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Microbial transformations of sulfur compounds in a stratified lake (Solar Lake, Sinai)[1]

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Abstract

Microbiological and chemical aspects of the sulfur cycle were studied in the chemocline of a tropical salt lake. Oxygen and sulfide coexisted in a 0–10-cm layer which migrated up and down during a diurnal cycle. Sulfide was rapidly oxidized by oxygen, with a half-life of 5–10 min, to produce mainly sulfate and thiosulfate. Thiosulfate and elemental sulfur had concentration maxima in the chemocline while polysulfide was abundant throughout the sulfide zone. Radiotracer experiments showed that the elemental sulfur was produced by anoxygenic photosynthesis in cyanobacteria. The elemental sulfur was further oxidized or again reduced, depending on the presence or absence of oxygen. Cyanobacteria in the chemocline shifted between anoxygenic photosynthesis in the morning and oxygenic photosynthesis in the afternoon. A high dark CO_2 fixation was found in the chemocline which could be stimulated by sulfide, elemental sulfur, and thiosulfate. The oxidation rate of sulfide in the chemocline was dependent on the presence of bacteria.

Anoxic, sulfide-bearing bottom waters characterize many lakes, marine basins, and fjords which are stratified in salinity or temperature (Richards 1965; Sorokin 1970, 1972). The chemocline (here defined as the interface between oxygen and sulfide) of these waters has attracted special interest. In this layer, sulfide transported upward reacts with oxygen transported downward. The sulfide contains a large part of the chemical energy transferred by bacterial sulfate reduction from the organic material which originally settled into the bottom water or was produced there. In the presence of oxygen this energy may become available to microorganisms which can catalyze the oxidation of sulfide; as a result, the chemical energy is partly preserved in the biomass of the bacteria. The chemocline is accordingly a zone of high microbial activity, detectable as, e.g. a peak of dark

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CO_2 fixation (Sorokin 1964, 1970, 1972; Tuttle and Jannasch 1973*a*).

Where light penetrates into the sulfide zone, high populations of photoautotrophic bacteria develop. In nearly all cases these are purple or green sulfur bacteria which photo-oxidize the sulfide to elemental sulfur and sulfate. In Solar Lake, however, cyanobacteria (blue-green algae) completely dominated the phototrophic organisms in the sulfide-rich hypolimnion. These organisms have been shown to be capable of shifting between oxygenic (algal-type) and anoxygenic (bacterial-type) photosynthesis, depending on the absence or presence of sulfide (Cohen et al. 1975*b*; Oren and Padan 1978). It was therefore of special interest to investigate the possible role of this physiological versatility in the lake.

In spite of the well known potential of many thiobacilli to grow autotrophically on sulfide, their participation in sulfide oxidation in the chemocline has still not been convincingly demonstrated (Kuenen 1975). The difficulty is largely because sulfide also reacts spontaneously with oxygen so that the bacterial oxidation cannot be studied separately. A combination of microbiological methods, radiotracer techniques, and chemical kinetics was necessary to attack the problem.

We have applied a number of such approaches in a study of the oxygen-sulfide interface of Solar Lake. This small lake on the east coast of Sinai, 20 km south of Elat, is fed by seepage from the sea through a narrow terrestrial bar. It has a high salinity, of seawater composition, which causes stratification during most of the year. An inverse thermocline with hot bottom water builds up during winter and sulfide accumulates in the hypolimnion. The general limnology, chemistry, and microbiology of the lake have been described elsewhere (Cohen et al. 1977*a,b,c*; Por 1969).

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Methods

Sampling—The study of the steep gradients of sulfur chemistry and microbial activity in the chemocline of Solar Lake required an extremely accurate sampling procedure. Water samples had to be collected with reproducible depth intervals of a few centimeters. This was possible only with an anchoring system that kept the sampling position horizontally fixed within ±10–20 cm even on windy days.

A small aluminum boat was fixed with three steel wires at 120° angles forming a triple-point anchor to the shore. Two of the wires were tied to large boulders; the third was attached to a car with which the whole system was stretched tight. Water samples were obtained with a peristaltic pump and a tube submersed to the sampling depth. The pump was mounted on an electric drill driven by a generator on shore. The inlet at the end of the 5-mm-wide PVC tube consisted of two solid cones spaced 1 cm apart (Fig. 1). Water from a defined depth flowed in between the cones and up through the central tube. The inlet was suspended from the boat on a steel chain with accurate depth marks. The pumping rate was 0.5–1 liter·min^{-1} and the total dead volume of tubes and inlet was 0.25 liter. For each new depth the pumping system was flushed for 1 min before samples were taken. Sampling bottles were similarly flushed with two bottle volumes overflow to avoid contamination with atmospheric oxygen. Bottles for incubation in the lake were temporarily submersed in a bucket of freshwater immediately after stoppering to avoid any entrance of air due to the rapid contraction of the hot brine as it cooled. When the bottles were then lowered to the original depth, the brine again expanded and displaced the top layer of freshwater. This procedure efficiently excluded air bubbles in all incubations.

The sampling accuracy in the chemocline was carefully checked as it is cru-

cial for the results. Continuous pumping never proceeded for more than 5 min at any depth. During this period the oxygen and sulfide concentrations showed no systematic change. We estimated the actual thickness of the water layer sampled to be 2–3 cm as judged from the chemical gradients. Up to 10-fold changes of H_2S concentration could be measured over a 2.5-cm depth interval. If a 2-cm-thick sampling layer is assumed, then the water is collected from a distance of <20 cm from the inlet even after 5 min of pumping. The depth at which the inlet was positioned was defined with ±1-cm accuracy. When the inlet was moved away from the chemocline and then immediately returned to the same depth the chemical concentrations measured were the same.

Chemical and physical determinations—Oxygen was measured in 150-ml samples by the Winkler technique (Strickland and Parsons 1968). Sulfide was measured spectrophotometrically after methylene blue formation (Pachmayr 1960). The 10- or 100-ml samples collected were fixed immediately by addition of 2% Zn-acetate solution. The sulfide concentration calculated from the absorbance was checked by iodine titration (Golterman 1971) in sulfide-rich samples.

When oxygen and sulfide coexisted they were determined in the same sample by the technique of Ingvorsen and Jørgensen (1979). Sulfide was precipitated as ZnS in alkaline solution and, after rapid settling, a subsample was collected from the supernatant for oxygen determination. The reagents for sulfide precipitation were made up in 20% NaCl to settle the precipitate. Thiosulfate, which was detected in the chemocline, may interfere with the determination. Our results indicate, however, that oxygen cannot be seriously underestimated at the sulfide boundary. Furthermore, the transient appearance of water layers with no oxygen and only traces of sulfide indicates that contamination by atmospheric oxygen during the analytical procedure must be insignificant.

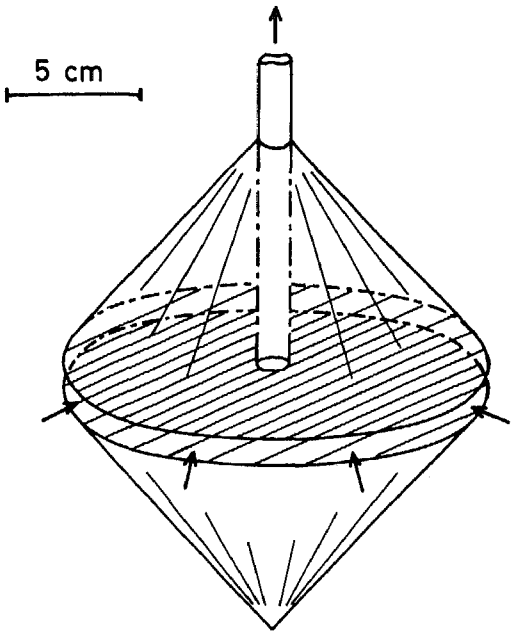

Fig. 1. Inlet of peristaltic water sampler.

Elemental sulfur present as suspended granules (S^0_{part}) was collected by immediate filtration of 50–100-ml samples through GF/F filters in the boat. The filters were then washed with 25 ml of water and placed in a vial with 10 ml of 5% Na_2SO_3. They were later steamed in an autoclave at 100°C for 2 h. During this treatment the following reaction takes place (Karchmer 1970): $S^0 + SO_3^{2-} \rightarrow S_2O_3^{2-}$. One milliliter of 2% Zn-acetate was added and the samples were again filtered and washed through GF/F filters. The filtrate was stirred and meanwhile 0.5 ml of 36% formaldehyde, 1 ml of glacial acetic acid, and 0.5 ml of 1% starch solution were added. The thiosulfate was then titrated with a standardized 10 mM iodine solution. Although sulfite was masked by formaldehyde, a small blank remained which was subtracted from the results. The specificity of the method for S^0 was shown by filtering a sample with a measured high S^0 content and then extracting the S^0 with acetone before continuing the procedure. This reduced the titration volume to the blank value. The possibility of interference from sulfide

could be excluded as sulfide assays on the first set of filters were negative.

Elemental sulfur coexisting with H_2S may dissolve as polysulfides (e.g. S_4^{2-} and S_5^{2-}: Chen and Gupta 1973). This S^0 fraction was separated by acidifying the filtered sample, thereby disintegrating the polysulfide into S^0 and H_2S. One milliliter of concd HCl was added to each 150-ml sample, which was then stoppered and left for 0.5 h before filtration of the generated S^0. The further analysis of S^0 proceeded as described above. The presence of polysulfide was also demonstrated by a slight change of the water from yellow to milky white upon acidification. The difference in absorption spectra of such samples when untreated and after acidification and centrifugation was typical of S_4^{2-} and S_5^{2-} in the range measured, from 320 to 500 nm (Chen and Gupta 1973).

Thiosulfate was analyzed by the rather unspecific method of iodine titration (Karchmer 1970). Sulfide was first precipitated with excess Zn-acetate and then completely removed by consecutive centrifugation and filtration. A 50-ml sample was titrated with 10 mM I_2 (starch indicator). The presence of thiosulfate was also demonstrated by boiling samples with excess $AgNO_3$; this converts the sulfane atom of thiosulfate into black AgS (Karchmer 1970), which can be recognized visually after filtration. The blackening of the filters was compared to that of standards made up from surface Solar Lake water with known additions of $Na_2S_2O_3$. This comparison agreed with the results of iodine titration in the thiosulfate-rich samples from the chemocline.

Sulfite was assayed by iodine titration with and without formaldehyde. Since formaldehyde did not reduce the titration values we concluded that sulfite was not present in significant quantities (<5 μM).

Sulfate was measured gravimetrically after precipitation with $BaCl_2$ (Am. Public Health Assoc. 1971).

Dissolved inorganic carbon was determined from the titration alkalinity (Strickland and Parsons 1968). The accuracy of this method in the Solar Lake brine was checked by Cohen et al. (1977*b*).

Salinity was determined conductimetrically after proper dilution, pH with a glass electrode on the lake or in the laboratory within a few hours after sampling. Temperature was measured with a thermistor (Yellow Springs Instr.) in the lake. Light penetration was measured with a quantameter (Lambda Instr. Corp.).

