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Temperature Limitation of Methanogenesis in Aquatic Sediments

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Microbial methanogenesis was examined in sediments collected from Lake Mendota, Wisconsin, at water depths of 5, 10, and 18 m. The rate of sediment methanogenesis was shown to vary with respect to sediment site and depth, sampling date, in situ temperature, and number of methanogens. Increased numbers of methanogenic bacteria and rates of methanogenesis correlated with increased sediment temperature during seasonal change. The greatest methanogenic activity was observed for 18-m sediments throughout the sampling year. As compared with shallower sediments, 18-m sediment was removed from oxygenation effects and contained higher amounts of ammonia, carbonate, and methanogenic bacteria, and the population density of methanogens fluctuated less during seasonal change. Rates of methanogenesis in 18-m sediment cores decreased with increasing sediment depth. The optimum temperature, 35 to 42 C, for sediment methanogenesis was considerably higher than the maximum observed in situ temperature of 23 C. The conversion of H_2 and [^{14}C]carbonate to [^{14}C]methane displayed the same temperature optimum when these substrates were added to sediments. The predominant methanogenic population had simple nutritional requirements and were metabolically active at 4 to 45 C. Hydrogen oxidizers were the major nutritional type of sediment methanogens; formate and methanol fermentors were present, but acetate fermentors were not observed. *Methanobacterium* species were most abundant in sediments although *Methanosarcina, Methanococcus,* and *Methanospirillum* species were observed in enrichment cultures. A chemolithotropic species of *Methanosarcina* and *Methanobacterium* was isolated in pure culture that displayed temperature optima above 30 C and had simple nutritional requirements.

Microbial methanogenesis is common in nature and is normally associated with the decomposition of organic matter. By and large, previous investigations on methanogenesis in both marine (11, 19, 21) and fresh water (10, 12, 13, 17) sediments have been concerned with detection and quantification of methane evolution from sediments. Relatively little is known about the environmental factors that influence methanogenesis or about the in situ microbial activities responsible for this process in aquatic sediments. Recently Cappenberg (9; T. E. Cappenberg, Microb. Ecol., in press) has reported on how microbial interactions between sulfate-reducing and methane-producing bacteria influence methanogenesis in bottom sediments of Lake Vechten, a man-made, eutrophic sandpit located in central Netherlands.

In 1972, we initiated a detailed investigation on the microbial ecology of methanogenesis in Lake Mendota, Wisconsin. Lake Mendota is perhaps the best-studied lake in the world, and methanogenic activity in its sediments was first reported in 1932 by Allgeier et al. (1). Recently, investigators (18) have demonstrated that the de novo rate of methane synthesis was greater in Lake Mendota sediments than in several soft-water, oligotrophic Wisconsin lakes. We report here on the general properties of methanogenic bacteria in Lake Mendota and on how temperature greatly limits their activity.

MATERIALS AND METHODS

Description of sampling sites. All samples for this investigation were obtained from three stations located on the southwestern portion of Lake Mendota in Madison, Wisconsin. Lake Mendota is a moderately eutrophic, hard-water lake of glacial origin in southern Wisconsin. It covers about 3,900 hectare, has an average depth of about 12 m and a maximum depth of 24 m, and is anaerobic below 10.5 m during stratification (5). Three sampling sites (5 m, 10 m, 18 m) were located on a transect between the University of Wisconsin Water Resources Building and Governors Island. The 5-m and 10-m sediment sites were located in University Bay under 5 and 10 m of water, respectively; the

18-m sediment site was located off Picnic Point under 18 m of water. This portion of the lake was chosen for study because several other investigations were performed in this area (2, 5, 10).

