*Limnol. Oceanogr.*, 26(5), 1981, 880–890

# The role of phototrophic bacteria in the sulfur cycle of a meromictic lake[1]

*T. B. Parkin*
Department of Crop and Soil Science, Michigan State University, East Lansing 48824

*T. D. Brock*
Department of Bacteriology, University of Wisconsin, Madison 53705

*Abstract*

During the summer months a dense population of green sulfur bacteria was observed in meromictic Knaack Lake, Wisconsin, at a depth where oxygen was not present. During the day, $H_2S$ was also absent at this depth but built up at night. $^{14}CO_2$ studies revealed that in the light the green bacteria were photosynthetically active and oxidized the $H_2S$. The ratio of $H_2S$ oxidized to $CO_2$ fixed was about 0.5, indicating that $H_2S$ was completely oxidized to sulfate. In the dark, $CO_2$ fixation did not occur and $H_2S$ accumulated. These results are related to the diurnal changes of $H_2S$ in the lake and to sulfate reduction rates in the water column.

Although there have been many studies of the dissimilatory sulfur metabolism of pure cultures of photosynthetic sulfur bacteria (Trüper and Schlegel 1964; Trüper and Pfennig 1966; Trüper 1964; van Gemerden and Jannasch 1971) and of the sulfur relationships of mixed pure cultures of photosynthetic bacteria and sulfate- or sulfur-reducing bacteria (van Gemerden 1967; Butlin and Postgate 1954; Biebl and Pfennig 1978), relatively few investigations of sulfur relationships in natural populations of photosynthetic sulfur bacteria have been made. In Lake Belovod sulfide concentrations at the chemocline decreased during the day and increased at night (Sorokin 1970). The decreases were thought to be due to photosynthetic oxidation by *Chromatium*, which was observed to migrate up and down the water column of the lake, following the diurnal changes of sulfide concentration. van Gemerden (1967) observed a similar situation in Lake Pluss. In the chemocline region, sulfide concentration decreased by day and increased at night. He proposed that the sulfide decreases were due to oxidation by purple sulfur bacteria and the increases to sulfate reduction. The sulfur cycle in Solar Lake has been elucidated (Jørgensen et al. 1979). At the chemocline in this highly stratified lake, oxygen and sulfide coexist in a 10-cm layer and changes in concentrations of these compounds were observed in a 20–30-cm layer over a diurnal cycle. During the day, sulfide was oxidized by cyanobacteria which carried out anoxygenic photosynthesis in the morning and oxygenic photosynthesis in the afternoon when sulfide concentrations had been reduced. From sulfate reduction rates in both the water column and the sediment and from the rates of sulfide oxidation, it was determined that most of the sulfide in the water at night was produced by the reduction of $S^0$ and $S_2O_3^{2-}$ which accumulated in the light. Photosynthetic sulfur bacteria were unimportant in these processes in Solar Lake.

Our study was designed to investigate the sulfur cycle at the thermocline of meromictic Knaack Lake with emphasis on how the photosynthetic bacteria in the lake affect and are affected by these transformations. During summer stratification there is a zone in the water column where neither sulfide nor oxygen is present, so that our study of sulfur transformations was relatively uncomplicated, as transformations by colorless sulfur bacteria and molecular oxygen did not have to be distinguished.

[1] This work was supported by National Science Foundation grant DEB 790603 and National Institutes of Health training grant 5T32GMO7215.

We thank C. Knaack for providing access to Knaack Lake and N. Parkin and T. Phelps for technical assistance.

## *Materials and methods*

*Sampling methods*—Water samples were collected over the deepest part of the lake with a peristaltic pump (Horizon Ecology Co.) (Winfrey and Zeikus 1979). The inflow tubing consisted of amber latex (0.48-cm i.d.) and was connected to a chain which prevented stretching of the tube and allowed sampling at very narrow intervals. Samples taken for diurnal changes in sulfide and bacteriochlorophyll were collected at 20-cm depth intervals about every 3 h, filtered immediately, and the filters stored on ice for bacteriochlorophyll assays done in the laboratory.

Light extinction in the lake was measured with a LiCor model-185 quantum meter.

*Lake chemistry*—Water samples (10 ml) for sulfide analysis were collected in screwcap test tubes containing 0.5 ml of a 2% zinc acetate in 0.2% acetic acid. Sulfide was determined by the methylene blue method (Pachmayr 1960; described by Parkin and Brock 1980*a*). The limit of detection of this assay is 0.05 mg·liter$^{-1}$.

Sulfate was determined by a modification of the reduction method of Johnson and Nishita (1952). Samples were collected in 500-ml Nalgene bottles and 5 ml of 20% zinc acetate was added to prevent the oxidation of sulfide present in some samples. In the laboratory the ZnS was removed by acidification with 5 ml of concentrated HCl and bubbling with $N_2$ for 1 h and the elemental sulfur was then removed by filtration through glass-fiber filters (Gelman GF/C). The water was concentrated to dryness with a rotary evaporator (Buchi Rotavapor-R). A reduction mixture (4 ml), prepared by mixing 15 g of red phosphorus, 125 ml of hydriodic acid, and 90 ml of formic acid and heating for 2 h under a stream of $N_2$ at 115°C, was added to the samples. Samples were reduced with this reagent in a distillation apparatus similar to that of Johnson and Nishita (1952) for 1 h under a stream of $N_2$ and the $H_2S$ gas formed was distilled into a test tube containing 5 ml of 2% zinc acetate. Sulfide was then assayed as above. The reduction efficiencies, checked in all samples by adding 1 $\mu$Ci of $Na_2{}^{35}SO_4{}^{2-}$ and determining the amount of $^{35}S^{2-}$ produced after the reduction was complete, ranged from 60 to 96%.

This procedure measures only soluble inorganic sulfur compounds including sulfate, thiosulfate, trithionate, tetrathionate, and sulfite. However, trithionate, tetrathionate, and sulfite are rarely present in natural waters (Pfennig 1975) and thiosulfate was undetectable in Knaack Lake (*see below*), so most of the sulfur detected by this method was probably in the form of sulfate. Organic sulfur compounds, both particulate and soluble, are not affected by this assay (Johnson and Nishita 1952).

