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Dynamics of Bacterial Sulfate Reduction in a Eutrophic Lake

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Bacterial sulfate reduction in the surface sediment and the water column of Lake Mendota, Madison, Wis., was studied by using radioactive sulfate ($^{35}SO_4^{2-}$). High rates of sulfate reduction were observed at the sediment surface, where the sulfate pool (0.2 mM SO_4^{2-}) had a turnover time of 10 to 24 h. Daily sulfate reduction rates in Lake Mendota sediment varied from 50 to 600 nmol of SO_4^{2-} cm^{-3}, depending on temperature and sampling date. Rates of sulfate reduction in the water column were 10^3 times lower than that for the surface sediment and, on an areal basis, accounted for less than 18% of the total sulfate reduction in the hypolimnion during summer stratification. Rates of bacterial sulfate reduction in the sediment were not sulfate limited at sulfate concentrations greater than 0.1 mM in short-term experiments. Although sulfate reduction seemed to be sulfate limited below 0.1 mM, Michaelis-Menten kinetics were not observed. The optimum temperature (36 to 37°C) for sulfate reduction in the sediment was considerably higher than in situ temperatures (1 to 13°C). The response of sulfate reduction to the addition of various electron donors metabolized by sulfate-reducing bacteria in pure culture was investigated. The degree of stimulation was in this order: H_2 > *n*-butanol > *n*-propanol > ethanol > glucose. Acetate and lactate caused no stimulation.

The importance of bacterial sulfate reduction in the mineralization of organic matter in marine sediments has been well established (12, 27). However, little is known about this process in freshwater ecosystems. The concentration of sulfate in freshwater (typically between 0.01 and 0.2 mmol of SO_4^{2-} $liter^{-1}$) (11, 22, 30, 31; A. Jassby, Ph.D. thesis, University of California at Davis, 1973) is 100 to 1,000 times lower than it is in seawater. Furthermore, in many eutrophic lakes, sulfate becomes depleted immediately below the sediment surface (33; R. L. Smith, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, N30, p. 184). Quantification of sulfate reduction by existing ^{35}S-radiotracer methods (13) presents analytical difficulties in determining the pool size of sulfate in the interstitial water of sediments. It is also difficult to assess in situ rates of bacterial sulfate reduction by mathematical modeling, which has been applied successfully in marine environments where partial or complete sulfate depletion occurs much deeper in the sediment (2, 14). Whole-lake labeling studies (30) with $^{35}SO_4^{2-}$ in Linsley Pond, Conn., provided important information on the movement and transformation of sulfur compounds, but this method is not generally applicable. Recently, a mass balance approach for sulfate and sulfide was used to estimate the rate of sulfate reduction in the anaerobic hypolimnion of Lake Mendota, Madison, Wis. during summer stratification (K. Ingvorsen and T. D. Brock, Limnol. Oceanogr., in press). Direct radiotracer measurements of sulfate reduction have been reported for various Russian lakes (11, 22), but most of these lakes contained high concentrations of sulfate. Data on bacterial sulfate reduction in low-sulfate environments are therefore essentially lacking.

The present study describes the dynamics of sulfate reduction in Lake Mendota. Rates of bacterial sulfate reduction were measured with $^{35}SO_4^{2-}$ in the water column during summer stratification and in the sediments throughout the sampling year. The effect of environmental parameters such as temperature, exogenous electron donors, and sulfate concentration on the rate of sulfate reduction is also reported.

MATERIALS AND METHODS

Sampling locality. All samples were from a 24-m-deep station (Central Station) in Lake Mendota. The water column at this station becomes anaerobic below the thermocline (10 to 12 m), and hydrogen sulfide and methane accumulate in the hypolimnetic water between July and September. The in situ sediment temperature varies from 1 to 13°C during the year.

Sediment sampling. Surface sediment samples were collected with an Eckman dredge. Upon opening the top of the dredge, we carefully removed the overlying water and carefully scooped off the top 2 to 3 cm of sediment with a spatula and transferred it to N_2-

gassed, 250-ml thick-walled glass bottles. The glass bottles were filled to the top and sealed anaerobically with butyl rubber stoppers. Excess sediment was allowed to escape from the bottles through an 18-gauge needle temporarily inserted through the stopper. Sealed bottles were transported to the laboratory in well-insulated containers and, if not processed immediately, were stored in the dark at in situ temperature. The black sediment at Central Station is strongly reducing, owing to its content of FeS and free sulfide, which protect the anaerobic bacteria from oxygen exposure during sampling (4, 33). Subsequent manipulations of sediment were done by using modified Hungate anaerobic techniques (9).

