatively little research has focused on these issues [11].

This study and an earlier one [5] have helped elucidate several factors which influence the anaerobic mineralization of organic matter in a shallow anoxic aquifer. Sulfate, pH, temperature, and a pH–temperature interaction have been identified as significant variables influencing aquifer methanogenesis. The picture that emerges from these studies is one where sulfate can limit areas of methanogenesis in the aquifer. Such is the case for site A, which is anaerobic, has relatively high levels of sulfate year round (Fig. 10), and no evidence for the production of methane 'in situ' despite the relative ease with which methanogens could be cultured from this portion of the aquifer (Figs. 3 and 5). The origins of the sulfate are as yet unknown, but refuse such as gypsum building materials placed in the landfill may create a localized plume of sulfate which differentially impacts ground water in a localized area. The extent of site A may be a function of the rate of sulfate input into the ground water and its rate of microbial consumption by dissimilatory respiration. Sulfate is often found as a constituent of landfill leachate [15] and many studies have noted the inhibitory influence of this anion on methanogenesis [1,5,23,29,44]. Similarly, the transient levels of sulfate detected at site B and C may help account for the interruption in methanogenic activity noted at these sites despite favorable aquifer temperatures and pH conditions.

A seasonal pattern of aquifer methanogenesis was observed at site B and C. This is in sharp contrast to the findings of Bingemer and Crutzen [6] who report a temperature of 35°C in the anaerobic portion of temperate landfills without much seasonal variation. Since the aquifer is shallow, it is influenced by ambient air temperatures. In winter, 'in situ' methane production was essentially undetectable and ground water pH values were minimal. Conceivably, the colder winter temperatures limited methanogenic activity and resulted in the accumulation of volatile fatty acids. These compounds are known constituents of landfill leachates [8,9,13,17,18,20,34,37] and their accumulation in winter could conceivably overwhelm the buffering capacity of the aquifer and account for the depressed ground water pH values. Greater concentrations of such volatile acids were noted in water samples taken from sites B and C, when 'in situ' methanogenic activity was minimal. The lack of a similar observation in water sampled from site A probably reflects the generally 10 fold lower amount of dissolved organic matter impacting the area compared to either site B [5] or site C. Presumably, most of the volatile acids were below detection limits in the unconcentrated samples from site A. Our finding of a pH-temperature interaction on methanogenesis draws attention to the multiple

abiotic factors which influence microbial activity in complex environments. While many studies have suggested either temperature or pH as an influence on methanogenic activity [4,10,12,19,22,24,31,36, 39,43,46], we are unaware of other reports which show a significant interaction term between the two variables.

The pH and temperature optima for methane production in the aquifer are similar to other aquatic environments. The temperature optimum for aquifer methanogenesis (35°C) is comparable to the 35°C–42°C values reported for anaerobic lake sediments [46]. However, unlike the latter study we were unable to demonstrate significant methane production by thermophilic methanogens at pH 7. The pH optimum of 8 for aquifer methanogenesis was much like that reported by DeLaune et al. [12] for Mississippi River deltaic peat (pH 7.7), but unlike the value of 6.0 reported by Williams and Crawford [43] in their study of the Minnesota peatlands. In further contrast to the latter study, the addition of acetate to aquifer slurries stimulated rather than inhibited methanogenesis. Further, many pure cultures of methanogens have a pH optima near neutrality (6.5–7.5) and require mesophilic temperatures for optimal growth (30–40°C;25).

It would be incorrect to assume that all the variation in field methane production is due solely to the above mentioned environmental variables. In fact, a comparison of the field and laboratory-derived methane production patterns reveals several months of sharp deviation (Fig. 8). There were several times of the year at which 'in situ' methane production was undetectable and yet environmental conditions favored its production. Such responses could perhaps be due to aquifer recharge events or variation in ground water flow characteristics which might alter the concentrations of fermentable substrates and/or availability of terminal electron acceptors. Such factors could have a drastic impact on aquifer methanogenesis, but were not considered in the present study.

Methane detection at sites adjacent to landfills has been attributed to its formation in refuse mounds and its subsequent diffusion through the soil overburden above the water table [35]. While this may be true, our data indicate that methanogens are also in the aquifer per se (see also [5]). If these organisms have the proper environmental conditions and suitable substrates, they are capable of forming methane 'in situ' which can contribute to the overburdened pools of methane. This study and others have found numerous methanogenic substrates in leachate impacted ground waters including acetate, formate, methylamines, methanol, and hydrogen [2,3,8,9,13,17,18,20,34,37]. Therefore, methane emanating from leachate impacted areas of the subsurface can also be a source of regulatory concern.

Lastly, it is interesting to compare the maximum methane production rates at sites B and C with other environments. The maximum methane flux at these aquifer sites approximates that of eutrophic lakes (625–771 μmol/m^2/h; [19]), but is about 10 times less than swamps (estimated 1484 μmol/m^2/h; 30) coastal sediments (2500 μmol/m^2/h; 24), and another hypereutrophic lake (1458–1542 μmol/m^2/h; 40). We also compared our methane production rate results to a sulfate-rich coral reef environment (0.2–2 μmol/m^2/h) for comparison. Like the sulfate-rich aquifer site A, methane production in the coral reef is also relatively low. Such comparisons are important in light of the belief that up to 80% of the atmospheric methane has been attributed to biological sources [39]. It has been suggested that methane can act as a 'greenhouse gas' and contribute to global warming trends [6,10,39]. Recent estimates of the contribution of landfills to atmospheric methane concentrations range from 6–18% [6]. However, these estimates may be conservative if they ignore the amount of methane formed during the fermentation of leachate constituents in terrestrial subsurface environments.

In conclusion, we have demonstrated microbial methane production from two sites in a shallow anoxic ground water aquifer polluted by leachate from a municipal landfill. A third site containing high levels of sulfate did not produce methane 'in situ' during the course of our study. However, methanogenic bacteria were easily detectable at this site. Laboratory studies of temperature, pH, temperature–pH interaction and the presence of sulfate

showed that any of these variables could limit aquifer methanogenesis. Sites producing methane showed seasonal temperature, and pH variations, and also transient accumulations of sulfate. Considering the 'in situ' methane production data, we can infer the involvement of at least one of these variables approximately 80% of the times when methane was not produced in the field.

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