The extraction methods of Pachmayr (1960) were not satisfactory for elemental sulfur analysis in Knaack Lake; organic materials in the water caused high blanks, reducing the sensitivities of the assays. We used an alternate method in which elemental sulfur was concentrated on glass-fiber filters (GF/C) and the sulfur on the filters analyzed by the reduction method described above. A standard curve was constructed by filtering colloidal sulfur solutions through GF/C filters and running these filters through the reduction procedure.

Thiosulfate in Knaack Lake was determined by the methylene blue bleaching method of Pachmayr (1960) (described by Trüper and Pfennig 1966). The detection limit of this assay was 0.064 mg $S_2O_3{}^{2-}$-S·liter$^{-1}$.

Total sulfur analyses were done on several samples, by a perchlorate digestion method, at the Soils and Plant Analysis Laboratory, Madison, Wisconsin.

Oxygen was determined by the azide modification of the Winkler assay (Am. Public Health Assoc. 1976). Samples for oxygen were collected in 60-ml glass-stoppered bottles and fixed in the field by adding 1 ml of the Mn reagent and 1 ml of the NaOH reagent. Titrations were

19395590, 1981, 5, Downloaded from https://aslopubs.onlinelibrary.wiley.com/doi/10.4319/lo.1981.26.5.0880 by University Of Wisconsin, Wiley Online Library on [25/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

done immediately on return to the laboratory.

Dissolved inorganic carbon (DIC) was measured by the gas stripping method of Rudd et al. (1974). Samples (5 ml) were collected in test tubes (25 ml) that had been sealed with butyl rubber stoppers and flushed with $N_2$. In the laboratory the samples were acidified with 0.5 ml of 6 N $H_2SO_4$ and 0.5 ml of gas from the headspaces of the tubes was injected into a Packard gas chromatograph (model 410) for $CO_2$ determinations.

We determined bacteriochlorophyll (Bchl) after filtering lake water through GF/C filters and extracting the filters overnight with 90% acetone at 4°C. Bchl concentrations were measured spectrophotometrically using the extinction coefficients of Takahashi and Ichimura (1970).

Samples for routine chemical analyses were collected about every 2 weeks.

*$CO_2$ fixation-sulfide oxidation*—Water was collected at the depth of peak concentrations of photosynthetic bacteria and incubated in the lake or under in situ conditions in the laboratory. For laboratory incubations, the water was collected in glass bottles (1,200 ml) and transported to the laboratory on ice, in the dark. $NaH^{14}CO_3$ (100 $\mu$Ci) was then added, the water dispensed into 60-ml glass-stoppered bottles (Parkin and Brock 1980*b*), and hydrogen sulfide was added in the form of a neutralized sodium sulfide solution (pH 6.8). The bottles were then incubated at a light intensity of 0.7 $\mu$Einst$\cdot m^{-2} \cdot s^{-1}$ at 10°C, with a red Plexiglas filter put between the bottles and the fluorescent bulbs to simulate the spectrum of light at 2.2 m in the lake (Parkin and Brock 1980*b*).

The effect of sulfide concentration on photosynthesis was determined by adding different amounts of a neutralized sodium sulfide solution to lake water collected in 60-ml glass-stoppered bottles. These bottles were incubated at 10°C with 4 $\mu$Ci $NaH^{14}CO_3$ for 6 h at 0.7 $\mu$Einst$\cdot m^{-2} \cdot s^{-1}$.

For the in situ incubations, water was collected in 60-ml glass-stoppered bottles, 4 $\mu$Ci $NaH^{14}CO_3$ added, and the bottles returned to the lake. At intervals, bottles were removed and sulfide concentrations and $^{14}CO_2$ incorporation into the cells measured. The latter was determined by filtering the contents of the bottles through 0.45-$\mu$m membrane filters (Gelman GN-6 GD); the filters were then fumed in HCl for 6 h, dried, and counted in toluene-based scintillation cocktail with a Packard Tri-Carb liquid scintillation counter (model 3375). Quench corrections were made by the channels ratio method.

All isotope solutions were prepared in water which had been boiled, then gassed with $N_2$ for 1 h. The headspaces of the isotope vials were then evacuated and flushed 10 times with $N_2$. These precautions ensured that $O_2$ contamination of the lake water from the isotope additions was minimal.

*Sulfate reduction*—In situ sulfate reduction rates were measured by collecting lake water in 60-ml bottles, adding $2.2 \times 10^6$ dpm of $Na_2{}^{35}SO_4$ which had a specific activity of 1,000 mCi$\cdot$mM$^{-1}$ (New England Nuclear Corp.) and incubating the bottles at collection depths for 3 h. The reactions were stopped by adding 1 ml of a 20% zinc acetate solution and 1 ml of a 20% zinc sulfide solution; this preserved the samples so that there was no loss of labeled sulfide. In the laboratory the samples were acidified in a distillation apparatus under $N_2$ and the $H_2{}^{35}S$ gas produced was trapped in 10 ml of 2% zinc acetate. The ZnS precipitate was then counted in 10 ml of Aquasol in a Beckman liquid scintillation counter. Efficiencies of this distillation procedure were >90%.

The effect of acetate or sulfate concentration on sulfate reduction rates was evaluated by incubating lake water for 6 h at 10°C with different concentrations of acetate or $Na_2SO_4$ and $1.11 \times 10^7$ dpm of $Na_2{}^{35}SO_4$ and then determining the amount of labeled $H_2S$ formed as described above. Where sulfate was added, the different specific activities at each

19395590, 1981, 5, Downloaded from https://aslopubs.onlinelibrary.wiley.com/doi/10.4319/lo.1981.26.5.0880 by University Of Wisconsin, Wiley Online Library on [25/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

sulfate concentration were corrected for by calculating the amount of sulfate reduced as

$$(H_2{}^{35}S/{}^{35}SO_4{}^{2-}) \times 1.06 \times \text{mg } SO_4{}^{2-}\text{-S} \cdot \text{liter}^{-1}$$

where $H_2{}^{35}S$ is the amount of radioactive $H_2S$ formed (in dpm), ${}^{35}SO_4{}^{2-}$ is the amount of radioactive $SO_4{}^{2-}$ added (in dpm), and $SO_4{}^{2-}\text{-S} \cdot \text{liter}^{-1}$ is the sulfate concentration of the lake water.