Sediment sampling procedures. Initially, grab samples were collected anaerobically with a modified Martex bottom sampler (Rigosha & Co., Ltd., Tokyo, Japan). The modification involved the addition of a rubber ring on the spring-loaded cap of the sampler, creating an airtight seal necessary for the maintenance of anaerobiosis during the collection of sediments. The sampling procedure was to first remove oxygen from the bottom sampler by gassing with purified nitrogen gas. The sampler was then dropped into the water and allowed to impact with the sediments, collecting a quantity of mud. The sampler was retrieved, and the sediments were transferred into 25-ml, heavy-walled Pyrex centrifuge bottles while being continuously gassed with oxygen-free nitrogen and sealed with a rubber bung to maintain anaerobic conditions during transport to the laboratory at in situ temperature. The collected sediment slurry samples were mixed well prior to distribution into experimental anaerobic tubes. Grab samples were also collected with an Eckman dredge and by scuba divers that employed anaerobic collection techniques. All sampling procedures described above yielded identical experimental results if the sediment was rapidly removed from the samples and placed into anaerobic containers in the field. Grab samples contained the top 8 cm of sediment and were thoroughly mixed prior to use in experiments. All manipulations of sediment were carried out anaerobically by using the technique described by Hungate (14), modified for sample collection and manipulation in the field and laboratory. Undisturbed sediment cores were taken at the 10- and 18-m sites by means of a Jenkins mud sampler (8). Immediately after sampling, the core was processed anaerobically in the laboratory, and subsamples were placed into anaerobic culture tubes that contained N_2.

Analysis of sediments. Measurements of temperature and dissolved oxygen in water immediately overlying the sediment were made in situ using a combination thermistor-polarographic oxygen probe (Yellow Springs Instruments, Yellow Springs, Ohio). Measurements were recorded on a YSI model 51A oxygen meter (Yellow Springs Instruments).

Determinations of sediment pH and Eh were performed immediately upon return to the laboratory with collected sediment. A glass combination pH electrode (Corning Scientific Instruments, Medfield, Mass.) and a platinum combination electrode (Orion Research Inc., Cambridge, Mass.) were placed in the sediment to determine pH and Eh, respectively, while an oxygen-free nitrogen atmosphere was maintained over the sample. Both pH and Eh values were recorded on a Corning model 12 research pH meter (Corning Scientific Instruments). The following standard analytical procedures were used for the chemical analysis of these sediments: $CaCO_3$ equivalent (23), organic N (4), NH_4N (15), and organic carbon (25).

Culture media. Basal medium was a buffered, mineral salts medium that contained cysteine and vitamins as sole organic addition. Basal medium was prepared as described previously (29). Enriched medium contained the following parts per liter of distilled water: $(NH_4)_2SO_4$, 0.45 g; NaCl, 0.9 g; $MgSO_4 \cdot 7H_2O$, 0.18 g; $CaCl_2 \cdot 2H_2O$, 0.1 g; NH_4Cl, 0.5 g; KH_2PO_4, 1.5 g; K_2HPO_4, 2.2 g; sodium acetate$\cdot 3H_2O$, 2 g; Trypticase, 2 g; yeast extract, 2 g; $Na_2S \cdot 9H_2O$, 0.5 g; cysteine, 0.5 g; trace mineral solution (27), 9 ml; vitamin solution (27), 5 ml; and 5% $FeSO_4$, 0.3 ml. The final pH of all media used was 7.0 to 7.2. Anaerobic culture tubes that contained enriched or basal medium were gassed with one of the following heated, copper-scrubbed, ultrapure gases: N_2, H_2-CO_2 (80:20), or N_2-CO_2 (95:5).

Enumeration procedures. Numbers of methanogens in sediments were estimated by serially diluting (1:10) 1 ml of collected sediment into anaerobic culture tubes that contained enriched medium, basal medium, or basal medium with 1% acetate and a H_2-CO_2 (80:20) or H_2-CO_2 (95:5) gas atmosphere. Determinations in any medium were done in duplicate, and identical, duplicate sets were incubated at in situ sediment temperature or 30 to 37 C. After a 30-day incubation period, the highest dilution that displayed methane formation and growth indicated the number of sediment methanogens. Numbers of methanogens are reported on a dry weight of sediment basis. All values reported represent identical results obtained for duplicate experiments performed in both basal medium and enriched medium, under H_2-CO_2, and incubated at in situ temperature.

