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# Photosynthetic activity and population dynamics of *Amoebobacter purpureus* in a meromictic saline lake

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*Abstract:* A dense population of the purple sulfur bacterium *Amoebobacter purpureus* in the chemocline of meromictic Mahoney Lake (British Columbia, Canada) underwent consistent changes in biomass over a two year study period. The integrated amount of bacteriochlorophyll reached maxima in August and declined markedly during early fall. Bacteriochlorophyll was only weakly correlated with the light intensity and water temperature in the chemocline. In the summer, bacterial photosynthesis was limited by sulfide availability. During this period the intracellular sulfur concentration of *A. purpureus* cells decreased. A minimum concentration was measured at the top of the bacterial layer in August, when specific photosynthetic rates of *A. purpureus* indicated that only 14% of the cells were photosynthetically active. With the exception of a time period between August and September, the specific growth rates calculated from $CO_2$ fixation rates of *A. purpureus* were similar to growth rates calculated from actual biomass changes in the bacterial layer. Between August and September 86% of the *A. purpureus* biomass disappeared from the chemocline and were deposited on the littoral sediment of Mahoney Lake or degraded within the mixolimnion. This rise of cells to the lake surface was not mediated by an increase in the specific gas vesicle content which remained constant between April and November. The upwelling phenomenon was related to the low sulfur content of *A. purpureus* cells and a low resistance of surface water layers against vertical mixing by wind.

*Key words:* Anoxygenic photosynthesis; Low-light-adaptation; Sulfur cycle; Sulfate reduction; Aggregation; Gas vesicle

## Introduction

Phototrophic sulfur bacteria form dense populations where light reaches sulfide containing bottom layers of lakes and sediments. Due to their anaerobic oxidation of sulfide, phototrophic sulfur bacteria constitute an important component of the sulfur cycle. In addition, anoxygenic photosynthesis can account for up to 90% of total photosynthetic $CO_2$ fixation in lakes [1]. Populations of phototrophic sulfur bacteria often persist during times of very low light intensity in winter. This has been attributed to low light adaptation of bacterial photosynthesis [2].

An extremely dense layer of the purple sulfur bacterium *Amoebobacter purpureus* was described for meromictic Mahoney Lake (British Columbia, Canada) and is present throughout the entire year [3,4]. The biomass concentration present in the chemocline (the layer of steep vertical

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concentration gradients of dissolved substances like oxygen, sulfide, sulfate and carbonate) exceeds all values reported so far [4] and is related to passive accumulation of gas-vacuolated cells. In recent years a large amount of purple aggregates has been observed floating at the lake surface in late summer, concurrent with a drastic decrease of *A. purpureus* biomass in the chemocline (M. Rodrigo, J.T. Beatty and K.J. Hall, unpublished).

As part of a comprehensive study of the sulfur and carbon cycles in Mahoney Lake, we studied sulfide limitation and low light adaptation of anoxygenic photosynthesis, respiratory activity and changes in cellular gas vesicle content in the bacterial layer, and their significance for the development of the population of *A. purpureus* over a two year period.

## Materials and Methods

*Sampling*

All measurements and incubations were performed on 12 dates between April 1992 and November 1993 at a permanent station located over the deepest site (14.5 m) in the center of the lake. Samples of the bacterial plate were collected at 2.5 cm intervals using a syringe sampler as described previously [4].

*Physical measurements*

Water temperature and conductivity were measured with a YSI model 33 meter (Yellow Springs Instr., Yellow Springs, OH), with calibration and correction of the conductivity values according to Hall and Northcote [5].

Global irradiance was recorded during sampling days on the southern lake shore with a Belfort pyrheliometer (Belfort Instrument Company, Baltimore, MD). Mahoney Lake is surrounded by hills, so global irradiance varies between different locations at the lake surface. Therefore the Belfort pyrheliometer was calibrated by measuring irradiance at the sampling station with a LiCor LI-200SB pyranometer sensor. Daily global irradiance data for 1992 and 1993 were measured with a Kipp & Zonen pyranometer model CM5 at the meteorological station of Agriculture Canada at Summerland (B.C.). The data from both locations showed good correlation ($r^2 = 0.85$, $n = 10$).

The vertical distribution of photosynthetically available radiation (PAR) within the water column was determined with a LiCor quantum meter (model 185A).

*Analytical procedures*

Sulfide concentrations above the biomass maximum of *A. purpureus* were measured spectrophotometrically [6].

