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Characterization of a dense, purple sulfur bacterial layer in a meromictic salt lake

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Abstract

In meromictic Mahoney Lake (British Columbia), purple sulfur bacteria formed a dense, 10-cm-thick layer at the primary chemocline. The dominant species (*Amoebobacter purpureus*) reached a maximal cell concentration of 4×10^8 cells ml^{-1}, 27% of which were viable. A maximal concentration of 20,900 μg bacteriochlorophyll *a* $liter^{-1}$ was determined—the highest concentration ever found in a natural body of water. Steep vertical gradients of conductivity, light, and sulfide concentration were detected across the bacterial layer, while the concentration of oxygen increased above it. Integrated primary production of the layer was 15 mg C m^{-2} h^{-1}. Bacteriochlorophyll-specific assimilation rates reached maxima at the top of the layer. Anoxygenic photosynthesis was limited by reduced sulfur compounds at the top of the layer, whereas strong attenuation of irradiance resulted in light limitation for most of the cell population below. The sulfide flux from below the layer accounted for <37% of the sulfide oxidized in the layer. Thus >63% of the sulfide oxidized during anoxygenic photosynthesis must have been produced within the layer. This calculation agrees well with sulfide production rates measured in situ (12.4 μmol $liter^{-1}$ h^{-1}). In contrast to other lakes, considerable uptake of [^{14}C]acetate by purple sulfur bacteria and comparatively high numbers of purple nonsulfur bacteria indicate an important role of dissolved organic matter and a short cycle of carbon and redox compounds in the dense purple microbial layer.

Where light reaches sulfide-containing water layers of lakes, phototrophic sulfur bacteria often occur in dense populations, so-called plates (e.g. Takahashi and Ichimura 1968; Biebl and Pfennig 1979; Parkin and Brock 1980*b*; Guerrero et al. 1985). The ecology of these bacteria has been investigated by studying the annual variation of growth rates and biomass changes (Mas et al. 1990), the influence of light quality on species composition (Parkin and Brock 1980*a*; Montesinos et al. 1983), and the physiology of bacteria within the plate (Guerrero et al. 1985; van Gemerden et al. 1985).

Overlapping, but at the same time opposing, gradients of light and sulfide limit the vertical extents of phototrophic bacterial plates and therefore require narrow sampling intervals across the layer. Through close-interval sampling, considerable vertical variation with respect to biomass distribution (Croome and Tyler 1984) and bacterial metabolism (Guerrero et al. 1985; van Gemerden et al. 1985) has been detected even at 5- or 10-cm intervals.

Earlier investigations of Mahoney Lake (British Columbia) revealed the presence of a very narrow layer of purple sulfur bacteria at the chemocline (Northcote and Hall

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1983). The bacteriochlorophyll (BChl) concentrations exceeded all values reported so far (Hall and Northcote 1990). However, the vertical extent of the phototrophic layer, its species composition, and the vertical gradients of abiotic factors and bacterial metabolism in the plate were not investigated at high resolution. The present study was done to assess possible causes for the extreme development of bacterial biomass in this lake.

Materials and methods

Mahoney Lake is at 49°14'N, 119°34'W and 470 m asl near Penticton in south-central British Columbia. It is a small (max length, 820 m; max width, 407 m; max depth, 17.8 m), saline lake, the meromixis of which has been examined over the last two decades (Northcote and Halsey 1969; Northcote and Hall 1983, 1990; Hall and Northcote 1990).

The lake was visited three times in September–October 1989. Samples were collected early in the morning over the deepest site in a syringe sampler modified after Baker et al. (1985). The device was equipped with 60-ml disposable plastic syringes staggered at 2.5-cm intervals. Sampling, incubation experiments, and underwater light measurements were performed with a calibrated stainless steel cable. With this cable, measurements were highly reproducible. The vertical distribution of photosynthetically usable radiation (PUR, 400–700 nm) was determined with a LiCor quantum meter (model 185A) and spectral distribution of underwater irradiance with a OS-1 spectrometer (Focal Marine Ltd.).

Temperature and conductivity were measured with a YSI model 33 meter (Yellow Springs Instr.). Calibration procedure and correction of conductivity values are reported elsewhere (Hall and Northcote 1986). The pH of lake water was determined with a Fisher model 119 pH meter fitted with a combination electrode. Alkalinity measurements were done after returning to the laboratory as described by Northcote and Hall (1983). To determine the concentrations of dissolved inorganic carbon (DIC), we analyzed triplicate samples with a Shimadzu total carbon analyzer (TOC-500) equipped with an autosampler (AS 1-502). The instrument was calibrated with a solution of Na_2CO_3.

Oxygen concentrations in the water column were assessed with a YSI model 57 meter. The electrode was calibrated at air saturation as well as in surface water purged with nitrogen. Sulfide concentrations were measured with the methylene blue method (Am. Public Health Assoc. 1985; detection limit, 3 μM sulfide). Sulfide samples were preserved with 2 N zinc acetate and 6 N NaOH. Absorbency was measured at 664 nm in a Bausch & Lomb Spectronic 88 spectrophotometer.

According to Fick's first law, vertical diffusion fluxes of sulfide J_z (at depth z) are calculated from the first derivative of the vertical sulfide concentration profile:

$$J_z\ (\mu\text{mol cm}^{-2}\ \text{s}^{-1}) = -D_{H_2S/HS^-} \cdot dC_z/dz. \quad (1)$$

Here D_{H_2S/HS^-} is the molecular diffusion coefficient for H_2S ($=1.52 \times 10^{-5}\ \text{cm}^2\ \text{s}^{-1}$) (Jørgensen and Revsbech 1983), and C_z is sulfide concentration (μM) at depth z (m). For comparison, the vertical eddy diffusion coefficient K_z for sulfide was estimated from the density gradient below the plate with the formula of Colman and Armstrong (1987). Density was calculated from conductivity values with the salt composition given by Northcote and Halsey (1969). The eddy diffusion coefficient was $K_z = 4.2 \times 10^{-5}\ \text{cm}^2\ \text{s}^{-1}$ which (considering the relatively high SE of the calculation) is not significantly different from the molecular diffusion coefficient.

