

## Spatial Variability in Biodegradation Rates as Evidenced by Methane Production from an Aquifer

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**Accurate predictions of carbon and energy cycling rates in the environment depend on sampling frequencies and on the spatial variability associated with biological activities. We examined the variability associated with anaerobic biodegradation rates at two sites in an alluvial sand aquifer polluted by municipal landfill leachate. In situ rates of methane production were measured for almost a year, using anaerobic wells installed at two sites. Methane production ranged from 0 to 560  $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{day}^{-1}$  at one site (A), while a range of 0 to 120,000  $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{day}^{-1}$  was measured at site B. The mean and standard deviations associated with methane production at site A were 17 and 57  $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{day}^{-1}$ , respectively. The comparable summary statistics for site B were 2,000 and 9,900  $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{day}^{-1}$ . The coefficients of variation at sites A and B were 340 and 490%, respectively. Despite these differences, the two sites had similar seasonal trends, with the maximal rate of methane production occurring in summer. However, the relative variability associated with the seasonal rates changed very little. Our results suggest that (i) two spatially distinct sites exist in the aquifer, (ii) methanogenesis is a highly variable process, (iii) the coefficient of variation varied little with the rate of methane production, and (iv) in situ anaerobic biodegradation rates are lognormally distributed.**

Groundwater supplies in the United States are limited, and withdrawal rates continue to increase. The accidental or deliberate release of organic chemicals has caused concern over the quality and quantity of drinking water supplies (21). This concern has prompted many studies on the fate (16, 40), transport (28), and risks (18) associated with organic pollutants in subsurface environments. Numerous computer models have been used to predict the migration of contaminants in groundwater (8, 22, 37). Many models consider only the physical and chemical interactions of the solute with the environmental matrix. However, the terrestrial subsurface is known to harbor a diverse microbiota (2, 15) capable of transforming a variety of pollutants (9, 16, 26, 27). Biotransformation processes often govern the destruction of contaminants from subsurface environments (7). Therefore, information on biodegradation kinetics is required for an accurate assessment of the dangers of subsurface chemical contamination.

Aquifer heterogeneities (39) and other unknown factors that affect biodegradation rates in aquifers make it difficult to extrapolate biodegradation kinetics from the laboratory to the field. Modeling efforts are often hampered because of the uncertainty in specific fate processes, the values of model parameters, and the spatial and temporal variations of these processes (1). One approach is to account for the natural heterogeneity of groundwater environments by describing them in a probabilistic fashion (12, 39). This often results in quantitative process predictions encompassing a range of estimates rather than absolute determinations. We decided to quantitatively assess the variability associated with in situ biodegradation rates. We chose to study the variability in methane production rates in a shallow, anoxic aquifer as a measure of the end product of anaerobic biodegradation activity. We found that (i) biodegradation rates were tempo-

rally variable and correlated with seasonal temperature fluctuations, (ii) coefficient of variations in biodegradation rates changed very little with the mean rate, (iii) hot spots of biodegradation existed within the aquifer, and (iv) biodegradation rates were lognormally distributed. Our results demonstrate that biodegradation activity was significantly different at two locations in a shallow aquifer.

### MATERIALS AND METHODS

**Site description and sampling.** The aquifer chosen for study receives leachate from a recently closed municipal landfill (2). Previous research indicates that two chemically and spatially distinct sites are located in close physical proximity within the aquifer (2). One site (site A) is characterized by a low concentration of dissolved organic carbon (80 to 160 ppm) in the groundwater, a high sulfate concentration (52 to 540 ppm), and a low rate of methane production (0.06 to 1.0  $\text{ppm} \cdot \text{day}^{-1} \cdot \text{g}^{-1}$ ). This assessment was made relative to another site (site B) that exhibited the opposite characteristics. That is, dissolved organic carbon was high (up to 1,100 ppm), sulfate values were often an order of magnitude less than those at site A and were generally undetectable during the summer, and methane production was several orders of magnitude higher most times of the year (2). The sediment at both sites is a quaternary recent alluvium, composed mostly of sand but also silt, clay, and gravel (38). Geologically, the aquifer is relatively uniform, with the top of the water table averaging 0.61 to 1.5 m below the soil surface. Our assumption is that variation in biodegradation rates as evidenced by methane production in more complex environments will be at least as variable as that measured in this relatively simple aquifer.

The sites were prepared by removing overlying vegetation and waste materials and leveling the ground surface. Sixteen bore holes, 1.8 m apart, were placed in a four by four grid pattern on the aquifer surface. The grids were centered around existing monitoring devices that have been in place since 1986. Polyvinylchloride anaerobic wells were installed in each bore-