High concentrations of polysulfides were found below the biomass maximum (Overmann et al., in preparation). The Cline method underestimates total sulfide if polysulfides are present [7]. Therefore sulfide concentrations at the biomass maximum of the plate and below were determined by polarography. A set of 15 ml tubes, each containing 0.3 ml 2 N NaOH, was sealed with butyl rubber septa and flushed with nitrogen for 10 min. Ten ml of lake water from each depth were filtered through 0.2 $\mu$m cellulose acetate filters and simultaneously injected through the septa of these tubes. The headspaces of all tubes were flushed with nitrogen and the tubes frozen on dry ice. Polarograms were run with a Metrohm 663VA dropping mercury electrode system equipped with a Princeton Applied Research model 174A analyzer and a X-Y recorder (Soltec VP-6415S; Soltec Valley, Sun Valley, CA). Linear sweep voltammetry was performed between 0 and $-1.5$ V at a scan rate of 5 $mV \cdot s^{-1}$ in the sampled DC mode. Water samples of 0.2 to 5 ml were diluted into 15 ml of a solution of 0.2 M $NaNO_3$/0.2 M $NaHCO_3$ (pH = 9.5), which permits determination of polysulfide S(2-) and S(0) without interference at the Hg electrode [7].

Vertical diffusion fluxes $J_z$ of sulfide were calculated from the first derivative of the vertical sulfide concentration profile:

$$J_z\ (\mu mol\ cm^{-2}\ s^{-1}) = -D_{H2S/HS^-} \cdot dC_z/dz \quad (1)$$

where $C_z$ is the sulfide concentration ($\mu$M) at depth z (m). Values of the molecular diffusion coefficient for sulfide, $D_{H2S/HS^-}$, at in situ water temperatures were taken from Table 2 of Broecker and Peng [8].

A modification of the method of Steinmetz and Fischer [9] was used to determine intracellular sulfur. Water samples were filtered through precombusted glass fiber filters (Whatman GF/A 25 mm), washed with 5 ml of $N_2$-flushed distilled water and frozen on dry ice. In the laboratory the filters were thawed, 0.1 ml of 1 M potassium phosphate buffer (pH 8.75), 3.75 ml distilled water and 0.1 ml 1.25 M KCN were added, and the samples incubated at 80°C for 20 min. After cooling the samples to 5°C, 0.5 ml of 1.5 M $Fe(NO_3)_3$ in 4 N $HClO_4$ were added, the samples were slowly warmed up to room temperature, centrifuged in an Eppendorf microfuge and the absorbance read at 460 nm.

Sulfate was determined turbidometrically after precipitation with $BaCl_2$ [10].

Dissolved inorganic carbon was measured in triplicate samples with a Shimadzu total carbon analyzer (TOC-500) equipped with an autosampler (AS 1-502). The instrument was calibrated with solutions of $Na_2CO_3$.

Bacteriochlorophyll (Bchl) *a* was determined after centrifugation of water samples and extraction of the pellet with acetone (4°C, 24 h). Absorbance was measured at 772 nm in a Hitachi UV-2000 spectrophotometer. Bchl *a* in water samples from the mixolimnion was concentrated by filtration of 2-l samples through a 54 $\mu$m Nitex screen. No Bchl *a* was found in the 0.2–54 $\mu$m fraction. Pigment concentrations were calculated from the equations given by Steenbergen and Korthals [11].

*Physiological parameters*

Photosynthetic $CO_2$ uptake in the bacterial layer was assessed at 5 cm intervals using the $^{14}C$ radiocarbon method and a special incubation rack described previously [4].

Throughout the year sulfide concentrations within the upper part of the bacterial layer decreased between sunrise and noon, and increased in the afternoon. In a preliminary experiment, sulfide limitation of anoxygenic photosynthesis during the incubation of a sample from the top of the layer was observed. Therefore $^{14}C$ incorporation in the layer was measured for 3 h around solar noon after spiking each sample with 200 $\mu$M $Na_2S$. Sulfide limitation was never observed under these conditions.

Sulfate reduction rates were determined at 5 cm intervals across the bacterial plate. After addition of 3 $\mu$Ci of $Na_2^{35}SO_4$ per ml the samples were incubated for 8 h in the same apparatus as described for the $^{14}C$ assimilation experiments above. After incubation, 0.5 ml samples were removed for determination of total radioactivity. Ten ml of the sample were injected through a rubber septum into a reaction vessel of an apparatus for anaerobic distillation. $H_2S$ was driven off at ambient temperature by flushing with nitrogen and collected in two traps placed in series, which contained 10 ml 2% $Zn(Ac)_2$ and 10 $\mu$l Antifoam C (Sigma, St. Louis, MO) each. After purging with $N_2$ for 5 min, the pH in each reaction vessel was adjusted to 1.0 by addition of concentrated HCl. Flushing continued for 15 min. The precipitate of both traps was combined and centrifuged to a final volume of 0.5 ml. Samples were counted in a Beckmann LS 6000 TA liquid scintillation counter in 20 ml Scintiverse II.

Respiration rates were determined in natural samples employing a Clark type oxygen electrode (Model 53, Yellow Springs Instruments, OH) in a thermostatted 10 ml-cuvette. For calibration, aliquots of oxygen-saturated potassium phosphate buffer (50 mM, pH 7.4) were injected. Respiration rates were calculated from the decrease of oxygen with time. Substrates (sulfide, thiosulfate and sulfite) were dissolved in degassed distilled water and stored under a nitrogen atmosphere prior to injection into the cuvette. Respiration rates were corrected for chemical sulfide oxidation.