Water sampling. Water samples were collected with a 4-liter polyvinyl chloride Van Dorn water sampler. Upon recovery, a thick-walled amber latex tube was connected to the outlet valve of the water sampler. The first 250 ml of sample was used to flush the tubing before samples were taken. All water samples were collected in 60-ml biological oxygen demand bottles with tapered ground-glass stoppers, and several bottle volumes were allowed to overflow before the stoppers were replaced. Great care was taken to avoid air contamination during sampling. Zinc acetate was added in the field to samples intended for sulfide and sulfate analyses. Samples for measurement of water column sulfate reduction were stored at in situ temperature in the dark. Isotope was added within 2 h of sampling. Water samples were occasionally collected by pumping for more precise resolution of depth (35).

Quantification of sediment sulfate reduction. The concentration of sulfate in surface sediments was below 1 $\mu g\ ml^{-1}$; approximately 85% of carrier-free $^{35}SO_4^{2-}$ was reduced to sulfide within 1 h after being added to this sediment. In suspensions of surface sediment mixed with anaerobic bottom water, sulfate became undetectable within 24 h. The rapid turnover of sulfate in this sediment indicated a high rate of sulfate reduction, and the following procedure was finally adopted to estimate rates of sulfate reduction in the surface sediment. Undiluted homogenized sediment (10 ml) was dispensed anaerobically into a 20-ml serum vial containing five glass beads to facilitate subsequent mixing. The headspace was then flushed with N_2-CO_2 (99:1) for an additional 2 min, stoppered with a black-lip butyl rubber stopper (Bellco Glass, Inc.), and crimped with an aluminum seal. The isotope was injected through the stopper with a 1-ml Glaspak syringe as a mixture of carrier-free $^{35}SO_4^{2-}$ diluted in an Na_2SO_4 solution. The total sulfate concentration of the isotope mixture was adjusted for each experiment to establish an initial sulfate concentration identical to that at the sediment surface when 0.1 ml of the solution was added. Combined addition of tracer and carrier sulfate was important to obtain an even distribution of both compounds within the sample. The sulfate concentration of the tracer solutions varied between 5 and 20 mM and contained 3 to 5 $\mu Ci\ ml^{-1}$. The sediment-isotope mixture was blended vigorously on a Vortex mixer and incubated in the dark at in situ temperature. After incubation, each vial was injected with 1 ml of 20% zinc acetate followed by submersion in an ethanol-dry ice mixture for 15 min and storage at −25°C until analysis was performed. Samples could be stored up to 14 days without any loss in sulfide radioactivity, and sulfate reduction did not resume when samples were thawed. After thawing took place, an anoxic sample (usually 5 ml) was removed from the sealed vial with a syringe and 18-gauge needle and injected anaerobically into a distillation apparatus similar to that described by Jørgensen and Fenchel (16). The sediment was acidified to pH 0 with 6 N HCl, and the liberated H_2S gas was quantitatively recovered in traps containing 15 ml of 2% zinc acetate solution. A sample (10 ml) from the zinc acetate trap was mixed with 10 ml of Aquasol scintillation cocktail (New England Nuclear Corp.) for determination of sulfide radioactivity. The slurry remaining in the reaction vessel was diluted to 100 ml with water and centrifuged. A 1-ml sample of the supernatant was used for quantification of sulfate radioactivity. Sulfate reduction was calculated as described by Jørgensen (13). Most incubations were stopped before 10% of the initial $^{35}SO_4^{2-}$ was converted to $^{35}S^{2-}$ (i.e., normally within 0.5 to 1 h of incubation) to ensure linearity of sulfate reduction. A Packard Tri-Carb 3375 liquid scintillation spectrophotometer was used to determine radioactivity. Quench corrections were made by the channel ratio method. Isotope recovery ($^{35}SO_4^{2-}$ + $^{35}S^{2-}$) from vials was >93% in samples and killed controls (zinc acetate treated and autoclaved).

