Ecological observations on phototrophic sulfur bacteria and the role of these bacteria in the sulfur cycle of monomictic Lake Vechten (The Netherlands).

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Abstract. Lake Vechten, located centrally in The Netherlands, was formed in 1941 by sand digging. Its total area is 4.7 ha, the maximum depth is 11 m and the mean depth is 6 m. The water balance is regulated by rainfall, evaporation and horizontal groundwater flow; the residence time is approximately 2 years. Lake Vechten can be classified as warm-monomictic in most years; the stable summer stratification starts in May and lasts till the end of October. The lake is meso-eutrophic; during its history the sulfate concentration has increased considerably. Sulfide is produced mainly in the sediment, its concentration in the bottom water is restricted by the occurrence of high amounts of ferrous iron. At present small quantities of free sulfide can be found and phototrophic sulfur bacteria develop at the oxygen-sulfide borderline.

Phototrophic sulfur bacteria occurred in a two layer pattern; the upper one at 6.5-7.0 m depth contained *Chloronema*-type filaments, the deeper one at 7.0-7.5 m depth consisted mainly of *Chromatium* and a brown-colored *Chlorobium*. During their maximal development in September the phototrophic sulfur bacteria contributed on an areal basis ca. 18 % to the total pelagic primary production of the lake. Using sediment traps it was shown that phototrophic sulfur bacteria sank throughout the season at a rate of ca. 0.20 $m.d^{-1}$. From this depletion rate and from biomass and production studies a tentative specific growth rate for the phototrophic sulfur bacteria of 0.22-0.51 ln $units.d^{-1}$ was calculated.

Studies on the response of the carbon fixation rate to increasing sulfide concentrations and light intensities suggested that the phototrophic sulfur bacteria in Lake Vechten may be sulfide rather than light limited. Furthermore, from a balance study it was proposed that the phototrophic sulfur bacteria played only a marginal role in the transformations of sulfur compounds in the lake.

Key words: Phototrophic bacteria, *Chloronema*, *Chlorobium*, *Chromatium*, cyanobacteria, sulfide production, light limitation, iron sulfide.

1. Introduction

Planktonic phototrophic sulfur bacteria are common in many meromictic as well as monomictic lakes with anaerobic bottom waters during stratification. As phototrophic sulfur bacteria use mainly reduced sulfur compounds as electron donors in their photosynthesis, they prevail at depths where a continuous supply of sulfide and sufficient light are available (Pfennig 1978). Sulfide is formed by mineralization of organic matter but also by sulfate-reducing bacteria in deeper layers of the water column but mainly in the sediment (Stuiver 1967, Ingvorsen *et al.* 1981, Parkin and Brock 1981, Kohler *et al.*

1984). Sulfate reduction rates vary greatly, the highest being found in saline environments (Trudinger 1979).

An important consequence of sulfide production is the precipitation of metals as sulfides. Ferrous ions may react with sulfide ions to form amorphous ferrous sulfides. Apparently, iron availability in the bottom water of a lake may largely control the concentration of free sulfide, a mechanism which appears to occur in monomictic Lake Vechten (Verdouw and Dekkers 1980).

In Lake Vechten, moderate sulfate reduction rates in the sediment result in complete sulfate depletion of the bottom waters in August (Hordijk *et al.* 1985). Although methanogenesis dominated in anaerobic mineralization in the lake sulfate reduction in sediment accounted for a mineralization of 8-13 % of the total carbon input (Cappenberg *et al.* 1984). Despite this, sulfide was detected in relatively low concentrations, and did by no means account for the sulfate depletion.

The phototrophic sulfur bacteria played a minor role in the annual pelagic primary production (ca. 4 %), but on a daily areal-basis their share at their maximum in September was ca. 18 % (Steenbergen 1982). Compared with the observations of Parkin and Brock (1980a) these high productivity values suggest that in Lake Vechten at least during September, light might not be the primary factor influencing photosynthetic bacterial production.

The present study aims to evaluate the growth conditions of the phototrophic sulfur bacteria in Lake Vechten with emphasis on the relationship between sulfide supply and the bacterial production.

2. Materials and methods

Sampling and measurements were carried out in the eastern basin of Lake Vechten from a raft anchored in the deepest area (Fig. 1A).

Temperature and oxygen concentrations were measured *in situ* at 0.5 m depth intervals using a temperature-oxygen probe (Yellow Spring Instruments, Model 5739; or Orbisphere, Model 2714). PhAR light intensity profiles were measured using an underwater sensor (Lambda Instruments, Model LI 192 S).

Water samples were taken before sunrise and transported immediately to the laboratory in a cool box. For this either a Friedinger-type water bottle or a hand driven peristaltic pump connected with a rubber tube to a weighted T-shaped stainless steel inlet (internal diameter, 1.0 cm) was used. The inlet was suspended from the raft on a steel chain graduated at 10 cm intervals allowing reproducible sampling.

The water was collected in 140 ml serum bottles, previously flushed with oxygen-free nitrogen. To avoid contamination of the sampled water with atmospheric oxygen and to exclude air bubbles the sampling bottles were overflowed and screwcapped under water using a bucket containing water from the sampling depth.

Sedimentation rates were measured at 2-week intervals using sediment traps suspended from a buoy about 30 m away from the raft at the deepest area. Sediment traps were made of plexiglass, 0.5 cm thick, 30 cm long with an internal diameter of 6.0 cm; for details of their use see Verdouw and Dekkers (1982). Upon retrieval the water was siphoned off from the sediment which was collected on pre-weighed glass fibre filters (Whatman GF/F) dried in vacuum beforehand. In these subsamples pigments were analyzed according to Steenbergen and Korthals (1982) and Korthals and Steenbergen (1985). From this the total daily downward flux of various pigments was calculated on areal basis. Sinking velocity was calculated by dividing the measured settling flux by the mean concentrations of pigments in a one metre water column above the traps.