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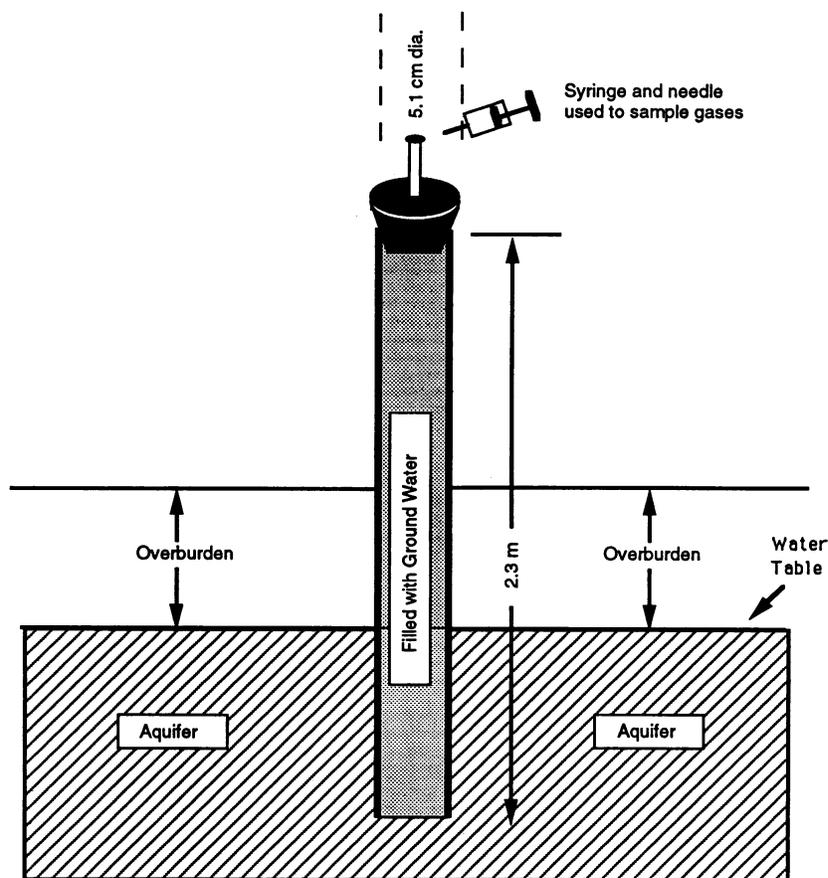


FIG. 1. Diagram of the anaerobic well used to collect gas samples from the aquifer.

hole, filled with groundwater, and closed at one end with a number 12 rubber stopper through which a severed balch tube was inserted (Fig. 1). The wells were placed 0.61 m below the water table to ensure that they would remain submerged despite fluctuations in the height of the water table. The wells were allowed to equilibrate for 3 months prior to the start of the study. Gases (0.5 to 5 ml) collecting at the top of the wells were sampled by syringe at about 2-week intervals for 9 months. The sampling procedure typically created a slight vacuum, so the syringe needle was momentarily stopped in the rubber stopper and allowed to equilibrate against atmospheric pressure. The gas volume was recorded, and then the sample was placed in a nitrogen-flushed anaerobic culture tube and transported to the laboratory for subsequent methane analysis. The volume of gas remaining in the anaerobic well was determined in a similar manner. Methane analysis was performed by using a Varian model 3300 gas chromatograph equipped with a flame ionization detector as previously described (2). Methane production rates are expressed as micromoles of methane  $\cdot$  meter<sup>-2</sup>  $\cdot$  day<sup>-1</sup>. When methane levels were below detection limits or too little gas was produced to sample, the detection limit of the assay for each site was substituted for the methane production rate in the anaerobic well. This value varied depending on the sampling time interval, but the minimum detection limits observed for sites A and B were 0.21 and 0.16  $\mu$ mol of CH<sub>4</sub>  $\cdot$  m<sup>-2</sup>  $\cdot$  day<sup>-1</sup>, respectively.

**Statistical analysis.** Comparison of the frequency distribu-

tions for methane production rates at the sites were made by using quantile-quantile (Q-Q) plots, a specialized probability-plotting method (11, 14, 45). These plots provide an effective way of comparing unknown frequency distributions and evaluating their potential relatedness through an assessment of linearity. The log-transformed biodegradation rates from each site were converted to standard normal deviates, and the empirical quantiles for site A were plotted against those at site B. The normality of the methane production rates at the two sites was assessed by ranking the biodegradation rates for each site and season and plotting the data against corresponding Gaussian quantiles (34). Normally distributed data yield a straight line in such plots, while lognormally distributed data will be linear in such plots after being logtransformed. Significant differences in methane production between the two sites were evaluated by using a two-tailed *t* test on untransformed data, a two-tailed *t* test on log-transformed data, and a mean confidence interval overlap method for lognormally distributed data (32). The confidence intervals about the means were calculated with a BASIC computer program (USDA Technology Transfer Document NSTL91-3) as described by Parkin et al. (33).