Measurement of water column sulfate reduction. Rates of sulfate reduction in water samples were measured in 60-ml biological oxygen demand bottles immediately upon return to the laboratory. The tapered glass stopper of a sample bottle was removed, and 0.050 ml of carrier-free $^{35}SO_4^{2-}$ solution (ca. 50 $\mu Ci\ ml^{-1}$) was rapidly injected near the bottom of the bottle. Anoxic distilled water (~1.5 ml) was carefully added with a syringe to avoid entrapping air when the stopper was replaced. The bottles were incubated in the dark within ±1°C of in situ temperature for 17 h. Sulfate reduction was found to be linear for at least 36 h. Bacterial sulfate reduction was stopped by addition of 1 ml of 20% zinc acetate, which also precipitated the sulfide. The stopper was then replaced, and the contents were mixed thoroughly before a 20-ml portion was pipetted into each of two 25-ml serum vials containing 1 ml of 20% (wt/vol) ZnS suspension. These vials could be stored unstoppered for weeks at 2 or −25°C before distillation without any loss in the $Zn^{35}S$ radioactivity. Use of formaldehyde as poison invariably resulted in low recoveries. The procedure for determining sulfate and sulfide radioactivities is described above; details can be found in reference 13.

Sediment-water chemistry analysis. For water samples, sulfide was determined by the method of Pachmayer (F. Pachmayer, Ph.D. thesis, University of Munich, 1960) for samples fixed with zinc acetate in the field. A modification of the method of Tabatabai (31) was used for sulfate determination. Samples were collected in 60-ml biological oxygen demand bottles, and 0.15 ml of 0.1 M zinc acetate was added in the field to precipitate sulfide. In the laboratory, each sample was filtered through a 0.45-μm HCl-washed membrane filter to remove ZnS; sulfate was then determined turbidimetrically in the filtrate (31). Oxygen assays were made by standard Winkler titration (29). Interstitial water for sulfate and sulfide analyses

was obtained from surface sediment by centrifugation under anaerobic conditions. The supernatant was passed through an HCl-washed 0.45-μm membrane filter before analysis. The dry weight of the sediment was determined after a weighed portion of fresh sediment was heated to constant weight at 105°C for 24 h.

Chemicals, gases, and stock solutions. All chemicals were of reagent grade. All stock solutions were prepared, sterilized, and stored in N_2-gassed serum vials sealed with black butyl rubber stoppers (Bellco Glass, Inc.). Additions were made aseptically with glass syringes. Traces of O_2 were removed from gases by being passed through a heated (340°C) copper column. Carrier-free $^{35}SO_4^{2-}$ (43 Ci mg^{-1}) was obtained from New England Nuclear Corp.

RESULTS

Distribution of oxygen and sulfur compounds during stratification. Figure 1 shows a profile of sulfide, sulfate, and oxygen concentrations at various depths toward the end of summer stratification. Hydrogen sulfide built up in the anaerobic hypolimnion, reaching a maximum concentration of 0.14 mM immediately above the sediment surface. Sulfate was depleted in the hypolimnion, and the sulfate profile was almost complementary to that of hydrogen sulfide; this was the case throughout the stratification period. Although sulfide may also originate from anaerobic degradation of protein-bound sulfur, the pronounced sulfate depletion in the hypolimnion can only be explained by sulfate reduction.

Comparison of sulfate reduction in the water column and sediment. The rate of H_2S formation from bacterial sulfate reduction in the anaerobic hypolimnion was measured several times during stratification. Sulfate reduction in the water column on 18 September 1979 first became detectable at 16 m, and the rate remained relatively constant down to 23 m (Fig. 2). The rate of sulfate reduction increased by a factor of 10 between 23 and 24 m. The deepest sample (24 m) (arrow in Fig. 2) contained visible amounts of suspended sediment. Sulfate reduction was not detected in samples from the aerobic epilimnion (5 and 10 m). Sulfate reduction rates in the top sediment were typically 10^3 times higher than those in the water column above 24 m. The sulfate reduction rates obtained in the surface sediment of Lake Mendota (Central Station) showed a relatively large variation which may have been due partly to seasonal differences (Table 1). However, much of the variability was probably due to the difficulty in obtaining reproducible surface mud by sampling the upper 2 to 3 cm of sediment collected with the Eckman dredge.