Carbon fixation rates were measured using the ^{14}C-technique according to De Kloet (1982), Steenbergen (1982) and Steenbergen and Korthals (1982), correcting for the dark uptake and subtracting the uptake of a formadehyde poisoned blank. Rates of CO_2 dark fixation ranged between 5-10 % of that of the CO_2 light fixation. Oxygenic and anoxygenic photosynthesis were distinguished using 3-3,4 dichlorophenyl-1, 1-dimethyl urea (DCMU) in the light bottles at a final concentration of 1.4 μM (cf. Jørgensen *et al.* 1979). Light and dark bottles were incubated either *in situ* (Steenbergen and Korthals 1982) or in the laboratory at *in situ* temperature (10-14 °C) for 3 hours at a light intensity of 10-14 $\mu Einst.m^{-2}.s^{-1}$ and in a linear light intensity gradient (Van Nes 1985). Light source was fluorescent tubes (Philips TL 33), with maximum emittance in the spectral range of 350-650 nm (Steenbergen 1974), resembling *in situ* light quality.

Alkalinity at the sampling depths was determined according to Golterman *et al.* (1978).

Rates of sulfate reduction were measured with $^{35}SO^{2}_{4}^{-}$ as tracer and subsequent microdistillation of the sulfide produced as described by Hordijk *et al.* (1985). Total sulfur was determined by the modified method of Tabatabai and Bremner (1970) and Hordijk and Van Engelen (unpublished). Sulfate was measured according to Hordijk *et al.* (1984). Sulfide was estimated either with a sulfide specific ion electrode (Orion, Model 94-16; see Steenbergen and Korthals 1982) or using Aquaquant kits 14416 and 14435 (E. Merck). Total reduced sulfur (S_{red}) was calculated as the difference between total sulfur and sulfate. Ferrous iron was estimated using Aquaquant kits 14438 and 14761 (E. Merck).

Dispersion coefficients were calculated from the temperature profiles by the Delft Hydraulics Laboratory (Delft, The Netherlands) using their Model DISPER, in which heat transfer from upper to lower regions is assumed to be due to dispersion.

Microscopic counts were performed with reflected-light fluorescence (Zeiss equipment) using the *in vivo* chlorophyll *a* fluorescence (see Steenbergen and Korthals 1982) and 4'6-diamidino-2-phenylindole (DAPI) as a fluorochrome stain.

3. Results

3.1. Study area

Different aspects of the limnology of Lake Vechten (52°04'N, 5°05'E) are described in the several papers in Gulati and Parma (1982) including the physiography (Steenbergen and Verdouw 1982).

The lake, situated centrally in The Netherlands, had its origin in excavation in 1941. It is divided by a ridge in the shallow area in the middle into two similar basins each with a maximum depth of ca. 11 m. The total area of the lake is 4.7 ha and its mean depth 6 m (Table 1).

Table 1. Morphometric data of Lake Vechten at a water level of 1.0 m above N.A.P. (Netherlands ordnance datum) (symbols according to Wetzel 1983).

	Whole lake[1]
Surface area A (x $10^3 m^2$)	47.15
Volume V (x $10^4 m^3$)	28.280
Maximum depth Z_m (m)	11.9
Mean depth $\bar{z}$ (m)	6.0
Depth of cryptodepression z_c (m)	10.9
Relative depth z_r	4.9 %
$\bar{z} : Z_m$	0.50
Length l (m)	470.0
Maximum breadth b (m)	130.0
Mean breadth $\bar{b}$ (m)	100.0
Shore line L (m)	1130.0
Shore line development D_l	1.47

[1] Data from Parma (1971); see Steenbergen and Verdouw (1982).

The relative depths (Z_r) of the two basins are 6.1 and 6.8 %. So the basins belong to the category of deep lakes with a relatively small surface area, which may explain the great stability of the summer stratification (Wetzel 1983). The lake has no surface inlet or outlet and is fed mainly by seepage and precipitation, its residence time is about 2 years. It is in a rural area, bounded by pastureland and bordered by willows, alders and poplars (Fig. 1A). Lake Vechten can be classified as meso-eutrophic with an average annual pelagic primary production of 0.28 g $C.m^{-2}.d^{-1}$ (De Kloet 1982). The winters being mild in The Netherlands, the lake is warm-monomictic in most years.

Summer stratification, with an anaerobic hypolimnion as well as increased concentrations of iron and manganese (maximally 17.4 and 4.4 $mg.l^{-1}$ at 10 m depth; Verdouw and Dekkers 1980), begins in May and lasts until mid-November. The thermocline develops in May at a depth of 4-5 m and steadily descends until turbulent mixing of the whole lake starts.

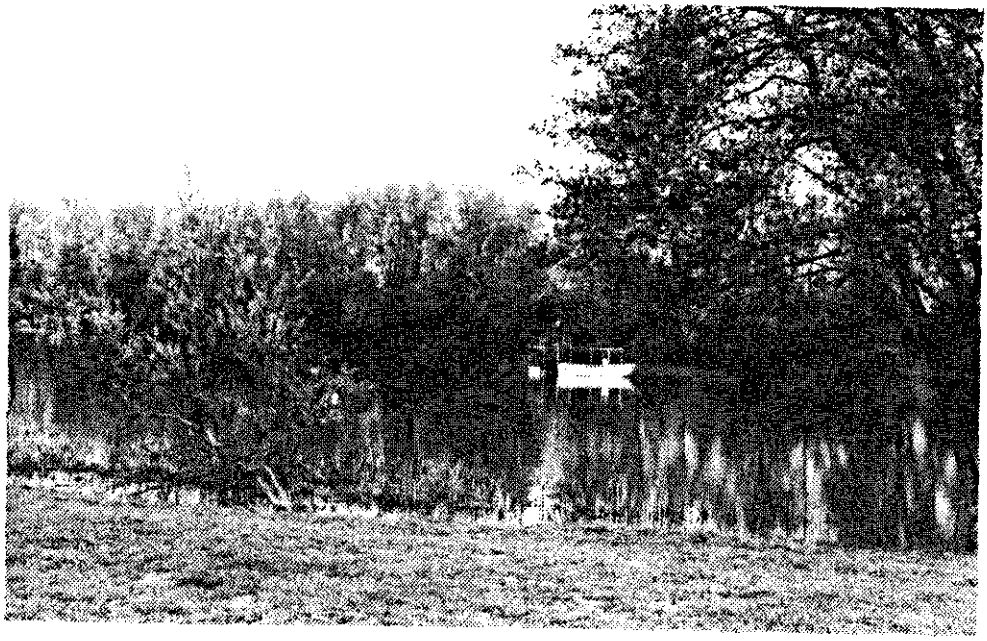

Fig. 1A. A view of eastern basin of Lake Vechten with the experimental raft.

