Biogeochemistry 1: 63–77 (1984)

Epilimnetic sulfate reduction and its relationship to lake acidification

C. A. KELLY

Biology Department, University of Winnipeg, Winnipeg, Manitoba R3B 2E9, Canada

J. W. M. RUDD

Freshwater Institute, Winnipeg, Manitoba R3T 2N6, Canada

Key words: sulfate reduction, sediment, lake acidification

Abstract. Sulfate reduction occurred from 0–3 cm below the surface of the epilimnetic sediments of three northwestern Ontario lakes, including L.223, which has been experimentally acidified by additions of sulfuric acid. Shallow water sites were conducive to SO_4^{2-} reduction because decomposition in these predominantly sandy sediments caused oxygen concentrations to decrease rapidly within mm below the interface. The occurrence of methanogenesis just below the depth of minimum SO_4^{2-} concentration demonstrated that availability of organic carbon was not a limiting factor for sulphate reduction.

Laboratory studies showed that SO_4^{2-} reduction rates in mixed sediments were lower at pH 4 than at pH 6. However, sulfate gradients in sediments indicated that there was no effect of acidification on sulfate reduction in situ. This was probably because microbial H^+ consumption in the epilimnetic sediments maintained steep pH gradients below the sediment-water interface. The pH increased from $\simeq$ 5.0 to 6.5 or higher by a depth of 3.0 cm into the sediments.

Introduction

During a whole lake acidification experiment at the Experimental Lakes Area in northwestern Ontario, a large portion of the sulfate added a sulfuric acid was lost within the lake, due to the activity of sulfate-reducing bacteria (Schindler et al., 1980; Schindler and Turner, 1982; Cook and Schindler, 1983). Over a five year period, 35% of the total sulfate input was reduced and lost to the sediments. In addition, this sulfate loss, resulted in a significant neutralization of the acid added. Of all the biological and chemical processes operating within the lake to neutralize acid inputs, sulfate loss could account for 84% of the total H^+ consumption (R.B. Cook, U. of Minnesota, Duluth, unpubl. data). The reactions which could be responsible for this acid neutralization are sulfate reduction followed by precipitation of the reduced sulfur with iron, or sequestration of sulfur in the sediments in elemental or organic forms (e.g. Schindler et al., 1980; Kelly et al., 1982).

*Contribution from a symposium on the role of sulfur in ecosystem processes held August 10, 1983, at the annual meeting of the A.I.B.S., Grand Forks, ND; Myron Mitchell, convenor.

While sulfate reduction is important in affecting the progress of lake acidification, little information is available about this process in epilimnetic (shallow water) sediments, which usually make up the largest sediment area in a lake. Previous studies have focused on the hypolimnion (e.g. Ingvorsen et al., 1981; Smith and Klug, 1981) where the water column often becomes anoxic and SO_4^{2-} reduction can be studied by measuring disappearance of SO_4^{2-} (Cook and Schindler, 1983; Kelly et al., 1982; Ingvorsen and Brock, 1982). This focus on hypolimnetic sediments is apparently due to a view which many workers have expressed that sulfate reduction would be confined to lakes with anoxic hypolimnia (e.g. Niragu and Coker, 1983). Epilimnetic sediments are overlaid by oxygenated water, and tend to be sandy material of low organic content, making them appear to be unlikely sites for anoxic microbial activities.

Research presented in this paper demonstrates that this sandy epilimnetic sediment is an active site of microbial activity in both control and experimentally acidified lakes, with conditions of anoxia close to the sediment-water interface. This environment is thus conducive to sulfate reduction, and because it constitutes a large area it is an important potential contributor to total sulfate reduction in these lakes.

Methods

Study sites: Cores and pore water samples were taken from 3 m and 4 m depth in L.223, from 1.5 and 3 m depth in L.302 North and L.302 South, and 2 m in L.114. Lake 223 has been experimentally acidified with sulfuric acid since 1976 (Schindler et al., 1980). The pH of the epilimnetic water has been lowered approximately one-quarter pH unit per year, to 5.0 in 1982, and held at 5.0 in 1983. Lake 114 has been experimentally acidified since 1979. Additions were equivalent to adjusting the pH of rain on the lake's surface downward by one pH unit with H_2SO_4 (Schindler and Turner, 1982). The pH of this lake has not changed (Schindler, unpublished data). Lakes 302 North and South are basins of one lake, separated by two shallow narrows which are closed with sea curtains. The epilimnetic sediments of L.223 and 302N and S are sandy. L.114 sediments are highly organic. Average summer temperatures of ELA epilimnetic sediments are about 22 °C.

In situ enclosure experiment: Porewater chemistry and sulfate reduction were monitored in four 5-m diameter enclosures which are sealed to the sandy epilimnetic sediment of L.302N at 2 m depth. Their construction and installation were as described in Rudd et al. (1980). Two of the enclosures were acidified with HCl to a pH of 5.2. The other two enclosures remained at ambient pH (6.8–7.0). Na_2SO_4 and $NaNO_3$ were added to all four of the enclosures, increasing the concentrations of SO_4^{2-} to about 90 μmol $\cdot$ L^{-1} (from 28 μmol $\cdot$ L^{-1}) and of NO_3^- present in acid deposition. Water samples were analyzed for sulfate concentration on a Dionex ion chromatograph

after filtering through 0.22 μ Nucleopore filters which had been rinsed with distilled water (Millipore Super Q) to remove traces of ions. pH was measured on freshly collected unfiltered samples using a Fisher glass combination electrode.