Effect of temperature on sediment sulfate reduction. Sediment samples used for temperature experiments were collected on 10 December 1979, when the in situ temperature was 2°C. A brief incubation period (15 min) was used to avoid a rapid depletion of sulfate at higher temperatures. The optimum temperature for sulfate reduction was near 37°C (Fig. 3), which was considerably higher than the temperature of

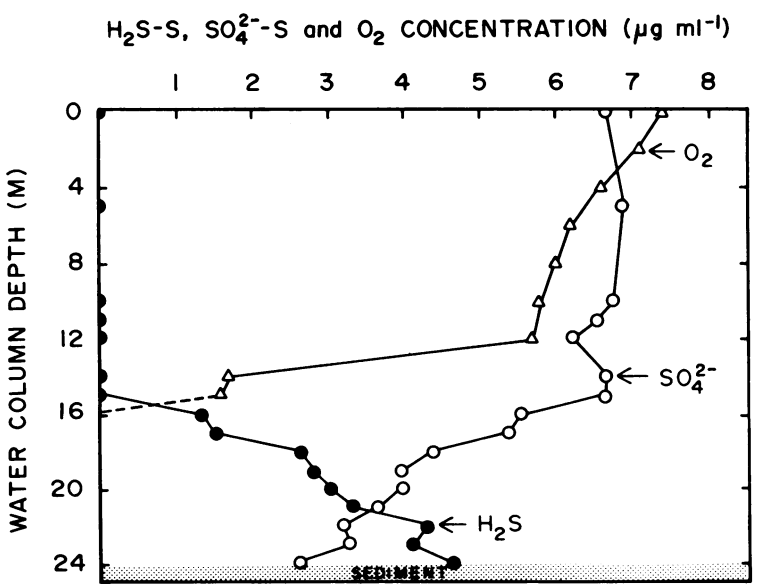

FIG. 1. *Distribution of sulfide, sulfate, and oxygen in the water column (Central Station) at the end of summer stratification (18 September 1979).*

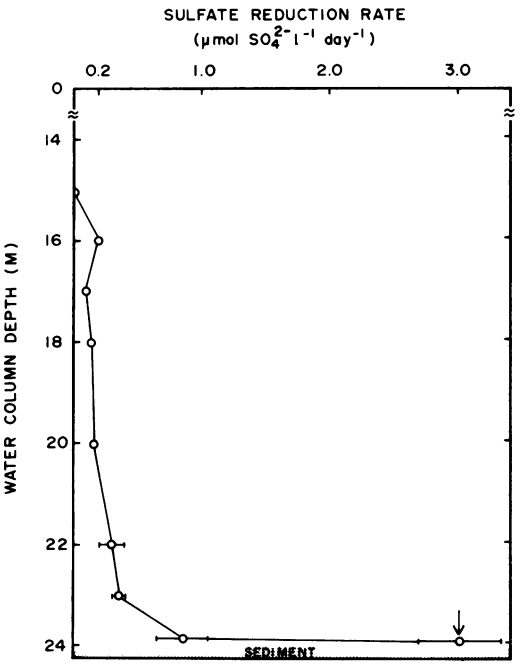

FIG. 2. *Rates of bacterial sulfate reduction (± standard deviation) in the hypolimnion (18 September 1979). ↓, Water samples contained small amounts of resuspended sediment.*

sampling or the maximum in situ temperature in summer (13°C). Sulfate reduction did not occur at 69°C, and a low daily rate of 40 nmol of SO_4^{2-} cm^{-3} was observed for samples incubated at 0.5°C. An Arrhenius plot of the data in Fig. 3 showed a linear response between 4 and 37°C and an activation energy (i.e., temperature characteristic of sulfate reduction) of 18 kcal (75 kJ) mol^{-1}, which corresponds to a Q_{10} of 2.9.

