Sulfhydrolase Activity in Sediments of Wintergreen Lake, Kalamazoo County, Michigan†

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The hydrolysis of p-nitrophenyl sulfate, p-nitrocatechol sulfate, and [^{35}S]sodium dodecyl sulfate was examined in anoxic sediments of Wintergreen Lake, Michigan. Significant levels of sulfhydrolase activity were observed in littoral, transition, and profundal sediment samples. Rates of sulfate formation suggest that the sulfhydrolase system would represent a major source of sulfate within these sediments. Sulfate formed by ester sulfate hydrolysis can support dissimilatory sulfate reduction as shown by the incorporation of ^{35}S from labeled sodium dodecyl sulfate into H$_2$^{35}S. Sulfhydrolase activity varied with sediment depth, was greatest in the littoral zone, and was sensitive to the presence of oxygen. Estimations of ester sulfate concentrations in sediments revealed large quantities of ester sulfate (~30% of total sulfur). Both total sulfur and ester sulfate concentrations varied with the sediment type and were two to three orders of magnitude greater than the inorganic sulfur concentration.

The sulfur cycle in the anoxic hypolimnion and sediment of lacustrine systems is generally described in terms of sulfate reduction, sulfide oxidation by bacterial photosynthesis, and degradation of the sulfur-containing amino acids. Although these processes are important, an additional process is required for a complete description. Hydrolysis of ester sulfates represents both an avenue for the degradation of a ubiquitous class of compounds and an input of sulfate into sediments.

Ester sulfates comprise a major fraction of the sulfur in soils and are present in significant quantities of freshwater sediments (2, 4, 5). These compounds are substrates for sulfhydrolases which catalyze the formation of sulfate and an alcohol from ester sulfates (see reference 5 for a review of sulfhydrolases). Sulfhydrolases have been found in a variety of soils and marine sediments and are considered important in the transformations and budget of soil sulfur (3–5, 8). The quantitative significance of ester sulfate hydrolysis has not been estimated with respect to the dynamics of the sulfur cycle in anoxic lacustrine sediments.

We present an initial characterization of the ester sulfate cycle in anoxic sediments of Wintergreen Lake (WGL), Michigan. WGL is a shallow, hard-water, hypereutrophic lake. The sediment pH is 6.5 to 7.0, water of surface sediments is >90%, and organic matter in the sediments

ranges from 20 to 40%. Water column sulfate concentration is approximately 20 mg of SO$_4$$^{2-}$ or less per liter, and interstitial sulfate concentrations are <2 mg of SO$_4$$^{2-}$ per liter (J. M. Molongoski and M. J. Klug, Freshwater Biol., in press). In situ sulfate reduction rates are quite rapid, and the turnover time of the interstitial sulfate pool is about 12 h (R. L. Smith, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, N30, p. 184). It is unlikely that such rapid rates of sulfate reduction could be maintained by diffusive input of sulfate alone. Ester sulfate was investigated as a potential source of sulfate.

MATERIALS AND METHODS

Ester sulfate hydrolysis was measured by the procedures of Tabitabai and Bremner (15) which assay arylsulfhydrolase activity, or the hydrolysis of aromatic sulfate esters. Model substrates, such as p-nitrophenyl sulfate (PNPS) and p-nitrocatechol sulfate (PNCS), were hydrolyzed to p-nitrophenol (PNP) and p-nitrocatechol (PNC), respectively. The latter compounds were determined spectrophotometrically. The sensitivity of the assay was 0.12 absorbance unit per μg of PNP and 0.08 absorbance unit per μg of PNC. The limit of detection was 0.2 μg each of PNP and PNC per ml. The coefficient of variation for sediment assays was <10% (n = 353).