Numbers of bacterial cells were determined in a Helber counting chamber. To examine the viability of phototrophic bacteria, we applied the most probable number technique (Am. Public Health Assoc. 1985), using a selective growth medium for Chromatiaceae (Pfennig and Trüper 1989) supplied with 1% NaCl, 0.15% $MgCl_2 \cdot 6H_2O$, 2 mM acetate, and 200 μM dithionite. Buoyancy of *Amoebobacter* cells was assessed by placing a drop of lake water in a Neubauer counting chamber (depth, 0.02 mm) and counting the cells that accumulated under the coverslip and those sinking to the bottom of the chamber after 5 min of incubation in the dark (at 20°C).

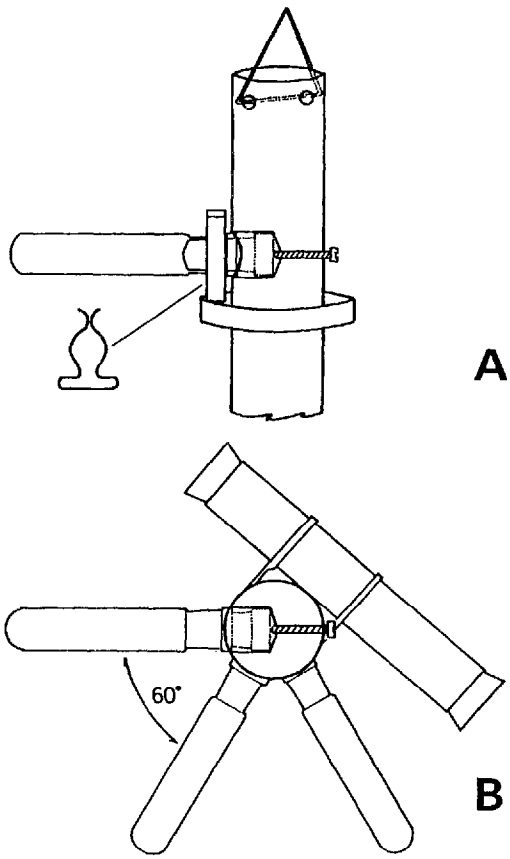

Fig. 1. Rack for incubation of water samples at 5-cm intervals. A. Ground-glass stoppers fit in holes in central Plexiglas bar (ϕ, 4 cm) and light tubes (11.5 ml) are held by a metal clamp. A plastic screw fixes the stoppers tightly. B. View from top. Arrangement of sequential light tubes at 60° angles and of a dark PVC tube (containing the dark control) opposite to the respective light tube reduces shading of lower tubes.

BChl *a* was determined after centrifugation of water samples and cold extraction of the pellet (99.5% acetone, 4°C, 24 h). Absorbance was measured at 772 nm in a Hitachi UV-2000 spectrophotometer. Pigment concentrations were calculated from the equations given by Steenbergen and Korthals (1982).

To assesss photosynthetic carbon uptake, we made an incubation rack that allowed placement of incubation tubes at a vertical distance of 5 cm, but avoided shading of the tubes by the overlying ones (Fig. 1). After sampling and distribution of water samples to the respective incubation tubes, 1 μCi of $NaH^{14}CO_3$ solution was added to each light and dark tube. On the basis of previous results (Northcote and Hall 1983), an incubation time of 6 h was chosen. Immediately after retrieving the incubation rack, 1 ml of each tube was pipetted into 10 ml of scintillation solution (Scintiverse 11, Fisher Sci.) for determination of total radioactivity. Thereafter, samples were filtered through membrane filters (cellulose nitrate; ϕ, 2.5 cm; pore size, 0.45 μm; Schleicher and Schuell) placed in a Nuclepore syringe filter assembly; the filters were washed with distilled water and subsequently placed in glass vials containing 6 drops concentrated HCl. After 2 h the vials were opened, the filters dried in air, and 10 ml of scintillation solution (120 g of naphthalene and 4 g of PPO in 1 liter of dioxane) added to each vial. All samples were counted in a Beckmann LS 7500 liquid scintillation counter.

To correct for the quenching of bacterial pigments, we determined counting efficiency for increasing amounts of phototrophic bacterial biomass on membrane filters in dioxane cocktail as well as for 1-ml water samples in Scintiverse 11. For calibration a [^{14}C]toluene standard (DuPont) was used.

Incorporation of [^{14}C]acetate (57.6 μCi μmol^{-1}; DuPont) was measured vertically through the plate at one solute concentration (0.38 μM) in the same way as outlined for $H^{14}CO_3^-$ assimilation experiments (incubation time, 2.5 h). To determine the dependence of uptake rates on substrate concentrations, we distributed a mixed water sample of the bacterial plate to four pairs of light and dark 60-ml BOD bottles. A different amount of [^{14}C]acetate was added to each pair, and all bottles were incubated under the same conditions in the bacterial plate.

Sulfide production rates were determined from the time-course of sulfide concentrations measured in 60-ml light and dark BOD bottles filled with lake water from the microbial plate and incubated in the middle of the layer. Sulfide concentrations in the bottles were measured at 12-h intervals. Water samples and incubation vessels were stored on ice in the dark and processed within a few hours on the same day.

Absorption spectra of whole cells were determined in the Shimadzu UV-300 spectrophotometer. To estimate the impact of bacterial pigments on the underwater light field, we determined the BChl-specific absorption coefficient k_B [m^2 (mg BChl *a*)$^{-1}$] from absorption spectra of pure cultures of *Amoebobacter purpureus* and the spectral distribution of underwater irradiance measured above the bacterial plate (cf. Bannister 1979). The BChl-specific absorption coefficient was calculated according to Eq. 2,

$$k_B = \frac{\int k_B(\lambda)\, E_q(\lambda)\, d\lambda}{\int E_q(\lambda)\, d\lambda}. \qquad (2)$$

Here $k_B(\lambda)$ is the BChl-specific absorption coefficient [m^2 (mg BChl *a*)$^{-1}$ nm^{-1}] as calculated from absorption at wavelength λ and BChl *a* concentration and E_q is the quantum flux at wavelength λ.

Amoebobacter purpureus strain ML1, *Thiocapsa roseopersicina* strain ML1, and *Prosthecochloris aestuarii* strain ML1 were isolated from the microbial plate and characterized as described by Overmann and Pfennig (1989). Light dependence of growth rates was measured in a light cabinet with daylight fluorescent tubes (Osram daylight 5000 de luxe) or green light (Göttinger Farbfilter plus infrared filter; maximal transmittance at 505 nm; spectral range, 440–580 nm). Cultures were incubated in 22-ml screwcap tubes and growth was followed by measuring the optical density at 650 nm of cultures free of sulfur globules.