Pore water profiles: Samples were collected using a pore water 'peeper' (Hesslein, 1976). Distilled water in the peeper cells was deoxygenated by equilibrating with nitrogen gas prior to placement in the sediment. The peepers were left to equilibrate with the pore water for one week. Pore water samples for pH were collected quickly after removal of the peepers from the sediment to prevent gas exchange with the atmosphere. Samples were drawn into glass syringes which had the dead volume filled with degassed (boiled) distilled water. Syringe samples were transferred to a closed container of the same size as the sample (3.5 mL) for measurement of pH. Sulfate samples were collected in rinsed plastic vials and analyzed using a Dionex ion chromatograph. Samples for H_2S analysis were collected in glass syringes equipped with a 3-way valve. The dead volume had been filled with degassed distilled water. Reagents for the H_2S analysis (Stainton et al., 1977) were injected through the three-way valve to prevent exposure of the samples to atmospheric oxygen. Samples for ferrous iron were collected in plastic vials to which 10 μL of 1N HCl were added to maintain the iron in solution. Iron which had crossed the peeper membrane during equilibration was considered to be ferrous. Samples for CH_4 and DIC (dissolved inorganic carbon) were taken quickly using a repeating Cornwall syringe and injected into evacuated serum vials which contained 10 μL of 8N H_2SO_4. The head space was sampled for CH_4 and CO_2, and appropriate calculations were made to determine the CH_4 and DIC concentrations in the samples.

Close interval pH and O_2 profiles: pH and oxygen profiles were obtained at 1 mm intervals using micro electrodes mounted on a threaded rod which allowed slow, precise penetration into freshly collected sediment cores (Flett Research Ltd., Winnipeg, Manitoba). The pH electrode was a flexible glass electrode (Microelectrodes, Inc., Londonderry, New Hampshire). The O_2 electrode was a platinum wire coated with epoxy, with 15 μ of exposed platinum at the tip of the probe (Flett Research Ltd.). The O_2 probe gave a linear response between 0–100% air saturation. Silver-chloride reference electrodes (Ingold Electrodes Inc., Andover Ma.) were held just above the sediment surface. Oxygen concentrations were expressed as a percentage of the surface water oxygen concentration.

Core incubations: Cores were taken from epilimnetic sediments by diver and brought immediately to the laboratory where they were incubed at in situ lake temperature. The water level in the core was adjusted to a depth of 4 cm above the sediment surface, leaving about 2 cm of air space above the water. The air space prevented oxygen depletion in the surface water during incubation. During incubation, the cores were tightly sealed with rubber stoppers and a coating of petroleum jelly to prevent evaporative loss.

In some cores, loss of SO_4^{2-} from the surface water was followed for several days at ambient and at elevated SO_4^{2-} levels. The long incubation times were used to ensure that steady state was reached. ^3H_2O diffusion measurements have shown that this occurs within 12 h in these cores (C. Kelly and R. Hesslein, unpubl. data). Sulfate samples were analyzed as described previously.

A second type of core incubation used $^{35}SO_4^{2-}$ (Amersham). The isotope was mixed into the surface water of cores taking care not to disturb the sediment. $^{35}SO_4^{-}$ was also injected at 1, 2, 3, and 4 cm below the core surface. The volume of label added at each depth was chosen to correspond to the total SO_4^{2-} at that depth. Because of problems in determining the specific activity of the SO_4^{2-} reduced during the incubation (see Discussion), all cores received the same ^{35}S additions and were incubated for the same length of time. Since SO_4^{2-} concentrations and profiles were similar in all the enclosures, the specific activity of the reduced S should have been similar in all the cores, but was unknown. Therefore, results were reported as dpm reduced ^{35}S rather than as μmoles reduced S. The cores were incubated for 24 hours and then sectioned. The sections were acidified with concentrated HCl and sparged with oxygen-free nitrogen to collect acid volatile sulfide (AVS = H_2S + FeS). The sulfide was trapped in a basic zinc acetate solution (Howarth and Teal 1979), solubilized with concentrated H_2SO_4, and oxidized quantitatively with iodine (Stainton et al., 1977). The remaining iodine was the back-titrated with thiosulfate to quantify the sulfide. A 3 mL aliquot of this solution was assayed for radioactivity after mixing with Instagel fluor (Packard Instruments Inc.). Unreacted $^{35}SO_4^{2-}$ was washed from the sediments by rinsing four times in lake water until radioactivity was at background levels. The rinsed sediment was digested in Aqua Regia to solubilize all remaining forms of reduced sulfur (Howarth and Teal, 1979).