Effect of sulfate on sediment sulfate reduction. The response of the in situ sulfate-reducing population to increasing sulfate concentrations was determined with short-term tracer experiments. Surface sediment (50 ml) was transferred anaerobically to 100-ml serum vials and stored at 4°C for a week to deplete the sulfate in the sediment below the detection limit of 15 μM SO_4^{2-}. The initial sulfate concentration was thus assumed to be that calculated from the addition of $^{35}SO_4^{2-}$ diluted with unlabeled Na_2SO_4 solutions of different molarities. The experimental vials were incubated on a shaker at 4°C, and a time course was run at each sulfate concentration by removing 5-ml samples of sediment through the serum stopper with a syringe at appropriate intervals. The specific activities of the tracer solutions were adjusted to avoid large differences in incubation periods. Sediment sulfate reduction did not follow Michaelis-Menten kinetics, as evidenced when the direct linear plot (6) was applied to the data in Fig. 4. Other experiments carried out at 12°C showed that sulfate reduction was independent of the sulfate concentration down to 0.1 mM SO_4^{2-}, which was the lowest concentration tested. It is unlikely that the brief incubation periods and the low temperature caused any enrichment for sulfate-reducing bacteria.

Effect of exogenous electron donors on sediment sulfate reduction. Electron donors metabolized in pure culture by sulfate-reducing bacteria were added to sediment samples to study their effect on sulfate reduction (Table 2). Sulfate was added at an initial concentration of 0.5 mM in all samples. Sediment receiving distilled water served as a control; the incubation period was kept at only 7 h to avoid extensive enrichment of sulfate-reducing bacteria during the experiment. Addition of acetate and lactate did not stimulate sulfate reduction. Hydrogen caused the greatest stimulation (Table 2), and this finding was confirmed in other experiments (results not shown) performed at different temperatures, sulfate concentrations, and H_2 concentrations. Hydrogen invariably caused a 30 to 80% stimulation of sulfate reduction in samples incubated on a shaker (to facilitate H_2 mixing into the sediment).

TABLE 1. *Sulfate reduction rates measured with $^{35}SO_4^{2-}$ in surface sediment of Lake Mendota (24 m)*

In situ temp (°C)[a]	Sampling date (mo/day/yr)	Sulfate concn[b] (mM)	Daily sulfate reduction rate[c] (nmol of SO_4^{2-} cm^{-3})
3.0	12/10/1979	0.22	83
3.0	12/10/1979	0.22	101
3.0	12/20/1979	0.22	365
2.5	02/09/1980	0.22	163
2.0	02/13/1980	0.22	420[d]
2.0	02/16/1980	0.22	554[d]
2.0	03/05/1980	0.22	174
3.0	03/26/1980	0.22	365
12.0	09/29/1980	0.22	279
12.0	09/29/1980	0.10	250

[a] The incubation temperature was within 2°C of the in situ temperature.

[b] Determined as mmol of SO_4^{2-} liter of wet sediment^{-1}. The water content of the samples varied from 87 to 90% (wt/vol).

[c] All samples were incubated for less than 1 h. Values represent means of triplicate or duplicate determinations. Standard deviation was <20%.

[d] Measured in sediment from 0- to 0.5-cm sediment interval.

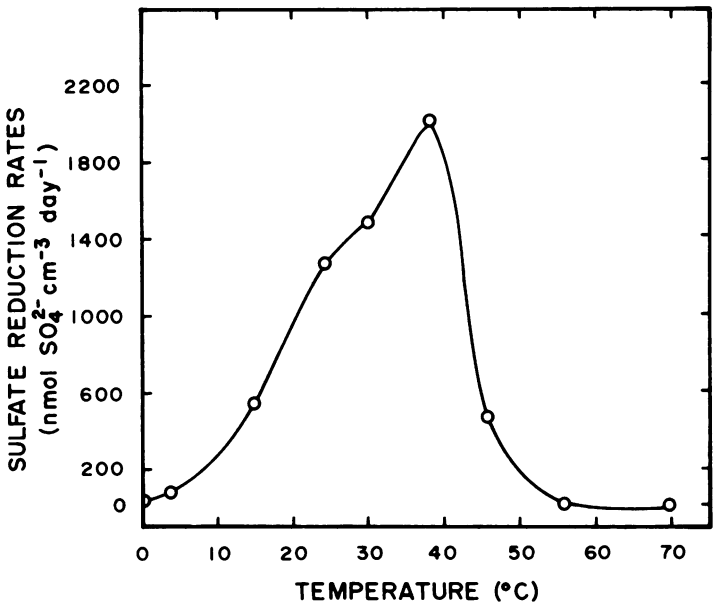

FIG. 3. *Temperature dependence of bacterial sulfate reduction in surface sediment from Central Station (24 m). The in situ temperature was 2°C (10 December 1979). The initial sulfate concentration was 0.25 mmol of SO_4^{2-} liter of wet sediment^{-1}. The incubation period was 15 min. (To obtain sulfate reduction rates per dry-weight gram of sediment, multiply by 12.5.)*