*Counts of sulfate-reducing bacteria*—Numbers of sulfate-reducing bacteria in Knaack Lake were estimated by a dilution tube method in Postgate's medium B to which both lactate and acetate had been added (Postgate 1979).

## Results

*Study area*—Knaack Lake is on farmland in central Wisconsin. It has a surface area of 1.1 ha, a maximum depth of 22 m, and mean depth of 7 m. The lake's physical and chemical limnology have been under investigation since 1976; it is biogenically meromictic.

In spring and summer, the lake is thermally stratified with a thermocline at 1.5–3.5 m. A chemocline (the zone where the greatest increase in conductivity with depth occurs) was present all year at 15 m. During stratification $H_2S$ is present up to the bottom of the thermocline. Because the thermocline is so shallow in spring and summer, light penetrates to the $H_2S$-containing waters and a population of photosynthetic sulfur bacteria develops. The light intensities reaching these organisms are low, 0.05–2 $\mu$Einst$\cdot m^{-2} \cdot s^{-1}$ (Parkin and Brock 1980*b*). The photosynthetic bacteria, containing bacteriochlorophyll *d*, are species of *Pelodictyon* and *Clathrochloris*. Peak concentrations of these organisms in spring are in the $H_2S$-containing waters where light is available. During summer there is a Bchl *d* maximum just above the $H_2S$-containing waters (Fig. 1); oxygen was undetectable at 2.0 m while $H_2S$ was first detected in the lake at 2.4 m, with maximum concentration of Bchl *d* at 2.2 m.

Sulfate concentrations were very low

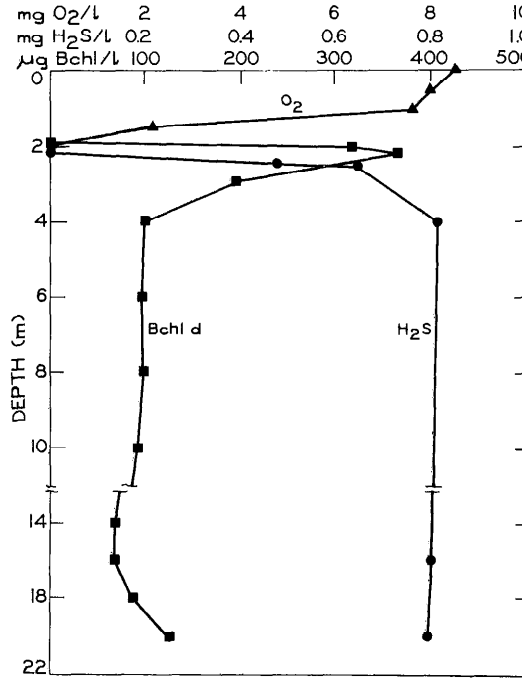


Fig. 1. Profiles of oxygen, bacteriochlorophyll *d*, and hydrogen sulfide in Knaack Lake, 21 June 1979.

at all times of year. In spring and summer, sulfate concentrations in the epilimnion ranged from 0.5 to 0.7 mg$\cdot$liter$^{-1}$ and in the hypolimnion from 0.1 to 0.2 mg $SO_4{}^{2-}\text{-S} \cdot \text{liter}^{-1}$. Below the chemocline, sulfate was undetectable. Elemental sulfur was found in the lake only during the partial mixing period in November, when 20–30 $\mu$g $S^0 \cdot \text{liter}^{-1}$ were present throughout the water column, probably produced by chemical oxidation of $H_2S$ by the molecular oxygen which was present down to 15 m at this time. Thiosulfate was never detected in the lake.

*Sulfide oxidation and $CO_2$ fixation*—Because the green sulfur bacteria in Knaack Lake develop maximum numbers at a depth where we could find no electron donors for photosynthesis, we did experiments to determine if this population was photosynthetically active (Fig. 2). When 2.2-m lake water was incubated in situ with $^{14}CO_2$, in the light, $CO_2$ was incorporated at a rate of 1.57 $\mu M \cdot h^{-1}$

19395590, 1981, 5, Downloaded from https://aslopubs.onlinelibrary.wiley.com/doi/10.4319/lo.1981.26.5.0880 by University Of Wisconsin, Wiley Online Library on [25/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

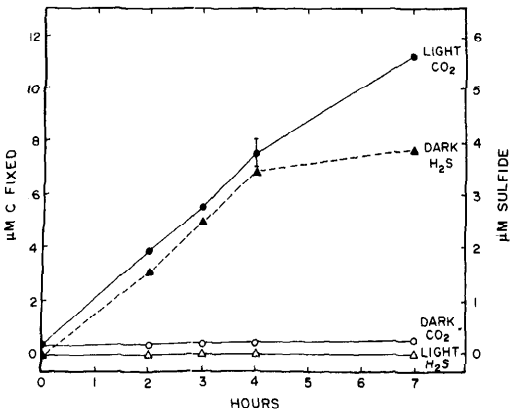


Fig. 2. Rates of $CO_2$ fixation and sulfide production by 2.2-m Knaack Lake population. Water collected on 7 July 1979. Sulfide and oxygen were not detected. Samples were incubated at 2.2 m in the lake. Sulfide was not detected in any light bottles. Error bar indicates range of duplicate bottles.