Laboratory experiments: Effects of reduced pH on sulfate reduction were studied in the laboratory using sediment containers in which the sediment pore water could be controlled at a precise pH (Furutani et al., in press). Briefly, the upper 1–2 cm of L.114 sediment were transferred from an Ekman dredge to gas tight containers. The sediment was then mixed and distributed in 1 L lots into replicate N_2-gassed containers with a 1.6 L nitrogen gas phase. After settling, there was a 1–2 cm layer of water above the sediments. Control containers received no acid additions and remained at the ambient pH of 6.3. Pore water pH of test containers was reduced to 4.2 by additions of HCl. HCl was used rather than H_2SO_4 or HNO_3 because the reduction of nitrate and sulfate would result in an elevation of pH (e.g. Kelly et al., 1982). Rates of organic carbon decomposition were determined by monitoring the accumulation of DIC plus CH_4 with time in each of the containers. Using this methodology it has been determined that a change in rate of 15% or greater between control and test containers is statistically significant at the 99% confidence level (Furutani et al., in press). Sulfate reduction rates were

monitored by following the decrease in sulfate concentration in the sediment porewater with time. Na_2SO_4 was added to the containers following an initial (10 day) period during which total rates of CO_2 and CH_4 production were measured to ensure that rates were linear. Disappearance of sulfate was followed in the containers over 1.4–14 day periods, depending on the rate of disappearance. Sediment samples were analyzed for pore water SO_4^{2-} concentrations by centrifuging, filtering the supernatant and analyzing as described previously.

Organic carbon content of sediment samples was measured using a CHN analyzer (Stainton et al., 1977).

Results

The epilimnetic sediments in Lakes 223 and 302N were sandy with a low organic carbon content (0.4–1.6% C). Surface sediments (0–1 cm) were 30–40% water. Deeper sediments (3–7 cm) were 18–24% water. Over this depth range the densities were 1.5–1.9 g (dry weight) $\cdot$ cm^{-3}. In contrast, the epilimnetic sediment of L.114 was highly organic (24% C), and flocculent (95–97% water), with a density of 0.04–0.07 g (dry weight) $\cdot$ cm^{-3}.

All of these sediments were sites of intense microbial activity which resulted in steep gradients of dissolved chemical species. Oxygen penetrated only 1–2 cm into the sediments (Figure 1a). The minimum concentration of sulfate occurred 0–1.5 cm below the depth of zero oxygen concentration (Figure 1b). Methane and iron concentrations increased rapidly below the depth of zero oxygen concentration (Figure 1c, d).

The effect of SO_4^{2-} concentration in the overlying water on the rate of flux of SO_4^{2-} into sediments was examined directly in cores incubated at ambient (55 μmole $\cdot$ L^{-1}) and at elevated (105 μmol $\cdot$ L^{-1}) concentrations. The higher concentration was related to a higher loss rate (Table 1).

In unacidified L.302N pH increased from 6.4–6.8 in the water column to 6.9 at 3.6 cm below the sediment surface (Figure 2). A pH minimum occurred near the sediment-water interface. This pH depression was characteristic of unacidified sediments and is thought to be a result of oxic CO_2 production. In acidified L.223, the pH increased rapidly within the sediments, reaching values of 6.0 or above by 1 cm depth (Figure 2).

Effects of acidification on rates of sulfate reduction: Comparison of sediment profiles in the acidified and non-acidified enclosures in L.302N showed that both SO_4^{2-} gradients and pH values within the sediments (deeper than 0.5 cm below the sediment surface) tended to be similar in all of the enclosures (Figures 3 and 4). The only difference between acidified and non-acidified enclosures was that pH was lower at or near the sediment-water interface in the acidified enclosures (Figure 4). The maintenance of near-neutral pH a short distance below the sediment-water interface of the epilimnetic sediments has also been observed in L.223, even after 8 years of acidification (Figure 2).

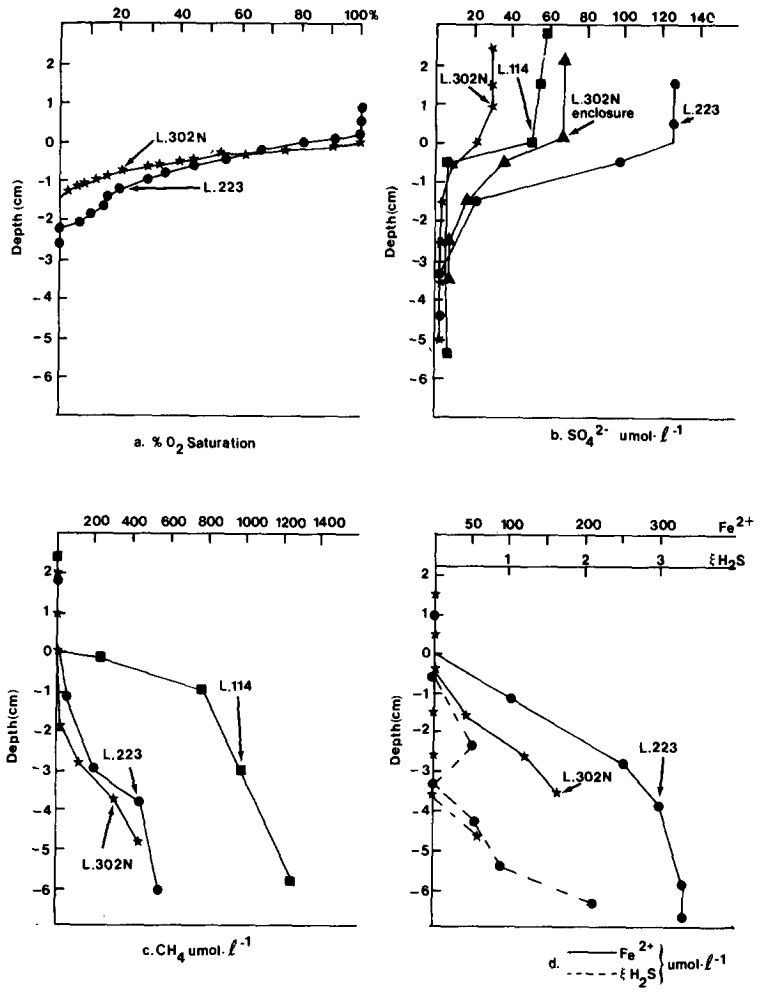

Figure 1. (a) Porewater profiles of oxygen concentration, as % of surface water oxygen concentrations, in epilimnetic sediments of L.223 at 3 m depth (June 1983) and L.302N at 3 m depth (August 1982).