Rates of arylsulfhydrolase activity were measured for WGL sediments collected wtih gravity cores or an Ekman grab sampler. Sediments were obtained from three areas in the lake—the profundal zone, the littoral zone, and a transition zone intermediate between the former sites. Sediments were taken to the laboratory and stored at 10°C before analysis (processing occurred within 24 h of collection). Triplicate subsamples

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(2.5 ml) were taken in a cutoff 5-ml syringe and injected into 50-ml Erlenmeyer flasks. A 10-ml amount of a 0.5 M acetate buffer and 0.6 ml of toluene were then added, and the flasks were stoppered with butyl rubber stoppers. The headspaces of the flasks were flushed with deoxygenated N_2 for 20 min; flushing was accomplished by piercing the stoppers with two 20-gauge needles that formed inlet and outlet pores. The sample flasks were equilibrated at the desired incubation temperature, after which 1.0 ml of a 5 mM solution of PNPS or PNCS was injected with a needle and syringe. The flasks were then incubated for appropriate intervals and opened, and the contents were extracted with 2.5 ml of 0.5 M $CaCl_2$ and 10 ml of 0.5 M NaOH. The flask contents were filtered through an AAP4700 membrane filter (Millipore Corp., Bedford, Mass.), the filtrate was collected, and absorbance in the filtrate was assayed with a Hitachi model 100-40 spectrophotometer at 407 or 510 nm for PNP or PNC, respectively. Controls for sediment absorbance were obtained by adding PNPS or PNCS to some sediment samples just before the extraction procedure. Mercuric chloride and autoclave treatments were used to establish the biological nature of the hydrolytic activity (see Table 2).

Hydrolysis and reduction of the sulfate moiety of an alkyl ester sulfate, sodium dodecyl sulfate (SDS), were monitored with [^{35}S]SDS (50 mCi/mmol; Amersham Corp., Arlington Heights, Ill.). Stock solutions of [^{35}S]SDS were diluted with unlabeled SDS (Sigma Chemical Co., St. Louis, Mo.) to a final concentration of 10 mM and an activity of 5 μCi/ml. Sediment samples were prepared as described for the arylsulfhydrolase assays with several modifications. Sediment was transferred into 50-ml serum bottles or Hungate (13 by 130 mm) tubes; 5 ml of sediment was used in the serum bottles, and 1 ml was used in the tubes. The serum bottles were sealed with Teflon-lined septa (Supelco, Inc., Bellafonte, Pa.) and aluminum crimp caps, and the tubes were sealed with similar septa and Hungate screw caps (Bellco Glass, Inc., Vineland, N.J.). All manipulations were performed in an anerobic glove box. The serum bottles or tubes were removed from the glove box and flushed for 20 min with deoxygenated N_2. After preincubation for 24 h at 10°C, 1 ml of the SDS stock solution was injected into the bottles or tubes by needle and syringe. After incubation, triplicate samples were "quick frozen" in a dry ice-acetone bath and stored at −70°C.

$H_2{}^{35}$S formed from the [^{35}S]SDS was assayed by thawing the samples and injecting 5 ml of a solution of 5% glutaraldehyde–4 N HCl. Glutaraldehyde was used as a killing agent, and the acid served to volatilize the sulfide. The sample vessels were connected during the injection of acidic glutaraldehyde to a bubble trap consisting of three scintillation vials (each containing 5 ml of 2% $CdCl_2$) joined in series by nylon tubing. The headspaces of the sample vessels were flushed into the scintillation vials with deoxygenated N_2. Flushing was continued for a minimum of 30 min. ^{35}S trapped in the scintillation vials was assayed by adding 10 ml of aqueous scintillation cocktail (Amersham Corp.) to the vials and determining radioactivity with a Beckman LS8000 scintillation counter (Beckman Instruments, Inc., Fullerton, Calif.).

[^{35}S]SDS remaining in solution was assayed by extracting the sediment-water suspension of the Hungate tubes with 5.0 ml of 1-octanol (Sigma Chemical Co.) after the stripping procedure. The octanol-sediment mixture was stirred on a Vortex mixer for 30 s and centrifuged in a clinical centrifuge (International Equipment Co., Div. of Damon Corp., Needham Heights, Mass.) for 5 min. The octanol phase was sampled, and the radioactivity in 0.25 ml was counted in 5 ml of aqueous scintillation cocktail. Total recovery of label was greater than 98%. Extraction efficiencies were approximately 70%, and the counting efficiency of octanol-[^{35}S]SDS-aqueous scintillation cocktail was greater than 95%. The counting efficiencies of the Cd^{35}S-aqeuous scintillation cocktail gels varied from 60 to 90%, depending on the amount of CdS in the samples. Quench corrections were made by the "H number" method developed for Beckman Instruments, Inc.