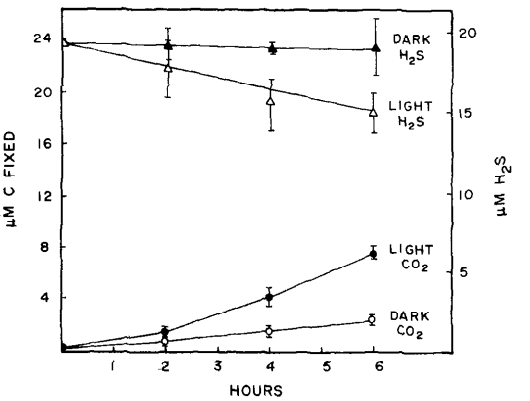


Fig. 3. Rates of sulfide oxidation and $CO_2$ fixation by Knaack Lake bacteria when sulfide had been added. After sulfide addition sulfide concentration was 17 $\mu$M. Error bars indicate ranges of duplicate bottles.

while the dark $CO_2$ fixation rate was 0.07. There was no $H_2S$ in any of the light bottles, but in the dark $H_2S$ accumulated at a rate of 0.88 $\mu M \cdot h^{-1}$ during the first 4 h. Thus, despite the absence of $H_2S$, elemental sulfur, and thiosulfate from these waters, the green sulfur bacteria at 2.2 m were photosynthetically active. Because $H_2S$ was produced in the dark, it was probably also produced in the light and served as the electron donor for bacterial photosynthesis but did not accumulate in the light because it was oxidized as rapidly as it was produced. If we assume that the rate of $H_2S$ production in the light was the same as in the dark, then the ratio of $H_2S$ oxidized to $CO_2$ fixed in the light would equal 0.57. This is very close to the theoretical ratio of 0.5 if the $H_2S$ were oxidized completely according to the equation:

$$H_2S + 2CO_2 + 2H_2O \rightarrow 2(CH_2O) + 2H^+ + SO_4^{2-}.$$

We measured rates of sulfide oxidation by the photosynthetic bacterial population directly by adding sulfide to lake water and following sulfide decreases in the light and $CO_2$ fixation rates (Fig. 3). In the dark, sulfide remained constant, while in the light it was oxidized at a rate of 0.57 $\mu M \cdot h^{-1}$. $CO_2$ was fixed in the light at a rate of 0.88 $\mu M \cdot h^{-1}$. The ratio of sulfide oxidized to $CO_2$ fixed in this experiment was 0.67, indicating the reactions described by the above equation.

Because it appears that $H_2S$ was oxidized completely to sulfate, and $S^0$ and $S_2O_3^{2-}$ could not be detected in the lake, the most likely source of the $H_2S$ production was from dissimilatory sulfate reduction.

*Sulfate reduction*—Sulfate reduction was measured in both the light and the dark using $^{35}SO_4^{2-}$ (Fig. 4). In the dark, the rate of sulfate reduction was maximal at 2.2 m (0.34 $\mu M \cdot h^{-1}$); in the rest of the water column sulfate reduction rates were low, from 0.011 to 0.007. In the photic zone, rates of sulfate reduction (sulfide production) in the light were lower than in the dark bottles, presumably because the sulfide produced was being reoxidized by the green sulfur bacteria.

At 2.2 m deep we found about $5 \times 10^3$ sulfate-reducing bacteria $\cdot ml^{-1}$; below this, numbers were an order of magnitude lower. Thus apparently the green sulfur bacteria at 2.2 m were being supplied with reducing power for photosynthesis from dissimilatory sulfate reduction at this same depth.

*Effect of $H_2S$ on photosynthesis*—To

19395590, 1981, 5, Downloaded from https://aslopubs.onlinelibrary.wiley.com/doi/10.4319/lo.1981.26.5.0880 by University Of Wisconsin, Wiley Online Library on [25/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

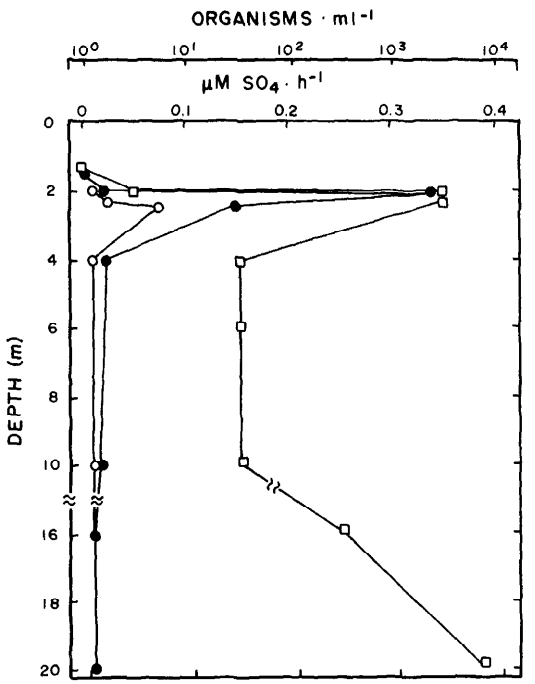


Fig. 4. Profiles of sulfate reduction rates and numbers of sulfate-reducing bacteria in Knaack Lake water column. Samples collected on 17 July 1979. ○—Light sulfate reduction rates; ●—dark sulfate reduction rates; □—sulfate-reducing bacteria.

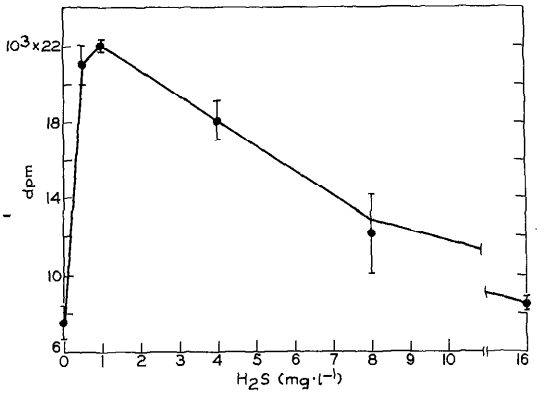


Fig. 5. Photosynthetic response of Knaack Lake green sulfur bacteria to increased sulfide concentrations. Lake water from 2.2 m collected on 7 July 1979. Sulfide and oxygen were undetectable. Sulfide concentrations were 0.0, 0.5, 1.0, 5.0, 10.0 and 20.0 mg·liter$^{-1}$. Error bars indicate ranges of duplicate bottles. Dark $^{14}CO_2$ uptake (not shown) was constant at 700 dpm at all sulfide concentrations.

determine if the rate of supply of $H_2S$ was high enough so that it did not limit bacterial photosynthesis, we incubated 2.2-m lake water with increasing concentrations of $H_2S$ and measured photosynthesis (Fig. 5). Even at the in situ sulfide concentration (undetectable) $CO_2$ was fixed; however, increasing sulfide concentrations stimulated the photosynthetic rate so that at 1.0 mg $H_2S$-S·liter$^{-1}$ there was a 3-fold increase. These results suggest that photosynthesis by the sulfur bacteria in the upper zone of the hypolimnion is sulfide limited and, since the sulfide is supplied by sulfate-reducing bacteria, that the green sulfur bacteria at 2.2 m are ultimately limited by the sulfate reduction rate.