(b) Porewater profiles of SO_4^{2-} concentration in epilimnetic sediments of L.223 at 4 m depth (August 1982), L. 302N enclosures (July 1982), L.302N at 1.5 m depth (August 1982) and L.114 at 2 m depth (September 1981).

(c) Porewater profiles of CH_4 concentration in epilimnetic sediments of L.223 at 4 m depth (August 1981), L.302N at 1.5 m depth (August 1982) and L.114 at 2 m depth (September 1981).

(d) Porewater profiles of ΣH_2S and Fe^{2+} concentrations in epilimnetic sediments of L.223 at 4 m depth (August 1981) and L.302N at 1.5 m depth (August 1982).

Undisturbed core incubations with $^{35}SO_4^{2-}$ in sediments taken from acidified and non-acidified lake enclosures showed no inhibition of sulfate reduction in the acidified enclosures (Table 2). There was some variability in SO_4^{2-} profiles from core to core, on the order of that seen in the profiles shown in Figure 3, and this would affect the specific acitivity of the reduced

Table 1. Rates of SO_4^{2-} loss from water overlying cores from L.302S (1.5 m), incubated at in situ temperature (23 °C) and at ambient and elevated SO_4^{2-} concentrations

Initial SO_4^{2-} $\mu mol \cdot L^{-1}$	Loss rate $\mu mol \cdot m^{-2} \cdot d^{-1}$
55	−100, −170
105	−270, −360

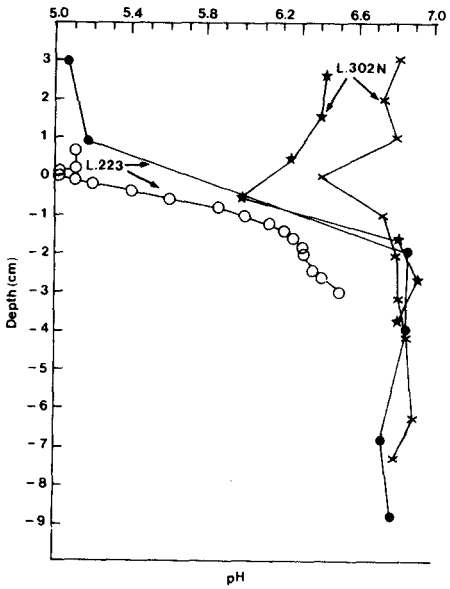

Figure 2. Porewater profiles of pH in epilimnetic sediments of L.223 at 3 and 4 m depth, June 1983 and August 1981 respectively, and in L.302N at 1.5 and 3 m depth, August 1982 and September 1981 respectively.

^{35}S. However, we took 2 cores from each enclosure (2 acid and 2 non-acid) and saw no trend in reduced ^{35}S which could be related to differences in water column pH (Table 2). Acid-volatile sulfide was a major end-product of sulfate reduction in these sediments, as were acid stable form(s) of reduced S (Table 2).

Analyses of total sulfur and acid volatile sulfur in L.302N and L.223 epilimnetic sediments showed that most of the sulfur was in acid stable forms (Table 3).

In the mixed sediment incubations, rate of SO_4^{2-} loss was much slower at pH 4 than at pH 6 (Table 4).

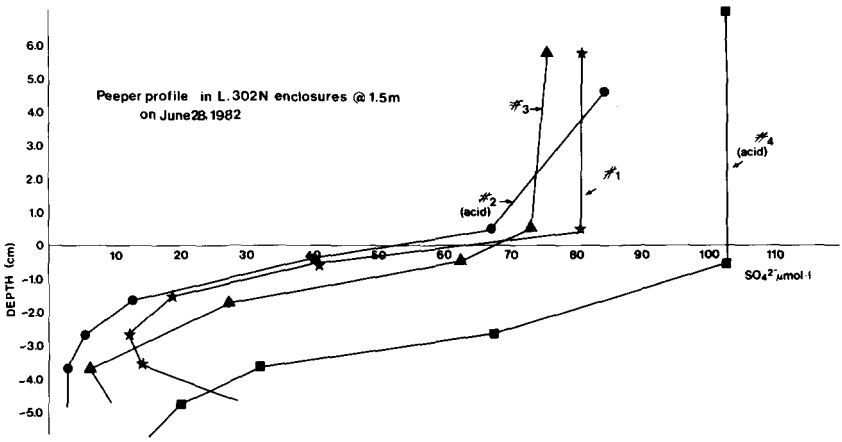

Figure 3. Porewater sulfate concentrations in epilimnetic sediments of four in situ enclosures in L.302N. The pH of two of the enclosures (2 and 4) was controlled at about 5.2 by the addition of HCl, beginning on June 1, 1982.