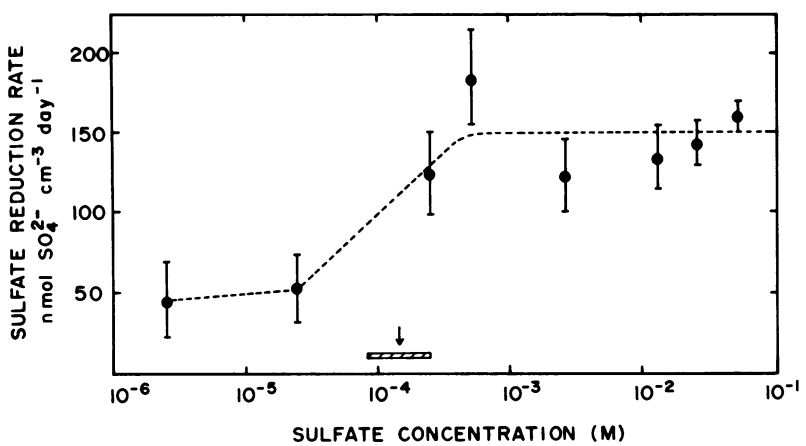

FIG. 4. *Plot of sulfate reduction rate versus initial sulfate concentration in Lake Mendota sediment (24 m). The incubation temperature was 4°C. Hatched bar (indicated by arrow) shows range of sulfate concentrations in the bottom water of Lake Mendota.*

DISCUSSION

The data clearly indicate that sulfate reduction is very active in Lake Mendota sediment and support the general hypothesis that sulfate reduction is an important process for anaerobic carbon mineralization in freshwater lakes. The sulfate reduction rates in Lake Mendota surface sediment, when compared on a dry-weight basis and normalized for temperature, fell in the upper range of rates for this process in marine environments (8, 16) and were of the same order of magnitude as those reported for a productive salt marsh (8) and bacterial mats of a saline tropical lake (15).

Recently, a mass balance model based on detailed chemical analysis of sulfur and previous carbon cycle data for Lake Mendota (7) indicated an average daily extrapolated sulfate depletion rate of 600 nmol of SO_4^{2-} cm^{-3} for a 1-

TABLE 2. *Effect of electron donors on sulfate reduction in Lake Mendota sediment (24 m)*[a]

Electron donor added[b]	% Stimulation of sediment sulfate reduction[c]
Acetate	0
Lactate	0
Glycerol	14
Choline chloride	14
n-Propionate	23
n-Butyrate	32
Glucose	32[d]
Yeast extract	32[d]
Ethanol	39[d]
n-Propanol	50[d]
n-Butanol	59[d]
H_2[e]	73[d]

[a] Results are expressed as percent change relative to controls receiving distilled water. Each value represents the mean of three replicates. The daily sulfate reduction rate in the control was 240 nmol of SO_4^{2-} cm^{-3} at 10°C.

[b] All electron donors were added, as neutralized solutions, at an initial concentration of 1 mM, except for yeast extract, which was added to give a concentration of 0.02% (wt/vol).

[c] Determined after 7 h of incubation at 10°C. Sulfate was added at an initial concentration of 0.5 mmol of SO_4^{2-} liter of wet sediment^{-1}.

[d] Significance was on a 1% level ($P = 0.01$), as compared with control, and was determined according to Dunnett's procedure for multiple comparisons with a control (3).

[e] Headspace of vials (10 ml) contained H_2-CO_2 (98:2 [vol/vol]). The equilibrium concentration of dissolved H_2 in the interstitial water was approximately 0.8 mM.

cm-thick surface sediment layer; the model also indicated that electron flow via sulfate reduction in the hypolimnion during summer stratification constituted at least 25% of the electron flow via methanogenesis (Ingvorsen and Brock, in press). Our $^{35}SO_4^{2-}$ tracer measurements of sulfate reduction in surface sediment (0 to 0.5 cm) are in satisfactory agreement with the rates calculated from whole-lake studies. It should be noted that the data reported here only represent approximate natural rates for sulfate reduction, since it was impossible to simulate in situ conditions in the surface sediment during laboratory incubations. More precise values await the development of techniques suitable for measurement of in situ activities per se in unconsolidated freshwater sediments.