*Effect of acetate and sulfate on sulfate reduction*—We determined the effect of sulfate concentrations on sulfate reduction indirectly by incubating 2.2-m lake water with increasing concentrations of sulfate and following changes in photosynthetic rate using $^{14}CO_2$ as a tracer. If sulfate concentration were limiting sulfide production, increasing sulfate should have stimulated sulfide formation and, therefore, photosynthesis by the green sulfur bacteria. However, $CO_2$ fixation rates did not increase over the range of sulfate concentrations that we tested (Table 1). Sulfate reduction rates at different sulfate concentrations were also measured directly: radioactive sulfate was added to lake water to which increasing concentrations of sulfate had also been added and $^{35}S^{2-}$ formation determined. Sulfate reduction rates were essentially

Table 1. Effects of sulfate concentration (mg·liter$^{-1}$) on photosynthesis and sulfate reduction ($\mu$g·liter$^{-1}$·h$^{-1}$).

| $SO_4^{2-}$-S concn | Photosynthesis (cpm) | $SO_4^{2-}$-S concn | $SO_4^{2-}$-S reduced |
|---|---|---|---|
| 0.3 | 3,190±570 | 0.42 | 3.07±0.31 |
| 0.8 | 2,800±169 | 10.4 | 2.71±0.60 |
| 1.3 | 2,530±254 | 50.4 | 3.45±0.15 |
| 5.3 | 2,660±300 | 100 | 3.60±0.85 |
| 10.3 | 2,650±300 | | |
| 20.3 | 2,410±170 | | |

19395590, 1981, 5, Downloaded from https://aslopubs.onlinelibrary.wiley.com/doi/10.4319/lo.1981.26.5.0880 by University Of Wisconsin, Wiley Online Library on [25/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Table 2. Effect of acetate concentration (mM) on sulfate reduction rates (cpm × $10^{-2}$) in the light and dark.

| Acetate concn | Sulfate reduction | |
|---|---|---|
| | light | dark |
| 0 | 150±50 | 275±31 |
| 0.01 | 400±40 | 420±25 |
| 0.1 | 360±55 | 750±150 |
| 1.0 | 420±20 | 750±45 |

identical at each sulfate concentration. Thus, despite the low sulfate concentrations in Knaack Lake, sulfate does not seem to be limiting sulfate reduction at 2.2 m.

The effect of acetate on sulfate reduction is shown in Table 2. In both light and dark, an acetate concentration of 0.1 mM increased sulfate reduction rates by a factor of 2.5. The lower rates in the light incubations are probably a function of two processes. Since green sulfur bacteria incorporate acetate into cell material if light and $H_2S$ are available (Pfennig and Biebl 1976; Sadler and Stanier 1960), there could have been competition for the added acetate. Also, some of the $H_2S$ formed by the sulfate-reducing bacteria could have been oxidized by the green sulfur bacteria.

*Diurnal changes on sulfide and Bchl*—To relate the rates of $CO_2$ fixation, sulfide oxidation, and sulfate reduction to the processes occurring in the lake, we followed diurnal changes in Bchl *d* and $H_2S$ concentrations. Concentrations of Bchl *d* in Knaack Lake remained relatively constant throughout the sampling period with peak concentrations always at 2.2 m (Fig. 6). The green sulfur bacteria are nonmotile and their gas vacuoles do not function in the fine regulation of buoyancy but simply serve to provide buoy-

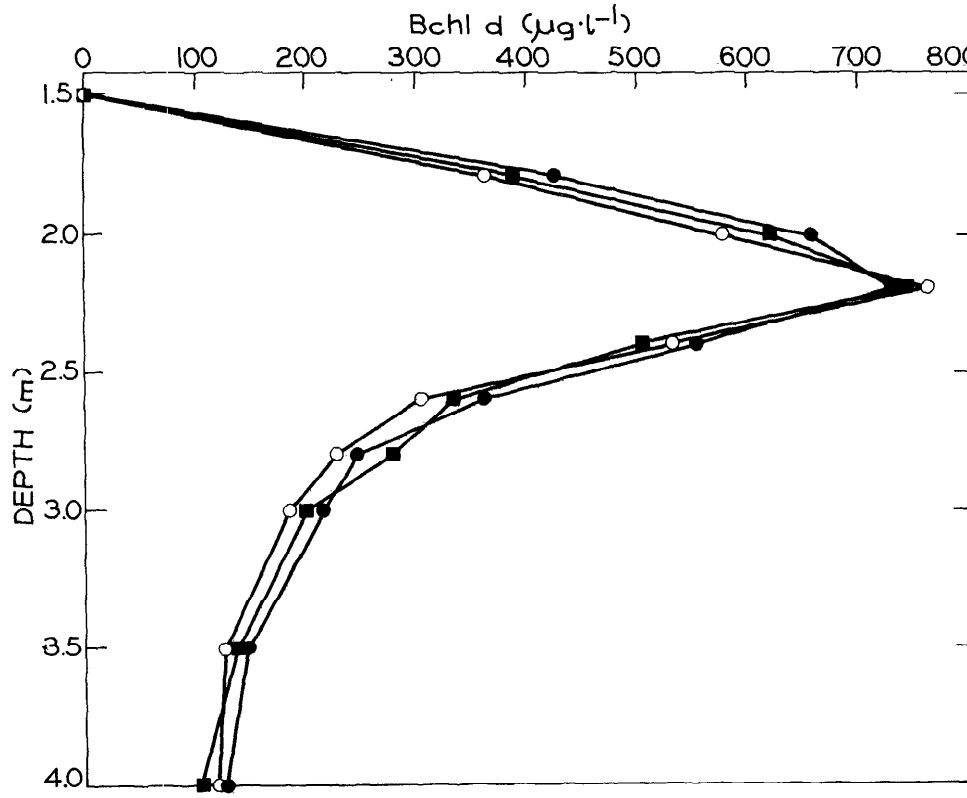


Fig. 6. Profiles of Bchl *d* in Knaack Lake at different times of day: ■—0900 hours; ○—1800 hours; ●—2400 hours.