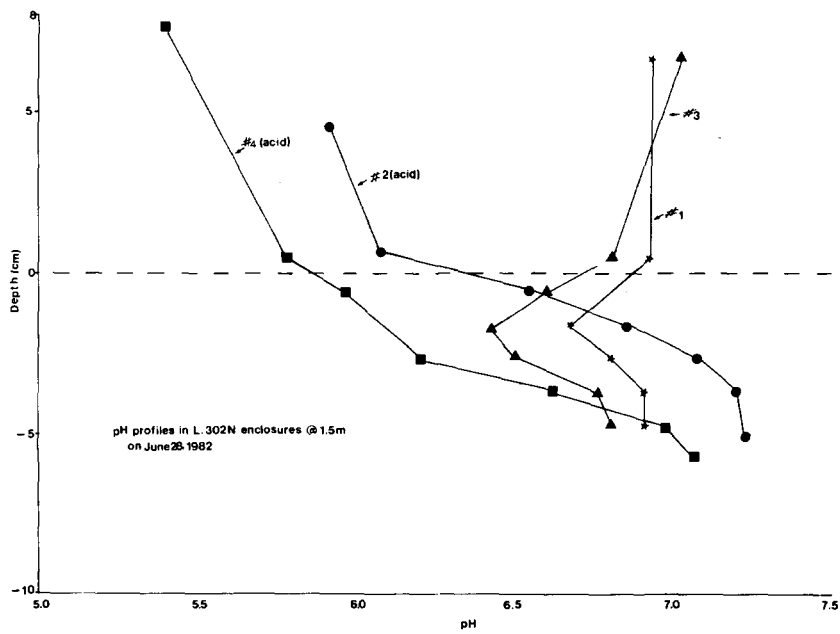

Figure 4. Porewater profiles of pH in epilimnetic sediments (1.5 m) of four in situ enclosures. The pH of the surface water in two of the enclosures (2 and 4) was controlled at about 5.2 by the addition of HCl, beginning on June 1, 1982.

Table 2. AV^{35}S and total reduced ^{35}S in 0–3 cm sections of intact cores obtained from 2 control and 2 acidified enclosures, 2 cores per enclosure, 21 July 1982[a].

Enclosure	AV^{35}S	Total^{35}S
	10^5 dpm	
Control (pH 6.8)	1.9, 1.8, 1.1, 0.6	–, 3.3, 3.0, 2.2
Acidified (pH 5.5–5.2)	2.6, 1.7, 1.2, 1.8	5.8, 3.5, 3.6, 3.2

[a]SO_4^{2-} concentrations in the surface water of the cores were similar (60–70 $\mu mol \cdot L^{-1}$).

Table 3. AVS (acid volatile sulfide) and total S in epilimentic sediments of L.302S and L.223, 0–3 cm

	AVS	Total S
	10^3 $\mu mol \cdot m^{-2}$	
L.302S (1.5 and 3 m)	2.2–13.4[a]	120–150[b]
L.223 (4 m)	12–21[c]	400–450[d]

[a]19 cores, summer 1982.
[b]4 cores, summer 1982.
[c]4 cores, 5 Aug 1982.
[d]2 cores, 6 July 1982.

Table 4. Rates of sulfate reduction in acidified and unacidified L.114 sediments incubated in the laboratory. Porewater pH was controlled by addition of HCl. Sediment was removed from the lake during the winter of 1983.

Initial $[SO_4^{2-}]$ $\mu mol \cdot L^{-1}$	Duration[a]			SO_4^{2-} reduction $\mu mol \cdot g^{-1} \cdot d^{-1}$ –
	pH	d	r^2	
45	6.3	1.4	0.96[1]	1.05
53	4.2	3.0	0.96	0.32
81	6.3	1.5	0.88	1.33
120	4.2	11.3	0.93	0.26
150	6.3	3.1	0.97	1.36
200	4.2	14.0	0.99	0.44

[a]Porewater concentration of SO_4^{2-} as a function of the time was fitted linearly. Disappearance of SO_4^{2-} was followed until concentrations were reduced to 2–6 $\mu mol \cdot L^{-1}$.

Discussion

The data presented in this article demonstrated the occurrence of intense microbial activities in epilimnetic sediments of low organic content (Figures 1–4). These activities resulted in the rapid depletion of oxygen and lead to the creation of an anoxic environment within 1–2 cm of the sediment-water interface which was suitable for sulfate and iron reduction and for methanogenesis. Sulfate and iron reduction consume hydrogen ions (Kelly et al., 1982) and this was probably largely responsible for the observed maintenance of porewater pH above the pH of the overlying water in acidified systems (Figures 2, 4).

It should be noted that the organic carbon content of the epilimnetic sediments of Lakes 223 and 302N was much lower (0.4–1.6%) than that of the hypolimnetic sediments (10–32%, Cook, 1981, and C. Kelly, unpubl. data). However, microbial decomposition rate has been shown to be related to the rate of sedimentation of fresh organic carbon onto the sediment surface (Kelly and Chynoweth, 1981; Robertson, 1978) rather than to the percent organic content of the sediment. If decomposition is rapid, organic inputs to epilimnetic sediments and reduction of dissolved electron acceptors in the sediments could be high even though accumulation rates of organic carbon are low.