The present study indicates that the process of bacterial sulfate reduction in Lake Mendota is very dynamic and varies with site, season, and temperature. Most of the sulfate reduction took place in the uppermost portion of the sediment. However, during summer stratification, sulfate reduction in the water column can account for 18% of the total sulfate depletion in the hypolimnion. This summer period is too short to allow the sulfate concentration in the water column to decrease below a concentration at which sulfate becomes limiting for sulfate reduction in the surface sediment (0 to 0.5 cm). However, owing to the rapid consumption of sulfate in the surface sediment, sulfate reduction is likely to be sulfate limited deeper in the sediment. The rates of sulfate reduction in the anaerobic water column are of the same order of magnitude as those reported for environments of moderate and high sulfate concentrations (10, 17, 22, 28) but do not approach the degree (70% of the total) of sulfate reduction per unit area shown in a permanently stratified estuary in Norway (10).

The rate of sulfate reduction in Lake Mendota was severely temperature limited. This phenomenon has been reported earlier for sediment methanogenesis (36). Values of Q_{10} ranging from 3.4 to 3.5 have been reported for sulfate reduction in coastal sediments (1, 12), and a value of 2.9 was obtained in the present study.

The data indicate that the sulfate-reducing population in surface sediment of Lake Mendota was not sulfate limited at sulfate concentrations of 0.1 to 0.2 mM, which is approximately 100 times lower than the average concentrations of sulfate in seawater. Previous reports on the effect of SO_4^{2-} on the rate of sulfate reduction indicated a nonlimiting substrate concentration of 0.3 to 3 mM in kinetic studies using different experimental systems (23, 24, 26). However, kinetic studies conducted at high concentrations of energy sources are difficult to relate to natural ecosystems, in which the bacteria may be alive but not multiplying during part of the year.

In addition, the sulfate-reducing population in Lake Mendota did not conform to Michaelis-Menten kinetics with respect to sulfate. It is conceivable that the sulfate-reducing bacteria were limited by more than one substrate or were severely temperature limited or both. In these cases, Michaelis-Menten kinetics may not apply; factors causing deviations from Michaelis-Menten kinetics in complex microbial communities have recently been described (20). It should be emphasized that in our studies, the initial sulfate concentrations were calculated from the amount of added sulfate, assuming that the in situ sulfate pool was infinitely small or that sulfate was not being produced in the anaerobic sediment. These assumptions may not be valid for the lowest concentrations tested, because recent studies have demonstrated significant daily rates of sulfate production (120 to 2,400 nmol of SO_4^{2-} cm^{-3}; 10°C) by sulfhydrolase activity in sedi-

ments of a eutrophic lake (19).

The observation that sulfate reduction in surface sediment was not electron acceptor limited suggests that electron donors were limiting the process, perhaps because of the lack of available organic matter or because of their consumption by other microbial populations. The electron donors for bacterial sulfate reduction in nature are not well known and may vary within the sediment ecosystem. The data presented here indicate that acetate and lactate, well-documented (21) electron donors for sulfate-reducing bacteria, did not enhance sulfate reduction rates, whereas hydrogen and other reduced carbon compounds that are either substrates (5, 18, 25; F. Widdel, Ph.D. thesis, University of Göttingen, 1980) for sulfate reducers or fermented to H_2 and acetate by other sediment anaerobes were stimulating.

Three possible explanations for lack of stimulation by acetate and lactate can be envisaged: (i) sulfate-reducing organisms able to utilize these substrates are not present in significant amounts; (ii) high concentrations of these substrates are already present—thus, further additions have no effect; and (iii) other bacteria with higher rates of consumption of these substrates compete successfully with the sulfate reducers. Further work will be needed to distinguish which of these explanations is correct. Previous studies of Lake Mendota demonstrated that hydrogen was not detectable in surface sediments and that sulfate reducers and methanogens competed for H_2 and acetate as electron donors (32–34).

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