Cyanobacterial chlorophyll *a* was analyzed according to Potts (in prep.) and bacteriochlorophyll *a* according to Gest et al. (1963). One-liter samples were filtered through GF/C filters. The filters were then extracted by boiling in 95% methanol for 3 min. Chlorophyll *a* and bacteriochlorophyll *a* were calculated from the absorbance at 665 and 770 nm. Protein was measured by the Folin phenol reagent method (Lowry et al. 1951). The 100–150-ml samples were filtered on GF/F filters and washed with absolute ethanol to remove elemental sulfur.

^{35}S-tracer experiments—In situ rates of bacterial sulfate reduction were measured with [^{35}S] SO_4^{2-} as a tracer. The water samples were collected in 150-ml dark bottles, 0.2 ml of radioactive solution was injected, and the bottle was immediately stoppered and lowered to incubate at the sampling depth. The incubation time and injected radioactivity were either 24 h and 5 μCi or 30 min and 50 μCi. Bacterial reduction was stopped by injection of 1 ml of 50 mM ZnS carrier, 1 ml of 1 M Zn-acetate, 1 ml of 1 M NaOH, and 1.5 ml of 36% formaldehyde. The samples were stored at 2°C for 1–2 days until analysis.

One milliliter of the supernatant was used to measure the [^{35}S]SO_4^{2-} radioactivity. Most of the supernatant was then removed and the remaining ZnS precipitate was used for isolation of [^{35}S]H_2S. The ZnS was acidified and labeled sulfide was transferred as H_2S gas into Zn-acetate traps in the apparatus described by Jørgensen and Fenchel (1974). The freshly precipitated [^{35}S]ZnS was used to measure sulfide radioactivity in a liquid

scintillation counter. The sulfate reduction rate was calculated as described by Sorokin (1962).

The sensitivity of the method was 0.1–0.2 μmol $SO_4^{2-} \cdot liter^{-1} \cdot d^{-1}$ or 0.001‰ of the sulfate pool per day. Contamination of $[^{35}S]SO_4^{2-}$ in the $[^{35}S]H_2S$ samples was not detectable, i.e. the carryover of label was $<1:10^7$. The linearity of the process was checked by incubating parallel samples from 4.5-m depth for 2, 4, 6, and 24 h. The calculated rate remained constant within ±20%.

The oxidation of sulfide was also studied with ^{35}S as a tracer. Labeled sodium sulfide (Negev Nuclear Center, Israel) was diluted into anoxic 1 mM NaOH with 1 mM H_2S as a carrier. This stock solution was stored at 2°C under N_2 from which O_2 was catalytically removed. On the day of the experiment, a subsample was transferred to a Vacutainer tube flushed with N_2. In the field, 0.2-ml portions containing 2 μCi of $[^{35}S]H_2S$ were injected into each 150-ml water sample. This caused a final increase in sulfide concentration of 1 μM H_2S, which was small compared to the natural concentration in the sulfide zone. The bottles were immediately stoppered after injection and lowered to the sampling depth for incubation.

Incubation was stopped by a physical separation of the following sulfur pools: H_2S, S^0_{part}, and S_{ox}. The S^0_{part} comprised the particulate fraction of elemental sulfur while S_{ox} contained the sulfuroxy ions such as $S_2O_3^{2-}$ and SO_4^{2-}. A 10-ml sample was rapidly filtered (on the lake) through a GF/F filter, followed by 10 ml of a carrier solution containing 50 mM each of H_2S, $S_2O_3^{2-}$, and SO_4^{2-}. The filter was used for counting $^{35}S^0_{part}$ radioactivity. The filtrate was collected in 5 ml of 5% Zn-acetate solution which fixed the sulfide. The ZnS was later separated from the supernatant by centrifugation and its radioactivity was counted separately. A subsample of the supernatant was used to count the $^{35}S_{ox}$ fraction.

In a few samples, the S_{ox} was further separated into polythionates, $S_2O_3^{2-}$, SO_3^{2-}, and SO_4^{2-}. The separation procedure is based on the differential solubility of the lead salts of these ions in various solutes (Mangan 1949).

The separation of S^0 by filtration collects only the particulate fraction, while polysulfide-S^0 combines with the ZnS pool. $[^{35}S]$polysulfide-S^0 was analyzed separately only in one control experiment where the sample was acidified before filtration.

The radioactivity of the different sulfur fractions was measured by liquid scintillation counting in a Insta-Gel (Packard):water mixture of 1:1. The results are expressed as percentage distribution of the label. A blank of oxidized sulfur in the $[^{35}S]Na_2S$ stock solution was subtracted.

The oxidation or reduction of elemental sulfur was also studied by tracer injections. The labeled S^0 was prepared from $[^{35}S]H_2S$ as follows. To a 10-ml subsample of a $[^{35}S]Na_2S$ stock solution was added one drop of 2 N HCl. The sulfide was then rapidly titrated with 10 mM iodine solution until a slight yellow color remained. The excess iodine was backtitrated with excess 10 mM $Na_2S_2O_3$. The milky suspension of $^{35}S^0$ was centrifuged, washed with distilled H_2O, and resuspended in 10 ml of distilled H_2O by sonication in an ice bath. Microscopical examination showed the sulfur to be well dispersed granules of appearance similar to that in natural samples from the chemocline. The sulfur granules and all radioactivity were quantitatively retained by a GF/F filter.

The $^{35}S^0$ suspension was used in the field within a few hours after preparation. For each 150-ml sample, 0.2 ml with 0.5 μCi of $^{35}S^0$ was injected. This caused a final increase in S^0 concentration of 2 μM S^0. The rest of the experiment was done as with labeled sulfide.

$[^{14}C]CO_2$ fixation—The rate of CO_2 fixation was measured in light and dark bottles with the ^{14}C technique (Sorokin and Kadota 1972; Steemann Nielsen 1952). Into each 150-ml sample, 1 ml (containing 10 μCi) of membrane-filtered $[^{14}C]HCO_3^-$ stock solution was injected. The bottle was immediately lowered to the

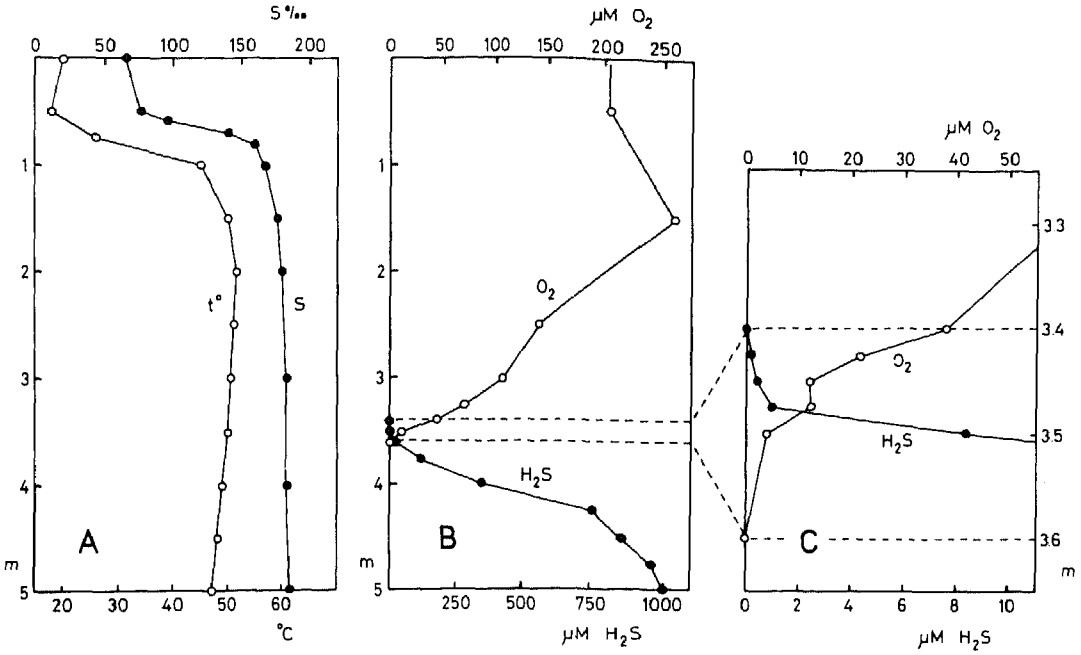

Fig. 2. A. Temperature and salinity in Solar Lake. B. Oxygen and sulfide gradients. C. Oxygen–sulfide interface from "B" shown with expanded scales to demonstrate zone of coexisting O_2 and H_2S (16 February).

sampling depth and incubated for 30 min or a few hours. The biological activity was stopped by injecting 1.5 ml of 36% formaldehyde. A 20–50-ml sample was filtered on a GF/F filter and the filter fumed with HCl and counted by liquid scintillation. Quenching was corrected for by the external standard method. Controls showed that the formaldehyde efficiently stopped [^{14}C]CO_2 fixation and that the GF/F filters quantitatively retained all particulate ^{14}C >0.2 µm. Light incubations were done both with and without DCMU [3-(3,4-dichlorophenyl)-1,1-dimethyl-urea] at a final concentration of 10 µM. DCMU inhibits algal (oxygenic) photosynthesis but does not affect bacterial (anoxygenic) photosynthesis. DCMU also inhibited dark CO_2 fixation in the lake by 15–40%, but this is not significant in interpreting the light experiments.

The effect of adding oxygen and reduced sulfur compounds on the rate of dark CO_2 fixation was studied in several experiments. Oxygen was added by injecting 20 ml of oxygen-saturated, membrane-filtered (0.22 µm) surface water into each bottle. A 10-ml sample was then withdrawn to leave an air pocket inside the bottle during incubation. Sulfide, elemental sulfur, or thiosulfate was injected in 10-ml portions from stock solutions. The elemental sulfur was prepared by dissolving analytical grade S^0 in hot 2 N NaOH and then neutralizing the solution just before use in the field. This produced well dispersed sulfur granules which only started to aggregate after a few hours.

Results

During February–May 1978, Solar Lake remained stratified with a sharp halo- and thermocline at 0.5–1-m depth (Fig. 2A). The salinity of the epilimnion was around 70‰ and the temperature 18–24°C. In the hypolimnion, the salinity reached 190‰; the maximum temperature at 3 m varied between 48° and 55°C.