The presence of methane near the depths of sulfate and oxygen depletion (Figures 1a, b, c) in all these lakes (223, 302N and 114) indicated that organic substrate was in excess of that needed to support aerobic decomposition and sulfate reduction. It has been shown in many experiments that CH_4 production is an indicator of excess flow of electrons (i.e. excess organic substrate) available for sulfate reducing activity, since electrons will flow preferentially to sulfate if it is available, at the expense of methane production (e.g. Bryant et al., 1977; Reeburgh and Heggie, 1977; Winfrey and Zeikus, 1977). The steepness of the CH_4 gradient may have been partly related to anoxic CH_4 oxidation by sulfate reducers (Devol, 1983; Reeburgh, 1980). However, whether this was occurring or not, the production and presence of methane below the depth of minimum sulfate concentration showed that organic material must have been present in excess of that required for SO_4^{2-} reduction. The same general relationships were shown in the hypolimnia of three ELA lakes where the excess of organic carbon was shown by the much higher flux rates of CH_4 and methanogenic CO_2 than of SO_4^{2-} (Table 5). This is in contrast to marine systems where sulfate concentrations are about 1000 times higher and sulfate reduction is limited by the supply of organic carbon (Westrick and Berner, 1984).

Table 5. Decomposition of organic carbon by sulfate reducing and methanogenic bacteria in the hypolimnia of three ELA lakes during the summer of 1979[a].

Lake	Anoxic decomposition[b] $\mu mol\, m^{-1}\, d^{-1}$	% Decomposition linked to SO_4^{2-} reduction	Methanogenesis[c]	Other anoxic microbial processes[d]
223	9500	20	72	8
226N	10000	16	75	8
227	12000	16	82	2

[a]Recalculated from data presented in Kelly et al. 1982.
[b]Decomposition by all anoxic microbial processes including CO_2 produced by reduction of NO_3^-, SO_4^{2-}, Fe^{3+}, Mn^{++} and $CH_4 + CO_2$ produced by methanogenesis.
[c]The sum of carbon produced as CH_4 and CO_2.
[d]Includes CO_2 produced during the reduction of NO_3^-, Fe^{3+} and Mn^{2+}.

When decomposable organic carbon is in excess, SO_4^{2-} reduction rates should be related to the concentration of SO_4^{2-} in the overlying water. There is some evidence that this was the case. For example, increasing the SO_4^{2-} concentration in the overlying water of replicate cores resulted in increased rates of SO_4^{2-} loss to the sediments (Table 1). A similar relationship between increasing SO_4^{2-} concentration in water overlying sediments and increasing SO_4^{2-} loss to sediments has also been observed in situ in the hypolimnion of L.223 where SO_4^{2-} concentrations have increased due to experimental acidification (Cook and Schindler, 1983). In addition, the epilimnetic pore water gradients shown here (Figure 1b) showed that the slope of the gradient in each case was related to the concentration of SO_4^{2-} in the overlying water, with higher concentraions related to steeper gradients. The porosities of these sediments were similar in all cases except for L.114 (see Results). Thus, the steeper gradients were indicative of faster flux rates into the sediments and therefore faster sulfate reduction rates.

We could not directly measure sulfate reduction rates in our freshwater sediments using recently developed $^{35}SO_4^{2-}$ core injection techniques (e.g. Jorgensen, 1978), because these methods were inappropriate for our freshwater systems. These core injection techniques were developed for marine sediments where there is not a significant consumption of labelled sulfate during the incubation period. In our freshwater systems much more than tracer quantities of $^{35}SO_4^{2-}$ (up to 50%) were consumed during a 4 hour incubation period. Also, the steep gradients of SO_4^{2-} meant that diffusion between injection intervals was significant, which is an unacceptable condition for this method (Jorgensen 1978). Other workers in freshwater sediments have discussed the difficulties of using $^{35}SO_4^{2-}$ to measure sulfate reduction rates (Smith and Klug, 1981; Ingvorsen et al., 1981). We are presently developing dual label (^3H_2O and $^{35}SO_4^{2-}$) diffusion methods which will overcome these problems of estimating rates of sulfate reduction in intact cores of freshwater sediments.

Even though the core injection technique could not be used to estimate absolute rates of sulfate reduction, the types of end-products formed during sulfate reduction in sandy epilimnetic sediments were partially identified by using $^{35}SO_4^{2-}$ added to the surface water of cores. In the cores taken from the experimental enclosures in L.302N, acid volatile sulfide was a major end-project (27–56%, Table 2). Acid volatile sulfides include both H_2S and FeS, but H_2S was not detectable or at extremely low levels (less than 2 $\mu mol \cdot L^{-1}$) in these sediments (Figure 1d), just as it was in L.223 sediments (Figure 1d). Therefore, most of the $AV^{35}S$ recovered was probably derived from $Fe^{35}S$. The AVS content of the epilimnetic sediments of both L.223 and L.302S (Table 3) was a smaller fraction of the total sulfur than the fraction of $AV^{35}S$ (Table 2) formed during the short term core incubations. This could be due to slow formation of pyrite from FeS (Berner, 1971) or to some other factor causing instability of the AVS on a long term basis.