Enrichment, isolation, and identification of methanogens. Procedures described previously (28, 29) were employed for enrichment and isolation of microorganisms. Methanogens were identified according to the criteria presented by Wolfe (26).

Analysis of methane. Methane was routinely detected with a Varian Aerograph model 600D gas chromatograph equipped with a Poropak R column connected to a flame ionization detector. All values of methane measurements are for total methane present in the anaerobic culture tube and are reported on a per gram, dry weight, basis.

[^{14}C]methane was quantified by the gas chromatograph-gas proportional counting procedure described by Nelson and Zeikus (20). No special preparative methods were employed prior to the injection of gas samples into the gas chromatograph. The procedure for sampling was to withdraw 0.4 cm^3 of atmosphere from an anaerobic culture tube and inject this into the chromatograph. Gas composition was determined and radioactivity was quantified within 3 min.

Radioactive chemicals. The following radioactive compounds were used: [U-^{14}C]glucose, specific activity, 7.3 mCi/mmol (ICN); and $Na_2{}^{14}CO_3$, specific activity, 60 mCi/mmol (Amersham/Searle, Arlington Heights, Ill.).

RESULTS

Characterization of sampling sites. Preliminary studies revealed that methanogenesis in Lake Mendota occured only in sediments. Sediments samples placed into anaerobic culture

tubes that contained a gas atmosphere of N_2, CO_2, argon, or helium generated equivalent quantities of methane with time. De novo synthesis of methane was inhibited when sediment samples were incubated under O_2 or air. In vitro sediment methanogenesis was terminated in control tubes by the addition of 5% formalin. No methane was formed when water samples, collected from various locations and depths throughout the water column, were incubated anaerobically in the presence of H_2-CO_2 or N_2-CO_2. Also, mixing sediment from each of the three sites with the adjacent overlying water resulted in inhibition of sediment methanogenesis. Grab samples of sediments collected from all sites were very homogeneous in physical composition, were brown-black in color, smelled of sulfide, and had a redox potential below −150 mV. Low levels of dissolved oxygen were repeatedly detected in the adjacent waters overlying sediments at the 5- and 10-m sites. The water overlying the 18-m sediments was depleted of dissolved oxygen throughout most of the sampling year. Sediments from the three sites varied with respect to certain physical and chemical parameters (Table 1). Sediments from 5 m were twice as dense as those from 10 or 18 m. All sediments sampled contained above-saturating levels of carbonate; somewhat higher levels of precipitated carbonate were detected in 18-m sediments. Total organic matter and organic nitrogen decreased from the shallower to deeper sites, whereas the amount of ammonium-nitrogen incresed. The pH values of all sediments were near neutral throughout the sampling year. To examine the variation in methanogenesis at each site, the rate of methanogenesis was determined for each of three samples collected from each site (Table 2). Rates of methanogenesis in subsamples within a given sample and different samples from each site did not vary significantly. The variability was greatest at the 5-m site and least at the 18-m site. The percent standard deviations for the 5-, 10-, and 18-m sites at in situ temperatures were 30, 16, and 11%, respectively. Furthermore, less variance occurred at 30 C than at in situ temperatures as the standard deviations for the 5-, 10-, and 18-m sites at 30 C were 24, 14, and 7%, respectively.

Methanogenic populations were observed in both mixed and pure cultures obtained from sediment enrichments. Enrichment cultures from all three sites were initiated by the addition of sediment to anaerobic culture tubes that contained enriched medium and H_2-CO_2, basal medium and H_2-CO_2, and basal medium with N_2-CO_2 and either 0.5% acetate, formate, or methanol. Methanogenic enrichments were transferred at 2-week intervals, and microscopy observations were performed after 3 months of incubation. Mixed cultures from all three sediment sampling sites contained species of *Methanobacterium, Methanospirillum, Methanococcus*, and *Methanosarcina*. *Methanosarcina* species predominated only in methanol enrichments. Identical methanogenic populations were observed when enrichments were initiated and maintained at in situ temperature or 30 C

TABLE 1. *Analysis of Lake Mendota sediments at different sampling sites*

Site[a]	pH	$CaCO_3$ equivalents (%)	Organic matter (%)	Organic N (μg/g)	NH_4N (μg/g)	Avg dry wt of sediment (g/ml)
5	7.1	32.5	17.75	1.621	20	0.33
10	7.1	32.5	16.39	1.019	34	0.16
18	7.1	36.5	15.48	0.547	127	0.16

[a] Depth in meters.