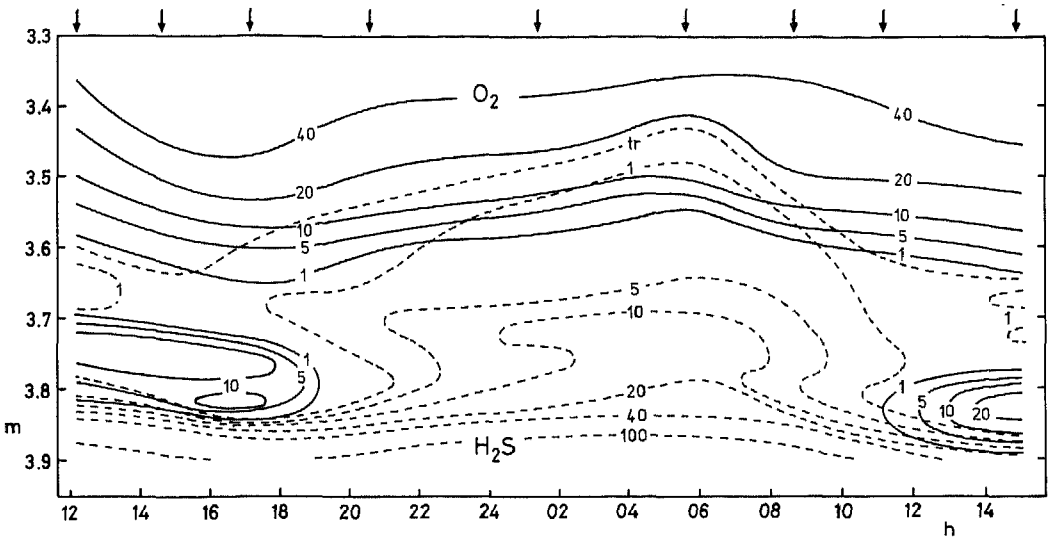

Fig. 3. Isopleths showing diurnal changes in distribution of oxygen (solid line) and H_2S (broken line) in chemocline. Numbers indicate μmol·liter^{-1} (tr = traces). Arrows indicate time of sampling. Sundown 1730 hours, sunrise 0600 hours (22–23 March).

Oxygen was present down to 2–4 m, below which H_2S accumulated. The exact position of the oxygen–sulfide interface as well as the chemistry and microbial metabolism of sulfur compounds varied significantly during the investigation. For practical reasons the many different approaches used in this study could not be applied at the same time. Conditions in the lake may therefore have changed between sampling periods, although the general pattern has been the same. This is important to note before making any quantitative comparisons between the following sets of data obtained on different dates.

Distribution of oxygen and of sulfur compounds—An example of dissolved oxygen and sulfide distributions in the lake is shown in Fig. 2B. The O_2 concentration at saturation is only 70 μM in the hot brine. Oxygen reaches over 200% supersaturation at 1.5-m depth, below which it decreases almost linearly to zero at 3.6 m. From about 3.5 m, H_2S increases rapidly to reach a concentration of 1 mM at the bottom. The oxygen–sulfide interface is shown with expanded scales in Fig. 2C. At 3.4–3.6-m depth there is an overlap between the O_2 and the H_2S gradients where the two compounds coexist.

Further studies of the oxygen–sulfide interface showed, however, a more complicated pattern which was only explained 5 weeks later after a detailed study of the diurnal variation of the two compounds. Throughout 28 h, we analyzed O_2 and H_2S every 2–4 h at 2.5–5-cm intervals from 3.35–3.90-m depth. The results are drawn as isopleths in Fig. 3. There is a general shift of the lower oxygen boundary between day and night. In the afternoon, at the end of the photosynthetic period, the isopleths reach 10 cm deeper than at dawn. Sulfide shows a complementary change between day and night. The sulfide can be traced 10 cm up into the oxygen region in the dark but disappears from the oxygen region during the brightest daytime. This picture is complicated by a secondary oxygen maximum which suddenly builds up within the sulfide zone in the late morning and disappears again at sundown. Thus at 3.82-m depth there is a change from 30 μM H_2S to 37 μM O_2 within the first 9 h of daylight. The buildup of a sulfide maximum at 3.7 m during the night

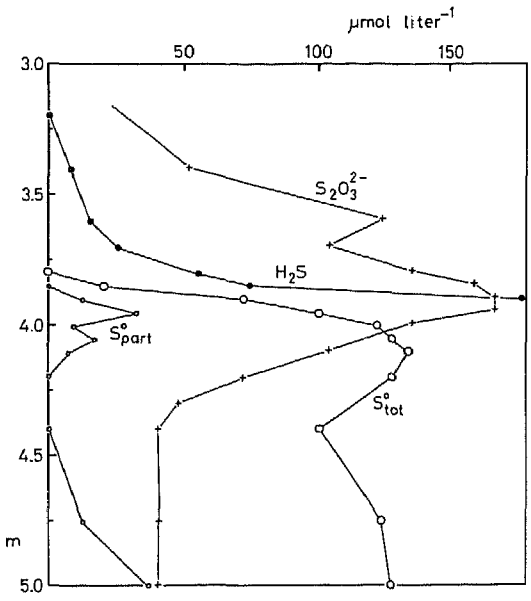

Fig. 4. Distribution of sulfur compounds in hypolimnion. Total elemental sulfur (S^0_{tot}) comprises particulate sulfur (S^0_{part}) and polysulfide-S^0 (27 April).

shows that H_2S is not only supplied by diffusion from below but is also produced in this layer. The evolution of oxygen within the sulfide zone must be due to intensive photosynthesis by the cyanobacteria which dominate the plankton in this layer. A diurnal migration of the oxygen–sulfide interface due to bacterial photosynthesis was also shown by Sorokin (1970) in meromictic Lake Belovod.

As a consequence of sulfide oxidation by oxygen and possibly also by direct photosynthetic oxidation, intermediate oxidation products of sulfide accumulate within and below the chemocline. The presence of elemental sulfur granules was indicated by a slightly milky appearance of the water from the chemocline. A more complete analysis of the distribution of sulfur compounds was made in late April when the oxygen–sulfide interface had ascended to 3.2-m depth (Fig. 4). The H_2S increased steeply below 3.8 m to a concentration of 4 mM at the bottom. Particulate S^0 showed a peak of 33 µmol $S^0 \cdot liter^{-1}$ at 4 m and was also present near the bottom. Large amounts of elemental sulfur were, however, present as polysulfides which constitute the difference between S^0_{tot} and S^0_{part} in Fig. 4. In the water below 4 m there was a constant, high polysulfide-S^0 concentration of around 100 µmol $S^0 \cdot liter^{-1}$. Thiosulfate showed a peak of 170 µM at the depth where sulfide began to build up rapidly. This is in accordance with its formation from the chemical (as opposed to the biological) reaction between O_2 and H_2S (Chen and Morris 1972; Cline and Richards 1969; Sorokin 1970, 1972). The concentration of SO_4^{2-} in the hypolimnion was around 130 mM. Its concentration is determined by the salinity, as the SO_4^{2-}:Cl^- ratio does not deviate significantly from that of seawater (Aharon 1974).

We did a 24-h study at the lake in May to see whether the intermediate oxidation products showed diurnal changes in pool size with those at the oxygen–sulfide interface. The distribution of oxygen, sulfide, particulate S^0, polysulfide-S^0, and thiosulfate together with pH were analyzed five times at 4–5-h intervals. Again the oxygen–sulfide interface moved up and down by 10–20 cm during night and day (Fig. 5A). Just below the chemocline, at 2.6 m, sulfide varied between 17 µM in the late afternoon and 89 µM in the early morning. Particulate sulfur showed a very pronounced diurnal variation (Fig. 5A). In the early morning S^0_{part} was hardly detectable in the chemocline. During the day a S^0_{part} peak of 39 µmol $S^0 \cdot liter^{-1}$ developed in the uppermost sulfide zone; at night the S^0_{part} disappeared again. Polysulfide-S^0 also reached maximum concentrations during the day in the upper sulfide zone and partly disappeared at night (Fig. 5B). The thiosulfate pool was more constant, with only a small increase by day and decrease at night. The pH, which is important for the degree of polysulfide formation from S^0_{part} (Chen and Gupta 1973), decreased down through the chemocline from an average of 7.7 at 2.0 m to 7.2 at 3.0 m. At any fixed depth in the chemocline the pH showed a diurnal variation of 0.1–0.2 units, with

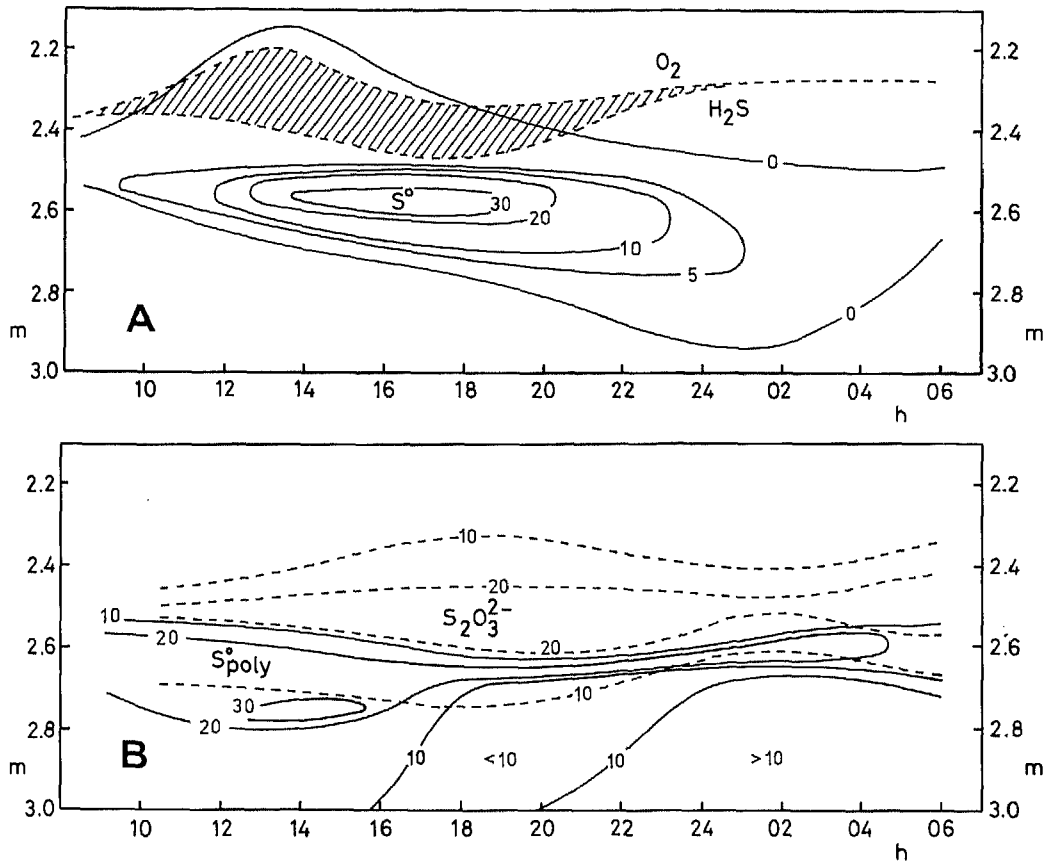

Fig. 5. A. Diurnal changes in oxygen–sulfide boundary (broken line) and coexisting oxygen and sulfide (hatched area). Isopleths show distribution of S^0_{part}. B. Isopleths showing diurnal changes in polysulfide (solid line) and thiosulfate (broken line) distribution. Numbers indicate μmol·liter^{-1} (18–19 May).

values lowest in the early morning and highest in the afternoon.

The diurnal variations, with a minimum of sulfide and maxima of particulate sulfur, polysulfide, and thiosulfate during the day and vice versa during the night, indicate that these sulfur compounds undergo quite dynamic transformations. There is a shift toward more oxidized forms during the photosynthetic period and toward the most reduced forms by dark. Due to its very high concentration, this shift is not detectable in the sulfate pool.