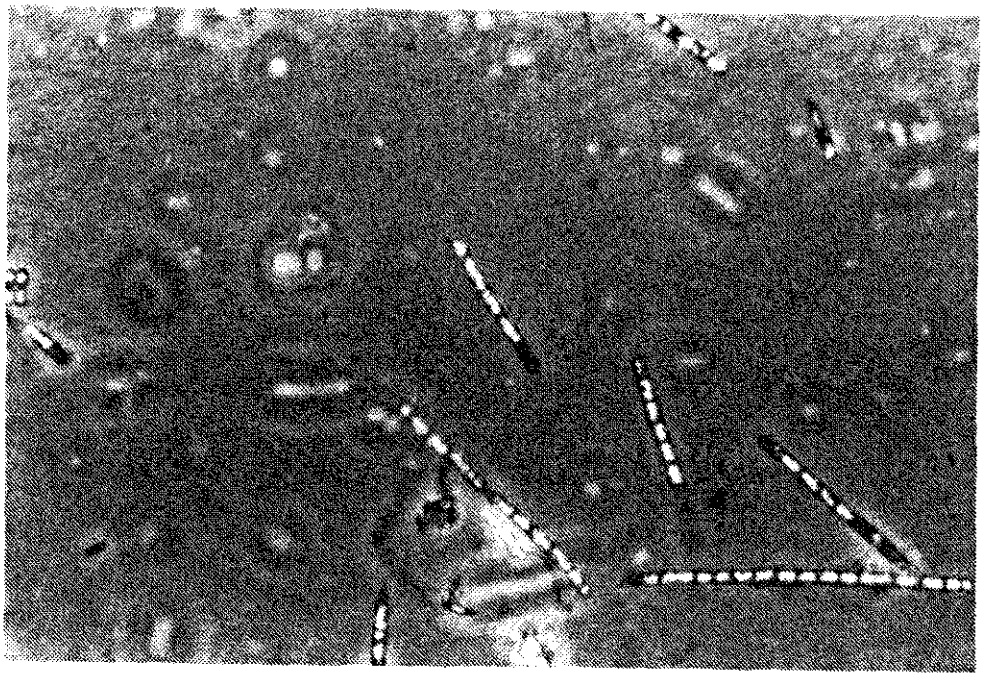

Fig. 1B. Chloronema-type filaments (800 x). Note free part of sheat visible at arrow.

The water is moderately hard; the concentrations of all the major cations is 3.5-6.4 meq.l^{-1} of which Ca accounts for 2.2-3.9 meq.l^{-1}. The concentration of total dissolved inorganic carbon is 2.6-5.5 meq.l^{-1}. Sulfate concentrations in Lake Vechten apparently increased during two decades from 150 μM SO_4 in the late sixties (Van Gemerden 1967, Verdouw and Dekkers 1980) to ca. 220 μM SO_4 in 1982 and 1983 (Steenbergen and Verdouw 1984, Hordijk *et al.* 1985). In summer the sulfate concentration below 8 m depth decreases due to bacterial sulfate reduction, becoming undetectable at the bottom by mid-August (Van Gemerden 1967; Verdouw and Dekkers 1980; Steenbergen and Verdouw 1984). Van Gemerden (1967) could not detect free sulfide in the hypolimnion of Lake Vechten, but in the last decade H_2S was detected in low concentrations and in the H_2S containing layers populations of phototrophic sulfur bacteria were observed (Steenbergen and Korthals 1982). The sulfide concentrations found during recent years were in the range of 3-12 μM H_2S, which by no means explains the sulfate disappearance (cf. Ingvorsen *et al.* 1981, Kohler *et al.* 1984) Sulfide may mainly precipitate with iron to form FeS (Verdouw and Dekkers 1980).

Light penetration in Lake Vechten is highest in May and in late August-September, with vertical extinction coefficients ($\varepsilon_{0\text{-}5}$) between 0.45 and 0.66 m^{-1} (Steenbergen and Verdouw 1982). The most penetrating component of the spectrum is in the green (480-570 nm) and yellow-orange (560-620 nm) regions ($\varepsilon_{0\text{-}5}$ = 0.38-0.82 m^{-1}). Red light (> 600 nm) penetrates less ($\varepsilon_{0\text{-}5}$ = 0.73-1.02 m^{-1}) and blue light (350-450 nm) the least ($\varepsilon_{0\text{-}5}$ = 0.69-1.42 m^{-1}) (Steenbergen 1974). The phototrophic bacterial populations have a marked effect on the extinction of light below 6 m depth; at 8 m depth light disappears almost completely (Fig. 2).

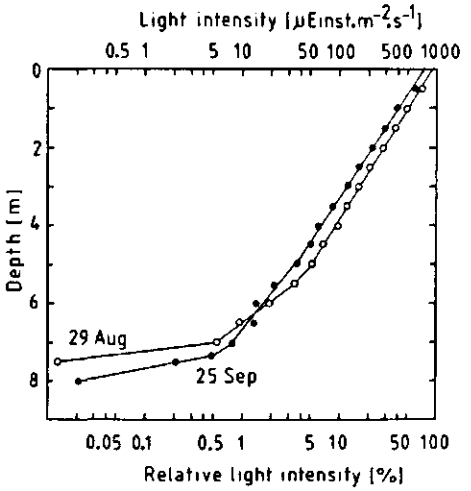

Fig. 2. Attenuation with depth of PhAR light intensity measured at midday in Lake Vechten on two clear days in late summer of 1979.