TABLE 2. *Variations in methanogenesis between samples collected at the same site and time, and between subsamples of a single-sediment sample, as a function of sediment sampling site and incubation temperature*[a]

Sample	5-m site				10-m site				18-m site			
	15 C		30 C		15 C		30 C		10 C		30 C	
	Rate	%s	Rate	%s	Rate	%s	Rate	%s	Rate	%s	Rate	%s
A	4.8	7	15.6	32	19.8	14	52.2	5	24.0	12	64.8	13
B	3.6	5	13.8	0	16.2	12	61.2	20	21.0	18	60.0	11
C	6.6	11	21.6	6	22.2	17	46.8	8	26.4	9	69.0	16

[a] Rates are expressed as nanomoles of CH_4 formed per hour per gram, dry weight, of sediment and represent the average of triplicate subsample experiments. A, B, and C were different samples collected at each site on 16 October 1975. %s, Percent standard deviation ($s/\bar{x} \times 100\%$).

throughout the sampling period. Hydrogen-oxidizing methanogens were the major nutritional type of methanogen present in sediments; formate fermenters were present, but acetate fermenters were not observed. A *Methanobacterium* species and a *Methanosarcina* species were isolated from sediments that grew in basal medium on H_2-CO_2. Both species had temperature optima for growth well above 30 C.

Seasonal variation in temperature and number of methanogens. Sediment temperature varied greatly from winter to summer months during a 3-year study period that extended from September 1972 to October 1975; the range for in situ sediment temperatures was 4 to 24 C at 5 m, 4 to 22 C at 10 m, and 4 to 18 C at 18 m. Table 3 illustrates the effect of seasonal variations on sediment temperature and number of methanogenic bacteria. A rise in sediment temperature from winter to summer months correlated with an increase in the number of methanogens; a decrease in sediment temperature from summer to winter months correlated with a decrease in numbers. All sampling sites differed with respect to total numbers of methanogens present and seasonal fluctuation in population density. The 18-m site contained the highest number of methane bacteria throughout the sampling period. The methanogenic population decreased from the 18-m to the 10-m to the 5-m sampling sites. Seasonal variation was most pronounced on the number of methanogens at the 5-m site. The methanogenic population fluctuated least at the 18-m site.

The medium used for enumeration of methanogens greatly influenced the total number of methanogens observed. Nearly identical numbers were obtained during the sampling period when enriched or basal medium that contained H_2-CO_2 was used for determinations. However, identical end-dilution tubes in enriched medium always contained higher quantities of methane than those with basal medium. The number of sediment methanogens decreased by 10^2 to 10^3 when enriched medium or acetate basal medium that contained N_2-CO_2 was used for determinations. The number of sediment methanogens remained constant when in situ or 30 to 37 C incubation temperatures were used.

Optimum temperature for methanogenesis. As increased sediment temperature caused by seasonal change was shown to greatly increase the number of methanogens, studies were initiated to determine the optimum temperature for methanogenesis. Sediment was collected from all sites and placed into anaerobic culture tubes that contained N_2-CO_2. The tubes were incubated at temperatures from 4 to 60 C, and methane was quantified after 6 days. Figure 1 illustrates that the optimum temperature for methanogenesis in sediment collected from all sites in January 1975 was considerably higher than in situ temperature (4 C). Similar results were obtained for sediment samples examined in May 1975 (Fig. 2). It is important to note here that incubation times longer than one week at temperatures above 50 C resulted in increased methanogenesis with time. This was the result of enrichment and proliferation of thermophilic methanogens present in very low numbers in sediments. Sediments collected from all sites throughout the sampling period had an optimum temperature for methane formation between 35 and 42 C.