Results

Chemical and physical measurements—The water was isothermal to a depth of 6 m apart from a higher value at the surface (Fig. 2). A distinct maximum was found at 6.8 m just underneath the layer of purple sulfur bacteria (cf. Fig. 6A, B). This phenomenon has been observed over several years (T. G. Northcote and K. J. Hall unpubl. data). Conductivity was constant (~24,000 μS cm^{-1}) in water layers above the purple sulfur bacterial plate, whereas a steep vertical gradient was found beginning precisely at the maximum of photosynthetic biomass and exceeding 50,000 μS cm^{-1} at 10 m deep.

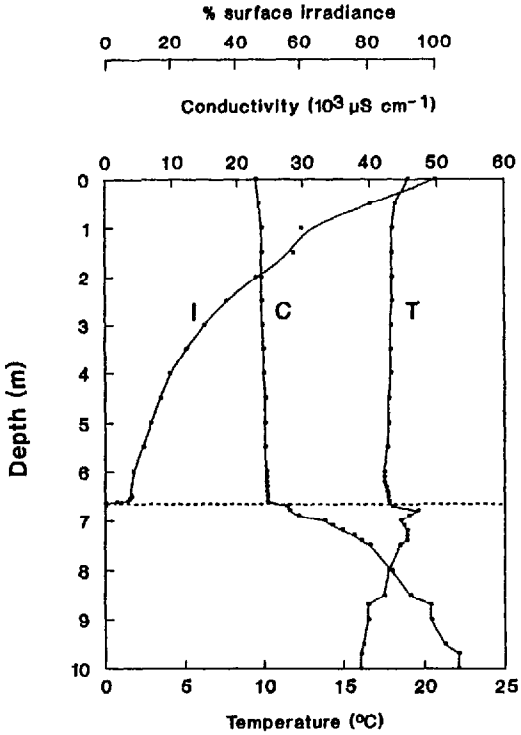

Fig. 2. Vertical profiles of water temperature (*T*), conductivity (*C*, standardized to 25°C), and percent light transmission (*I*) (1200 hours 23 September 1989). The dashed line in this and subsequent figures indicates the position of the bacterial plate.

Oxygen concentration was 250–263 μM (95–100% saturation) between 0- and 6.4-m depth and decreased exponentially to zero between 6.4 and 6.8 m (Fig. 3). As the concentration gradient is stable for weeks (Northcote and Hall 1990) and a vertical increase of eddy diffusion between 6.4 and 6.8 m is very unlikely in the lake, this vertical oxygen distribution strongly indicates high respiration rates from 6.4- to 6.5-m depth and decreasing rates below. A slight response of the oxygen electrode to sulfide was observed at 6.8 m. Because of the high sulfide concentration at this depth, oxygen concentration was assumed to be zero.

Below 6.65 m, concentrations of H_2S increased linearly, reaching values of several mmol $liter^{-1}$ below the bacterial plate. If a steady state during the main part of the light

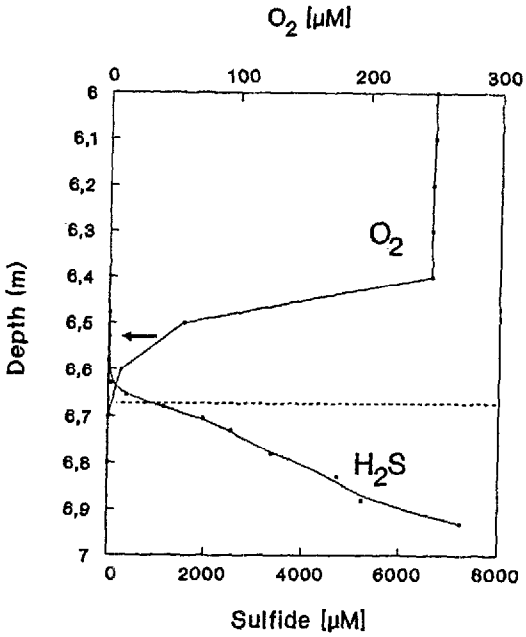

Fig. 3. Vertical profiles of oxygen and sulfide gradients across the bacterial plate. Arrow indicates position of a small peak of sulfide concentration (*see text*) (23 and 24 September 1989).

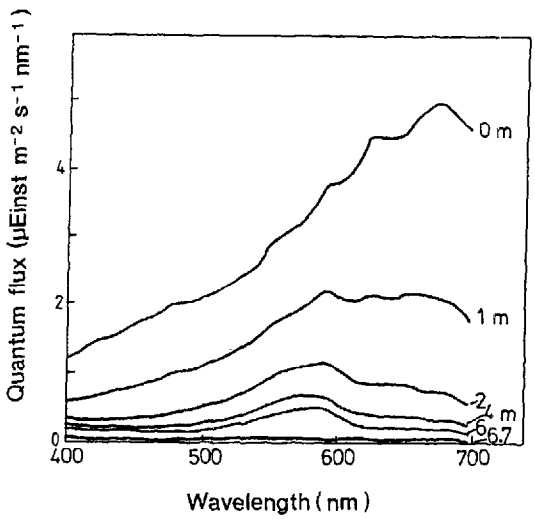

Fig. 4. Spectral distribution of quantum flux (linear scale) at different depths (2 October 1989).

period is assumed, we calculate a vertical sulfide flux of 123 µmol m^{-2} h^{-1} (Eq. 1) for this lower part of the lake. A small peak of 12.5 µM sulfide (compared to 6.2 µM both above and below) was detected at 6.53 m (*see arrow in Fig. 3*). The pH values ranged between 8.8 at 6.5 m and 7.8 at 7 m. The alkalinity at these depths was 31.9 and 43.8 meq $liter^{-1}$.

In the upper water column, quantum fluxes decreased exponentially, following Beer's law. A vertical light attenuation coefficient of $k_D = 0.399$ m^{-1} was calculated between the surface of the lake and the top of the bacterial plate (6.5 m), where 7.8% of surface irradiance was measured. The remaining light was absorbed within a depth interval of 5 cm (Fig. 2). Because of the progressive narrowing of the spectrum with depth, light composed mainly of wavelengths between 525 and 600 nm reached the layer of purple sulfur bacteria (Fig. 4).