In addition to AV^{35}S, a significant portion of the reduced ^{35}S was found in an acid-stable component (Table 2). This could have been elemental sulfur, organic sulfur, and/or pyrite. Howarth (1979) has shown that rapid formation of FeS_2 is possible under conditions where the Fe^{2+} and S^{2-} ion activity product exceeds the solubility product of FeS_2, but is lower than the Ksp's for the iron monosulfides (Giblin and Howarth, 1984). This seems possible in L.223 and L.302 because Fe^{2+} concentrations are high near the surface (greater than 100 μmol $\cdot$ L^{-1}, e.g. Figure 1d) where H_2S is undetectable (Figure 1d). In order to predict the most likely precipitation product, however, we need to know the H_2S concentration more accurately within the range 0–1 μmol $\cdot$ L^{-1}. Preliminary analyses for pyrite using a chromium reduction technique (Howarth and Jorgensen, submitted) indicate that a major sulfur form L.302 epilimnetic sediments is pyrite, and we are currently investigating the possibility of its rapid formation with the use of ^{35}S.

Lake acidification appeared to have had no effect on epilimnetic sulfate reduction in situ. This was demonstrated by the similarity of sulfate profiles in the acidified and unacidified L.302N enclosures (Figure 3), and in acidified L.223, and unacidified L.302N (Figure 1b). This was also shown by the similar ^{35}S incubation results in cores taken from the acidified and unacidified enclosures (Table 2). This lack of effect was apparently due to maintenance of porewater pH's above epilimnetic pH (Figures 2, 4). When sediment was mixed and the pH was artificially lowered and maintained at a low level (pH = 4.2), sulfate reduction rates were obviously inhibited (Table 4). Inhibition has also been shown for sulfate reducers in soils, with activity falling rapidly at pH's below 6.8 (Connell and Patrick, 1968). However, in its natural, unmixed state, only about the top 1 cm of the sediment in L.223 was at a pH less than 6.0, after 8 years of acidification (Figure 2). Thus, eight years after the commencement of experimental acidification, most of the zone of sulfate reduction (0–3 cm) in the epilimnetic sediments has remained at a pH above surface water pH (Figure 2). Another possible reason for the lack of effect is that the sulfate reducing population in L.223 and in the acidified enclosures adapted to the lower pH at and near the sediment surface. Sulfate reduction occurs in acid bogs at pH's as low as 3.8 (Hemond, 1980).

During the initial years of acidification, steep chemical gradients in surface sediments could be dependent upon the chemical buffering and cation exchange capacity of the sediments as well as microbial H^+ consumption. To see if microbial H^+ consumption could maintain porewater pH on the long-term (i.e. $>$ 8 yr), we have recently examined sediments from Adirondack lakes which have been atmospherically acidified for several decades. In Dart Lake in the Adirondacks, the pH and sulfate gradients in the epilimnetic sediments were even steeper than in L.223, indicating that longterm maintenance of porewater pH by microbial activity is very possible (unpublished data).

A continued supply of organic substrate is necessary for formation of all

forms of reduced S from SO_4^{2-}. Lake 223 is oligotrophic (primary production = 17–50 g C · m^{-2} yr^{-1}; Schindler, 1980), but there could be lakes of extremely low productivity where it is conceivable that organic supply, rather than diffusion of sulfate into the sediments, could be a major factor in determining rates of sulfate reduction.

The buffering produced by reduction of sulfate to any end-product will only last as long as that product remains in a reduced form. One possible pathway of re-oxidation could be the diffusion of dissolved sulfide out of the sediments to oxygenated water. It is unlikely that this pathway could be of major importance in L.223 because dissolved sulfide concentrations were so low (Figure 1d). Secondly, bioturbation could mix reduced sulfur upward to depths with higher oxygen concentrations. Oxidation of reduced particulate sulfur to dissolved SO_4^{2-} could also occur during periods of low microbial activity which would lead to increased penetration of oxygen into the sediments. This phenomenon has been observed in marsh sediments (Giblin and Howarth, 1984). The data available for our sediment showed that during winter there was a slightly deeper penetration of SO_4^{2-} into the sediments, but the same concentration gradient (unpublished data). Even if some re-oxidation is occurring, SO_4^{2-} budgets for Lakes 223 and 114 (Schindler and Turner, 1982) indicate that there is an appreciable net SO_4^{2-} loss on an annual basis. Nevertheless, it will be important to study factors controlling the balance between suflur reduction and re-oxidation processes in the future because this balance will contribute to the rate of acidification and recovery of lakes.

Acknowledgements

R.H. Hesslein and R. Howarth provided invaluable advice on sediment pore water and sulfur chemistry methodologies, and provided helpful review. V. St. Louis, E. Schindler and K. Beaton collected and analyzed numerous field samples. A. Furutani expertly produced the laboratory sulfate reduction data. R.J. Flett generously provided advice on microelectrode techniques. Lake water chemistry samples were collected by the ELA sampling team headed by D. Cruikshank and J. Penny. These samples were analyzed by the ELA analytical group headed by G. Linsey and J. Prokopowich. This work was supported by the Department of Fisheries and Oceans, Government of Canada and NSERC grant A2671.

References

Berner, R. A.1971. Principles of Chemical Sedimentology. McGraw-Hill. 240 p.

Bryant, M. P., L. L. Campbell, C. A. Reddy, and M. R. Crabill. 1977. Growth of Desulfovibrio in lactate or ethanol media low in sulfate in association with H_2-utilizing methanogenic bacteria. Applied and Environmental Microbiology 33: 1162–1169.