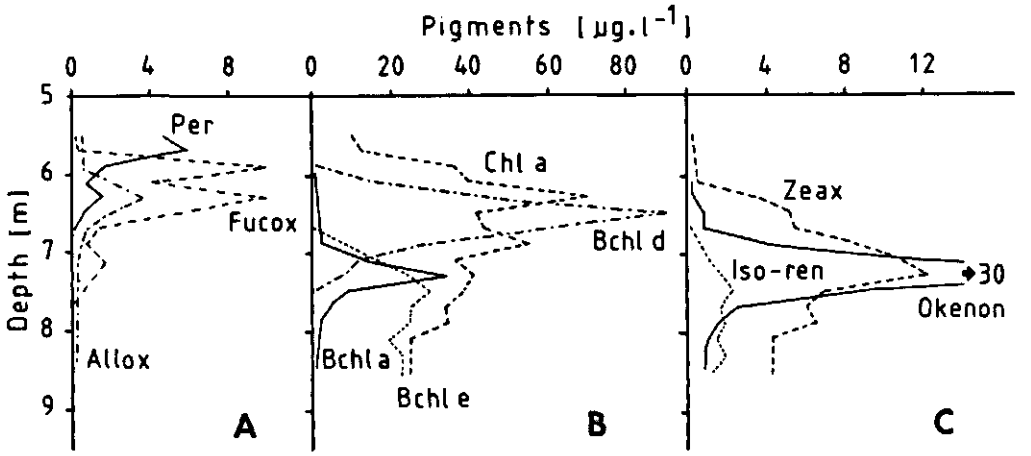

Fig. 3. Vertical distribution of photosynthetic pigments on 29 August 1985 in metalimnion and hypolimnion of Lake Vechten.

3.2. Distribution patterns

Phototrophic populations in Lake Vechten have been estimated using photosynthetic pigments as a chemo-taxonomical tool (Steenbergen and Korthals 1982, Korthals and Steenbergen 1985). Profiles of major signature pigments during the bloom of phototrophic sulfur bacteria are shown in Fig. 3. Algal signature pigments were still found in very small amounts at 7 m depth (Fig. 3A); they indicate the abundance of *Cryptomonas* spp. (alloxanthin), *Mallomonas caudata* (fucoxanthin) and *Ceratium hirundinella* (peridinin) (see Blaauboer 1982; Blaauboer *et al.* 1982).

Prokaryotic pigments were found at various levels below the eukaryotic ones (Fig. 3B, C). Bacteriochlorophyll *d* that peaked at a depth of 6.5 m represents a population of green colored Chlorobiaceae i.e. gas-vacuoles containing *Chloronema*-type filaments (Fig. 1B) (cf. Dubinina and Gorlenko 1975). Maximum numbers of 3 x 10^7 filaments.l^{-1} correspond to bacteriochlorophyll *d* concentrations of 80-90 µg.l^{-1}. Bacteriochlorophyll *a* was always found in combination with okenone, indicating the presence of *Chromatium* spp. which were found maximally (35 µg Bchl *a*.l^{-1}) at a depth of 7.2 m, at which depth they formed a typical bacterial plate at densities of 0.1 to 0.3 x 10^9 cells.l^{-1}. *Thiopedia* spp. were present in very low numbers. Finally, below a depth of 7 m bacteriochlorophyll *e* (28 µg.l^{-1}) was found in combination with isorenieratene. These pigments pointed to the occurrence of brown-colored Chlorobiaceae and very probably to *C. phaeobacterioides.* In addition to the sulfur bacteria a population of cyanobacterial *Synechococcus*-type cells, maximum density of ca. 3 x 10^9 cells.l^{-1}, was present as indicated by the presence of zeaxanthin and Chl. *a.* (see Fig. 3B, C). Filamentous cyanobacteria were encountered sporadically in Lake Vechten.

So, during summer stratification in Lake Vechten two distinct plates of phototrophic sulfur bacteria are found, that peak approximately 1 m apart. The upper one contained exclusively *Chloronema*-type filaments, maximally at 6.5 m depth, while the deeper one contained *Chromatium* cells, maximally at 7.2 m depth and *Chlorobium* cells more or less uniformly distributed below 7 m.

This two layer pattern is consistent with other recent findings where brown-colored Chlorobiaceae occupied a position below populations of Chromatiaceae and green-colored Chlorobiaceae (Montesinos *et al.* 1983, Kohler *et al.* 1984).

The periodicity of the phototrophic sulfur bacteria studied earlier (Steenbergen and Korthals 1982) showed that all the populations occurred maximally during late August and September. These findings were confirmed recently (see Fig. 4). From mid-September onwards the bacterial plates gradually disappeared as they came within range of a turbulent aerobic environment and by mid-November signature pigments of phototrophic sulfur bacteria could be no longer detected in the lake (Korthals and Steenbergen 1985).

3.3. Sedimentation studies

During thermal stratification when turbulence in bottom layers is minimal estimates for downward transport of particles obtained using sediment traps may be realistic (Bloesch and Burns 1980). In Lake Vechten one such study was carried out from June through the end of stratification and the sedimented material was analyzed for photosynthetic pigments (cf. Steenbergen and Verdouw 1984; Fig. 5). Signature pigments of the phototrophic sulfur bacteria were always found in sediment traps at 7.2 and 9.6 m; it may be inferred that from July onwards the flux of phototrophic sulfur bacteria towards the bottom of the lake was continuous.

In this study the errors due to mineralization effects cannot be excluded, although the exposure time never exceeded 14 days and temperature was between 8-14 °C. Under these conditions the decomposition of entrapped organic material may amount to ca. 10 % (Bloesch and Burns 1980).

Results of mean sinking velocities for 4-weekly intervals over the experimental period are shown in Table 2. Considering the mineralization effects settling fluxes might have been underestimated. Therefore the calculated sinking velocities must be considered as minimal. However, these compare well with those obtained by Rathke *et al.* (1981) for phytoplankton in the hypolimnion of Lake Erie.