All known methanogenic bacteria are capable of chemolithotrophic metabolism and will reduce CO_2-HCO_3^- to CH_4 in the presence of hydrogen (26). Previous studies that determined the optimum temperature for sediment methane formation measured the complex of microbial activities that result in methanogenesis from the decomposition of organic matter. Additional experiments were initiated to determine the optimum temperature for CO_2-HCO_3^- reduction by sediment methanogens. Samples were collected from the 5-m site in June and November 1974, and 5 ml of sediment was placed into anaerobic culture tubes that contained H_2-CO_2. $Na_2{}^{14}CO_3$ (5 μCi) was added to each tube, and the tubes were incubated at

TABLE 3. *Seasonal variation in temperature and number of methanogens in Lake Mendota sediments*

Date	5-m Site		10-m Site		18-m Site	
	Temp (C)	Methanogens/g	Temp (C)	Methanogens/g	Temp (C)	Methanogens/g
6-17-74	17	10^4	16	10^5	15	10^6
7-8-74	21	10^5	19	10^6	16	10^6
8-29-74	22	10^5	21	10^6	16	10^6
11-19-74	10	10^2	10	10^4	10	10^5
1-28-75	4	10^2	4	10^4	4	
3-11-75	4		4		4	10^4
5-13-75	13	10^4	11	10^5	9	10^5

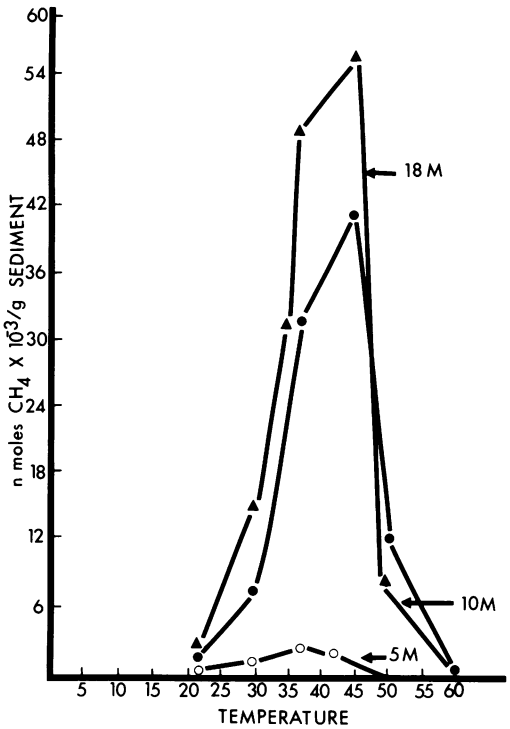

FIG. 1. *Effect of temperature on sediment methanogenesis in samples collected on 17 January 1975. Results represent the average of duplicate experiments.*

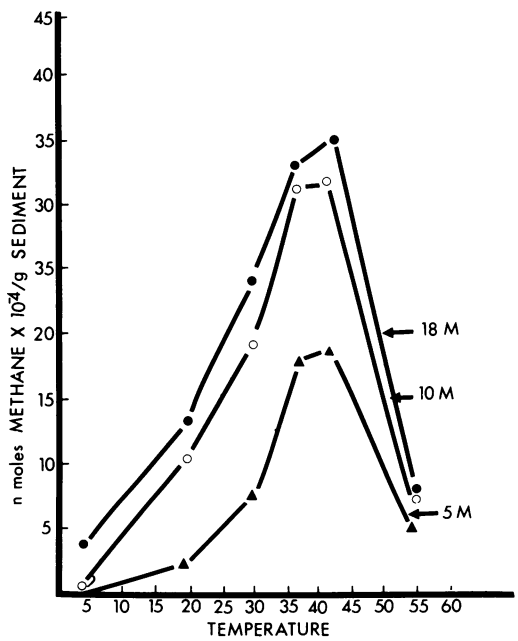

FIG. 2. *Effect of temperature on methanogenesis in sediments collected on 26 May 1975. Results represent the average of duplicate experiments.*

temperatures that ranged from 5 to 50 C. $^{14}CH_4$ was quantified after 5 days of incubation, and the results are shown in Fig. 3. The optimum temperature for reduction of CO_2-HCO_3^- to methane by methanogens was 35 C in 5-m sediments collected in June or November 1974.