Transformations of sulfur compounds—The rate of H_2S formation from bacterial sulfate reduction was measured on several occasions. Figure 6 shows the results of two series of experiments incubated *in situ* for 24 h. In February, sulfate reduction started within the chemocline at 3.5-m depth where initial oxygen and sulfide concentrations were 4 and 8 μM (Fig. 2). In March the reduction rate showed a minimum at 3.75 m, at which depth a secondary oxygen maximum developed in the light. Sulfate reduction reached a maximum near the bottom of 4.1 and 6.9 μmol $SO_4^{2-}\cdot$liter$^{-1}\cdot d^{-1}$.

The radiotracer incubations in February were also done in light bottles at 3.5-, 4.0-, and 4.5-m depth. At 3.5 m the measured rate decreased fivefold in the light bottle compared to the dark bottle. Since even the light bottle was in the dark during the night, the lack of detectable

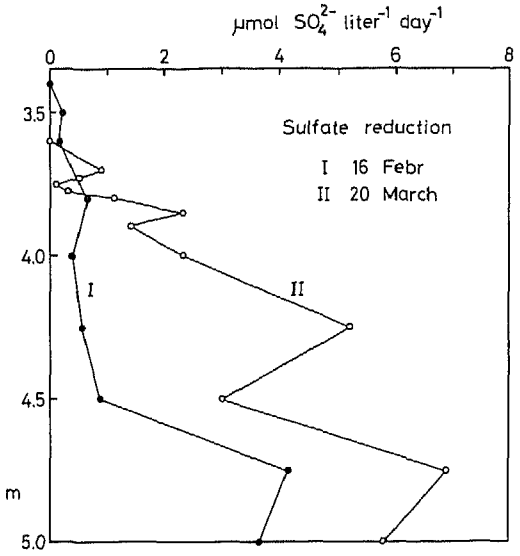

Fig. 6. In situ rates of bacterial sulfate reduction from radiotracer measurements.

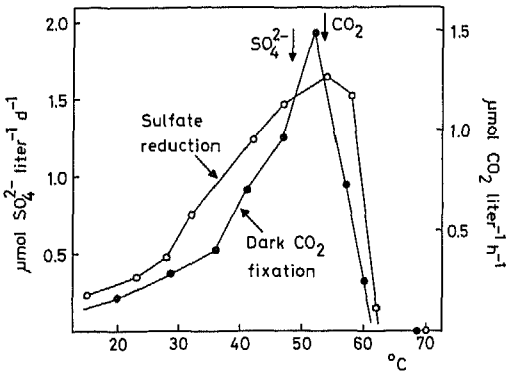

Fig. 7. Temperature dependence of bacterial sulfate reduction in 4.5-m samples and of dark [^{14}C]CO_2 fixation in oxygen- and sulfide-enriched chemocline samples. Arrows indicate in situ temperature (16 February—SO_4^{2-}; 7 April—CO_2).

[^{35}S]H_2S must have been due to rapid reoxidation of the H_2S produced in the light. At 4.0 and 4.5 m there was no significant difference between light and dark incubation, showing that there is not a rapid stimulation of sulfate reduction by photosynthate formation during the day.

The sulfate reduction in Fig. 6 took place at temperatures above 45°C. In order to study the temperature tolerance of the sulfate-reducing bacteria, we collected fresh samples at 4.5-m depth and incubated them with [^{35}S]SO_4^{2-} for 24 h in the laboratory at 10 different temperatures ranging from 15°–70°C. The in situ temperature was then 48°C. Figure 7 shows that the maximum rate of metabolism is reached at 55°C. Above 60°C the rate declines rapidly and at 70°C sulfate reduction is no longer detectable.

To characterize these thermophilic sulfate reducers further, we studied their response to additions of lactate and acetate. The results of two such experiments are shown in Table 1. Sulfate reduction is stimulated by addition of acetate at 10^{-5} M or above in both experiments. Lactate only stimulates the reduction rate in one experiment and is less effective than acetate. The experiment does not show whether acetate serves as a direct electron donor for the sulfate reducers. However, the preferential stimulation by acetate and the thermophilic temperature curve both suggest the presence of bacteria of the *Desulfotomaculum acetoxidans* type (cf. Widdel and Pfennig 1977).

Table 1. Effect of adding Na-lactate or Na-acetate on rate of sulfate reduction in water samples from 4.5-m depth. Incubation at 42°C for 24 h.

Added substrate concn (M)	Sulfate reduction rate (µmol·liter^{-1}·d^{-1})			
	12 Feb		21 Feb	
	Lactate	Acetate	Lactate	Acetate
0	1.25	1.25	1.30	1.30
10^{-6}	1.22	1.14	1.34	1.30
10^{-5}	1.25	1.89	1.46	1.78
10^{-4}	1.27	4.84	2.02	4.53
10^{-3}	—	—	2.35	4.56

Both chemical and radiotracer techniques were used to study the rates and products of sulfide oxidation in the field. Due to the constantly changing oxygen and sulfide gradients (Figs. 3 and 5A) it was difficult to select a sampling depth where the two compounds were known to coexist. The highly dynamic balance between oxygen and sulfide also made timing of the experiments difficult.

We measured the time-course of sul-

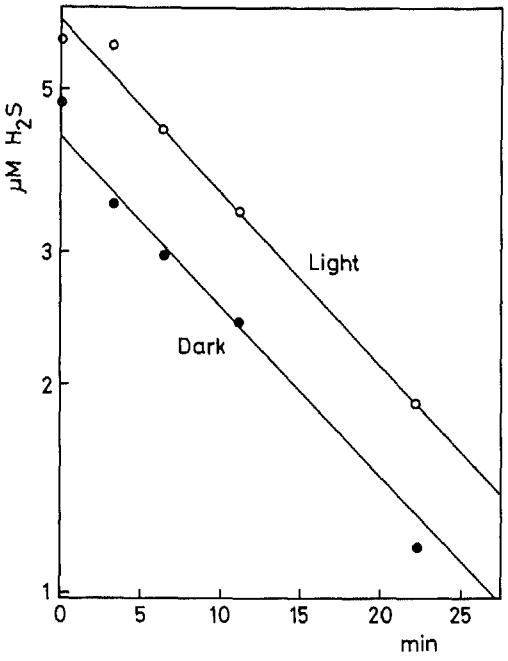

Fig. 8. Exponential disappearance of sulfide at oxygen–sulfide interface. In situ incubation at 53°C (15 March).

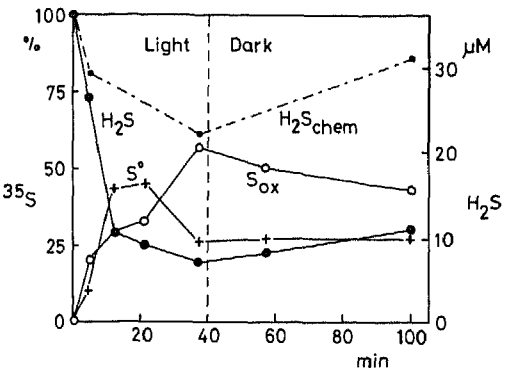

Fig. 9. Oxidation of ^{35}S-labeled sulfide in water samples from just below chemocline (3.95 m). Parallel samples were incubated in situ for up to 40 min in light followed by up to 60 min in dark. Percentage distribution of label is shown as well as concentration of sulfide (H_2S_{chem}) (30 March).

fide oxidation in the chemocline by collecting water samples in 15-ml screwcap tubes and incubating them in situ. Air contamination was carefully avoided. At intervals a tube was recovered and the sulfide concentration determined. Both light and dark tubes were used. Figure 8 shows in a semilog plot the result of one experiment with samples collected exactly at the oxygen–sulfide interface. The water initially contained about 5 μM H_2S and between 5 and 10 μM O_2. Sulfide disappeared exponentially at the same rate in light and dark. The half-life was 12 min and the initial rate of disappearance was 9 μmol $H_2S \cdot liter^{-1} \cdot h^{-1}$.

The experiment was repeated 5 days later. The oxygen–sulfide interface was then at 3.75 m with both oxygen and 3 μM H_2S present. The rate of sulfide disappearance was again similar in light and dark, with a half-life of only 3–5 min and an initial rate of disappearance of 12–20 μmol $\cdot liter^{-1} \cdot h^{-1}$. Five centimeters deeper, at 3.8 m, there was no sulfide disappearance in the dark; in the light, sulfide had a half-life of 50 min. The H_2S concentration here was 16 μM and there was no oxygen. The initial rate of disappearance in the light was 13 μmol $\cdot liter^{-1} \cdot h^{-1}$. These results demonstrate how extremely dynamic the processes are at the oxygen–sulfide interface.

The products of sulfide oxidation in the chemocline were studied by the use of ^{35}S-labeled H_2S. Figure 9 shows the results of a time-course experiment where $[^{35}S]H_2S$ was added to a number of 150-ml samples from the uppermost part of the sulfide zone. The in situ sulfide concentration was 36 μM. Oxygen was not measured. The temperature was 50°C. In situ incubation was done in the light for 40 min and then continued in the dark.

The labeled sulfide disappeared rapidly in the light with a half-life of 9 min. The oxidation products were elemental sulfur and a nonspecified pool of sulfuroxy anions (S_{ox}), in roughly equal proportions. During the dark period some of the labeled sulfide was recovered from the oxidized pool while S^0 remained constant. Since the sulfate concentration is much too high to cause a significant decrease in the amount of labeled sulfate by reduction, the source of regenerated $[^{35}S]H_2S$ in the dark must be an intermediate oxidation product. Thiosulfate was

Table 2. *In situ transformations of* ^{35}S-labeled H_2S and S^0 at five depths in lake. Water samples were incubated in light with 1 μM [^{35}S] H_2S or 2 μM $^{35}S^0$ for 15 min or 2.5 h. Percentage distribution of label is shown for three groups: H_2S—sulfide and polysulfide: S^0_{part}—particulate elemental sulfur; S_{ox}—sulfuroxy ions (24 April).

	Depth (m)				
	2.50	3.50	3.75	4.00	4.20
	Initial conditions				
t°C	53	55	55	54	53
O_2 (μM)	94	14	11	0	0
H_2S (μM)	0.0	0.0	0.4	170	500
	Products of [^{35}S]H_2S, 15 min (%)				
H_2S	83	59	35	71	97
S^0_{part}	0	0	0	5	0
S_{ox}	17	41	65	24	3
	Products of [^{35}S]H_2S, 2.5 h (%)				
H_2S	6	15	18	69	93
S^0_{part}	0	0	0	7	0
S_{ox}	94	85	82	24	7
	Products of $^{35}S^0_{part}$, 15 min (%)				
H_2S	1	1	10	43	71
S^0_{part}	55	55	42	30	29
S_{ox}	44	44	48	27	0
	Products of $^{35}S^0_{part}$, 2.5 h (%)				
H_2S	1	1	10	43	95
S^0_{part}	1	2	5	23	5
S_{ox}	98	97	85	34	0

present in sufficient concentration (Fig. 4) to account for both the chemical and radiotracer increase of sulfide in the dark. The experiment therefore indicates that thiosulfate was being reduced to sulfide at this depth.