## RESULTS

**Temporal variability in methane production.** Typically, about 1 to 50 ml of gas accumulated in each well at each sampling, although a range from <1 to 200 ml of gas was

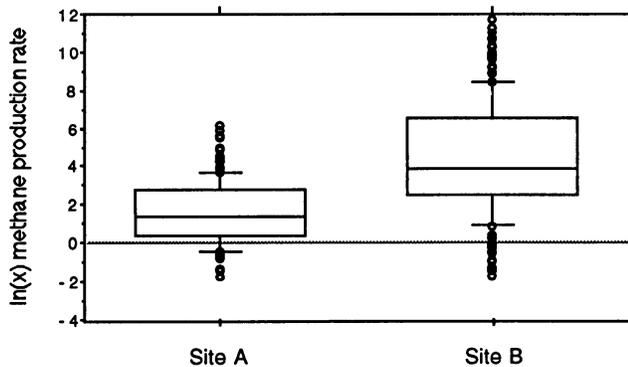


FIG. 2. Box-and-whisker plots of the natural logarithms of the methane production rates integrated over time for sites A and B. The bottom of the box represents the 25th percentile, and the top represents the 75th percentile. The middle line of the box represents the 50th percentile, or the median. The whiskers at the bottom and top of the box represent the 10th and 90th percentile, respectively. The small open circles represent the 10% lowest and highest methane production rates.  $x$  is in units of micromoles of  $\text{CH}_4 \cdot \text{meter}^{-2} \cdot \text{day}^{-1}$ .

occasionally measured. Some gas samples had no detectable methane content or the wells produced such a small gas volume ( $<1.0$  ml) that reliable sample measurement was precluded. These methane values are reported as being below the detection limit and are referred to as left censored data (17). Various recommendations exist for analyzing censored data sets (17, 19, 36, 44). Helsel (19) notes that the substitution of 0 for the censored value produces means that are biased low, while substitution of the detection limit produces means that are biased high. We calculated the mean of the data set by replacing the censored values with either 0 or with the detection limit of the methane analysis. The means calculated in the latter fashion were an average of 0.53% higher than those calculated by the former method. The largest difference in means was 2.2%. These small differences were due to the wide range and magnitude of methane production rates that were measured. We considered these differences small and therefore used the detection limit for the censored data.

Methane production in the wells located at site B ranged from 0.16 to 120,000  $\mu\text{mol}$  of methane  $\cdot \text{m}^{-2} \cdot \text{day}^{-1}$ , while site A had a narrower range of 0.21 to 560  $\mu\text{mol}$  of methane  $\cdot \text{m}^{-2} \cdot \text{day}^{-1}$ . The median (50th percentile) methane production rate at site B was greater than that observed for site A (Fig. 2). The coefficient of variation for both sites was  $>340\%$  even though site A had a mean methane production rate 210-fold less than that measured at site B.

A seasonal comparison of the methane production rates is shown in Fig. 3. The middle 50% of the distribution in the methane production rates at site B was greater than that of site A in both the summer and the fall. Generally, only the lower methane production rates for site B ( $<25\%$  percentile) overlapped with the middle 50% of the rates at site A at these times of the year. During the spring, however, a large degree of overlap in biodegradation rates between the two sites was evident (Fig. 3).

Both sites exhibited seasonal trends in methane production rates with the highest rates observed in the summer, followed by the fall and spring (Table 1). Despite the seasonal trends in the rates of biodegradation, the relative variation associated with these rates remained extremely high (Table 1). That is, the coefficient of variation was  $>340\%$  for site B in the summer and spring even though there was a 130-fold differ-

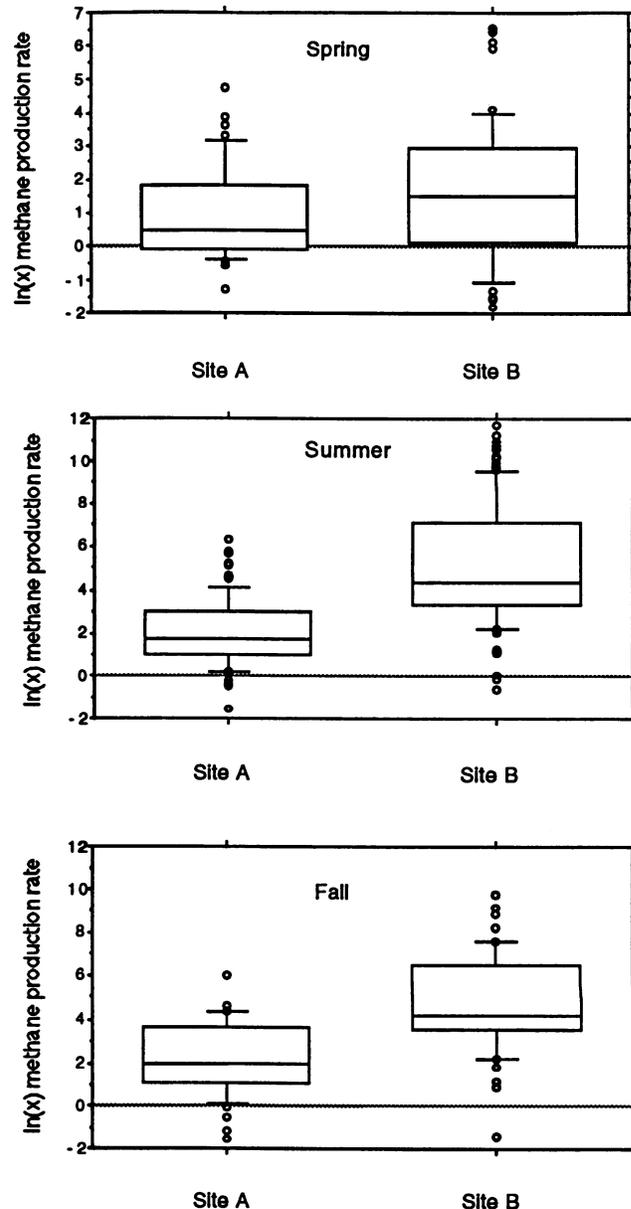


FIG. 3. Box-and-whisker plots of the natural logarithms of the methane production rates. (Top panel) Box plots for sites A and B during the spring season; (middle panel) box plots for sites A and B for the summer season; (bottom panel) box plots for sites A and B for the fall season.  $x$  is in units of micromoles of  $\text{CH}_4 \cdot \text{meter}^{-2} \cdot \text{day}^{-1}$ .