Species composition, vertical distribution, and viability of phototrophic bacteria—The absorption spectrum of an untreated water sample taken from the bacterial layer exhibited peaks at 877, 826, 585, and 365 nm (Fig. 5), which can be attributed to BChl *a*. The broad absorption band at 515 nm is typical of the carotenoid okenone. Light microscopic examination revealed only one morphological type of purple sulfur bacteria, which contained gas vacuoles and intracellular sulfur globules. On the basis of its cell morphology, the possession of gas vacuoles, and the capability of pure cultures to use glucose, acetate, and pyruvate in the presence of sulfide for photoheterotrophic growth, the numerically dominant species of the phototrophic bacteria in Mahoney Lake was identified as *A. purpureus*. In contrast to other strains of this species described so far (Eichler and Pfennig 1988), the Mahoney Lake strains exhibited high salt tolerance. Growth rates decreased in culture media containing zero or >2.2% NaCl and no growth was observed at concentrations >4.5% NaCl.

Total cell numbers, viable cell counts, and BChl *a* concentrations reached maxima at a depth of 6.68 m (Fig. 6A, B), exactly where these maxima had been detected on a previous visit 2 weeks before: the vertical position of the layer of purple sulfur bacteria in the lake remained constant over several weeks even within the centimeter range. The maximal cell density of *A. purpureus* was 4.0 × 10^8 ml^{-1}, 27% of which (i.e. 1.1 ×

10^8 cells ml^{-1}) were viable under the growth conditions applied. The diameter of *Amoebobacter* cells remained almost constant with depth (Fig. 6A). A mean value (±1 SD) of 2.69±0.22 μm (n = 180) and a cell volume of 10.19 μm^3 were calculated.

The maximal concentration determined for BChl *a* was 20,880 μg $liter^{-1}$. This value is the highest concentration ever measured in a natural body of water. The total amount of BChl *a* beneath a lake surface area of 1 m^2 was 1,050 mg. A small peak of BChl *a* was measured at 6.53 m (80 μg $liter^{-1}$ vs. 15 and 34 μg $liter^{-1}$ above and below, *see arrow in Fig. 6B*).

In water samples taken from the upper part of the bacterial plate, large, purple cell aggregates were visible. Microscopic examination revealed aggregates of cells that remained firmly attached to each other. The considerable microheterogeneity of biomass distribution of *Amoebobacter* in the lake resulted in high standard deviations of total and viable cell counts. As the volume of lake water used for BChl *a* determination is much larger than that used for total and viable cell counts, errors due to heterogeneous distribution of biomass are much smaller for BChl *a* than for cell numbers. Therefore, the former was chosen as a more reliable measure of biomass of phototrophic bacteria.

After brief incubation (5 min) in the counting chamber, the percentage of cells floating was about the same as the percentage of cells sinking to its bottom. No differences were observed with respect to sampling depth. In contrast, cell aggregates from the upper part of the plate settled when, in a second experiment, a vertical series of samples was obtained in the early afternoon on a sunny day (10 September 1989) and incubated in glass vials in the dark at 4°C for 4 d. This incubation time was required to establish clear differences in buoyancy. The biomass of floating, suspended, and sinking cells at each depth was determined by measuring the amount of BChl *a* in the top, middle, and bottom part of each vial (Fig. 7). Similarly, formation of aggregates and sedimentation was obtained with sulfur-depleted cells in outgrown pure cultures; cells resuspended within seconds when H_2S

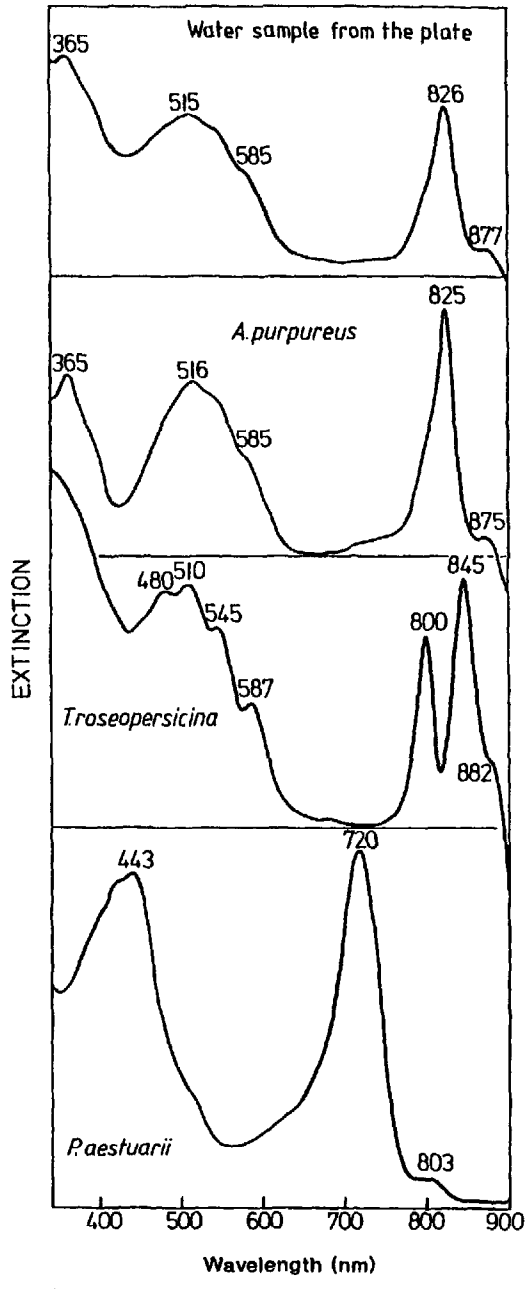

Fig. 5. Absorption spectra of whole cells in a water sample from the bacterial plate and for isolated strains of *Amoebobacter purpureus* strain ML1, *Thiocapsa roseopersicina* strain ML1, and *Prosthecochloris aestuarii* strain ML1.