Comparison of the radiotracer results with the changes in the chemical concentration of H_2S shows that the labeled sulfide disappeared relatively faster in the light than did the total pool of sulfide. Thus, 80% of the [^{35}S]H_2S was gone after 40 min of light incubation, H_2S concentration decreased only 39%. This shows that, concurrent with its oxidation, the H_2S was being produced from a pool of low specific label. A similar conclusion can be drawn from the dark incubations, where the relative increase in total H_2S concentration is larger than the relative increase in labeled H_2S. The curves in Fig. 9 are therefore the net result of simultaneous oxidation and reduction processes, with elemental sulfur as an important intermediate product.

This complex pattern of sulfur transformation was studied in more detail a month later. Pairs of light and dark bottles were incubated at five depths with [^{35}S]H_2S and $^{35}S^0$ as the initial tracer compound. Two of the sampling depths were within the oxic zone, one exactly at the oxygen–sulfide interface, and two within the sulfide zone. The samples were incubated in situ for 15 min or 2.5 h.

The results of the light incubations are shown in Table 2. In the oxic layers the labeled sulfide was transformed into the S_{ox} pool with no detectable S^0 formation. The percentage of [^{35}S]H_2S oxidized after 15 min increased with depth toward the chemocline from 17% at 2.5 m to 65% at 3.75 m. Since the oxygen concentration decreased with depth, the oxidation rate cannot be governed by oxygen. It is more probably regulated by catalysis, by bacteria or perhaps chemically. At 4.0 m, elemental sulfur granules were present and a small percentage of the labeled sulfide was oxidized into this pool. The chemical concentration of particulate S^0 at this depth was 26 μmol $S^0 \cdot liter^{-1}$, with an additional 24 μmol $S^0 \cdot liter^{-1}$ dissolved as polysulfide. Polysulfide-S^0, which is included in the [^{35}S]H_2S fraction, may also contain a part of the label. At 4.2 m very little [^{35}S]H_2S was oxidized after 15 min due to the large pool size of nonlabeled sulfide. After 2.5 h the [^{35}S]H_2S in the oxic layers was rather completely oxidized, most completely at the highest oxygen concentration.

The results of the dark [^{35}S]H_2S incubations were quite similar to those of the light incubations in the oxic layers. At 4.0 m there was no significant $^{35}S^0_{part}$ formation but some $^{35}S_{ox}$ still appeared despite the lack of oxygen or light. No sulfide oxidation could be detected at 4.2 m in the dark.

Separation of the $^{35}S_{ox}$ fraction into specific sulfur compounds showed that it consisted of about 50% [^{35}S]sulfate. The rest was mainly thiosulfate, some sulfite, and a little polythionate. There was no

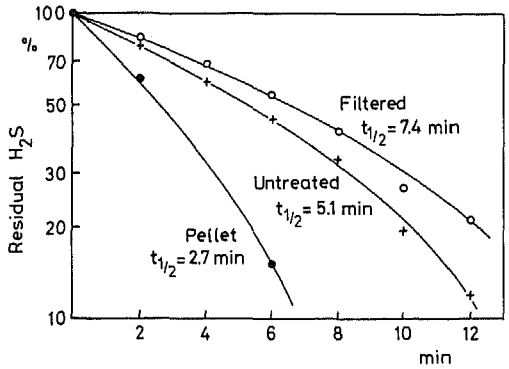

Fig. 10. Sulfide oxidation in oxic water samples from chemocline incubated in the laboratory at 45°C. Effect of filtration and of concentrating particulate fraction is shown.

consistent difference in this pattern between light and dark, different depths, or 15-min and 2.5-h incubations.

The $^{35}S^0$ incubations again showed rapid oxidation in the oxic zone. The initial oxidation rate was slightly higher in the chemocline than in the layers above, indicating catalysis, possibly by bacteria. After 2.5 h, the S^0 oxidation was most complete at the highest oxygen concentration. Significant oxidation took place at 4.0 m, although oxygen was not detectable. At 4.2 m, S^0 was not oxidized. The degree of S^0 transformation into the H_2S and polysulfide pool increased with depth. Since polysulfide formation is not a reductive process, it is of interest to separate it from H_2S formation. This was unfortunately not done in this experiment. Control experiments in the laboratory a few days later indicated that polysulfide formation was the dominating process but that some $^{35}S^0$ was transformed into $[^{35}S]H_2S$. Water samples from 4.0-m depth were incubated with $^{35}S^0$ for 1 h at 54°C. The subsequent fractionation showed that 50% of the label had gone into the H_2S and polysulfide pool. Of this, only 3–4% was actually present as $[^{35}S]H_2S$, while the rest was $^{35}S^0$ dissolved as polysulfide. The production of $[^{35}S]H_2S$ need not however be a biological, reductive process. Voge (1939) demonstrated a rapid, chemical isotope exchange between H_2S and S^0 at 100°C in the presence of polysulfide.

Separation of the $^{35}S_{ox}$ into specific compounds showed that 60% was present as SO_4^{2-}. The rest was mainly $S_2O_3^{2-}$, with some SO_3^{2-} and polythionate.

The dark $^{35}S^0$ incubations gave similar results to the light incubations. However, at 3.75 and 4.0 m, significantly more ^{35}S was found in the H_2S and polysulfide pool in the dark than in the light.

O_2 and H_2S laboratory experiments—The field studies of H_2S oxidation by oxygen in the chemocline do not show whether this process is mainly biologically or chemically catalyzed. To investigate the potential importance of microorganisms for sulfide oxidation in the lake, we collected samples from the oxygen–sulfide interface and brought them to the laboratory.

In one type of experiment the kinetics of H_2S disappearance were studied in untreated and in centrifuged or filtered samples. Samples were placed in conical flasks with airspace above and incubated aerobically with shaking at the in situ temperature. Sulfide was added in ecologically relevant concentrations (5–10 μM) and its disappearance was monitored on subsamples by sulfide assay at regular intervals.

The results of one experiment are shown in Fig. 10. The water was collected at 3.7–3.8-m depth and samples were prepared as follows: untreated; membrane-filtered (0.22 μm); centrifuged (10,000 rpm for 45 min at 37°C), supernatant used; centrifuged, pellet resuspended in 1/20 of original volume of membrane-filtered water from the same depth. Sulfide was added to a final concentration of 6 μM to give similar starting conditions in all samples for both H_2S and O_2. H_2S was present at the sampling depth in the lake in this order of magnitude at night but was replaced by oxygen during the day. Figure 10 shows that the rate of sulfide oxidation decreased when the water was filtered to remove bacteria. When the bacteria were concentrated by centrifugation and resuspension (pellet) the sulfide oxidation rate was strongly stimulated, corresponding to a twofold reduction in half-life. The actual rates of

Table 3. Oxidation rate of sulfide in dark and light incubated, oxic chemocline samples. Untreated and treated samples compared in the laboratory.

Sample type	Sulfide oxidation rate (μmol·liter^{-1}·h^{-1})	
	Dark	Light
Untreated	16	25
Supernatant	11	11
Concd pellet	22	33

Table 4. Sulfide-dependent oxygen uptake rate of untreated and treated chemocline samples in the dark.

Sample type	Oxygen uptake rate (μmol·liter^{-1}·h^{-1})
Untreated	99
Boiled	63
Filtered (0.22 μm)	45
Concd pellet	235
Concd pellet, boiled	92

oxidation were 24, 35, and 67 μmol $H_2S \cdot liter^{-1} \cdot h^{-1}$ in the filtered sample, the untreated sample, and the concentrated pellet. The oxidation rate in the supernatant after centrifugation did not differ significantly from that in the untreated sample, probably because the centrifugation of bacteria was rather ineffective in the dense brine.

The experiment in Fig. 10 was repeated three times with similar results. The half-life of H_2S in centrifuged samples varied from 4.6 min in a 5–12-fold concentrated pellet to 55 min in the supernatant. In one experiment, H_2S disappearance was measured in closed tubes without airspace in both dark and light (Table 3). The initial oxygen and sulfide concentrations were 10 and 20 μM. Light stimulated the oxidation rate by 35% in both the untreated sample and the concentrated pellet, but it had no effect in the supernatant. This shows that the photosynthetic organisms, mainly cyanobacteria which are more efficiently spun down than other bacteria, are involved in the oxidation of H_2S, either directly or indirectly.

The rate of H_2S disappearance in samples from the chemocline (3.9 m) was also compared to the rate in the oxic zone at 2.5 m under similar oxygen and sulfide conditions. In untreated samples the rate was 14% faster in the chemocline than at 2.5 m and in the equally concentrated pellets it was 80% faster. Thus, the bacterial fraction from the chemocline has a much stronger catalytic effect than that from the oxic water. Even at 2.5 m, however, the H_2S disappearance rate was 35% greater in the concentrated pellet than in the untreated sample.

The effect of H_2S addition on the rate of oxygen consumption in the samples was studied in an oxygen uptake chamber. The samples were incubated in the dark at 45°C. After the endogenous oxygen consumption rate was determined, a concentrated sulfide solution was injected to give a final concentration of 50 μM and the initial rate of oxygen disappearance read on a recorder. Table 4 shows the results of one experiment. The initial O_2 concentration was about 60 μM in all samples. The endogenous oxygen uptake rate was negligible in the untreated sample. The sulfide-dependent oxygen consumption was 99 μmol $O_2 \cdot liter^{-1} \cdot h^{-1}$. Boiling the sample for 10 min significantly decreased the uptake rate, filtration through a membrane filter (0.22 μm) decreased it even further. This indicates that the sulfide-dependent oxygen uptake is stimulated by living microorganisms. It also shows that either the boiled bacteria have retained part of their sulfide-oxidizing capacity or other particulate matter has some catalytic effect. When the bacteria and other particles are concentrated by centrifugation, the oxygen uptake increases strongly upon addition of sulfide. When the concentrated pellet is first boiled for 10 min, the rate remains low but is still higher than that of the nonconcentrated, boiled sample.

In summary, the H_2S and O_2 disappearance experiments show that sulfide oxidation and the sulfide-dependent rate of oxygen consumption are highly stimulated by the particulate fraction containing living microorganisms. We consider this strong evidence that the process of sulfide oxidation with oxygen is directly

influenced by microorganisms present in the chemocline.

The oxygen uptake experiments in Table 4 were run until the uptake rate had returned to the endogenous rate. The final oxygen uptake was slightly more than 1 mol O_2 taken up per 1 mol H_2S. This indicates that a considerable part of the sulfide must have been oxidized beyond S^0, which would need 0.5 mol O_2 only, but also that the oxidation does not go completely to sulfate (during the period of the experiment), which would need 2 mol O_2. This stoichiometry agrees with the radiotracer results.