ence in the methane production rate between the two seasonal means. This large relative variation also remained high when the two sites were compared. For example, the coefficient of variation was  $>290\%$  for sites A and B in the summer even though site B had a 170-fold-greater methane production rate than site A (Table 1).

**Spatial variability in methane production rates.** The spatial variability of the methane production rates is most apparent when mean values in each well within each site are considered (Fig. 4). These values varied from 1 to 97 and from 16 to 10,500  $\mu\text{mol}$  of methane  $\cdot \text{m}^{-2} \cdot \text{day}^{-1}$  at sites A and B, respectively. This corresponds to a 74-fold difference in the minimum and

TABLE 1. Seasonal trends in methane production rates at sites A and B

Season	Site	Methane production ( $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{day}^{-1}$ )		Coefficient of variation (%)
		Mean	Median	
Summer	A	26	4.4	290
	B	4,400	56	340
Fall	A	14	0.21	330
	B	550	1.3	410
Spring	A	4.6	0.62	320
	B	33	1.0	370

maximum methane production rates observed at site A, while a 670-fold difference was noted at site B. Such values demonstrate the extreme spatial variability in anaerobic biodegradation activity. To exemplify, a mean methane production rate of  $10,500 \mu\text{mol}$  of methane  $\cdot \text{m}^{-2} \cdot \text{day}^{-1}$  was observed in one well at site B, while an adjacent well had a comparable rate of only 40, a 260-fold difference in biodegradation activity. This

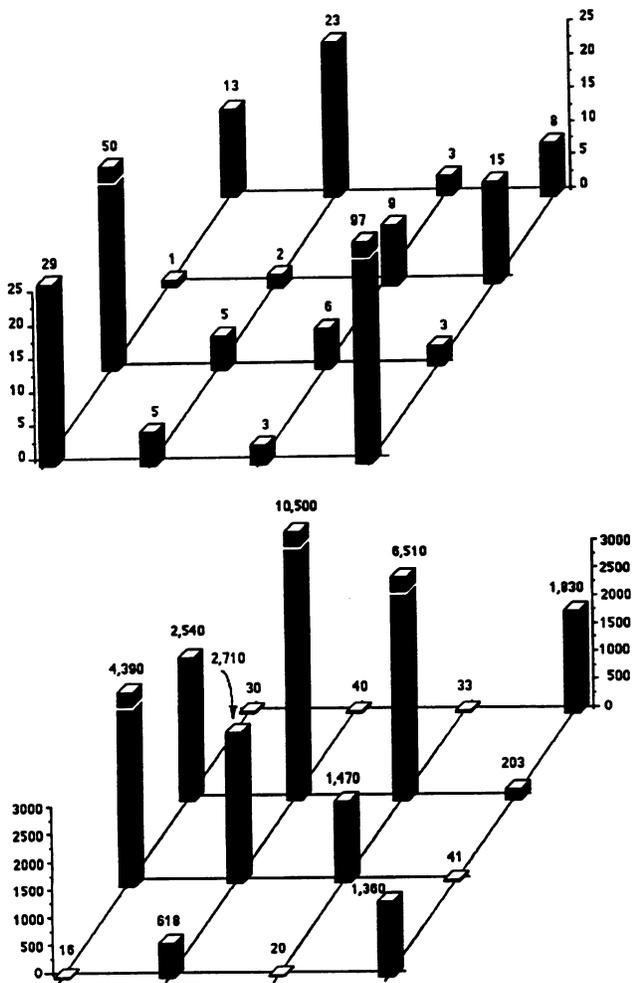


FIG. 4. Spatial plot demonstrating the extreme variability of the methane production rates for sites A (top) and B (bottom). The column represents the mean methane production rate observed during the study period, with the actual rate above each column. Methane is reported as micromoles of  $\text{CH}_4 \cdot \text{meter}^{-2} \cdot \text{day}^{-1}$ .