Concentrations of total sediment sulfur were determined by a modification of the method of Tabitabai and Bremner (16). Lots of approximately 50 mg of air-dried sediment obtained from gravity cores by the methods of Molongoski and Klug (in press) were weighed into 50-ml beakers. Three milliliters of a solution of sodium hypobromide was added, the mixtures were swirled, and the beakers were placed in an oven regulated to 250°C. Approximately 30 min after the solution had evaporated, the beakers were removed from the oven and 3 ml of 2 N HCl was added to each residue. The liquid was boiled away at 250°C, and, after cooling, the residue in each beaker was dissolved in 20 ml of 0.1 M Sorenson glycine buffer, pH 2 (12). Approximately 10 mg of Dowex AG-50 (H^+ form) was added to each beaker, and the mixture was incubated overnight in a refrigerator. The solutions were decanted and centrifuged for 5 min in a clinical centrifuge. A 2-ml sample of the supernatant was diluted into 18 ml of glycine buffer, and the sulfate content was determined by the turbidimetric assay of Tabitabai (14). Recovery of added methionine and comparison of the hypobromide oxidation with another combustion procedure (13) showed that the method quantitatively converted sediment sulfur to sulfate. The sensitivity of the assay of Tabitabai (14), which was used for total sulfur and ester sulfate determinations, was 0.002 absorbance unit per μg of $SO_4{}^{2-}$-S. The limit of detection was 30 μg of S per 50 mg (dry weight) of sediment for total sulfur and ester sulfate. The coefficient of variation for sediment samples was <10% ($n = 315$).

Ester sulfate was determined by modifying a method of Freney (6). Approximately 50-mg samples of sediment (as above) were weighed into screw-cap test tubes. Five milliliters of 5 N HCl was added to each sample, and the resulting slurry was autoclaved for 5 h. Afterwards, the slurries were cooled, mixed with 4 ml of 5 N NaOH, and held at room temperature overnight. Sulfate that formed from ester sulfates by acid hydrolysis was measured turbidimetrically (14). Standardization of this procedure with PNPS, PNCS, and choline-O-sulfate showed that the hydrolysis and recovery were quantitative. Cysteine and methionine did not interfere. The lability of the C-O-SO$_3$ function in acid has been noted before (11).

RESULTS

PNPS and PNCS hydrolyses were substantial for both substrates in the profundal and littoral samples (Table 1). PNPS hydrolysis was greatest in the littoral zone, whereas PNCS hydrolysis was most rapid in profundal samples. PNPS hydrolysis occurred to a greater extent than PNCS hydrolysis in all samples. The replacement time of the interstitial sulfate pool was 0.4 to 1.1 h in the profundal zone. This interval was calculated by assuming a nominal concentration of 2 mg of SO_4^{2-} per liter (Molongoski and Klug, in press) and by using the average of PNPS and PNCS rates shown in Table 1. The analogous replacement time in the littoral zone was 0.2 to 0.8 h.

PNPS sulfhydrolase activity was found to vary with depth (Fig. 1). Activity at the profundal site on 2 October 1978 was greatest at an intermediate depth in the 0- to 10-cm profile, whereas activity at the transition and littoral sites decreased with depth. Maximum activity on 4 April 1979 (data not shown) was located at an intermediate depth at all three sites. Rates of activity for each site, integrated over the 0- to 10-cm interval, were greatest in the littoral site, followed by the profundal and transition sites. PNCS activity, assayed on 2 October 1978, decreased with increasing depth at all sites. PNPS activity was greater than PNCS activity at all depths and sites (Fig. 1).

The sediment enzyme system of both profundal and littoral samples exhibited Michaelis-Menton kinetics (Fig. 2). Kinetic parameters of the systems, estimated from double-reciprocal plots, were: $V_{max} = 37.6$ mg of SO_4^{2-} released per liter of sediment per h and $K_{app} = 2.1$ mM for the profundal zone; $V_{max} = 12.5$ mg of SO_4^{2-} released per liter of sediment per h and $K_{app} = 1.0$ mM for the littoral zone. A determination of optimum activity in sediments at various temperatures and pH revealed a temperature optimum of 50°C and a pH optimum of 5.5 for both littoral and profundal sediments. At a concentration of 20.8 mM, $HgCl_2$ completely inhibited PNPS-arylsulfhydrolase activity (Table 2). Autoclaving had a similar effect (Table 2). PNPS hydrolysis was also sensitive to oxygen (Table 2); samples incubated aerobically had approximately one half of the activity found for anaerobic incubations.