19395590, 1981, 5, Downloaded from https://aslopubs.onlinelibrary.wiley.com/doi/10.4319/lo.1981.26.5.0880 by University Of Wisconsin, Wiley Online Library on [25/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

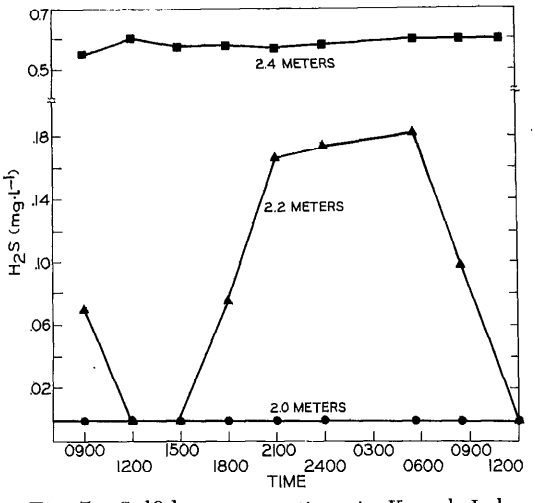


Fig. 7. Sulfide concentrations in Knaack Lake during a 24-h period.

ancy so that sinking is substantially reduced (Clark and Walsby 1978).

Sulfide concentrations at 2.2 m, however, showed a regular fluctuation (Fig. 7). At midday sulfide was undetectable. During the night, sulfide increased to a maximum concentration of 0.18 mg·liter$^{-1}$ and then decreased to about 0.05 in the early morning. The increase of sulfide in the dark was probably due to a combination of two factors: sulfate reduction at 2.2 m and diffusion of sulfide from below. The decreased sulfide concentrations in the light are presumably due to oxidation by the green sulfur bacteria. Sulfide was undetectable at 2.0 m; concentrations at 2.4 m remained relatively constant. Oxygen was not present at 2.0 m at any time of day.

## *Discussion*

From the primary production measurements of Culver and Brunskill (1969), Pfennig (1975) calculated that the photosynthetic bacteria in Fayetteville Green Lake oxidized 336 g $H_2S \cdot m^{-2} \cdot yr^{-1}$. The participation of the purple sulfur bacteria in the interactions of sulfide and oxygen at the chemocline of Lake Pluss have been examined by van Gemerden (1967), the sulfide oxidation and $CO_2$ fixation rates by *Chromatium* in Lake Belovod by Sorokin (1970). Jørgensen et al. (1979) followed the diurnal migrations of sulfide and oxygen at the chemocline of Solar Lake; although photosynthetic sulfur bacteria were not important in the oxidation of sulfide, the cyanobacteria in the lake played a very important role.

We wanted to determine how the photosynthetic bacteria participate in the sulfur cycle of a meromictic lake and how the sulfur transformations affect these organisms. The sulfur cycle at the thermocline region of Knaack Lake is significantly different from those of lakes mentioned above. In summer, oxygen and sulfide are separated in the water column, which makes study of the effects of sulfide on the photosynthetic bacteria considerably less complicated, as transformations by the colorless sulfur bacteria and molecular oxygen need not be considered. The photosynthetic sulfur bacteria reached maximum population densities at the depth where neither $H_2S$ nor $O_2$ were detected and these organisms carried out photosynthesis in this environment where other electron donors for photosynthesis were chemically undetectable. We determined that the $H_2S$ produced in this layer by sulfate reducers served as the electron donor. Although sulfate concentrations in Knaack Lake were low, most of this sulfide was produced from dissimilatory sulfate reduction and not from the reduction of elemental sulfur or thiosulfate.

Biebl and Pfennig (1978) described a syntrophic relationship between the sulfur-reducing *Desulfuromonas acetoxidans* and green sulfur bacteria in which a minimum sulfide concentration of 4 mg·liter$^{-1}$ was required to support the growth of the mixed culture. This relationship depends on the incomplete oxidation of $H_2S$ by the photosynthetic component. If the green sulfur bacterium oxidized the $H_2S$ completely to $SO_4^{2-}$ then $S^0$ was not available to the *Desulfuromonas*. However, Pfennig (1975) reported that at low levels of sulfide, green sulfur bacteria oxidized sulfide to sulfate with no accumulation of elemental sulfur. The low sulfide concentrations in

19395590, 1981, 5, Downloaded from https://aslopubs.onlinelibrary.wiley.com/doi/10.4319/lo.1981.26.5.0880 by University Of Wisconsin, Wiley Online Library on [25/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Knaack Lake favor oxidation of sulfide to sulfate and, therefore, a sulfur-reducing organism would be at a disadvantage. This is in accord with the absence of elemental sulfur from the water during stratification.

The highest rate of sulfate reduction in Knaack Lake was at the depth where the density of photosynthetic sulfur bacteria was greatest and coincided also with the depth having the maximum concentration of sulfate-reducing bacteria. Despite the fact that sulfide was being supplied to the photosynthetic sulfur bacteria in the light, added sulfide stimulated photosynthesis, indicating that the rate of sulfide formation was limiting the rate of bacterial photosynthesis. A similar observation by van Gemerden (1967) was that in mixed pure cultures of *Desulfovibrio* and *Chromatium* the growth rate of the *Chromatium* was limited by the growth rate of the *Desulfovibrio,* which was in turn limited by the concentration of electron donor.