Effect of temperature on rate of methanogenesis. Sediment samples were collected from all sites in January and May 1975 to examine the effect of temperature and seasonal variation on the rate of methanogenesis. Sediment slurry (5 ml) was added to anaerobic culture tubes that contained N_2 gas. Duplicate sets were incubated at in situ (4 C) or near-optimal (30 C) temperatures for January sediments and at 16 or 30 C for May samples. Total methane produced in each tube was quantified during an 8-day period. The results of these experiments are shown in Fig. 4A and B. The rate of methanogenesis at in situ temperatures in May was much greater than that for January. When compared with the January rate, methanogenesis in May increased by 400-fold at 5 m, 160-fold and 10 m, and 100-fold at 18 m. Although the rate of methanogenesis at in situ temperatures for both sampling dates was significantly less than the 30 C incubation rate, the difference between in situ and near-optimal temperature for methanogenesis was less in May than in January samplings. The rate of methane formation in sediments at in situ temperatures varied greatly with respect to sampling date and site. The rate of methanogenesis in January was 40 times greater at the 18-m site and 10 times greater at the 10-m site than the 5-m site rate of 50 nmol of CH_4/g of sediment per day. The rate of methanogenesis at in situ tem-

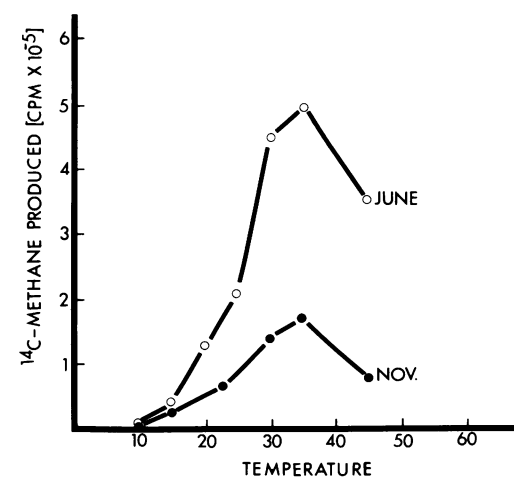

FIG. 3. *Effect of temperature on the production of [^{14}C]methane from H_2 and $Na_2{}^{14}CO_3$ added to 5-m sediments.*

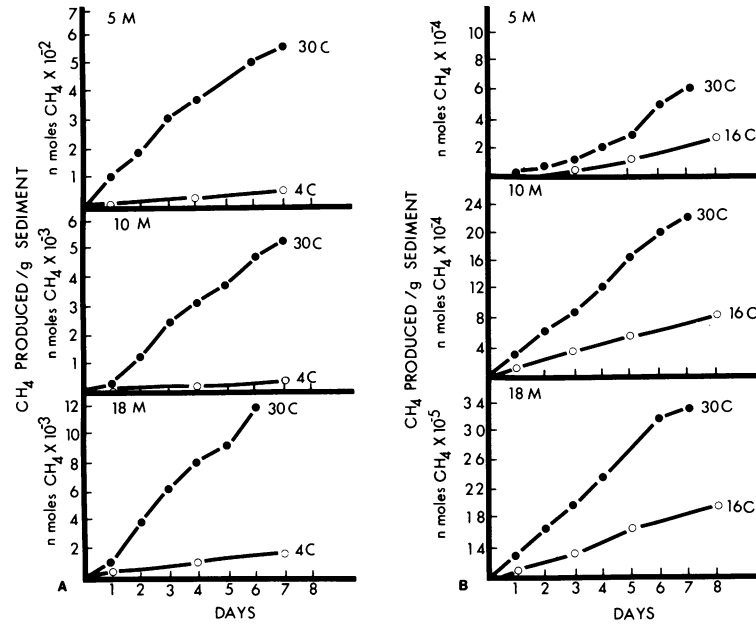

FIG. 4. *Effect of temperature on the rate of methanogenesis in sediments collected on 17 January 1975 (A) and 26 May 1975 (B).*

peratures in May was 10 times greater at the 18-m site and 4 times greater at the 10-m site than the 5-m site rate of 2×10^4 nmol of CH_4/g of sediment per day.