Rates of formation of $H_2^{35}S$ from [^{35}S]SDS were rapid in both profundal and littoral sediments (Fig. 3). $H_2^{35}S$ evolution was linear over an initial 4-h period in the incubations with 5 ml of sediment (r^2 littoral = 0.99 [$n = 12$]; r^2 profundal = 0.95 [$n = 12$]). Longer incubations (48 h) of 1 ml of sediment produced greater variability, yet evolution remained a linear function (r^2 littoral = 0.98 [$n = 12$]; r^2 profundal = 0.95 [$n = 12$]). Rates of $H_2^{35}S$ formation were similar for both the 5- and 1-ml sediment incubation systems. In both incubations, [^{35}S]SDS conversion was two to four times more rapid in littoral sediments. Rates of profundal sulfate reduction calculated from these data were similar to rates observed by Smith (Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, N30, p. 184) for in situ reduction. Rates of [^{35}S]SDS conversions were slower, however, than the rate of SDS hydrolysis (Fig. 3). Extractable [^{35}S]SDS decreased rapidly within the first 2 to 4 h of incubation to a level approximately 20 to 30% of that of the original and remained fairly constant thereafter. During the initial 4 h of incubation, [^{35}S]SDS hydrolysis was faster in the littoral samples. Control experiments (data not presented) demonstrated that the observed SDS hydrolysis was not due to abiological activity in the sediment or to the addition of acid before SDS extraction. SDS hydrolysis is thought to be the result of alkylsulfhydrolases (9, 10).

A comparison of the concentrations of total sediment sulfur and ester sulfate sulfur in pro-

TABLE 1. *Arylsulfhydrolase activity in WGL sediment[a]*

| Site | Substrate | Rate on following day: | | | |
		2 October 1978	3 December 1978	4 April 1979	30 April 1979
Profundal	PNPS	4.3 (1.3)	4.0 (0.8)	1.4 (0.2)	7.4 (2.8)
	PNCS	0.9 (0.3)	2.4 (1.1)		
Littoral	PNPS	8.0 (4.6)	5.8 (0.7)	1.9 (0.3)	8.3 (1.7)
	PNCS	0.5 (0.4)	1.4 (0.8)		

[a] Rates are milligrams of SO_4^{2-} released per liter of surface sediment (0 to 10 cm; pH 6.7; 10°C) per hour. Numbers within parentheses are ±1 standard error ($n = 3$).
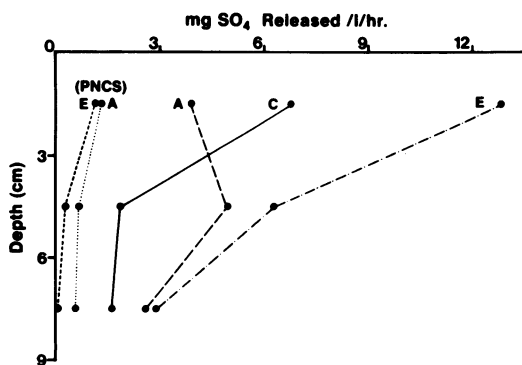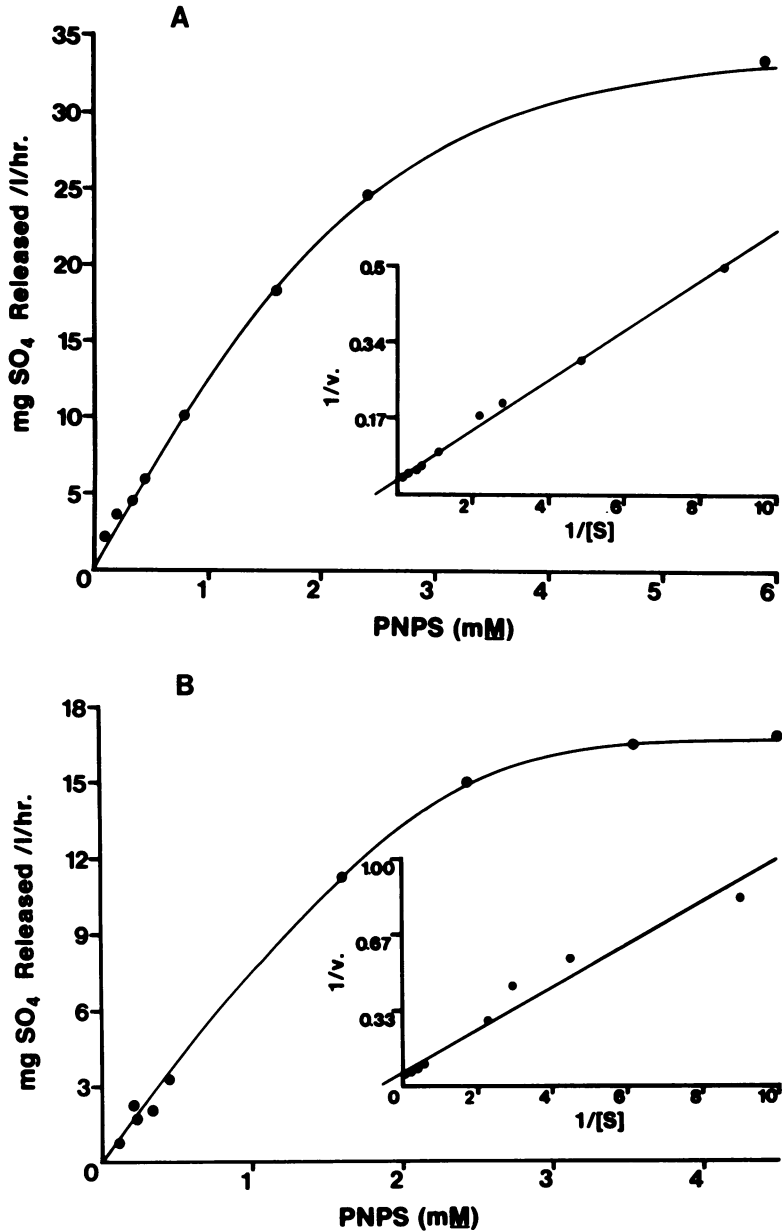