Although sulfate concentrations in Knaack Lake are very low, increased sulfate did not stimulate sulfate reduction rates. This is also consistent with the findings of van Gemerden (1967) that a low total sulfur content did not limit the growth of either the sulfate reducer or the photosynthetic bacterium in mixed culture. The concentration of electron acceptor did not seem to be limiting sulfate reduction rates in Knaack Lake. However when we increased the concentration of electron donor (acetate), sulfate reduction rates increased. Sulfate reduction in Solar Lake responded in a similar manner, and it was suggested that an organism such as *Desulfotomaculum acetoxidans* was responsible for the observed reactions (Jørgensen et al. 1979).

Since populations of sulfate-reducing bacteria appear to be limited by the availability of electron donors, it is important to consider what the possible sources of donors to these organisms are. The spatial relationships between the sulfate-reducing bacteria and the photosynthetic bacteria in Knaack Lake and in other lakes as well (van Gemerden 1967; Sorokin 1970; Indrebo et al. 1979) suggest that the latter group of bacteria may play a role in supplying sources of carbon, energy, or both to the former. Sirevag and Ormerod 1977) found that the polyglucose formed during photosynthesis by the green sulfur bacterium *Chlorobium thiosulfatophilum* was oxidized in the dark and excreted into the culture medium as acetate, propionate, caproate, and succinate. Photosynthetic bacteria in lakes excrete from 15 to 30% of the carbon fixed during photosynthesis as soluble organic material (Czeczuga and Gradzki 1973). This, also, suggests the possibility of "cross feeding" between sulfate-reducing and photosynthetic bacteria, but direct evidence of such reactions in Knaack Lake is lacking. Parkin and Brock (in press) found none of the $^{14}CO_2$ incorporated by the Knaack Lake green sulfur bacteria in the light in the lake water as soluble organic carbon compounds. However, if the excretion of organic material by photosynthetic bacteria were the step limiting the rate of its utilization by the sulfate-reducing bacteria, no accumulation of soluble $^{14}C$ organic matter in the lake water would be expected.

Although acetate stimulated the sulfate-reducing activity of the Knaack Lake bacteria, it is unlikely that acetate is the only electron donor used in sulfate reduction or that the photosynthetic bacteria are the only "suppliers" of compounds used by the sulfate-reducing bacteria. Algal production in the epilimnion of Knaack Lake was estimated to be 342 g C fixed$\cdot m^{-2}\cdot yr^{-1}$; of this only 20% reached the sediments, the rest being remineralized in the water column (Parkin and Brock in press). Thus, organic compounds liberated from the decomposition of the algae could also function as carbon and electron sources, driving the process of sulfate reduction.

The diurnal changes in sulfide concentration in Knaack Lake reflect the processes described above. The rapid decrease of sulfide at 2.2 m in the morning occurs as a result of photosynthetic oxidation by the green sulfur bacteria. Rates

19395590, 1981, 5, Downloaded from https://aslopubs.onlinelibrary.wiley.com/doi/10.4319/lo.1981.26.5.0880 by University Of Wisconsin, Wiley Online Library on [25/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

of $CO_2$ fixation and $H_2S$ oxidation suggest that $H_2S$ is oxidized completely to $SO_4^{2-}$. At night sulfide accumulated in this layer at a rate of 0.89 $\mu M \cdot h^{-1}$. However, the sulfate reduction rate measured at 2.2 m was only 0.34. Since in situ sulfate reduction cannot completely account for the $H_2S$ increase at night, diffusion of $H_2S$ into this layer may also be responsible for part of the sulfide increase.

We estimated the vertical diffusion of $H_2S$ in Knaack Lake by applying Fick's first law. Using the eddy diffusion coefficient of sulfate ($1.4 \times 10^{-3}$ $cm^2 \cdot s^{-1}$) calculated by Stuiver (1967) for Linsley Pond, we found a rate of sulfide increase at 2.2 m in Knaack Lake of 0.19 $\mu M \cdot h^{-1}$. We consider this to be a maximum value, because the coefficient of vertical eddy diffusion (or eddy conductivity) for a given lake appears to be related to the lake's surface area and depth (Hutchinson 1957), and Knaack Lake is deeper and has a smaller surface area than Linsley Pond. Sulfide might also be formed from the decomposition of sulfur-containing organic matter and might be transported by horizontal diffusion. Sulfide formation by photosynthetic sulfur bacteria (van Gemerden 1967) or by cyanobacteria (Jørgensen et al. 1979) is unlikely in Knaack Lake as neither elemental sulfur nor thiosulfate was detected in the water.

Unlike the *Chromatium* in Lake Belovod (Sorokin 1970), the green sulfur bacteria in Knaack Lake remain at a fixed depth and do not follow the changes of sulfide concentration in the water column. Clark and Walsby (1978) reported that the gas vacuoles contained by many species of green sulfur bacteria do not function in the fine regulation of buoyancy but simply serve to provide buoyancy to the organisms so that their sinking rate is greatly reduced. Since these organisms remain stationary in the water column of Knaack Lake, when sulfide becomes depleted during the day their photosynthesis is limited by the rate of sulfide supply. This region would perhaps be more habitable by motile purple sulfur bacteria which carry an electron donor for photosynthesis ($S^0$) intracellularly, but the nonmotile green sulfur bacteria are able to exist without competition because the large amounts of dissolved humic and tannic materials in the lake essentially exclude the carotenoid-rich purple sulfur bacteria. Only red light (680–720 nm) penetrates to the anaerobic zone and it is these wavelengths that are used by the green sulfur bacteria (Parkin and Brock 1980*b*). The light intensities at 2.2 m were in the range of 0.5–2 $\mu Einst \cdot m^{-2} \cdot s^{-1}$; however, at the depth where sulfide was present during the day (2.4 m) light intensities were much lower (0.05–0.5 $\mu Einst \cdot m^{-2} \cdot s^{-1}$). Parkin and Brock (1980*b*) showed that the photosynthetic bacterial population in Knaack Lake was light limited at intensities of light $<0.5$ $\mu Einst \cdot m^{-2} \cdot s^{-1}$ but that light intensities $>0.5$ were saturating for photosynthesis. Thus these bacteria in Knaack Lake are present in maximum numbers at a depth where there is a very delicate balance between the limitation of photosynthesis by sulfide availability where they are and the limitation by light that would be imposed if they were deeper where sulfide concentrations are higher.