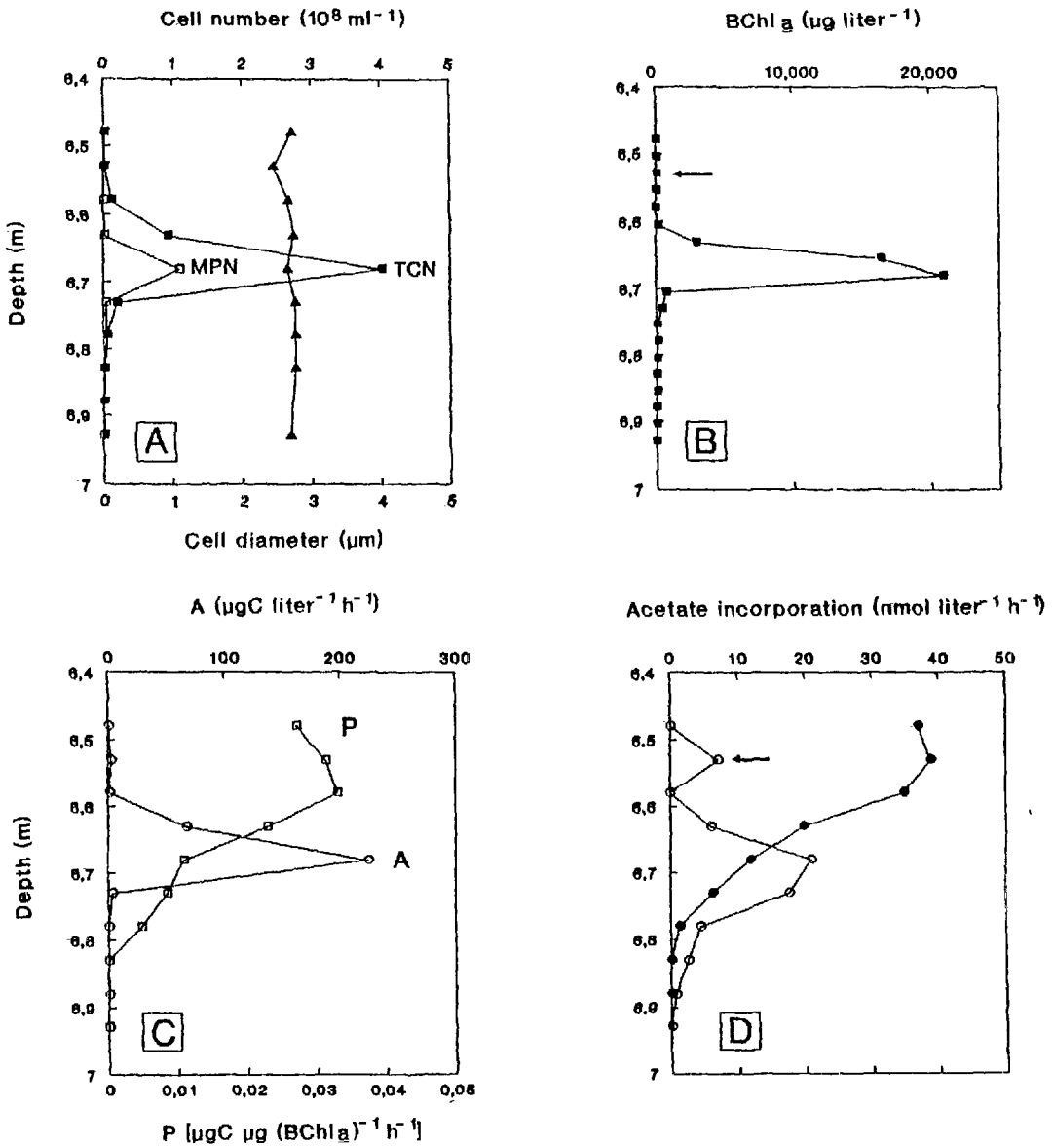

Fig. 6. A. Total cell number (TCN), most probable number (MPN), and cell diameter (▲) of *Amoebobacter purpureus* in the bacterial plate of Mahoney Lake. B. Vertical distribution of BChl *a*. C. $^{14}HCO_3^-$-assimilation rate *A* (light minus dark values) and BChl-specific assimilation rates *P* across the plate. D. Light (○) and dark (●) [^{14}C]acetate assimilation rates (23 and 24 September 1989). Arrows indicate the position of a small peak of BChl (*see text*).

was added. This response indicates a shortage of electron-donating substrates in the upper part of the plate, where light microscopy revealed sulfur globules in fewer *Amoebobacter* cells than at greater depths.

In the first tubes of the deep agar dilution series prepared from the purple layer, pinkish colonies (ratio to purple colonies of *A. purpureus*, 1 : 50) were present, which were identified as *T. roseopersicina*. Additionally, high numbers of *Rhodobacter capsulatus* (a purple nonsulfur bacterium) and *Chlo-*

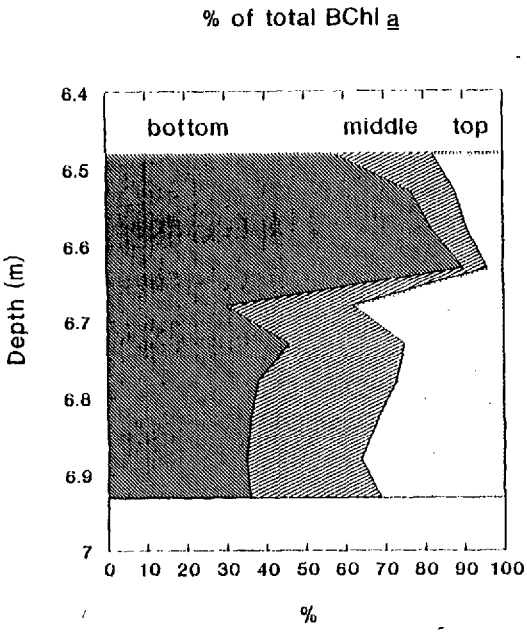

Fig. 7. Percentage of cells (measured as BChl *a*) sinking to the bottom, suspended (middle), and floating (top) after a 4-d incubation (4°C, dark) of water samples from different depths.

roherpeton thalassium (Chlorobiaceae) were counted in the first tubes, forming ~20,000 and 2,000 colonies ml^{-1} of lake water. Other green sulfur bacteria were obtained by enrichment culture with selective medium (Pfennig and Trüper 1989) and light conditions (fluorescent light). From these enrichments, two strains of *P. aestuarii* were isolated.

Although highly selective light conditions (incubation in green light) and different culture media were tested, all attempts to enrich brown species of the Chlorobiaceae from water samples of the bacterial plate or from anoxic sediment samples failed. Selectivity of the conditions was tested with sediment samples from Lake Konstanz (Germany) where brown *Chlorobium* species could be enriched, although green species predominate in the phototrophic bacterial community.