Glucose is not used as a substrate for methane formation by methanogenic bacteria (26). Thus, the decomposition of glucose to methane in sediments requires the combined activities of several microbial species that include chemoorganotrophs and chemolithotrophs. Experiments were initiated to determine the effect of temperature on the rate of glucose conversion to methane by sediment anaerobes. Sediment was collected from 18 m in May 1975, and 5 ml of slurry was added to anaerobic culture tubes that contained N_2 gas and 0.5 μCi of [U-^{14}C]-glucose. The tubes were incubated at in situ temperatures (16 C) or 30 C, and $^{14}CH_4$ production was quantified during a 17-day period. Figure 5 illustrates that the rate of glucose conversion to methane in 18-m sediments was temperature limited. The rate of methanogenesis from glucose was nearly linear for the first 3 days, but first-order kinetics were not observed at longer incubation times. The rate of methanogenesis at in situ temperature from glucose was 3.5-fold lower than the 30 C rate.

Sediment cores were obtained from 10- and 18-m sites during January and May 1975 to examine the influence of sediment depth on methanogenesis. It was not possible to obtain sediment cores from the 5-m site. Slurry (5 ml)

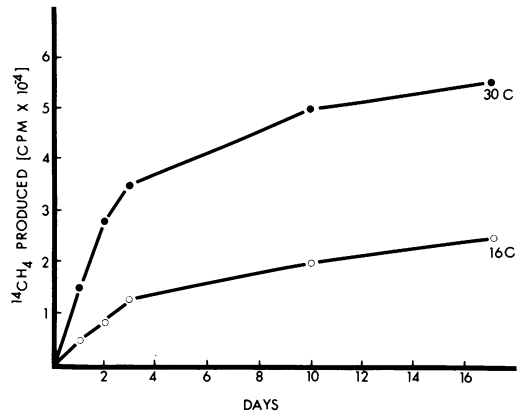

FIG. 5. *[^{14}C]methane production from radioactive glucose added to 18-m sediments.*

obtained from various depths of segmented sediment cores was placed into anaerobic culture tubes that contained N_2 gas. The tubes were incubated at 30 C, and methane formation was quantified for an 8-day period. The rate of methanogenesis in sediment cores varied with respect to the date of sampling, sediment site, and depth (Table 4). The rate of methanogenesis was greater for all sediment depths at the 18-m site than that for equivalent depths at the 10-m site. In addition, the rate of methane formation for all sediment depths at the 10-m site was greater in May than in January. At the

TABLE 4. *Effect of sediment depth on rate of methanogenesis*

Sediment depth (cm)	CH_4 produced (nmol/h per g of sediment)		
	10 m		18 m
	Jan. 1975	May 1975	Jan. 1975
0–1	29.6	260	458
1–2	97.8	2,390	369
2–4	60.3	1,560	80.1
4–6	41.6	1,030	47.3
6–8	45.8	712	47.3
8–12	19.2	182	29.6
12–18	3.1	42	10.9

18-m site, the highest rate of methanogenesis was found at a sediment depth of 0 to 1 cm, and the rate decreased with increasing sediment depth. In 10-m sediments, the rate of methanogenesis was significantly greater at various samplings between 1 and 8 cm than at a depth of 0 to 1 cm. The rate of methanogenesis in 10-m sediments was highest for the 1 to 2-cm layer and, except for the 0- to 1-cm layer, the rate decreased with increasing sediment depth. Enumeration studies of 18-m cores revealed higher numbers of methanogens at 4 to 8 cm than in surface sediments.