FIG. 1. *Depth profiles of arylsulfhydrolase activity in WGL sediments, 4 October 1978. A, Profundal; C, transition; E, littoral.*

FIG. 2. *Rates of PNPS hydrolysis versus concentration in (A) profundal and (B) littoral sediments.*

fundal and littoral sediments (Table 3) indicated that ester sulfate at all sites was 20 to 30% of total sulfur. Ester sulfate concentration and total sulfur were highest in the littoral zone. Inorganic sulfur in all sites was only a small fraction of ester sulfate total sulfur. In fact, inorganic sulfate concentrations were approximately three orders of magnitude less than ester sulfate concentrations. Carbon-bonded sulfur, as in methi-

onine, cysteine, or sulfolipids, was calculated to represent about 70% of total sediment sulfur.

DISCUSSION

The data presented demonstrate the potential role of ester sulfates in sulfur cycling in anoxic, lacustrine sediments. Sulfate concentrations in lacustrine sediments are generally reported as low or undetectable, yet sulfate reduction has

TABLE 2. Aspects of PNPS-sulfhydrolase activity in surface sediment

Site	Treatment	Rate[a]
Profundal	None	7.4 (2.8)
Littoral	None	8.3 (1.7)
Profundal	Autoclaved[b]	0.0
Littoral	Autoclaved[b]	0.0
Profundal	8 mM HgCl$_2$	4.7 (0.8)
Profundal	136 mM HgCl$_2$	0.5 (0.1)
Profundal	20.8 mM HgCl$_2$	0.0
Profundal	Aerobic	4.4 (0.6)
Littoral	Aerobic	3.5 (0.8)

[a] Milligrams of SO_4^{2-} released per liter of sediment per hour (±1 standard error; $n = 3$ samples).
[b] Twenty minutes at 121°C; 15 lb/in^2.

been proposed as an important process with respect to carbon and sulfur transformations (1, 17). Sources of sulfate that can maintain sulfate reduction as an active process include the following: (i) sediment-water column exchange; (ii) groundwater intrusion; and (iii) ester sulfate hydrolysis. Mechanisms i and ii are likely to represent variable, diffusion-regulated inputs to the sediment in many systems. Ester sulfate hydrolysis could represent an equally important input occurring in the lebensraum of the sulfate-reducing bacteria.