At the lower dilution steps of the MPN series, growth of Chl *a*-containing coccoid green algae was observed. Chl *a* was recognized by its red fluorescence in UV light. Viable algal cells reached a maximum of 1.5×10^5 cells ml^{-1} at 6.68-m depth (depth of maximal BChl *a*). Between 6.6 and 6.8 m the green algae were present in numbers <1% of the viable cell number of *A. purpureus*. From a depth of 6.5–6.8 m only BChl *a* could be detected spectrophotometrically in acetone extracts. Below this depth there was a smaller absorption peak at 663 nm. BChl *c* and Chl *a* in acetone both show absorption maxima at this wavelength. Because considerable numbers of both Chlorobiaceae and green algae were detected in the bacterial layer (Table 1), the absorption at 663 nm in acetone was probably caused by a mixture of BChl *c* and Chl *a*. Between 6.8 and 6.9 m, a concentration of the sum of both pigments of 12–14 μg $liter^{-1}$ was calculated. No BChl *e* could be detected in acetone extracts, thereby confirming the absence of brown species of Chlorobiaceae.

Physiology of purple sulfur bacteria in the natural environment—Similar to the vertical distribution of phototrophic bacterial biomass, the net assimilation rates of $H^{14}CO_3^-$ fixation in the light exhibited a distinct maximum at 6.68 m (Fig. 6C). Primary production integrated over the whole bacterial plate amounted to 15 mg C m^{-2}

Table 1. Species composition of the bacterial plate in Mahoney Lake as determined from MPN and deep agar dilution series of water samples from the center of the plate.

	Cells ml^{-1}	% of total
Amoebobacter purpureus	1.1×10^8	97.9
Thiocapsa roseopersicina	2.2×10^6	2.0
Rhodobacter capsulatus	2.0×10^4	0.02
Chloroherpeton thalassium	2.0×10^3	0.002
*Prosthecochloris aestuarii**	$<2.0 \times 10^3$	<0.002
Coccal green algae	1.5×10^5	0.14

* As judged from light microscopic examination and comparison with cell numbers of *C. thalassium*.

h^{-1}. The vertical distribution of BChl-specific assimilation rates (P) reached highest values above 6.6 m (max P_{opt} at 6.58 m) and decreased in lower water layers (Fig. 6C).

On the basis of the relationship between cell volume and carbon content of bacterial cells reported by Watson et al. (1977) (1.21×10^{-13} g C μm^{-3}), total cell counts of *A. purpureus* were converted to grams C per cubic meter and doubling times calculated from C fixation rates. Calculations were performed for depths of 6.63 and 6.68 m, as only in these depths of high cell densities were the standard deviations of total cell numbers small. For 6.63 m the doubling time of the whole population was 98 d; for 6.68 m, it was 126 d. To estimate the corresponding value for the depth of maximal P, we applied the ratios of C to BChl *a* obtained for 6.63 and 6.68 m to the BChl *a* value at the depth of P_{opt}. By this method, we calculated doubling time of 51 d for this metabolically most active part of the photosynthetic bacterial plate.

A mean value of $k_B = 0.050$ m^2 (mg BChl $a)^{-1}$ of the BChl-specific attenuation coefficient was determined for *A. purpureus* if gas vesicles were collapsed before measurement. This value is considerably higher than for Chl *a*-containing algae and cyanobacteria [*Chlorella pyrenoidosa*: 0.011 m^2 (mg Chl $a)^{-1}$; Bannister 1979]. The amount of BChl *a* necessary to reduce underwater irradiance at the top of the phototrophic bacterial layer ($I_T = 64$ μEinst m^{-2} s^{-1} around noon) to a value of $I_L = 0.4$ μEinst m^{-2} s^{-1} (lowest irradiance of green light to support growth of pure cultures; cf. van Gemerden et al. 1989) was estimated according to Beer's law

$$I_L = I_T \exp(-k_B B) \quad (3)$$

$$B = \ln(I_T/I_L)/k_B = 5.08/k_B = 101.5 \text{ mg Bchl } a \text{ m}^{-2}.$$

If this value is compared to the amount of BChl *a* in the lake (1,051 mg m^{-2}), it would seem that only 9.7% of the *Amoebobacter* cells in the plate grow at a light intensity >0.4 μEinst m^{-2} s^{-1}.

Beginning at 6.6-m depth, dark uptake rates of [^{14}C]acetate decreased monotonically with increasing depth (Fig. 6D). Maximal light-dependent uptake (light minus dark values) was found at 6.68 m and another small peak at 6.53 m (arrow in Fig. 6D). After 2.5 h of incubation, 68–81% of the [^{14}C]acetate added to the top three dark bottles of the vertical series had been assimilated. At greater depths, substrate depletion occurred at a much lower rate (41% in the dark bottle at 6.63 m after 2.5 h). Uptake rates increased linearly with substrate concentration (Fig. 8). At the concentrations used (40–160 nM), substrate saturation was not observed. The plateau indicated in Fig. 6D therefore appears to be caused mainly by the rapid substrate depletion at the top of the plate.

Dark incubation of a water sample in the middle of the plate yielded a sulfide production rate (minus the sulfide oxidation by chemical processes and chemolithotrophic bacteria) of 12.4 μmol $liter^{-1}$ h^{-1}. In the light bottle, a slow increase of sulfide concentration occurred even during the day, indicating net production despite photooxidation by *Amoebobacter* at this depth.

Experiments with pure cultures—*A. purpureus* and *P. aestuarii* exhibited maximal growth rates at a temperature of 29°C. Within the bacterial plate, only minor changes in water temperature were measured (17.7°–19.0°C), whereas a steep vertical gradient of irradiance was found in the upper part of the layer. Therefore, the dependence of growth rates on light intensity and light quality was determined for *A. purpureus, T. roseopersicina,* and *P. aestuarii.*

At low light intensities of daylight fluorescent tubes, *A. purpureus* reached higher growth rates than *T. roseopersicina*; *P. aestuarii* used low light intensities most efficiently (Fig. 9). Both species of the purple sulfur bacteria ceased to grow below 0.6–1 μEinst m^{-2} s^{-1}, but *P. aestuarii* still grew at these intensities. If greenlight was used, light-limited growth rates remained unchanged for *P. aestuarii* but increased for *A. purpureus* and *T. roseopersicina* (Fig. 10A). Under this condition, the lower limit of phototrophic growth of *P. aestuarii* was higher (1 μEinst m^{-2} s^{-1}) compared to *A. purpureus* and *T. roseopersicina* (0.4 μEinst m^{-2} s^{-1}). Nevertheless light-limited growth rates of

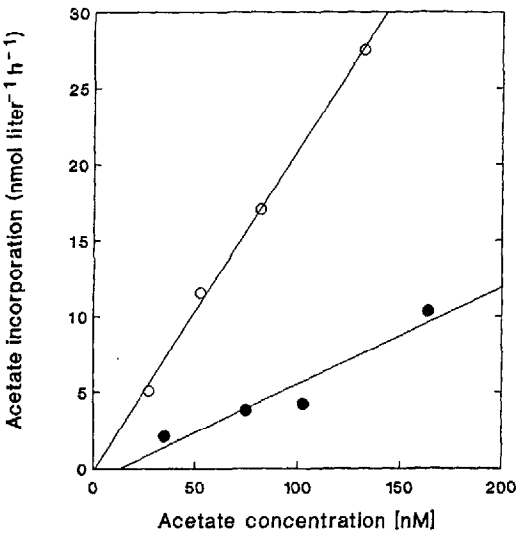

Fig. 8. Dependence of acetate incorporation in light (○) and dark (●) bottles on the [^{14}C]acetate concentration applied.