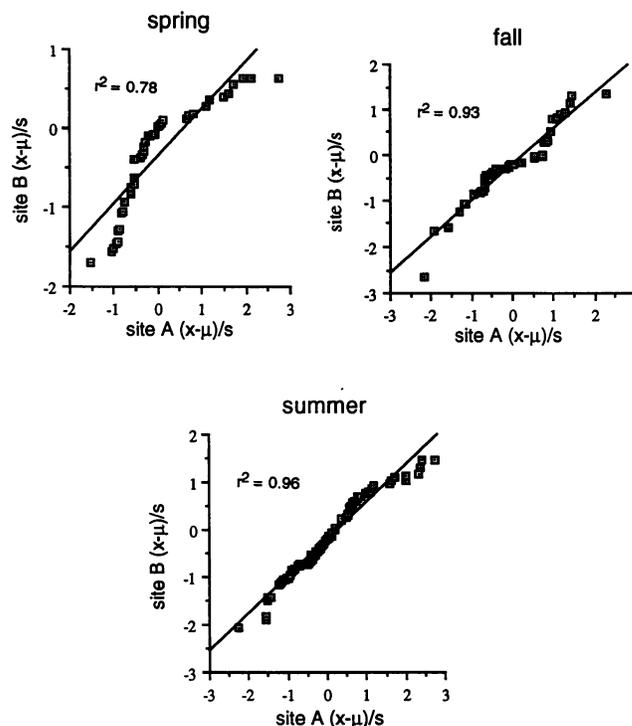


FIG. 5. Q-Q plots comparing the methane production rate frequency distribution between sites A and B for each season.  $(x - \mu)/s$  is the normal standard deviation of the natural log-transformed methane production rate.  $r^2$ , observed coefficient of determination between the normal standard deviations.

spatial variability was also evident at site A (Fig. 4). A mean methane production rate of  $50 \mu\text{mol}$  of methane  $\cdot \text{m}^{-2} \cdot \text{day}^{-1}$  was observed in one site A well, while an adjacent well had a mean rate of  $1 \mu\text{mol}$  of methane  $\cdot \text{m}^{-2} \cdot \text{day}^{-1}$ . The coefficients of variation for the means at sites A and B were 148 and 145%, respectively, so the relative variabilities in biodegradation activity at the two sites were similar.

Hot spots of high biodegradation activity occurred at both sites and accounted for a substantial portion of total methane production. For example, two of the anaerobic wells accounted for 54 and 53% of the total methanogenic activity observed at sites A and B, respectively (Fig. 4). Conversely, cold spots of biodegradation activity were also noted where the rates were relatively low. Cumulative methane production from eight other wells accounted for only 10 and 3% of the total methane production at sites A and B, respectively.

**Biodegradation frequency distributions.** While the rates of methane production were greater at site B than site A, both sites exhibited a similar degree of variability in methane production. The similarity in the distribution of methane production between the two sites was evaluated with Q-Q plots. Figure 5 shows that the frequency distributions of methane production rates at sites A and B are similar in both summer and fall ( $r^2 = 0.96$  and  $0.93$ , respectively). However, the similarities in the two underlying methane frequency distributions at the two sites are less pronounced in the spring, as evidenced by the deviations from linearity ( $r^2 = 0.78$ ) (Fig. 5). Thus, even though the rate of methane production is generally greater at site B than site A, the observed frequency distributions at the two sites are similar for most of the study period.

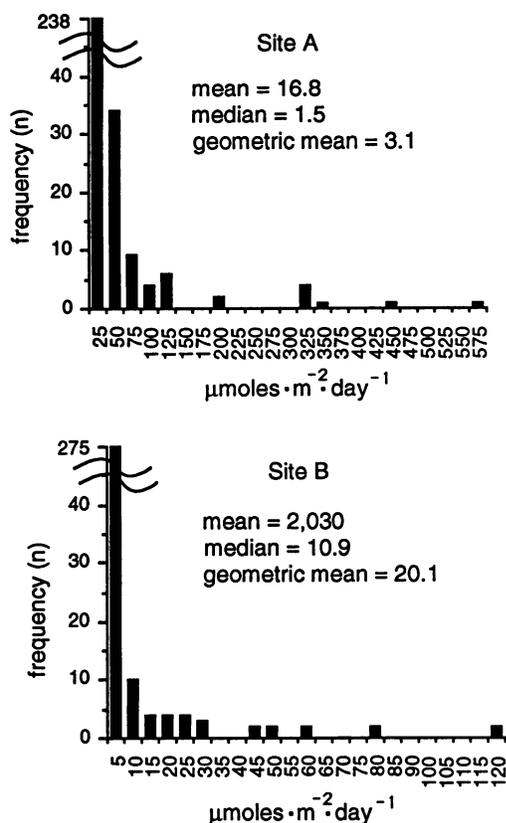


FIG. 6. Histograms of the methane production rates from sites A and B. Methane is reported as micromoles of methane · meter<sup>-2</sup> · day<sup>-1</sup>. Site B, 10<sup>3</sup>.