The present evidence suggests an average depletion rate from the bacterial plates in Lake Vechten to equal sinking velocities of 0.16-0.27 $m.d^{-1}$. Thus, the phototrophic bacteria may disappear from the plates within 4-5 days; at a constant sinking velocity of 0.20 $m.d^{-1}$ a population in a water column of 1 m would be depleted by 20 % d^{-1} had there been no growth and it would remain static at a specific growth rate of 0.22 ln units.d^{-1} (cf. Reynolds 1976). Clearly, a growth rate of 0.22 ln units.d^{-1} may be

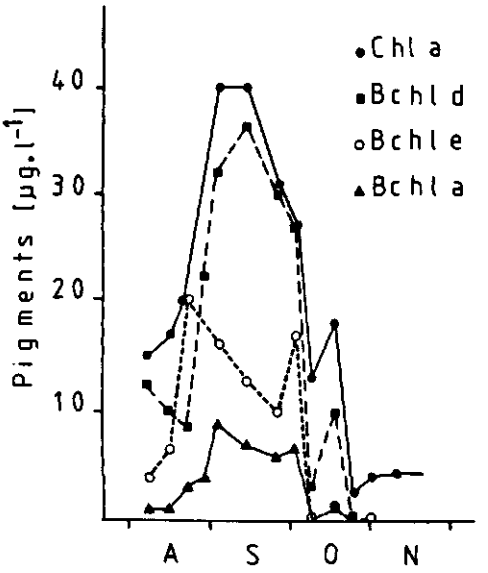

Fig. 4. Course of chlorophyll concentrations in the 6.5-7.5 m stratum of Lake Vechten, eastern basin, 1984.

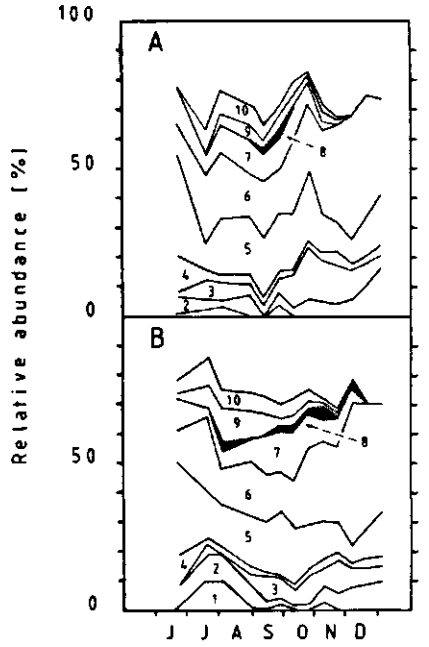

Fig. 5. Relative abundance of major photosynthetic pigments in sediment traps suspended at 7.2 (A) and 9.6 (B) m depths in eastern basin of Lake Vechten in 1982. Pigmentcodes are: (1) peridinin; (2) fucoxanthin; (3) alloxanthin; (4) Chl *b*; (5) Chl *a*; (6) chlorophyll derivatives; (7) zeaxanthin; (8) okenone; (9) Bchl *e*; (10) Bchl *d*.

Table 2. Sinking velocities ($m.d^{-1} \pm$ SD) of photosynthetic pigments measured by sediment traps suspended at 7.2 and 9.6 m depths in the eastern basin of Lake Vechten during July-November 1982; n denotes number of observations.

Pigments	n	Metalimnion	Hypolimnion
Chlorophyll *a*	6	0.13 ± 0.06	0.17 ± 0.06
Chl-derivates	6	0.80 ± 0.74	0.77 ± 0.44
Bacteriochlorophylls	6	0.27 ± 0.09	0.16 ± 0.07
Fucoxanthin	2	1.50 ± 1.51	
Alloxanthin	6	0.44 ± 0.38	0.36 ± 0.11
Peridinin	2	0.80 ± 0.79	
Zeaxanthin	6	0.24 ± 0.20	0.44 ± 0.40

tentatively regarded as the minumum specific growth rate of the bacterial populations.

Recently, we extended our pigment studies to the top layer of the sediment of Lake Vechten. Preliminary observations during winter indicated trace amounts of specific pigments of phototrophic sulfur bacteria. In addition, *Chloronema*-type filaments were observed together with motile, sulfur globules containing *Chromatium* cell, *Thiopedia* colonies and a dense *Beggiatoa* population. It is plausible that members of the bacterial populations hibernate on the sediment surface, at very low light intensities in a sulfide rich environment as the bacterial sulfate reduction in the anaerobic surface layer of the sediment continues and is maximal during that period (Hordijk *et al.* 1985).

3.4. Carbon fixation

Using DCMU as a specific inhibitor of oxygenic photosynthesis, it was shown that the phototrophic sulfur bacteria in Lake Vechten contributed significantly to the pelagic primary production of the lake (Steenbergen 1982). In the period of their abundance the phototrophic sulfur bacteria accounted for 1.5-14.1 % of the primary production (see Table 3).

Table 3. Ranges (means in parenthese) of daily photsynthetic CO_2 fixation for two strata in the eastern basin of Lake Vechten measured on eight sampling dates during June-October 1980. Daily production rates expressed as kg C per respective volumes of the strata.

Stratum	Production rates of strata	% of total	Type of photosynthesis
0 – 5 m	3.585 – 21.100 (9.857)	45.6 – 91.3 (67.3)	Oxygenic
5 – 9 m	0.152 – 10.576 (3.667)	1.1 – 38.6 (20.3)	Oxygenic
	0.204 – 2.197 (0.979)	1.5 – 14.1 (7.8)	Anoxygenic
	0.399 – 4.203 (1.846)		Oxygenic[1]
0 – 9 m	4.266 – 33.873 (15.359)		Oxygenic + Anoxygenic

[1] Production rates as measured under fully aerobic conditions (see text).

Synechococcus-type cells were largely responsible for the metalimnetic oxygenic photosynthesis, which contributed 1.1-38.6 % to the total production (Table 3). De Kloet and Steenbergen (1981) found that, for the primary production measurements, if incubated under fully aerobic conditions leaving an air pocket inside the bottles, the photosynthetic rate of the *Synechococcus* cells was drastically reduced (ca. 50 % reduction; see Table 3). Similar observations were made by Morris and Glover (1981) using marine *Synechococcus* strains. Clearly, this factor may drastically alter rates of oxygenic photosynthesis when no precautions are taken.