[^{14}C]CO_2 fixation—We measured the rate of dark CO_2 fixation in vertical profiles in the lake to see whether a peak in microbial activity could be detected in the chemocline. If bacteria were present that could gain energy from the oxidation of reduced sulfur compounds with oxygen this should be demonstrable by an increase in the specific CO_2 fixation rate at the oxygen–sulfide interface.

To determine a proper incubation time, we first did time-course experiments. Samples from the chemocline were injected with [^{14}C]CO_3^- and incubated in situ in dark bottles. An example of the results is shown in Fig. 11. The dark CO_2 fixation is linear for 80 min only and then levels off, but with a large air bubble in the bottle, the linearity continues beyond 100 min. Thus inhibition of dark CO_2 fixation after 80 min seems to be due to the lack of oxygen required by the microorganisms. Repetition of the experiment on different dates and different depths showed that linearity was always maintained for at least 1 h and often for several hours or more. Light CO_2 fixation was always linear for more than 2 h. For the following determinations of in situ dark fixation rates an incubation time of 30 min was used.

We made a detailed field study of the diurnal variation in light and dark CO_2 fixation in the chemocline. At 16 depths, from 3.40 to 3.95 m, water samples were collected four times during a period of 32 h. For each depth, one dark and two light bottles were injected with [^{14}C]CO_3^-. To

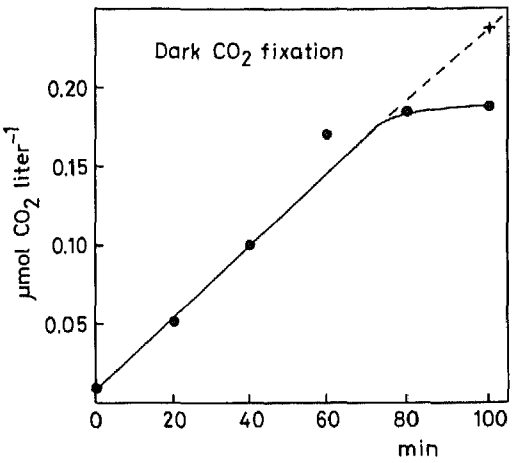

Fig. 11. Time-course of dark CO_2 fixation in water samples from chemocline (solid line). Linearity is sustained beyond 80 min by added oxygen (broken line).

one of the light bottles DCMU was added to inhibit oxygenic photosynthesis. The bottles were then immediately lowered back to the sampling depth for incubation. Additional water samples were collected for combined assay of oxygen and sulfide and for determining protein and chlorophyll. To allow time for sample preparations in the boat, we started the incubations at 10-min intervals; thus, there is a total time lag of 2–3 h between incubations at 3.40 and at 3.95 m.

The results of three sampling series are shown in Fig. 12. Around midnight (2300–0100 hours) the oxygen–sulfide interface was at 3.6–3.7 m. The dark CO_2 fixation had a broad maximum with a peak just at the upper range of sulfide. The high CO_2 fixation in the chemocline is presumably due to the presence of both oxygen and reduced sulfur compounds which are used for bacterial energy metabolism. Within the sulfide zone, H_2S may serve as the most important energy source. Just above the sulfide zone, where dark CO_2 fixation was also high, another reduced compound such as thiosulfate may be the energy source (cf. Fig. 4). The zone of high dark CO_2 fixation extended from 3.4 m downward just to the lower range of oxygen. These lim-

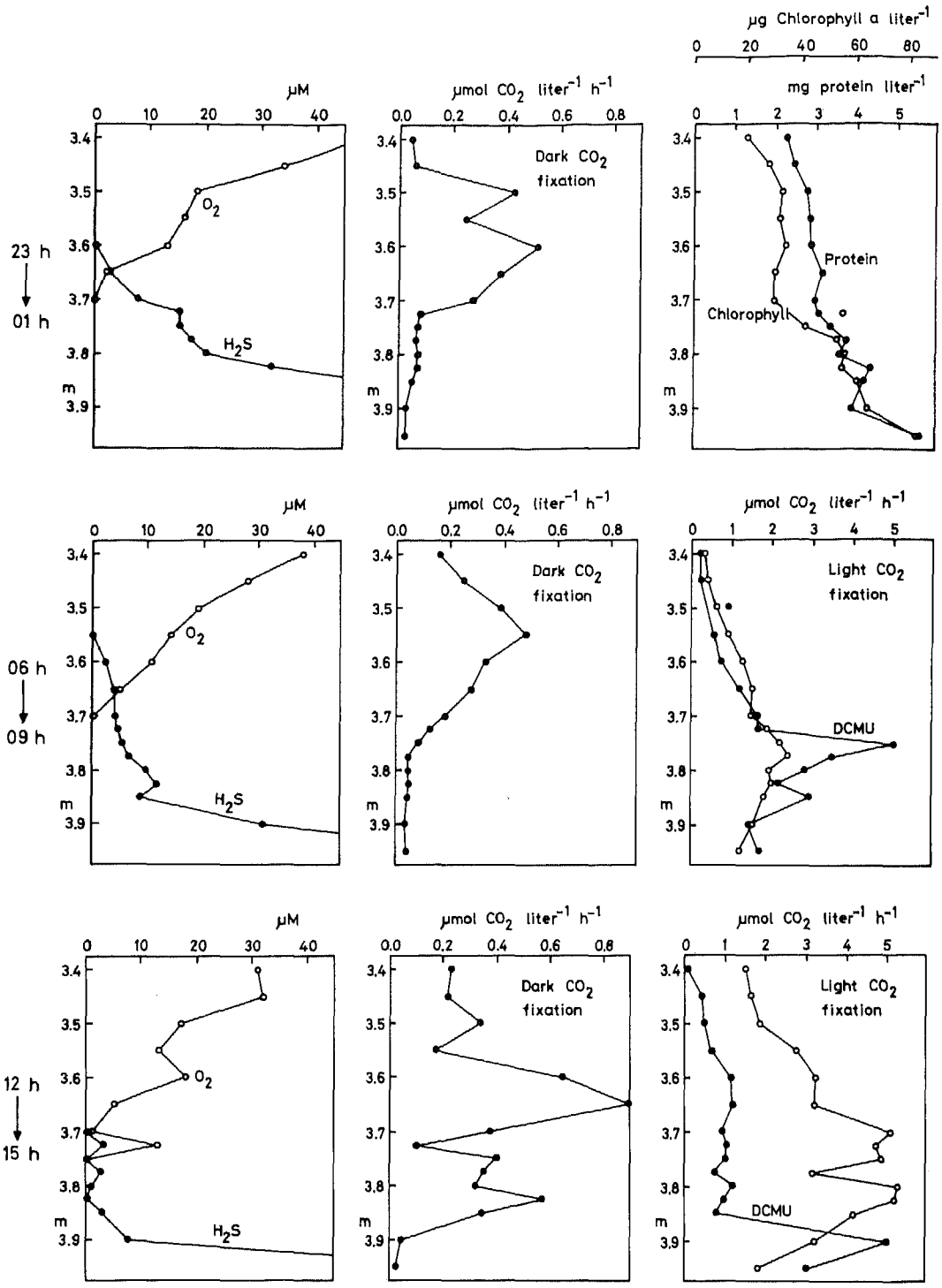

Fig. 12. Distribution of oxygen and sulfide, dark CO_2 fixation, and light CO_2 fixation with or without DCMU in chemocline. In situ incubations for 30 min at different hours. Protein and chlorophyll *a* gradients also shown (28–29 March).

its are explained by the lack of electron donor above and acceptor below this zone.

In the early morning (0600–0900 hours) the sulfide level decreased as H_2S was now rapidly being oxidized in the light. The dark CO_2 fixation had a maximum just above the sulfide zone, with lower rates in the layer of coexisting oxygen and sulfide. The light CO_2 fixation without DCMU increased gradually with depth to a maximum at 3.75 m. In the presence of DCMU, photosynthesis was slightly inhibited down to 3.7 m, below which inhibition was suddenly changed into strong enchancement. The photosynthetic organisms which completely dominated within and below the chemocline were filamentous cyanobacteria (*Microcoleus* sp. and *Oscillatoria* sp.), in densities of up to 10^5 filaments per ml. In addition to oxygenic photosynthesis they can conduct anoxygenic photosynthesis under sulfide conditions (Cohen et al. 1975*b*). The release of DCMU inhibition, at the depth of 5 μM H_2S, indicates that the cyanobacteria shifted from oxygenic photosynthesis above to anoxygenic photosynthesis below this depth. Thus the lower boundary of photosynthetic oxygen production coincided with the extension of the dark CO_2 fixation peak into the sulfide zone.

In the afternoon (1200–1500 hours) the sulfide level was further reduced and a secondary O_2 peak appeared at 3.73 m. Dark CO_2 fixation was now also high at 3.75–3.85 m, although oxygen was not detectable. The light CO_2 fixation measurements, however, showed strong DCMU inhibition down to 3.85 m, below which the inhibition was suddenly released. Consequently, photosynthetic oxygen production took place down to 3.85 m at a high rate, according to the light CO_2 fixation results, and the lack of detectable oxygen must be due to rapid consumption. Thus the bacteria which caused the high dark CO_2 fixation at 3.75–3.85 m may use the oxygen, which is then rate-limiting, as an electron donor, and use sulfide as the energy source. The sharp minimum in dark CO_2 fixation at 3.73 m coincided with a peak in both O_2 and H_2S. This supports the above conclusions, since a low rate of bacterial chemosynthesis would lead to less efficient O_2 and H_2S removal and thus to accumulation of both.

The release of DCMU inhibition below 3.85 m in the afternoon coincided with the depth of 5–10 μM H_2S. Cyanobacteria below 3.7 m which were not DCMU inhibited in the morning now showed inhibition down to 3.85 m. In the 3.7–3.85-m layer, the cyanobacteria must therefore have shifted from anoxygenic to oxygenic photosynthesis during the morning in accordance with the decreasing sulfide level.

A fourth series of dark CO_2 fixation measurements which were made just after sundown at 1800–2000 hours showed a pattern similar to that of the 2300–0100 hours series. The oxygen–sulfide interface was 5 cm deeper and the high dark fixation also reached 5 cm deeper than at midnight.

The chlorophyll *a* and protein distributions in the chemocline showed a regular increase with depth and did not change between day and night (Fig. 12). The sharp peaks of dark CO_2 fixation were thus not associated with peaks in total biomass. This means that the specific rates of dark fixation will have depth profiles similar to those in Fig. 12. The peaks of specific dark CO_2 fixation are 0.18, 0.17, and 0.28 μmol $CO_2 \cdot$mg protein$^{-1} \cdot h^{-1}$ at 2300–0100, 0600–0900, and 1200–1500 hours; above and below the chemocline, the specific rates are only 0.05 at 3.40 m and 0.005 at 3.95 m.

The specific rate of photosynthesis also showed a depth distribution similar to that in Fig. 12. The maximum specific rate of light CO_2 fixation (0.16 μmol $CO_2 \cdot \mu$g Chl $a^{-1} \cdot h^{-1}$) was reached at noon at 3.7 m, where the light intensity was 100 μeinst$\cdot m^{-2} \cdot s^{-1}$—20% of the surface value. The highest chlorophyll *a* concentration (180 μg$\cdot$liter^{-1}) was reached at 4.0 m, below which it decreased to 60 μg$\cdot$liter^{-1} at the bottom.