Connell, W. E. and W. H. Patrick, Jr. 1968. Sulfate reduction in soil: Effects of redox potential and pH. Science 159: 86–87.

Cook, R. B. 1981. The biogeochemistry of sulfur in two small lakes. Ph.D. thesis, Columbia University. 234 p.

Cook, R. B. and D. W. Schindler. 1983. The biogeochemistry of sulfur in an experimentally acicified lake. Environmental Biogeochemistyr Ecological Bulletin (Stockholm) 35: 115–127.

Devol, A. H. 1983. Methane oxidation rates in the anaerobic sediments of Saanich Inlet. Limnology and Ocenaography 28: 738–742.

Furutani, A., J. W. M. Rudd, and C. A. Kelly. In press. A method for measurement of the response of sediment microbial communities to environmental perturbations. Canadian Journal of Microbiology.

Giblin, A. E. and R. W. Howarth. 1984. Porewater evidence of a dynamic sedimentary iron cycle in salt marshes. Limnology and Oceanography 29: 47–63.

Hemond, H. F. 1980. Biogeochemistry of Thoreau's Bog, Concord, Massachusetts. Ecological Monographs 50: 507–526.

Hesslein, R. H. 1976. An in situ sampler for close interval pore water studies. Limnology and Oceanography 21: 912–914.

Howarth, R. W. 1979. Pyrite: its rapid formation in a salt marsh and its importance in ecosystem metabolism. Science 203: 49–51.

Howarth, R. W. and B. B. Jorgensen. Submitted manuscript. Formation of pyrite and elemental sulfur in coastal marine sediments (Limfjorden and Kysing Fjord, Denmark) during short-term $^{35}SO_4^{2-}$ reduction measurements.

Howarth, R. W. and J. M Teal. 1979. Sulfate reduction in a New England salt marsh. Limnology and Oceanography 24: 999–1013.

Ingvorsen, K. and T. D. Broack. 1982. Electron flow via sulfate reduction and methanogenesis in the anaerobic hypolimnion of Lake Mendota. Limnology and Oceanography 27: 559–564.

Ingvorsen, K., J. G. Zeikus, and T. D. Brock. 1981. Dynamics and bacterial sulfate reduction in a eutrophic lakes. Applied and Environmental Microbiology 42: 1029–1036.

Jorgensen, B. B. 1978. A comparison of method for the quantification of bacterial sulfate reduction in coastal marine sediments. Geomicrobilogy Journal 1: 29–47.

Kelly, C. A. and D. P. Chynoweth. 1981. The contribution of temperature and organic input in controlling rates of sediment methanogenesis. Limnology and Oceanography 26: 891–897.

Kelly, C. A., J. W. M. Rudd, R. B. Cook, and D. W. Schindler. 1982. The potential importance of bacterial processes in regulating rate of lake acidification. Limnology and Oceanography 27: 868–882.

Nriagu, J. O. and R. D. Coker. 1983. Sulphur in sediments chronicles past changes in lake acidification. Nature 303: 692–694.

Reeburgh, W. S. 1980. Anaerobic methane oxidation: rate depth distributions in Skan Bay sediments. Earth and Planetary Science Letters 47: 345–352.

Reeburgh, W. S. and D. T. Heggie. 1977. Microbial methane consumption reactions and their effect on methane distribution in freshwater and marine environments. Limnology and Oceanography 22: 1–9.

Robertson, C. A. 1978. Natural rates of methane production and their significance to carbon cycling in two small lakes. Ph.D. Thesis, Univ. of Michigan. 180 p.

Rudd, J. W. M., M. A. Turner, B. E. Townsend, A. Swick, and A. Furutani. 1980. Dynamics of selenium in mercury-contaminated experimental freshwater ecosystems. Canadian Journal of Fisheries and Aquatic Sciences 37: 848–857.

Schindler, D. W. 1980. Experimental Acidification of a Whole Lake: A Test of the Oligotrophication Hypothesis. Proceedings of an International Conference on the Ecological Impact of Acid Precipitation. Norway 1980, SNSF Project.

Schindler, D. W. and M. A. Turner. 1982. Biological, chemical and physical responses of lakes to experimental acidification. Water, Air and Soil Pollution 18: 259–271.

Schindler, D. W., R. Wagemann, R. B. Cook, T. Ruszczynski, and J. Prokopowich. 1980. Experimental acidification of Lake 223, Experimental Lakes Area: Background data and the first three years of acidification. Canadian Journal of Fisheries and Aquatic Sciences 37: 342–354.

Smith, R. L. and M. J. Klug. 1981. Electron donors utilized by sulfate-reducing bacteria in eutrophic lake sediments. Applied and Environmental Microbiology 42: 116–121.

Stainton, M. P., M. J. Capel, and F. A. J. Armstron. 1977. The chemical analysis of freshwater. 2nd Ed. Canadian Fisheries and Marine Service Miscellaneous Special Publication 25. 166p.

Westrich, J. T. and R. A Berner. 1984. The role of sedimentary organic matter in bacterial sulfate reduction: the G. model tested. Limnology and Oceanography 29: 236–249.

Winfrey, M. R. and J. G. Zeikus. 1977. Effect of sulfate on carbon and electron flow during microbial methanogenesis in freshwater sediments. Applied Environmental Microbiology 33: 275–281.