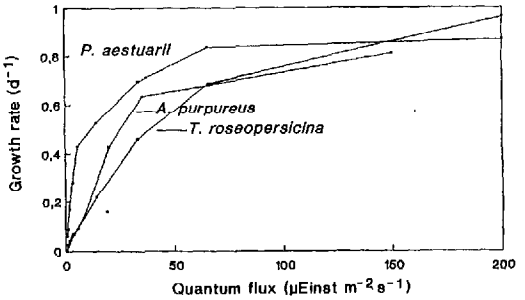

Fig. 9. Dependence of growth rate on quantum fluxes for three different species of phototrophic sulfur bacteria (fluorescent light source, Osram daylight 5000 de luxe).

P. aestuarii in green light were higher than those of both species of purple sulfur bacteria (as shown in Fig. 10B for a light intensity of 4 µEinst m^{-2} s^{-1}).

Discussion

Physiology of bacteria in the plate—Anoxygenic photosynthesis was at a maximum at the very top of the bacterial plate and declined rapidly below 6.6 m. At 6.6-m depth a flux of 60 µEinst m^{-2} s^{-1} was measured. The onset of light limitation for growth of pure cultures of *A. pupureus* was found at similar light intensities (Fig. 9). Similar light intensity values were reported by Takahashi and Ichimura (1968) and Mas et al. (1990). The decline of sulfide concentrations toward the top of the plate is due to sulfide oxidation during anoxygenic bacterial photosynthesis. The low cell sulfur content, formation of cell aggregates, and sedimentation similar to sulfur-depleted cells in cultures strongly indicate limitation of bacterial photosynthesis by the low sulfide concentrations at the top of the plate.

As indicated by the vertical sulfide profile (Fig. 3), the sulfide flux from below must be even lower than calculated for the bottom of the plate. Thus, sulfide limitation of bacterial photosynthesis at the top of the plate obviously represents a natural phenomenon and not an artifact caused by enclosure of water samples in incubation bottles. Temperature limitation of bacterial photosynthesis can be excluded in this lake. At the temperature measured in the bacterial layer and at nonlimiting sulfide concentrations, maximal BChl-specific assimilation rates of >0.2 µg C (µg BChl *a*)$^{-1}$ h^{-1} have been measured for an *A. purpureus* population in another lake (J. Overmann unpubl. results). This value is much higher than the one determined in Mahoney Lake [0.033 µg C (µg Bchl *a*)$^{-1}$ h^{-1}].

Only 10% of the total amount of BChl *a* determined in the bacterial plate is needed to reduce the quantum flux measured above the bacterial plate to the lower limit of phototrophic growth (0.4 µEinst m^{-2} s^{-1}). If the BChl concentrations are integrated beginning at the top of the plate, this limit should be reached at 6.63 m. As net photosynthetic C uptake was observed in deeper layers (Fig. 6C), the lower limit of anoxygenic photosynthesis must be reached at lower quantum fluxes.

A total of 15 mg C m^{-2} h^{-1} was determined for the primary production of the bacterial plate. If we assume complete oxidation of sulfide to sulfate, a minimum of 0.53 mol sulfide is oxidized per mole of C assimilated during photosynthesis:

$$17\ H_2S + 32\ CO_2 + 8\ NH_3 + 20\ H_2O \rightarrow 17\ H_2SO_4 + 8\ \langle C_4H_8O_2N \rangle.$$

Therefore a minimum of 664 µmol sulfide m^{-2} h^{-1} must have been oxidized in the

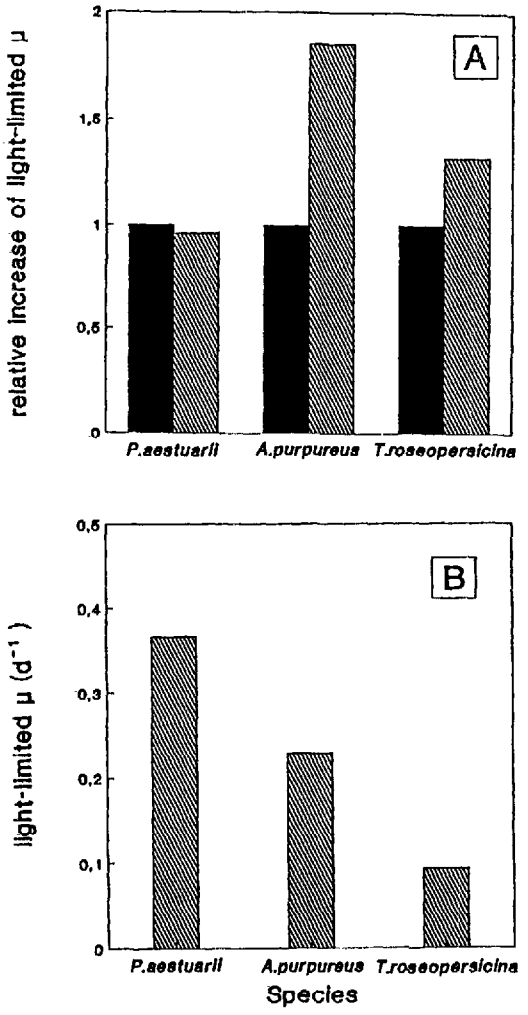

Fig. 10. A. Relative increase of light-limited growth rates in green light (hatched bars) as compared to those in fluorescent daylight (black bars) for the three different species. The initial slopes of specific growth rate μ vs. quantum flux curves (*see Fig. 9*) were compared. B. Respective growth rates determined at 4 μEinst m^{-2} s^{-1} in green light.

whole plate. An upward vertical flux of sulfide of 123 μmol m^{-2} h^{-1} was calculated for water layers below the bacterial plate. If the high sulfide flux remained constant at night, 246 μmol m^{-2} h^{-1} (37% of the sulfide oxidized) would reach the photosynthetic layer from below, whereas 63% must have been produced in the plate itself. On the basis of the sulfide production rate measured at 6.64 m, this amount of sulfide would be produced in a layer 3.4 cm thick. As the bacterial plate extends over > 10 cm, this value is very reasonable. At night, however, photosynthetic sulfide oxidation ceases, and the flux of sulfide from below will decrease as a consequence. Thus more than two-thirds of the sulfide oxidized during anoxygenic photosynthesis is produced within the bacterial plate itself. Our results agree with earlier observations of considerable sulfate reduction in the free water column of lakes (Sorokin 1970; Jørgensen et al. 1979).