**Lognormal distribution of biodegradation activity.** The methane production rates at the two sites were highly skewed to the right (Fig. 6). A graphical method with greater diagnostic power was used to assess the fit of the data to normal and lognormal distributions. The ordered untransformed and log-transformed data were plotted against the probits of a standard normal distribution (11, 14, 34, 45); the linearity of the data points is an indication of how well the data follow the normal or lognormal distribution, respectively. Since the transformed data of lognormally distributed variables are themselves normally distributed, a straight line on this curve is an indication of a lognormal distribution (34, 42). The probit plots clearly indicate the methane production rates in the summer, fall, and spring were not normally distributed (Fig. 7). An approximately linear relationship is observed when the empirical quantiles of the logtransformed data are plotted against the probits of the normal distribution. For example, both sites appear to be well approximated by a lognormal distribution in the summer and fall, as indicated by the straight line when the log-transformed data are plotted against the corresponding probits of the normal distribution (Fig. 7). The coefficient of determination ( $r^2$ ) for both sites in the summer and fall is equal to or greater than 0.96. In the spring, the values for site B also appear to be approximated by a lognormal distribution ( $r^2 = 0.966$ ), while site A exhibits more deviations away from this distribution ( $r^2 = 0.887$ ). These deviations suggest that site A may have an even more complex frequency distribution (Fig. 7).

Although earlier studies suggested that there were two

chemically and spatially distinct sites in the aquifer (2, 3), there was no a priori reason to assume that there were statistically significant differences in the rates of methanogenesis between the sites. For example, if the rates in methane production were highly variable, differences between the sites could easily be obscured. For example, a Student *t* test detected only 1 significant difference of 18 comparisons between the two sites when their mean methane production rates were compared at the  $P = 0.05$  level of significance (data not shown). This is undoubtedly due to the highly skewed data set which reduces the power of the *t* test for detecting differences (32). However, there was a low statistically associated probability with the observed *t* values for all the comparisons made. With one exception, all comparisons exhibited a probability of 0.25 or less. These consistently low probabilities suggest that a significant difference in the mean rate of methane production between sites A and B was likely. Eight significant differences between the two sites were observed when a mean confidence interval overlap method (one-sided 95% confidence limits) was used to evaluate differences in the methane production rates (33). Since the data were shown to be lognormally distributed, the assumption of normality can be met if a log transformation is performed on the data (42). A *t* test performed on the log-transformed data resulted in 12 significant differences of 18 comparisons (67%) at the  $P = 0.05$  level of significance. However, it should be clear that the median, rather than the mean, of the two sites was compared in this analysis.

## DISCUSSION

To accurately model and predict the fate of xenobiotic compounds in the environment, reliable information on rates of biodegradation and on the variability associated with those rates is needed. A variety of methods to measure rates of biodegradation exist. We chose to measure the rate of methane production, since this gas is a relatively insoluble end product of anaerobic biodegradation. The study site was a shallow anoxic aquifer impacted by leachate from a municipal landfill. Although it appears that this alluvial sand aquifer is relatively geologically and hydrologically simple, the spatial variability associated with the rate of methane production was high. Several lines of evidence suggest that methane production and therefore biodegradation rates are lognormally distributed: (i) the coefficients of variations were extremely high, (ii) large differences were observed between the sample means and geometric means, (iii) histograms of the rates of methane production were highly skewed, and (iv) the logarithms of the biodegradation rates appeared to be normally distributed.

Our results support the earlier contention that there are two spatially distinct sites located in the aquifer with respect to methane production and other physicochemical characteristics of the aquifer (2, 3). The mean, median, and geometric mean of site B exceeded the same summary location parameter estimates for site A for all comparisons except one. The reason for this exception is as yet unknown. However, the phenomenon occurred in the spring of the year, when we have historically measured high sulfate concentrations in the groundwater (3). The intrusion of sulfate at site B may have depressed aquifer methanogenesis to rates that were comparable to those measured at site A. As sulfate gets utilized at site B, the flow of carbon and energy through methanogenesis likely returns in later portions of the year. This possibility will be investigated in subsequent studies. Although we do not know the cause(s) of the variability, we were able to quantitate it and note that it was similar at both sites.