Photosynthetic sulfur bacteria, which were abundant in the hypolimnion in

Table 5. Effect on dark CO_2 fixation of addition of H_2S, S^0, and O_2 expressed as percentage change in fixation rate relative to untreated sample. In situ incubation of samples from just above and below oxygen–sulfide interface (11 April).

Substrate added	Depth (zone)	
	3.55 m (O_2)	3.75 m (H_2S)
H_2S, 5 μM	+120	−47
H_2S, 25 μM	+26	−91
H_2S, 100 μM	−83	−92
O_2, 10 μM	−9	+88
O_2; H_2S, 5 μM	+34	+49
O_2; H_2S, 25 μM	+86	+12
O_2; H_2S, 100 μM	−76	−83
S^0, 200 μM	+109	−19

previous years (Cohen et al. 1977*b*), were not present in significant numbers on this occasion; microscopy of centrifuged samples and assays for bacteriochlorophyll *a* both gave negative results throughout the sulfide zone.

One month later, however, when the results of Fig. 5 were obtained, a bloom of purple sulfur bacteria (*Chromatium* sp.) had developed just below the chemocline. Bacteriochlorophyll *a* peaks of 20 $\mu g \cdot liter^{-1}$ were found at 2.5 and 2.8 m and a chlorophyll *a* peak from cyanobacteria reached 40 $\mu g \cdot liter^{-1}$ at that depth.

To investigate some of the environmental factors which regulate or limit the rate of dark CO_2 fixation, we studied the effects of temperature and of addition of energy substrates.

The temperature dependence of the dark CO_2 fixation rate is shown in Fig. 7. The highest fixation rate was found at 52°C, close to the in situ temperature of 54°C. Above this temperature the microbial activity decreased steeply and around 62°C stopped completely. The temperature curve is very similar to that for the sulfate-reducing activity and characterizes the CO_2-fixing organisms as thermophiles.

The effect of addition of energy substrates for the dark CO_2 fixation was studied in the lake on several occasions. Samples from different depths were incubated in situ for 2 h in dark bottles with and without the addition of O_2, NO_3^-, H_2S, S^0, or $S_2O_3^{2-}$ in various combinations, and the stimulating or inhibiting effect of these substrates was used to indicate whether reduced sulfur compounds could serve as electron donors. An example of the results is shown in Table 5. The two sampling depths were just above and below the chemocline. At 3.55 m, oxygen was present but not sulfide and the dark fixation rate was 0.55 $\mu mol\ CO_2 \cdot liter^{-1} \cdot h^{-1}$ in samples with no substrate additions. At 3.75 m there was 8 μM H_2S and dark fixation was 1.45 $\mu mol\ CO_2 \cdot liter^{-1} \cdot h^{-1}$ in untreated samples.

In the oxic layer, the addition of H_2S stimulated the dark CO_2 fixation strongly at low concentration. At high concentration the dark fixation was inhibited, probably because the large H_2S pool rapidly depleted the available oxygen. Addition of oxygen alone had no significant effect since it was already present. The addition of both oxygen and sulfide showed the most stimulation at the intermediate sulfide concentration, probably because the two substrates coexisted for the longest period. Elemental sulfur also had a strong stimulating effect, indicating that it can serve as an energy substrate. Although 200 $\mu mol\ S^0 \cdot liter^{-1}$ was added, the S^0 concentration dissolved in the water was only in the order of 5 μM due to the low solubility of sulfur (Chen and Morris 1972).

In the sulfide zone, the addition of H_2S caused only inhibition of the endogenous, high CO_2 fixation rate; thus a small pool of available electron acceptors seems to be present here which is depleted by the addition of sulfide. The strong stimulation by oxygen shows that it is the electron acceptor which is limiting for the fixation rate. This explanation is supported by the combined effects of oxygen and sulfide. When sulfide is added to oxygen it causes inhibition because it removes the oxygen. Elemental sulfur has no significant effect here, in accordance with the previous conclusion that it is the electron acceptor which is limiting.

The general conclusions of this and five other substrate addition experiments can be summarized as follows:

At 2.5-m depth, in the oxic zone far from the chemocline, the dark CO_2 fixation is low. H_2S, S^0, and $S_2O_3^{2-}$ cause some stimulation. So does NO_3^-, which is probably used as a nitrogen source rather than as an electron acceptor.

At 3.5 m, just above the oxygen–sulfide interface, dark fixation is high. All three reduced sulfur compounds cause stimulation while O_2 and NO_3^- have little or no effect.

At 3.7 m, just within or below the interface, dark fixation is still high. There is mostly stimulation by O_2, sometimes also by the reduced sulfur compounds.

At 3.9 m, below the interface, dark fixation is low. The pattern of substrate influence is irregular. There is sometimes stimulation by O_2 and sometimes also by reduced sulfur compounds.

At 4.2 m, deep within the sulfide zone, dark fixation is even lower. There is little consistent stimulation—mainly by NO_3^-, if any.

In addition to this depth variation in the pattern of dark CO_2 fixation, there was a general increase in the fixation rate throughout spring from February to May.

Discussion

The sulfur cycle of the chemocline—From the results of the chemical, radiotracer, and microbiological studies that have been presented here, an integrated picture of the sulfur cycle emerges which will now be discussed.

The chemocline was defined by the oxygen–sulfide interface, situated between 2 and 4 m. The actual layer in which the two compounds coexisted at a given time was only 0–10 cm thick (Figs. 2, 3, 5, and 12). During the diurnal light and dark cycle, this narrow horizon migrated up and down by 20–30 cm. Thus, the layer in which oxygen and sulfide were both present within 24 h had a thickness of about 30 cm. In this chemocline the day and night rhythm produces a very dynamic sulfur cycle. In the daytime, oxidizing processes predominate due to photosynthesis, while at night these processes are to a large extent reversed by heterotrophic activity. The cyanobacteria were found to be of general importance for the processes of sulfide oxidation and especially for the sulfur cycle in the chemocline. The two dominating forms were filamentous, nonheterocystous species of *Microcoleus* and *Oscillatoria*. Photosynthetic sulfur bacteria were not of significance during most of the study.

Pure cultures of *Oscillatoria limnetica* and *Microcoleus* sp. isolated from the Solar Lake hypolimnion have the capacity for anoxygenic photosynthesis when grown with sulfide in the medium (Cohen et al. 1975*b*; Garlick et al. 1977). Thus *Oscillatoria* is able to switch from oxygenic to anoxygenic photosynthesis in the light when the environment changes from oxic to sulfide conditions. An induction period of about 2 h is needed (Oren and Padan 1978). If all the sulfide is ultimately consumed, *Oscillatoria* immediately switches back to the oxygenic metabolism, and the capacity for anoxygenic photosynthesis is gradually lost during subsequent growth. When grown under anoxygenic conditions, *Oscillatoria* uses H_2S as an electron donor for CO_2 reduction; the H_2S is oxidized only to elemental sulfur which precipitates as small granules around the filaments (Cohen et al. 1975*a*). Facultative anoxygenic photosynthesis is a common feature among the cyanobacteria; it was demonstrated in 16 out of 30 different strains tested by Garlick et al. (1977). Together with their extreme sulfide tolerance (8.5 mM H_2S in *O. limnetica*) this explains the selective advantage of the cyanobacteria in high sulfide environments such as the hypolimnion of Solar Lake and in environments with shifting oxygen and sulfide conditions such as the chemocline.

The experiments with DCMU inhibition of light CO_2 fixation in Solar Lake demonstrated a shift from oxygenic above to anoxygenic photosynthesis below the

chemocline. The shift took place at the level where the sulfide concentration exceeded 5–10 μM (Fig. 12). This is much lower than the 0.1–1 mM H_2S needed to induce anoxygenic photosynthesis in laboratory experiments. At 3.7–3.85-m depth the cyanobacteria performed anoxygenic photosynthesis in the early morning but shifted to oxygenic after they had reduced the sulfide concentration below 5 μM (Fig. 12). At this stage a large oxygen pool could suddenly build up (Fig. 3). The change in the type of photosynthesis seemed to be a true induction of the bacteria and not due to vertical migration since there was no difference in the protein and chlorophyll *a* distributions between morning and evening.

The photosynthetic oxidation of H_2S to S^0 explains the abundance of particulate elemental sulfur and of polysulfide sulfur in the hypolimnion. S^0 formation in the light was detectable mainly just below the chemocline where anoxygenic photosynthesis was highest and where a peak of particulate S^0 developed during the day (Table 3, Fig. 5). The high degree of S^0 formation may also be characteristic of other stratified lakes and fjords with a photic sulfide zone where purple and green sulfur bacteria usually are the dominating phototrophic microorganisms. These bacteria differ from the cyanobacteria, however, in being able to photo-oxidize the sulfide completely to sulfate, with elemental sulfur only as an intermediate oxidation state (Pfennig 1975).

In the absence of oxygen, the elemental sulfur is not oxidized further (Table 2), but it may disappear again during the night together with polysulfide (Fig. 5). Radiotracer experiments indicated that S^0 was partly reduced to H_2S (Table 2). Several types of anaerobic, heterotrophic bacteria can utilize S^0 as an electron acceptor (Biebl and Pfennig 1977; Pfennig and Biebl 1976; Wolfe and Pfennig 1977). Of these, *Desulfuromonas acetoxidans* has been isolated from Solar Lake by N. Pfennig. Van Gemerden (1967) showed that purple sulfur bacteria (*Chromatium*) in the dark are able to reduce intracellular S^0 to H_2S with a concomitant oxidation of storage carbohydrate to poly-hydroxy-butyrate. A similar dark meta olism in cyanobacteria would explain tl efficient reduction of S^0 during the nigl and studies of *O. limnetica* grown anc ygenically in the light have shown th this is indeed the case. In the dark, *limnetica* can respire anaerobically by r ducing extracellularly deposited sulfur H_2S (Oren and Shilo in prep.). Intrace lular polyglucose is concomitantly o: dized to CO_2. The cyanobacteria in tl lake may therefore carry out both tl shift from H_2S to S^0 during the day ar from S^0 to H_2S at night. At the interfa where oxygen and sulfide coexist in tl chemocline, a rapid oxidation of sulfi takes place: the half-life of H_2S (and O_2) was 3–12 min. This is a very rap turnover; the general half-life in seawat ranges from 10–20 min to several da (Chen and Morris 1972, Östlund ar Alexander 1963). The explanation is pa ly the high temperature of 50°–55°C Solar Lake and perhaps also the partic pation of bacteria.

Since the bacterial oxidation of sulfi with oxygen is always accompanied by spontaneous, chemical reaction, it is di ficult to distinguish the separate role the bacteria. From the similarity of su fide oxidation rates in poisoned and u altered water samples, several worke have concluded that bacteria play no si nificant role in the initial step of the pr cess (Sorokin 1970, 1972; Wheatlan 1954). Sorokin (1970, 1972) found a bi logical catalysis only in the last oxidatic step from thiosulfate to sulfate; the fir step seemed purely chemical.