Many species of cyanobacteria are facultatively anoxygenic (Garlick *et al.* 1977). Steenbergen and Korthals (1982) postulated that the *Synechococcus* type cells may only marginally participate in the anoxygenic photosynthesis in Lake Vechten. Firstly there is spatial separation of the cyanobacteria and the phototrophic sulfur bacteria in the period June-August. Most importantly is that anoxygenic photosynthetic fixation of CO_2 by these type of cells is supported only by relatively high concentrations of sulfide (25-200 μM H_2S; see Peschek 1978), which are very unlikely to occur in Lake Vechten. Besides, Garlick *et al.* (1977) in their screening test for anoxygenic photo-assimilation of CO_2 found their *Synechococcus* strain to be negative. Although, cyanobacteria may contribute to the anoxygenic photosynthesis, we believe it to be only a minor portion as the phototrophic sulfur bacteria undoubtedly have a greater affinity for sulfide than does *Synechococcus*.

From the results obtained on photosynthetic rates and biomass of the phototrophic sulfur bacteria (see Steenbergen and Korthals 1982) an average specific growth rate can be tentatively calculated. Given an average total biomass of 69 $mgC.m^{-3}$ and an average CO_2 fixation rate of 46 $mg\ C.m^{-3}.d^{-1}$ the specific growth rate would be 0.51 ln $units.d^{-1}$. In view of the calculated minimum specific growth rate of 0.22 ln $units.d^{-1}$ to maintain a static population as a result of sedimentation losses (section 3.3.) the value of 0.51 ln $units.d^{-1}$ certainly seems realistic, in which losses from predation by heterotrophic flagellates and ciliates and respiration are not taken into account.

3.5. Limiting factors

In an earlier study we considered light intensity and sulfide concentration as factors which may limit carbon fixation rate of the phototrophic sulfur bacteria in Lake Vechten (Steenbergen and Korthals 1982). The deeper bacterial plate at 7.2 m depth occupied a favourable position adjacent to the sulfide gradient (cf. Fig. 3C). The *Chromatium* cells from this layer always contained sulfur globules and were swimming actively. This led us to assume that these cells, at least during September when sulfide concentrations were highest, did not suffer from electron donor limitation, but more likely from light limitation.

Recently, the effect on the carbon fixation rate of increased sulfide concentrations was investigated for the green-colored Chlorobiaceae from the upper bacterial plate

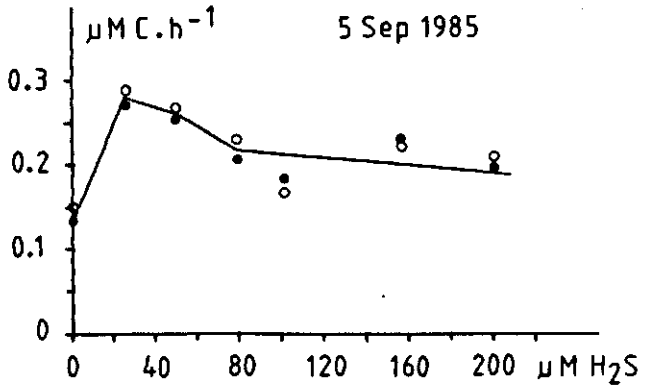

Fig. 6. Photosynthetic response of *Chloronema*-type filaments in Lake Vechten to increased sulfide concentrations.

(6.5 m depth; see Fig. 3). These *Choronema*-type filaments peaked at about 1 m above the depth with detectable amounts of sulfide (cf. Parkin and Brock 1981). We incubated 6.5 m lake water with DCMU at increasing concentrations of H_2S and measured the carbon fixation rates (Fig. 6). At the *in situ* undetectable sulfide concentration carbon fixation occurred at rates of about 0.15 µmol $C.l^{-1}.h^{-1}$ at average Bchl *d* concentration of 82 µg.l^{-1}. However, increasing sulfide concentrations stimulated the photosynthetic rate so that at 30 µM H_2S there was a ca. 2-fold increase (Fig. 6). Inhibition was observed at H_2S concentrations exceeding 60 µM. These results suggest that photosynthesis by the *Chloronema*-type filaments is limited by the sulfide supply.

Light conditions for the two bacterial plates differed substantially; at 6.5 m light intensities on clear days in September ranged from 8 to 16 µEinst.$m^{-2}.s^{-1}$ (see Fig. 2; cf. Steenbergen and Korthals 1982) and those at 7 m in the top layer of the *Chromatium* plate (cf. Fig. 3) from 5 to 8 µEinst.$m^{-2}.s^{-1}$, which is ca. 1.5 % and ca 1 % of surface light at the respective depths (Fig. 2). Also, the light quality between the two plates changed; the green and yellow-orange regions of the spectrum were reduced from 4 to 2 %, the red light from 0.8 to 0.3 % and the blue light from 0.5 to 0.1 % of the intensity at surface (Steenbergen and Verdouw 1982). So, at a depth of 7 m light from the spectral region 420-620 nm strongly predominated.

We tested the effect of increasing light intensities on the carbon fixation rate of the *Chloronema*-type filaments. To ensure that sulfide did not limit the photosynthetic activity the samples were enriched with sulfide to obtain 40 µM (cf. Fig. 6). We incubated lake water from 6.5 m with DCMU in a linear light intensity gradient and measured the carbon fixation rates. The results of one series are shown in Fig. 7. The population was light saturated at light intensities of 10-20 µEinst.$m^{-2}.s^{-1}$ showing a CO_2 fixation rate of 0.22 µM $C.h^{-1}$ at Bchl *d* concentration of 30 µg.l^{-1}. The light saturation value (I_k) was ca. 7 µEinst.$m^{-2}.s^{-1}$; I_k according to Talling (1957) is the light intensity at which a photosynthetic rate equivals the maximum (P_{max}) if light saturation behaviour were absent. So, a low I_k value may suggest a greater efficiency of low light utilization (see Parkin and Brock 1980). Our results suggest that at 6.5 m the filamentous green