The biodegradation rates at both sites also varied temporally

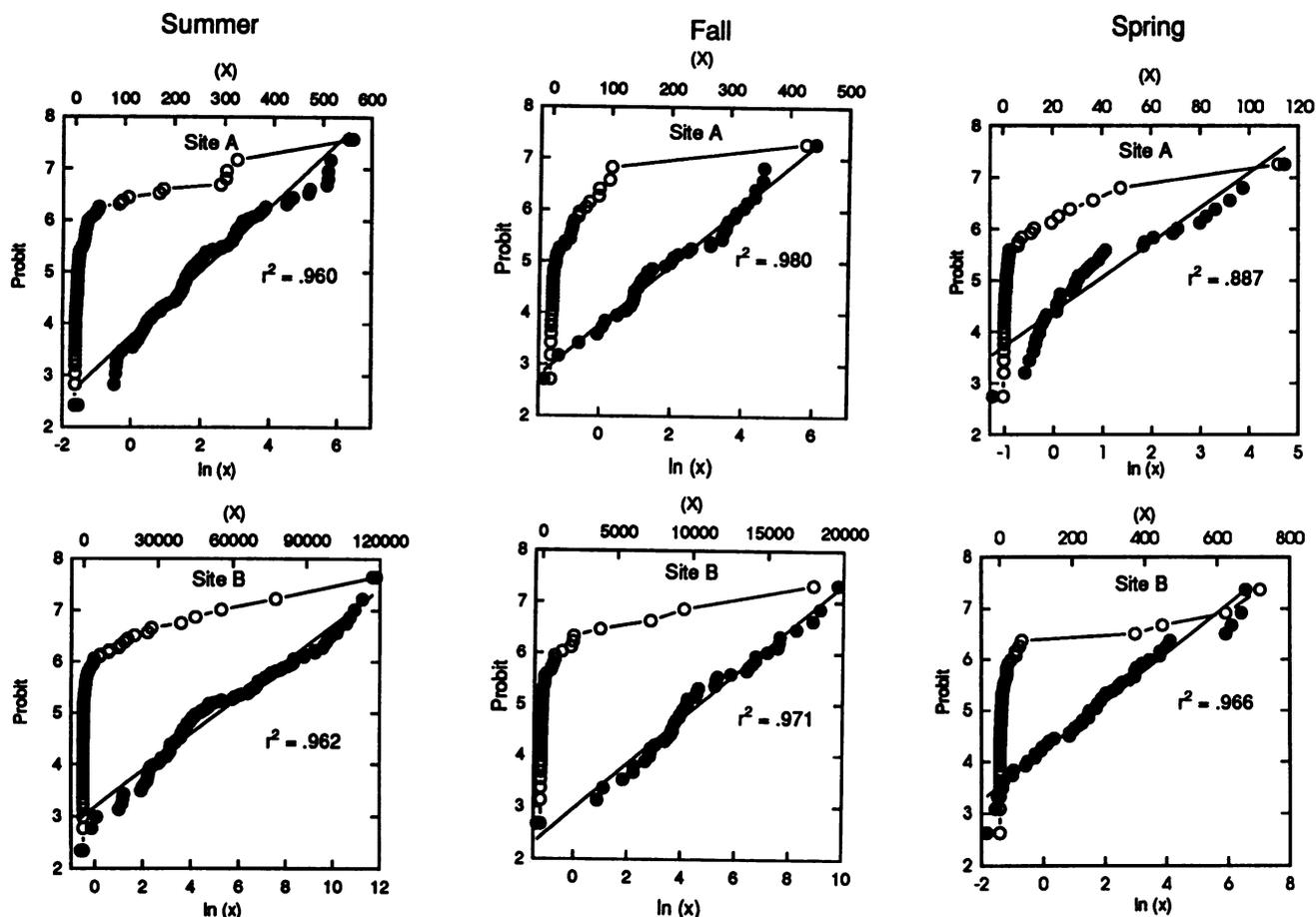


FIG. 7. Probit plots for sites A (top panels) and B (bottom panels) during the summer (left panels), fall (middle panels), and spring (right panels) seasons. The untransformed methane production rates (open circles) and the log-transformed methane production rates (closed circles) were plotted against the probits corresponding to the cumulative probabilities of the normal distribution. Cumulative probabilities were calculated according to  $(i_n - 0.5)/n$ , where  $i$  is the numerical rank of the  $i$ th sample, and  $n$  is the number of samples.  $r^2$ , observed coefficient of determination between the probits and ordered data;  $x$ , untransformed methane production rate in units of micromoles of  $\text{CH}_4 \cdot \text{meter}^{-2} \cdot \text{day}^{-1}$ .

and spatially. The highest methane production rates for both sites were seen in the summer, followed by the fall and spring. Methane production rates in these sediments were shown to be significantly influenced by pH, temperature, and sulfate content, although additional variables also influenced biodegradation rates (3). Similar variations in methane production rates in other environments have been observed. For example, seasonal variations in methane releases have been observed in freshwater sediments (24, 35, 47) and soils (25). A large amount of this variation was attributed to temperature changes (5, 25, 46, 47) and to a combination of temperature and sedimentation events (35, 41). A high spatial variability in methane production has also been observed in other environments. Pedersen and Saylor noted high spatial variability in methane production in freshwater sediment samples and between replicate subsamples (35). King and Wiebe (25) noted high spatial variability in methanogenesis from a salt marsh, while Dise (6) suggested that methane fluxes in peatlands were lognormally distributed. King and Wiebe attributed 20% of the variation in methane production to the heterogeneous distribution of organic matter (25). The temporal and spatial variabilities of biodegradation rates observed at our sites as evidenced by methane production appear to be common phenomena.

Other microbial activities in sediments are also known to be highly variable. For example, the spatial variability associated with bacterial hydrolase activities were found to be very high with coefficient of variations ranging from 43.3 to 87.6% (43). Federle et al. (10) investigated the spatial distribution of microbial biomass, microbial activity, and the biodegradation of linear alkylbenzene sulfonate and linear alcohol ethoxylate in the subsurface. They found up to a 100-fold decrease in the distribution of microbial activities below 2 to 3 m in the vadose zone, as evidenced by the rate of fluorescein diacetate hydrolysis and incorporation of thymidine. Linear alkylbenzene sulfonate was mineralized in the upper 2 m of the vadose and in the saturated zone with little to no mineralized between 2 and 14 m. Linear alcohol ethoxylate mineralization decreased with depth, similar to the activity measurements. Biodegradation rates of methanol, phenol, and tert-butyl alcohol also were shown to vary considerably over small distances, both horizontally and vertically (20). Denitrification is a biological process that is also extremely spatially variable. Coefficients of variation of >100% are typical and often are several hundred percent (4). A variety of biological activities appear to be highly spatially variable in the subsurface environment. It is likely that geological, hydrological, and microbiological varia-

tion all contribute to our observed spatial variation in biodegradation rates.