Rates of PNPS and PNCS hydrolyses in WGL sediments indicates that an active, constitutive arylsulfhydrolase population was present in all sites examined. Those rates may or may not mimic natural hydrolysis, as the natural array of substrates and their concentrations are unknown. However, since most arylsulfhydrolases hydrolyze PNPS and PNCS (11), the data obtained from WGL sediments described the natural enzyme system qualitatively and provide insights for further work.

For example, rates of PNPS hydrolysis were greater in littoral than profundal samples at the PNPS concentration routinely used for assays; however, estimates of V_{max} for PNPS were greater in the profundal zone. This discrepancy is due, in part, to the observed differences in dv/dS (Fig. 2) for the littoral and profundal zones over the pseudo-first-order interval and also to differences in K_{app}. The littoral enzyme system was saturated sooner than the profundal system; however, littoral rates in the pseudo-first-order region were higher than corresponding profundal rates. Since the PNPS concentration routinely used was 1 mM (and therefore within the region of pseudo-first-order kinetics), the rates typically observed were biased in favor of the littoral samples. Therefore, it would be instructive to compare littoral and profundal activities
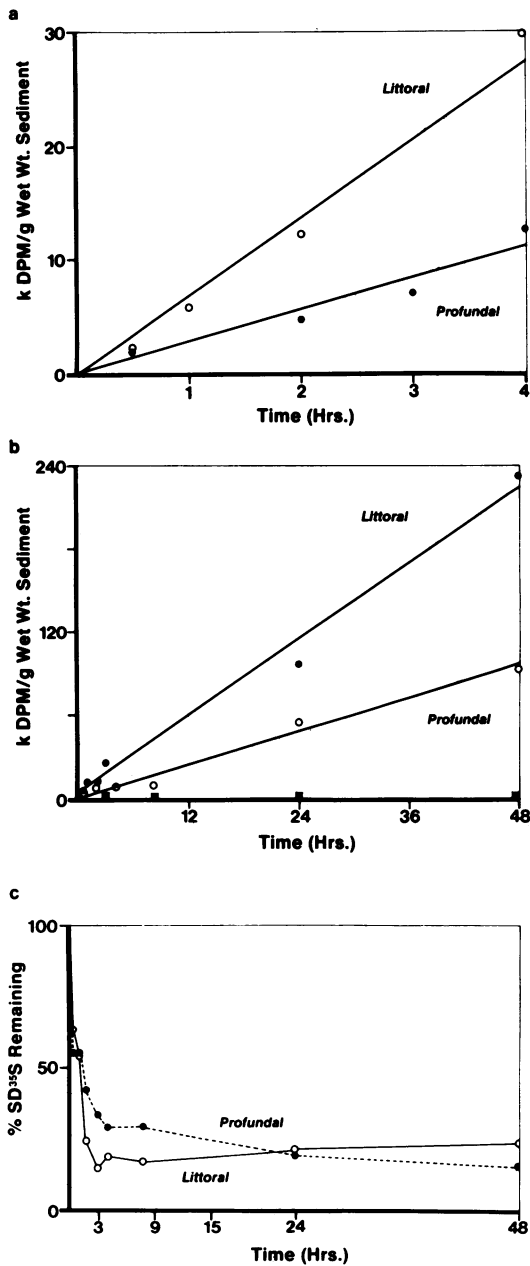
FIG. 3. $[^{35}S]SDS$ hydrolysis and $H_2^{35}S$ formation. (a) $H_2^{35}S$ formation from $[^{35}S]SDS$ in 5 ml of sediment (disintegrations per minute per gram [wet weight] of sediment). Symbols: O, littoral samples; ●, profundal samples. (b) Linear regressions of $H_2^{35}S$ formation from $[^{35}S]SDS$ in 1 ml of sediment (disintegrations per minute per gram [wet weight] of sediment). Symbols: O, profundal samples; ●, littoral samples; ■, killed controls. (c) $[^{35}S]SDS$ hydrolysis in profundal (O) and littoral (●) sediments.

TABLE 3. *Sulfur in WGL sediment*[a]

Site	Total sulfur	Ester sulfate-S	Ester sulfate (%)
Profundal	6.5 (0.1)	2.1 (0.1)	32.3
Transition	7.6 (0.2)	2.6 (0.1)	34.2
Littoral	12.2 (1.0)	2.7 (0.2)	22.1

[a] Milligrams of S per gram (dry weight) (±1 standard error); values are for surface sediments (0 to 3 cm).

with in situ substrate concentrations to clearly elucidate the differences between enzyme systems at the two sites.