In contrast to other lakes (van Gemerden et al. 1985), uptake of acetate by phototrophic bacteria was significant in Mahoney Lake. Dark uptake of this compound seems, however, to be related to the availability of dissolved oxygen (Fig. 6D). Besides the acetate incorporation measured in the phototrophic bacterial layer, high sulfide production rates and high cell numbers of the mainly organotrophic purple nonsulfur bacteria show the importance of organic matter for microbial metabolism in the plate. In many other lakes, purple nonsulfur bacteria were found only in insignificant amounts (Biebl and Drews 1969; Steenbergen and Korthals 1982). Our results point to a short cycle of C and redox compounds in the bacterial layer of Mahoney Lake.

A certain part of the population was able to maintain a vertical position above the main layer of purple sulfur bacteria as indicated by the small maxima of sulfide and BChl *a* concentrations, and rates of light-dependent acetate incorporation (arrows in Figs. 3 and 6B, D). Large aggregates of *Amoebobacter* cells were visible in water samples from this depth. Each time the lake was visited, dense, purple cell aggregates with diameters up to 1 cm also were observed at the lake surface. They may originate in the top of the plate and rise to the surface due to formation of more readily rising aggregates of gas vacuole-containing *Amoebobacter* cells. Higher sulfide concentrations in these aggregates will prevent rapid lysis of the cells at higher oxygen concentrations.

Species composition of photosynthetic bacteria in the plate—Brown species of the

Chlorobiaceae could not be detected in Mahoney Lake even by three different methods. Biomass of the green species was very low, as indicated by the low BChl *c* plus Chl *a* concentrations detected in the lower part of the purple sulfur bacterial layer. Cell numbers of Chromatiaceae species with carotenoids other than okenone were lower by a factor of 50 compared to *A. purpureus*. Light-limited growth rates of *A. purpureus* were more enhanced by green light than were those of *T. roseopersicina*. *P. aestuarii* still grew faster than the purple sulfur bacteria species under these conditions. In contrast to the filter used in the laboratory experiment, mainly light with wavelengths between 525 and 600 nm reached the phototrophic bacteria in the lake (Fig. 4). As the absorption spectrum of *P. aestuarii* exhibits its minimum at these wavelengths, light conditions in the layer seem more favorable for *A. purpureus* (Fig. 5). Light quality is of selective importance for the dominance of either Chromatiaceae or Chlorobiaceae in lakes (Trüper and Genovese 1968; Parkin and Brock 1980*a*). When purple sulfur bacteria predominate, the relative numbers of green species of Chlorobiaceae are greater than those of their brown counterparts (Montesinos et al. 1983). Similarly, our results indicate that the enormous standing biomass of *A. purpureus* in Mahoney Lake prevents growth of the brown Chlorobiaceae and keeps the biomass of the green species at very low levels.

Extreme development of purple sulfur bacteria—The maximal BChl concentration determined for the bacterial plate in Mahoney Lake was 9 times higher than respective values reported so far for natural water bodies all over the world (max, 2,325 μg liter^{-1}; Parker et al. 1983; *see* Hall and Northcote 1990). Similarly, the total amount of BChl *a* per square meter represents the highest value ever found in nature.

Main factors determining growth of purple sulfur bacteria in lakes are light and sulfide concentrations (e.g. Parkin and Brock 1980*b*; Guerrero et al. 1985; Steenbergen and Korthals 1982). Both factors are advantageous for the development of phototrophic bacteria in Mahoney Lake. Attenuation of underwater irradiance was lower than in many other lakes (Tilzer et al. 1975; Overmann and Tilzer 1989) and resulted in light saturation of bacterial photosynthesis at the top of the plate. Photosynthesis of sulfur bacteria in a large number of lakes studied was found to be directly related to the percentage of surface irradiance available for the bacteria (Parkin and Brock 1980*b*). Sulfide production rates within the purple layer were comparatively high (van Gemerden 1967; Sorokin 1970; Jørgensen et al. 1979; van Gemerden et al. 1985) and stoichiometrically consistent with total C assimilation measured at the same time. The high sulfide concentrations correspond to high sulfate concentrations (215 mM at 9-m depth, Northcote and Halsey 1969) measured in the monimolimnion of the lake.

Light intensity and sulfide concentration cannot be the sole causes, however, of the high cell density seen in Mahoney Lake. Comparable quantum fluxes and high sulfide production rates were determined for holomictic Lake Cisó (Guerrero et al. 1985), but the respective BChl *a* concentrations (max, ~1,000 μg liter^{-1}) represent only ~5% of the Mahoney Lake values. The tremendous amount of BChl *a* in Mahoney Lake is 10 times higher than the theoretical value calculated from the light absorption in the bacterial layer. Furthermore, the minimal doubling time of *Amoebobacter* cells in the lake calculated from specific C assimilation rates (50 d) and the low viable cell counts show that most of the *Amoebobacter* cells of the plate maintain their vertical position in the lake without significant growth. This observation agrees with laboratory results: when pure cultures of *A. purpureus* are incubated at 4°C in the dark, the whole population accumulates at the top of the culture vessel and stays there for >6 months. The population density of algae and bacteria observed in nature is controlled not only by growth rates of the organisms but also by rates of decay, sedimentation, and grazing (Tilzer 1984). During circulation, loss rates due to sedimentation and washout increased significantly in Lake Cisó (Mas et al. 1990). The permanent and very stable stratification of Mahoney Lake keeps bio-

mass losses due to sedimentation and decay of *Amoebobacter* at very low levels and may be the major factor that accounts for the high concentrations of cells in the purple sulfur bacterial plate.

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