## *References*


AMERICAN PUBLIC HEALTH ASSOCIATION. 1976. Standards for the examination of water and wastewater, 14 ed.

BIEBL, H., AND N. PFENNIG. 1978. Growth yields of green sulfur bacteria in mixed cultures with sulfur and sulfate reducing bacteria. Arch. Mikrobiol. **117:** 9–16.

BUTLIN, K. R., AND J. R. POSTGATE. 1954. The microbiological formation of sulfur in Cyrenaican lakes, p. 112–122. *In* Biology of deserts. Inst. Biol., London.

CLARK, A. E., AND A. E. WALSBY. 1978. The occurrence of gas-vacuolate bacteria in lakes. Arch. Mikrobiol. **118:** 223–228.

CULVER, D. A., AND G. J. BRUNSKILL. 1969. Fayetteville Green Lake, New York. 5. Studies of primary production and zooplankton grazing in a meromictic marl lake. Limnol. Oceanogr. **14:** 862–873.

CZECZUGA, B., AND F. GRADZKI. 1973. Relationship between extracellular and cellular production in the sulfuric green bacterium *Chlorobium limicola* Nads. as compared to primary production of phytoplankton. Hydrobiologia **42:** 85–95.

HUTCHINSON, G. E. 1957. A treatise on limnology, v. 1. Wiley.

INDREBO, G., B. PENGERUD, AND I. DUNDAS. 1979.


19395590, 1981, 5, Downloaded from https://aslopubs.onlinelibrary.wiley.com/doi/10.4319/lo.1981.26.5.0880 by University Of Wisconsin, Wiley Online Library on [25/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Microbial activities in a permanently stratified estuary. 2. Microbial activities at the oxic-anoxic interface. Mar. Biol. **51:** 305–309.

JOHNSON, C. M., AND H. NISHITA. 1952. Microestimation of sulfur. Anal. Chem. **24:** 736–742.

JØRGENSEN, B. B., J. G. KUENEN, AND Y. COHEN. 1979. Microbial transformations of sulfur compounds in a stratified lake (Solar Lake, Sinai). Limnol. Oceanogr. **24:** 799–822.

PACHMAYR, F. 1960. Vorkommen und Bestimmung von Schwefelverbindungen in Mineralwasser. Ph.D. thesis, Univ. Munich.

PARKIN, T. B., AND T. D. BROCK. 1980*a*. Photosynthetic bacterial production in lakes: The effects of light intensity. Limnol. Oceanogr. **25:** 711–718.

———, AND ———. 1980*b*. The effects of light quality on the growth of phototrophic bacteria in lakes. Arch. Mikrobiol. **125:** 19–27.

———, AND ———. In press. Photosynthetic bacterial production and carbon mineralization in a meromictic lake. Arch. Hydrobiol.

PFENNIG, N. 1975. The phototrophic bacteria and their role in the sulfur cycle. Plant Soil **23:** 1–16.

———, AND H. BIEBL. 1976. *Desulfuromonas acetoxidans* gen. nov. and sp. nov., a new anaerobic, sulfur-reducing, acetate-oxidizing bacterium. Arch. Mikrobiol. **110:** 3–12.

POSTGATE, J. R. 1979. The sulphate-reducing bacteria. Cambridge Univ.

RUDD, J. W., R. D. HAMILTON, AND N. E. CAMPBELL. 1974. Measurement of the microbial oxidation of methane in lake water. Limnol. Oceanogr. **19:** 519–524.

SADLER, W. R., AND R. Y. STANIER. 1960. The function of acetate in photosynthesis by green bacteria. Proc. Natl. Acad. Sci. **46:** 1328–1334.

SIREVAG, R., AND J. G. ORMEROD. 1977. Synthesis, storage, and degradation of polyglucose in *Chlorobium thiosulfatophilum*. Arch. Mikrobiol. **111:** 239–244.

SOROKIN, Y. I. 1970. Interrelations between sulphur and carbon turnover in meromictic lakes. Arch. Hydrobiol. **66:** 391–446.

STUIVER, M. 1967. The sulfur cycle in lake waters during thermal stratification. Geochim. Cosmochim. Acta **31:** 2151–2167.

TAKAHASHI, M., AND S. ICHIMURA. 1970. Photosynthetic properties and growth of photosynthetic bacteria in lakes. Limnol. Oceanogr. **15:** 929–944.

TRÜPER, H. G. 1964. Sulphur metabolism in Thiorhodaceae. 2. Stoichiometric relationship of $CO_2$ fixation to oxidation of hydrogen sulphide and intracellular sulphur in *Chromatium okenii*. Antonie van Leeuwenhoek J. Microbiol. Serol. **30:** 385–394.

———, AND N. PFENNIG. 1966. Sulphur metabolism in Thiorhodaceae. 3. Storage and turnover of thiosulfate sulfur in *Thiocapsa floridana* and *Chromatium* species. Antonie van Leeuwenhoek J. Microbiol Serol **32:** 261–276.

———, AND H. G. SCHLEGEL. 1964. Sulfur metabolism in Thiorhodaceae. 1. Quantitative measurements on growing cells of *Chromatium okenii*. Antonie van Leeuwenhoek J. Microbiol. Serol. **30:** 225–238.

VAN GEMERDEN, H. 1967. On the bacterial sulfur cycle of inland waters. Ph.D. thesis, Univ. Leiden.

———, AND H. W. JANNASCH. 1971. Continuous culture of Thiorhodaceae: Sulfide and sulfur limited growth of *Chromatium vinosum*. Arch. Mikrobiol. **79:** 345–351.

WINFREY, M. R., AND J. G. ZEIKUS. 1979. Microbial methanogenesis and acetate metabolism in a meromictic lake. Appl. Environ. Microbiol. **37:** 213–221.

*Submitted: 29 July 1980*
*Accepted: 21 January 1981*

19395590, 1981, 5, Downloaded from https://aslopubs.onlinelibrary.wiley.com/doi/10.4319/lo.1981.26.5.0880 by University Of Wisconsin, Wiley Online Library on [25/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License