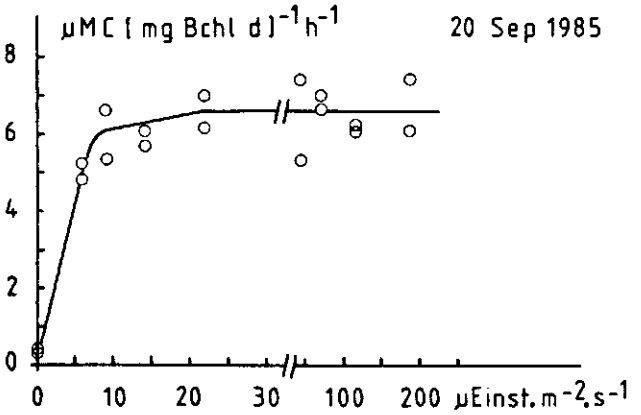

Fig. 7. Photosynthetic response of *Chloronema*-type filaments in Lake Vechten to increased light intensities.

sulfur bacteria were well adapted to the prevailing light intensities and may generally not be light limited.

In Lake Vechten a sharp light gradient was observed in the deepest bacterial layer between 7 and 8 m (Fig. 2). At the top of the layer primarily green and yellow light at intensities between 5 and 8 µEinst.m^{-2}.s^{-1} penetrated, which according to the results of Parkin and Brock (1980b), might be saturating for green and purple sulfur bacteria. In contrast, Guerrero *et al.* (1985) found evidence that *Chromatium* cells in Lake Cisó were light limited at 24 µEinst.m^{-2}.s^{-1}. Microscopic observations revealed that the *Chromatium* cells sampled from the top layer of the plate in Lake Vechten contained only a few sulfur globules, but those at the peak of the layer were heavily packed with sulfur globules. So, in this layer a similar situation may exist as described by Van Gemerden *et al.* (1985) for the bacterial layer in Lake Cisó, where from the top to the bottom of the layer there was a strong decrease in cellular activities due to increasingly severe light limitation. From the evidence now available we assume that apart from the top layer the greater part of the *Chromatium* population in Lake Vechten is light limited. For the population of *C. phaeobacterioides* we have as yet no information.

4. Discussion

The sulfate ion is the third most abundant anion in Lake Vechten (Steenbergen and Verdouw 1982). Its concentration in the lake water increased by about 40 % during the last twenty years. The depletion of sulfate by mid-August near the bottom of the lake can only be explained by the activity of sulfate reducing bacteria in the sediment (Ingvorsen *et al.* 1981, Steenbergen and Verdouw 1984, Hordijk *et al.* 1985). Close to the sediment the number of sulfate reducing bacteria was approximately 4 x 10^4 l^{-1}, but in the surface sediment it was about 10^7 l^{-1} of wet mud (Van Gemerden 1967;

Cappenberg 1972, 1974). Recently, comparable numbers of sulfate reducers were found in the water and sediment of eutrophic Rotsee by Kohler *et al.* (1984).

These findings agree with the sulfate reduction activities measured. Until now sulfate reduction in the water column of Lake Vechten was undetectable with the methods used and in the surface sediment amounted to 3.6 mM $m^{-2}.d^{-1}$ (Hordijk *et al.* 1985). Thus the bulk of sulfide built up in the anaerobic hypolimnion must have been produced mainly in the sediment.

Sulfide reached maximum concentrations of 10-12 µM during September at ca. 8 m depth, which by no means was complementary to the sulfate depletion (cf. Ingvorsen *et al.* 1981). Apparently, H_2S accumulates only after most of the Fe^{++} has been precipitated as FeS and as ferri-oxides formed by oxidation with O_2 in the upper layers of the hypolimnion and in the reduction of manganese (Mn) oxides by ferrous ions (Verdouw and Dekkers 1980). The mechanism of FeS formation in the deepest part of the lake may also explain the typical form of the H_2S profile, with a peak at 8-8.5 m, which developed during August-September (Steenbergen and Korthals 1982; see Fig. 8).

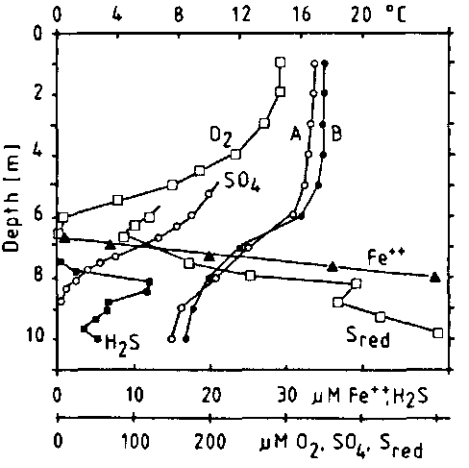

Fig. 8. Vertical distribution on 24 September 1985 of oxygen, ferrous iron, sulfate, total reduced sulfur and sulfide in eastern basin of Lake Vechten. Temperature profiles of 5 (A) and 24 (B) September are shown.

Quantitative aspects of the role of phototrophic sulfur bacteria in the sulfur cycle of lakes have been evalueted by several workers (Sorokin 1970, 1972; Jørgensen *et al.* 1979; Parkin and Brock 1981; Van Gemerden 1985). This concerned mostly meromictic lakes, with relatively high sulfide concentrations (25-600 µM H_2S) and dense populations of phototrophic bacteria i.e total Bchl concentrations of 400-2300 µg.l^{-1} (see also Lawrence *et al.* 1978; Guerrero *et al.* 1985; Lindholm *et al.* 1985). In contrast, monomictic Lake Vechten densities were relatively modest with Bchl concentrations of 30-90 µg.l^{-1}. Therefore, significant changes in concentrations of sulfur compunds due to bacterial activities in the water column were hard to find (Van Nes, unpublished). The

role of phototrophic sulfur bacteria in the transformation of sulfur is expected to be marginal in Lake Vechten. Their impact on the sulfur cycle may be estimated by comparing the supply of sulfide with the rate of sulfide oxidation using the data obtained in 1980 (Steenbergen and Korthals 1982) and in the present work (Fig. 8).