Some of the spatial variability that was encountered in our study was evidenced by the presence of hot spots of biodegradation activity in a few of the wells. For example, at both sites A and B, >50% of the total biodegradation activity was observed from only 12% of the wells. Similar hot spots of microbial denitrification activity were observed by other investigators (30). Denitrification hot spots wherein 25 to 85% of the total denitrification activity of intact soil cores was associated with particulate organic matter representing from 0.4 to 0.08% of the total mass of the soil cores were observed. Similar high heterogeneities in biodegradation activity were also observed in sediments (23). The occurrence of hot spots of biodegradation activity does not appear to be unusual and may be a frequently occurring phenomenon.

The variability in biodegradation activity remained high for both sites throughout the year. This is evident by a coefficient of variation of >290% for sites A and B throughout the study period despite a maximum difference of 950-fold in the methane production rates. This finding was somewhat surprising, since we hypothesized that the variability and skewness in biodegradation rates would become less when (i) methane production activity was minimal because of seasonal temperature and pH fluctuation and/or (ii) when all variables for methane production were near optimal, as in summer (3). A varying relative variability was observed with denitrification (30). Parkin observed highly skewed field denitrification rates with a coefficient of variation of 410%, while the relative variability of denitrification enzyme activity was only 48%. Since major controlling factors have been optimized in the latter assay, the variability associated with these denitrification measurements is due only to the dispersion of potentially active denitrifying enzymes in the soil. Christensen et al. also found the relative variability of denitrification to change, and in some cases the probability distribution was changed (4). For example, denitrification was less skewed in soil with a moisture content above the water-holding capacity than in soil held at the water-holding capacity. Similarly, the addition of high concentrations of organic matter changed the probability distribution so it conformed to a normal distribution. In both of the preceding investigations the relative variability of denitrification decreased when the factors controlling the process were optimized. At our two sites, the constant relative variability observed in biodegradation rates might suggest that the controlling factors are not optimal, even in the summer.

This constant high relative variability throughout the study period at sites A and B suggested a similarity between the two sites. The similarity between the two sites was compared by using probability plotting methods and normalizing the biodegradation rates by the standard deviation estimates. This demonstrated that the underlying distributions of methane production rates at sites A and B were similar and suggests that biodegradation rates are not random. Since histograms of the biodegradation rates were highly skewed to the right and the mean of the biodegradation rates was greater than the median, we hypothesized that the rates might be lognormally distributed (34). Probit plots were used to assess the lognormality of the biodegradation rates. These plots demonstrated that the biodegradation rates were better approximated by a lognormal distribution than a normal distribution. Many environmental variables (13) and biological activities (31) are known to be lognormally distributed.

The high variability of natural processes translates into a high degree of uncertainty regarding statistical estimation and inferences (34). Often, attempts are made to estimate biodeg-

radation rates by (i) disregarding the variability and assuming a Gaussian distribution, or (ii) using surrogate numbers such as total biomass or total cell numbers. However, it is difficult to accurately quantify the active biomass. For example, in a marine sediment over 90% of the sediment-water interface community was not actively growing (29). Ignoring the variability and making statistical inferences assuming a Gaussian distribution leads to inaccurate or incorrectly made decisions. Knowing the underlying distribution of biological processes allows one to choose more appropriate summary location parameters and methods for calculating the confidence interval about the mean, leading to more accurate estimates of biodegradation rates (34). Ignoring the variability in the biodegradation rates and applying parametric tests based on a different distribution leads to incorrect conclusions. For example, *t* tests are often used in hypothesis testing for detecting differences in rates of biodegradation. We have shown that using the *t* test is clearly inadequate for detecting differences in biodegradation rates between sites A and B. The assumption of normality for the *t* test is invalidated because of the lognormal distribution of the biodegradation rates. A mean confidence interval overlap method which has more power to detect differences in means from lognormal distributions was able to detect eight differences in methane production between the two sites. The greatest number of differences between the two sites was observed when the median methane production rates were compared. However, the mean is a more appropriate location parameter when evaluating the magnitude of a microbial process (34) such as methane production rates.

Including biodegradation coefficients into stochastic modeling efforts of environmental pollutants in the subsurface environment requires information on the variability of this process. Understanding and quantitating the variability in biological processes begin to lay the foundation to more accurately predict in situ biodegradation rates with a certain level of confidence. Realizing that biodegradation rates in the field are lognormally distributed will aid in selecting more appropriate methods for calculating confidence intervals about the mean, leading to more accurate predictions of biodegradation rates. Assuming that biodegradation rates are normally distributed could result in significant over- or underestimates of the predicted values. Many factors interact to control and cause variability in biological processes. To begin to identify and understand the effects of these factors requires information on the variability of that process. At our two sites we have quantitated this variability and demonstrate that the anaerobic biodegradation rates are better approximated by a lognormal than a normal distribution. Our tacit assumption is that the variability associated with biodegradation rates in deeper and more complex geological settings will be at least as high.

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