The pH and temperature optima, although unusual when the ranges of in situ conditions are considered, are within reported ranges (11). However, it is not clear whether only one type of enzyme is involved in arylsulfate hydrolysis or from whence the enzyme(s) originates. Furthermore, controls of the enzyme system are unknown, as is the function of the enzymes; for instance, in many microorganisms, sulfatases are produced in response to a sulfur deficiency (5, 11). WGL sulfhydrolases may be produced as a response to a sulfur limitation experienced by one or more types of bacteria. Oxygen sensitivity has not been reported before, even though sulfhydrolase activity has been assayed in anoxic sediments (3, 8). This may be because oxygen sensitivity has been previously overlooked as an important parameter. Arylsulfhydrolase activity in anoxic soils and sediments may have been underestimated in the past. The high concentration of $HgCl_2$ necessary for sulfhydrolase inhibition is possibly a function of high sediment sulfide levels (~10 mg/liter). Inhibition of sulfhydrolase activity by high concentrations of $HgCl_2$ has also been observed by Oshrain and Wiebe (8) for salt marsh soils containing sulfide.

The work with [^{35}S]SDS clearly demonstrates that ester sulfates are hydrolyzed in sediment and that the resulting sulfate can be utilized by sulfate reducers. The rapid conversion of [^{35}S]SDS to $H_2{}^{35}$S is the result of [^{35}S]SDS hydrolysis and the subsequent dissimilatory reduction of sulfate. Coupling of the two processes appears to be indirect, as [^{35}S]SDS hydrolysis is approximately 70 to 80% complete in 2 to 4 h. Thus sulfhydrolase activity seems independent of sulfate reduction. Under in situ conditions, the rates of sulfate release and reduction may be more nearly equal (highly coupled), thereby preventing any accumulation of sulfate and allowing for the existence of a small sulfate pool size and rapid rates of turnover. Differences in $H_2{}^{35}$S production in littoral and profundal samples are most likely due to differences in sulfate reduc-

tion and not [^{35}S]SDS hydrolysis. Incomplete extraction of SDS from sediment and the constant level of SDS remaining after approximately 4 to 8 h of incubation may be due to the association of [^{35}S]SDS with sediment organic matter in such a way as to render it unavailable for extraction and hydrolysis.

[^{35}S]SDS hydrolysis in WGL sediment demonstrates tentatively the presence of a second type of sulfhydrolase, that is, an alkylsulfhydrolase. This particular class of enzymes has not been studied as extensively as have the arylsulfhydrolases. Payne et al. (9, 10), Fitzgerald (5), and Huddleston and Alfred (7) have reviewed some aspects of the enzymes. The demonstration of alkylsulfhydrolase activity coupled with sulfate reduction in anoxic sediments may be significant when considering the fate of alkyl sulfates, especially detergent wastes, in lake or stream systems.

The concentrations of ester sulfate observed in WGL profundal and littoral sediments provide a striking example of the potential in situ significance of ester sulfates. Concentrations of ester sulfate are as much as three orders of magnitude greater than sulfate and dwarf all forms of inorganic sulfur that have been assayed (Smith, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, N30, p. 184). In fact, ester sulfates form a significant part of the organic sulfur fraction. The large percentage of ester sulfate is similar to that found in some soils (5) and in an Okefenokee Swamp peat (about 40% of organic sulfur and 25% of total sulfur) (2). At this time, it is not clear what types of compounds constitute the ester sulfate pool or how fast the pool turns over. It is very likely, though, that inputs of ester sulfate to the sediment are a function of the highly productive phytoplankton or *Nuphar* populations in the lake; Roy and Trudinger (11) have observed that sulfate esters are produced by some algae and plants.