Therefore it was assumed that sulfide was supplied by vertical diffusion across the 8 m plane and in the 6.5-7.5 m stratum was oxidized completely to sulfate by the phototrophic bacteria, such that the ratio of sulfide oxidized to CO_2 fixed in the light would equal 0.5 as found by Parkin and Brock (1981). Both chemical and bacterial oxidations of reduced sulfur compounds were considered negligible as oxygen was depleted at about 6 m depth and the rates and profiles of CO_2 dark fixation provided no evidence for a measurable activity of e.g. *Thiobacilli* (cf. Sorokin 1972; Jørgensen *et al.* 1979).

We estimated vertical diffusion of sulfate and sulfide from the slopes of the respective profiles (Fig. 8) using Fick's first law of diffusion:

$$F = -D^{dc}/dx$$

where F is flux in $mol.cm^{-2}.s^{-1}$, D the vertical dispersion coefficient in $cm^2.s^{-1}$, and dc/dx the existing concentration gradient in $mol.cm^{-3}.cm^{-1}$. An average D of 0.32×10^{-2} $cm^2.s^{-1}$ was used (Steenbergen and Verdouw 1984; Verdouw *et al.* 1985). In addition the concentration of total sulfur was measured and the reduced sulfur calculated (Fig. 8). Particulate organic sulfur (POS) was calculated from the average concentration of particulate organic carbon at 8 m depth of 3000 mg $C.m^{-3}$ and an organic carbon:sulfur mass ratio of 0.01. To calculate the sedimentation rate of sulfur the same carbon:sulfur ratio was used together with the average sedimentation rate of 800 mg $C.m^{-2}.d^{-1}$ (Steenbergen and Verdouw 1984). An average value for dimethylsulfide (DMS), as the main volatile product of cyanobacteria and microbial decomposition was taken from Bechard and Rayburn (1979) and from Wakeham *et al.* (1984).

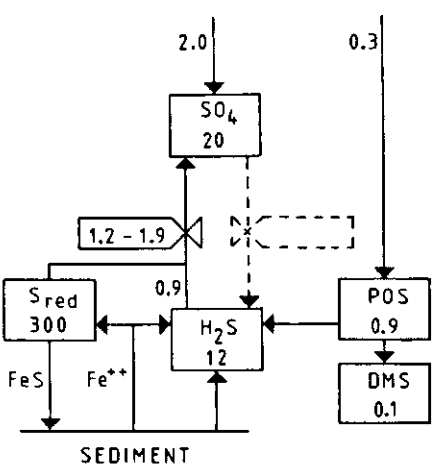

Fig. 9. Proposed relational diagram of pool sizes ($mmol.m^{-3}$) and fluxes ($mmol.m^{-2}.d^{-1}$) of sulfur compounds at 8 m depth; furthermore transformation ($mmol.m^{-3}.d^{-1}$) of sulfide to sulfate in the 6.5-7.5 m stratum of Lake Vechten during late summer.

From the simplified scheme constructed from our calculations (Fig. 9) it can be envisaged that on a daily basis the average vertical flux of H_2S (ca. 1.0 mmol.m^{-2}.d^{-1}) and the transformation of H_2S to sulfate by the phototrophic sulfur bacteria (ca. 1.5 mmol.m^{-3}.d^{-1}) were in the same order of magnitude. This suggests that the phototrophic bacteria in Lake Vechten might be sustained mainly by the daily flux of sulfide from below 8 m. Additional reduced sulfur compounds might be obtained from the main reduced sulfur pool, as indicated in the figure. Apparently, this might contradict our earlier calculations made on a hourly basis indicating a great discrepancy between sulfide supply by diffusion and its oxidation rate (Steenbergen and Korthals 1982). However, the present calculation includes the sulfide supply during the night as well.

Obviously, our calculations may be liable to serious errors due to uncertainties, the great variation in the vertical dispersion coefficient being important in this respect as only average values based on twice monthly temperature measurements are available. Another reservation must be made for the ratio H_2S oxidation: CO_2 fixation of 0.5. Pfennig (1978) proposed a ratio of 1.4, but Van Gemerden *et al.* (1985) found that in natural populations of *Chromatium* this ratio was ca. 2, which suggests that the cells grow by the oxidation of sulfide to elemental sulfur. In fact, an average turnover rate by the phototrophic sulfur bacteria in Lake Vechten of 1.5 mmol H_2S m^{-3}.d^{-1}, i.e. a transformation of approximately 0.5 % of the total sulfur pool indicates only a minumum value. However, this value compares favourably with those reported by Parkin and Brock (1981) for green-colored Chlorobiaceae from Knaack Lake. From their data a sulfide oxidation rate of approximately 6 mmol H_2S m^{-3}.d^{-1} can be estimated, while at the peak of the bacterial plate in Knaack Lake the Bchl concentration was about 6 times as high as that in Lake Vechten.

Our data suggest that the supply of H_2S to the layers where light penetrates and the oxidation of the H_2S in these layers are delicately balanced.

The available information allows construction of a tentative concept on the development of phototrophic sulfur bacteria in Lake Vechten. Due to the absence of H_2S in the hypolimnion, densities of phototrophic sulfur bacteria in Lake Vechten were rather low (Van Gemerden 1967). Increased sulfate concentrations in the lake, probably caused by acid precipitation, may have stimulated the sulfate reduction in the sediment resulting nowadays in free H_2S, which has escaped from FeS formation. The H_2S concentration build-up in the late summer period supports the present phototrophic bacterial populations.

So, our hypothesis is that, because of the favourable hypsometry of the lake basin, together with an increased H_2S supply, the densities of the bacterial populations will steadily increase. Consequently, light might become growth limiting temporarily during the year because of selfshading of the cells (cf. Pedrós-Alió *et al.* 1983; Van Gemerden *et al.* 1985).

5. Acknowledgements

The editorial comments of R.D. Gulati and the remarks of H. Verdouw and Th.E. Cappenberg were greatly appreciated. M.J.B. Bär-Gilissen counted the organims; C.A. Hordijk and H. van Engelen measured sulfate reduction and total sulfur. The sampling device was constructed by P. Schouten; H. Roon aided in the fieldwork and C.C.C. Kroon typed the manuscript. M.D.M. Trommel is thanked for her kind support.

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