DISCUSSION

These data indicate that microbial methane formation in Lake Mendota is greatly influenced by temperature. Temperature severely limited sediment methanogenesis; the maximum in situ temperature (23 C) attained during seasonal change was far below the temperature optimum (35 to 42 C) observed for in vitro methanogenesis. ^{14}C-tracer studies confirmed that chemolithotrophic metabolism of sediment methanogens displayed a similar temperature optimum. Increased in situ temperatures correlated with increased rates of methanogenesis; a change of 12 C during seasons was associated with a 100- to 400-fold increase in the rate of methanogenesis. The number of sediment methanogens was shown to vary with seasonal change; increasing sediment temperatures resulted in higher numbers. Thus, the increased rate of methanogenesis that was associated with seasonal change is in part a reflection of increased numbers of methanogens and increased rate of metabolic activity when sediment temperatures more closely approximate the optimum temperature for methanogenesis.

These results support other findings (3) that bacterial decomposition processes in aquatic sediments are temperature limited. Although the metabolic activity of sediment methanogens in Lake Mendota will never approximate the optimal rate because of temperature limitation, this fact should not be interpreted to mean that these microbes are not optimally adapted to their environment. It should be of far greater importance for an organism to adapt to environmental changes in temperature, nutrient supply, etc., than to proliferate at the maximal physiological growth rate.

The variation observed in microbial methanogenesis at different sampling sites was related to physical and chemical properties of the sediments. Increased numbers of methanogens correlated with increased amounts of available ammonia. Ammonia is the form of nitrogen that methanogens require for growth (7). The highest number of methanogens were found in the 18-m sediments, which also contained the most carbonate. Recent studies (11) suggest that precipitated carbonates in anaerobic sediments are, in part, a result of methanogenic activity. Oxygen inhibition may explain the lower number of methanogens and greater seasonal variation in population density than was observed in the shallower sampling sites. Surface sediments at the 18-m site are not exposed to continual oxygen as are the 5- and 10-m sites. Our results revealed that the highest rate of methanogenesis occurred at the surface layer (0 to 1 cm) of 18-m sediment cores, whereas the rate of methanogenesis was greatest at depths below the 0- to 1-cm layer in 10-m sediments. However, the highest number of methanogens in 18-m cores was found at sediment depths greater than 0 to 1 cm. One might expect to find the highest rate of methanogenesis to occur at the sediment surface because of increased nutrient concentrations, unless other factors influence methanogenesis. The general pattern observed in sediment cores of lower rates of methanogenesis with increasing depth may be an indication of nutrient depletion.

Methanosarcina has been considered (22) to be the most abundant methanogen in aquatic sediments although detailed studies on the diversity of methane-producing bacteria in these habitats have not been reported. The data presented here demonstrate that all known methanogenic genera (26) are found in Lake Mendota sediments, with *Methanobacterium* species predominating. Thus, methanogenic diversity in aquatic sediments approximates that found in sewage sludge digestors (24). Results of enumeration and enrichment-isolation studies indicate that the majority of sediment methanogens observed have minimal nutrient requirements. Hydrogen-oxidizing methanogens were most numerous, although

methanol- and formate-fermenting species were also present. Cappenberg reported that acetate-fermenting methanogens were the most numerous nutritional type in Lake Vechten (8) and that these species were inhibited by high levels of sulfide (Cappenberg, in press). We have not been able to isolate acetate-fermenting methanogens from Lake Mendota in several kinds of acetate-containing media that had varying concentrations of sulfide or no sulfide. No obligately psychrophilic methanogens were isolated from Lake Mendota although thermophilic species are present in very low numbers. Other reports (3, 6) indicate the absence of psychrophilic bacteria in sediments of northern lakes and caves. The methanogenic population has a wide temperature range for growth as methanogenesis in that both in vitro sediments and enrichment cultures could be maintained at temperatures between 4 and 45 C.

In summation, the results presented here should have an impact on further ecological studies of sediment methanogenesis. The numerous parameters shown to influence methanogenesis in Lake Mendota indicate the dynamic nature of the sediment ecosystem. Thus, comparative studies that aim to establish the rates of anaerobic carbon decomposition or define in situ carbon precursors and microbial interactions associated with methanogenesis must employ sediments nearly identical with respect to sample site and depth, to date of collection and processing, and to in vitro experimental conditions, including anaerobiosis and length and temperature of incubation.

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