The demonstration of an active sulfhydrolase system and significant quantities of ester sulfate in WGL raises many questions about the sulfur cycle in lakes. Some of these questions are shown in Fig. 4. One important aspect of this model is that it provides a mechanism for the maintenance of an active population of sulfate reducers when inputs of sulfate are low. A number of investigators (1, 17) have hypothesized that sulfate reduction is an important process in freshwater sediments, yet sulfate in the various systems examined has been quite low. This apparent paradox may be solved by the presence of an ester sulfate system such as that described for WGL. If the rapid rates of sulfate reduction found in WGL (Smith, Abstr. Annu. Meet. Am.
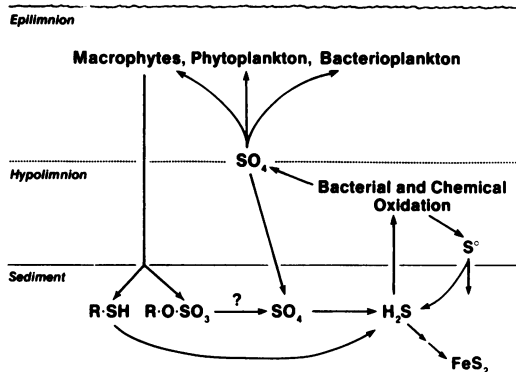
FIG. 4. *Proposed model for lacustrine sulfur cycling.*

Soc. Microbiol. 1979, N30, p. 184) are similar for other low-sulfate lake sediments and if ester sulfate hydrolysis provides a readily available source of sulfate, one might well find that anaerobic lacustrine carbon metabolism is intimately tied to sulfate reduction and methanogenesis.

LITERATURE CITED

1. **Cappenberg, Th. E.** 1974. Interrelation between sulfate-reducing and methane producing bacteria in bottom deposits of a fresh-water lake. 1. Field observations. Antonie van Leeuwenhoek J. Microbiol. Serol. **40**:285–306.
2. **Cassagrande, D. J., and K. Siefert.** 1977. Origins of sulfur in coal: importance of ester sulfate content of peat. Science **195**:675–676.
3. **Chandranrohan, D., K. Devenshan, and R. Natarajan.** 1974. Arylsulfatase activity in marine sediments. Mar. Biol. **27**:89–92.
4. **Cooper, P. J. M.** 1972. Arylsulfatase activity in northern Nigerian soils. Soil Biol. Biochem. **4**:333–337.
5. **Fitzgerald, J. W.** 1976. Sulfate ester formation and hydrolysis: a potentially important yet often ignored aspect of the sulfur cycle of aerobic soils. Bacteriol. Rev. **40**:698–721.
6. **Freney, J. R.** 1961. Some observations on the nature of organic sulfur compounds in soil. Aust. J. Agric. Res. **12**:424–432.
7. **Huddleston, R. L., and R. C. Alfred.** 1967. Surface-active agents: biodegradability of detergents, p. 343–370. *In* A. D. McLaren and G. H. Peterson (ed.), Soil biochemistry, vol. 1. Marcel Dekker, New York.
8. **Oshrain, R. L., and W. J. Wiebe.** 1979. Arylsulfatase activity in salt marsh soils. Appl. Environ. Microbiol. **38**:337–340.
9. **Payne, W. J., and V. E. Feisal.** 1963. Bacterial utilization of dodecyl sulfate and dodecyl benzene sulfonate. Appl. Microbiol. **11**:339–344.
10. **Payne, W. J., J. P. Williams, and W. R. Mayberry.** 1965. Primary alcohol sulfatase in a *Pseudomonas* species. Appl. Microbiol. **13**:698–701.
11. **Roy, A. B., and P. A. Trudinger.** 1970. The biochemistry of inorganic compounds of sulphur. Cambridge University Press, London.
12. **Sober, H. A. (ed.).** 1968. Handbook of biochemistry: selected data for molecular biology. The Chemical Rubber Co., Cleveland.
13. **Steinbergs, A., O. Jismao, J. R. Freney, and N. J. Barrow.** 1962. Determination of total sulfur in soil and plant material. Anal. Chim. Acta **27**:158–164.
14. **Tabitabai, M. A.** 1974. Determination of sulfate in water samples. Sulphur Inst. J. **10**:11–13.
15. **Tabitabai, M. A., and J. M. Bremner.** 1974. Arylsulfatase activity of soils. Soil Sci. Soc. Am. Proc. **34**:225–229.
16. **Tabitabai, M. A., and J. M. Bremner.** 1975. An alkaline oxidation method for the determination of total sulfur in soils. Soil Sci. Soc. Am. Proc. **34**:62–65.
17. **Winfrey, M. R., and J. G. Zeikus.** 1977. Effect of sulfate on carbon and electron flow during microbial methanogenesis in freshwater sediments. Appl. Environ. Microbiol. **33**:275–281.