We also used the kinetics of sulfide o idation in water samples from the ch mocline to detect a possible effect of ba teria. The sterile control was prepare not by poisoning but by membrane filtr tion or centrifugation. The results clear showed a consistent decrease of the ra of sulfide oxidation or sulfide-depende oxygen consumption in sterile sample The presence of the normal bacteri population increased the reaction rate b 35–100% (Fig. 10, Tables 3 and 4). E concentrating the bacteria by centrifug

tion and resuspension in a smaller volume, we obtained a strong stimulation of the reaction rate. These effects are shown by the whole particulate fraction and cannot directly be attributed to the bacteria alone. However, the involvement of bacteria is strongly indicated by the observation that the particulate fraction lost 80% of its stimulating effect upon heat sterilization (Table 4). The fact that the oxidation rate of trace amounts of sulfide is highest in the chemocline in spite of the low oxygen levels there (Table 2) also points toward the participation of bacteria in this layer.

Measurements of dark CO_2 fixation in the lake (Fig. 12) show that the bacteria had a maximum specific fixation rate in the layer where oxygen coexists with sulfide and thiosulfate. Above the sulfide zone, this rate is strongly stimulated by the addition of only 5 μM H_2S. Since a much larger pool of thiosulfate is already present at this depth (Fig. 4), the stimulating effect would not be detectable after chemical oxidation of the sulfide to thiosulfate.

It is not possible from our results to know whether true thiobacilli are active in the chemocline. The hot brine will exclude most strains, but thermophilic thiobacilli have been isolated which will grow up to 55°C (Williams and Hoare 1972) or even 75°C (Roux et al. 1977). The sulfide-dependent dark CO_2 fixation in the lake showed a peak at 52°C (Fig. 7).

The actual presence of thermophilic, sulfide-oxidizing autotrophs was demonstrated by enrichment techniques. Samples from the chemocline were inoculated into a chemostat at 22° or 45°–50°C in a medium of Solar Lake water enriched with inorganic nutrients. Bacteria grew at high cell densities at a dilution rate of $0.05 \cdot h^{-1}$. Oxygen was 10–20% of air saturation and sulfide or thiosulfate was the only energy source (plus some organic matter in the Solar Lake water which was insignificant relative to cell yield). Both substrates were oxidized to sulfate at 22°C. At 45°C thiosulfate was also oxidized to sulfate, while sulfide was oxidized only to elemental sulfur with a fourfold reduction of the cell yield.

The products of sulfide oxidation with oxygen in the chemocline were mainly sulfate and thiosulfate, with sulfite and polythionates being less important. This is in accord with the results of Chen and Morris (1972), of Sorokin (1970, 1972), and of Cline and Richards (1969) who found the sulfide oxidation products in seawater to be 50–60% SO_4^{2-}, 30–35% $S_2O_3^{2-}$, and 10–15% SO_3^{2-}. Thiosulfate, sulfite, and polythionate are not normal end products of bacterial sulfide oxidation and their formation in the lake indicates that a significant fraction of the oxidation process is chemical.

The oxidation products of elemental sulfur were similar to those of sulfide although more went completely to sulfate. It was not shown whether the process was bacterial or chemical. Mosser et al. (1974), however, found no chemical S^0 oxidation in Formalin-killed water samples in the temperature range of 35°–90°C. The high degree of H_2S transformation into S^0 in the lake may thus increase the efficiency of bacterial utilization of the chemical energy bound in reduced sulfur.

As a result of the H_2S and S^0 oxidation, a peak of thiosulfate builds up in the chemocline. The thiosulfate may serve as an energy source for aerobic bacteria which oxidize it to sulfate or perhaps to tetrathionate (Sorokin 1970, 1972; Tuttle and Jannasch 1973*a*, 1977). Cyanobacteria (*Anacystis nidulans*) can also photo-oxidize thiosulfate to sulfate (Utkilen 1976). Sulfite is also produced but does not reach detectable concentrations.

We did not demonstrate a reduction of thiosulfate back to sulfide in the anoxic zone but inferred it from the radiotracer experiment in Fig. 9. In addition to the obligately anaerobic, sulfate-reducing bacteria, some facultatively anaerobic marine bacteria and cyanobacteria can also reduce thiosulfate to sulfide (Sheridan and Castenholz 1968; Tuttle and Jannasch 1973*b*).

In conclusion, we have found a very dynamic sulfur cycle to be connected

with the chemocline. Oxidative and reductive processes alternate with depth and with time. Elemental sulfur and thiosulfate function as intermediates which may be either oxidized or reduced. A major diurnal cycle exists between sulfide and elemental sulfur and to some extent also thiosulfate. About 50–60% of the oxic reactions lead to sulfate formation; since sulfate reduction is of little significance within the chemocline, this causes a constant loss of reducing power from the cycle. A continuous inflow of sulfide from below maintains the bacterial and chemical processes.

Quantitative aspects—The sulfate reduction rate per square meter of the water column can be calculated from the data in Fig. 6. The rates over the deepest part of the lake (5 m) are 2.4 mmol $SO_4^{2-} \cdot m^{-2} \cdot d^{-1}$ in February and 4.6 in March. These rates are comparable to the 10 $mmol \cdot m^{-2} \cdot d^{-1}$ calculated by Sorokin (1962, 1972) for the Black Sea. However, due to the great depth of the Black Sea, the intensity of sulfate reduction there is much lower. The maximum reduction rate of 0.3 $\mu mol \cdot liter^{-1} \cdot d^{-1}$ found near the Black Sea bottom is 20-fold lower than the maximum rates in Solar Lake. In meromictic Lake Belovod, maximum rates of 1–5 $\mu mol \cdot liter^{-1} \cdot d^{-1}$ were found (Sorokin 1970).

The rate of sulfide accumulation in the water column of Solar Lake can be calculated from the measured sulfide concentrations. Between February and April, the average rate of accumulation is 38 mmol $H_2S \cdot m^{-2} \cdot d^{-1}$, 10 times the rate of production in the water. Consequently the main sulfide source must be the sediments, where a sulfate reduction rate of 70 $mmol \cdot m^{-2} \cdot d^{-1}$ was measured in the cyanobacterial mats (Jørgensen and Cohen 1977). This is sufficient to account both for the rate of accumulation and for an oxidation loss of sulfide in the chemocline. The upper limit of sulfide oxidation rate in the chemocline, which can be calculated from the production minus accumulation, is 70 + 4 − 38 = 36 mmol $H_2S \cdot m^{-2} \cdot d^{-1}$. Actually it is somewhat lower, because the deepest sediments had <70 $mmol \cdot m^{-2} \cdot d^{-1}$ of sulfate reduction. Furthermore, a part of the H_2S is being oxidized to S^0 by phototrophic organisms within the sulfide zone, so that the accumulation rate of total S^0 including polysulfide-S^0 (2–5 $mmol \cdot m^{-2} \cdot d^{-1}$) should be subtracted. This leaves an estimated 20–30 mmol $H_2S \cdot m^{-2} \cdot d^{-1}$ to be oxidized in the chemocline.

The rate and mechanism of sulfide oxidation in the chemocline varies with depth and time in a very complex manner. A calculation of the total oxidation rate per unit area and time is therefore not possible from the directly measured sulfide oxidation. Still, the chemical and radiotracer results give some idea of the order of magnitude of the process. The diurnal changes in sulfide concentration in the chemocline must give a minimum estimate of its rate of transformation. The time-course in oxygen and sulfide at the depth of maximum difference between day and night (3.83 m) is shown in Fig. 13 (data extracted from Fig. 3). Sulfide accumulates in this layer during the night at an average rate of 3 μmol $H_2S \cdot liter^{-1} \cdot h^{-1}$. When the photosynthetic oxidation starts in the morning it disappears again at a rate of up to 9 $\mu mol \cdot liter^{-1} \cdot h^{-1}$. When sulfide is almost depleted, oxygen accumulates at a similar rate, 10 μmol $O_2 \cdot liter^{-1} \cdot h^{-1}$. The stoichiometry of anoxygenic photosynthesis by the cyanobacteria is: $CO_2 + 2\ H_2S \rightarrow CH_2O + 2\ S^0 + H_2O$. The maximum photosynthetic rates of 2–5 μmol $CO_2 \cdot liter^{-1} \cdot h^{-1}$ (Fig. 12) are therefore equivalent to the oxidation of 4–10 μmol $H_2S \cdot liter^{-1} \cdot h^{-1}$, which is close to the observed rate of disappearance. Elemental sulfur showed similar maximum rates of transformation when studied 2 months later (Fig. 5A). In the middle of the day particulate S^0 accumulated at a rate of 9 μmol $S^0 \cdot liter^{-1} \cdot h^{-1}$ and disappeared again at night at a rate of 5 μmol $S^0 \cdot liter^{-1} \cdot h^{-1}$.

In the narrow zone where oxygen and sulfide coexist, the rate of sulfide disappearance in incubated samples was largely independent of light. From the initial rate of sulfide disappearance in Fig. 8 an

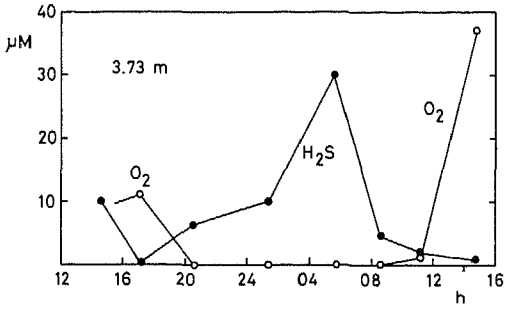

Fig. 13. Diurnal changes in oxygen and sulfide concentration in chemocline (22–23 March).

oxidation rate of 9 μmol·liter^{-1}·h^{-1} was calculated. Two other experiments showed rates of 10–20 μmol H_2S·liter^{-1}·h^{-1}. Thus the in situ oxidation rate by oxygen is of the same or slightly higher magnitude as the maximum photosynthetic oxidation rate.

The diurnal changes in the total pool of sulfide in the chemocline can be used as a minimum estimate of the amount of sulfide oxidized per day. From Fig. 3 we calculate that the sulfide pool decreases by 13 mmol $H_2S \cdot m^{-2}$ during the 9 h from 0600 to 1500 hours. If this rate of oxidation were sustained during the full diurnal cycle (which is probably not the case), it would be 35 mmol·m^{-2}·d^{-1}. The sulfide oxidation rate must thus be between 13 and 35 mmol·m^{-2}·d^{-1}. This fits well with the sulfide oxidation rate of 20–30 calculated from the sulfate reduction data.

Calculations of the increase in the particulate S^0, polysulfide-S^0, and $S_2O_3^{2-}$ pools between day and night (Fig. 5) yield 3, 5, and 1 mmol·m^{-2}. The concomitant decrease in H_2S was then 12 mmol·m^{-2}. This seems to indicate that a significant fraction of the sulfide oxidation during the daytime stops at the oxidation level of elemental sulfur and thiosulfate, to be regenerated from these pools the following night. The other fraction goes completely to sulfate.

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