*Cellular gas vesicle content*

When samples of the chemocline were examined microscopically, gas vesicles were detected only in *A. purpureus* cells. The gas space of gas vesicles was measured with a capillary compression tube [12]. In order to prevent gas bubble formation, samples were incubated prior to the measurement for 20 min at the same temperature used for the pressure tube. The sample was pressurized momentarily to 700 kPa and the volume of the gas vesicle gas space calculated from the

change in the position of the meniscus in the capillary. The maximum pressure required for the complete collapse of the gas vesicles in natural samples was determined by nephelometry [13] to be 500 kPa.

*Sedimentation rates*

*A. purpureus* cells which sedimented out of the mixolimnion and the bacterial plate were collected in traps positioned in the lake at 5.5 m and 8 m (vertical positions of openings). Three 55 cm long, 10 cm diameter PVC tubes were suspended at each depth. The traps at 5.5 m were preloaded with 60 ml 37% formaldehyde [14], as preliminary experiments showed a rapid decline of Bchl in non-preserved traps at that depth.

## Results

*Population dynamics of Amoebobacter purpureus*

In 1992 and 1993 the total biomass of *A. purpureus* in the chemocline increased during spring and reached maximum values in the month of August. The biomass in the layer was only weakly correlated with the mean water temperature in the layer ($r^2 = 0.544$, $n = 12$; Fig. 1A) and the average daily light intensity during the previous growth interval ($r^2 = 0.407$, $n = 11$; Fig. 1B).

The maximum concentration of Bchl *a* was always found near the chemocline (Fig. 2A) and reached 27470 and 24650 $\mu g\ l^{-1}$ in August 1992 and 1993, respectively. At these times the bacterial layer extended further into the mixolimnion than during the rest of the year. Concomitantly, a considerable fraction of these cells was thriving in water layers that contained less than 100 $\mu M$ sulfide. The upper boundary of the plate in Fig. 2A is defined by a concentration of 10 $\mu g$ Bchl*a* $l^{-1}$ (the detection limit) and the lower limit by 200 $\mu g$ Bchl*a* $l^{-1}$, as concentrations up to 200 $\mu g$ Bchl*a* $l^{-1}$ were also present between 7.5 and 14 m depth. The intracellular sulfur concentration in *A. purpureus* cells sampled above the Bchl maximum decreased by a factor of 7.4 between May and August 1993 (Fig. 2B). Employing the maximum Bchl *a* content of 9.36 $\mu g$ Bchl*a* (mg dry cell mass)$^{-1}$ determined for a pure culture of *A.*

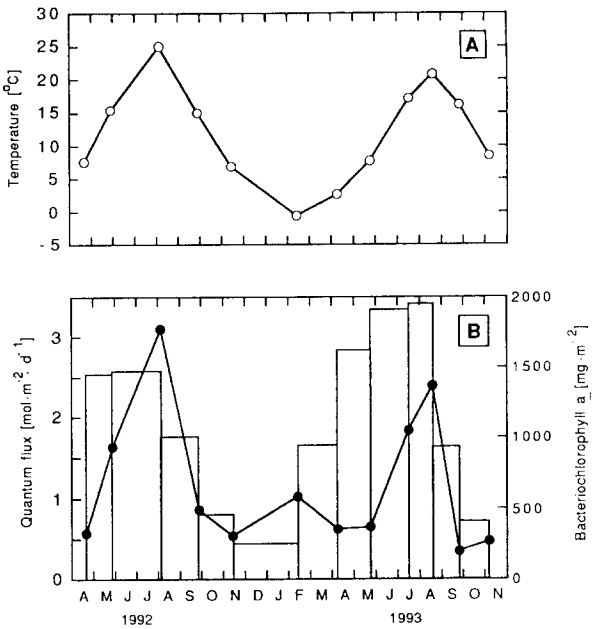


Fig. 1. Development of the purple sulfur bacterial layer in Mahoney Lake and physical parameters during the study period. **(A)** Mean water temperature in the layer. **(B)** Integrated biomass of *A. purpureus* measured as bacteriochlorophyll (filled circles) and mean daily quantum flux (bars) at the upper boundary of the layer between sampling dates.

*purpureus* under light limitation, the specific sulfur content varied between 3.5 and 33.8% dry cell mass. The latter value is close to the maximum sulfur content of 30% observed in a pure culture of *Chromatium* [15].

Daily integrals of potential anoxygenic photosynthesis for the entire layer were calculated from photosynthetic rates measured in the presence of excess sulfide at each depth of the plate. The values were converted to mmol of redox equivalents required for photosynthesis assuming a mean biomass composition of $\langle C_4H_8O_2N \rangle$ [16], thus obtaining an average of 4.25 redox equivalents needed per $CO_2$ reduced (Fig. 3, stippled bars). The maximum amount of redox equivalents available from oxidation of sulfide that either diffused from below into the plate (densely hatched bars), or which was generated by sulfate reduction within the plate (lightly hatched bars), was calculated for comparison. The demand of redox equivalents required for anoxygenic photosynthetic $CO_2$ fixation exceeded the supply from

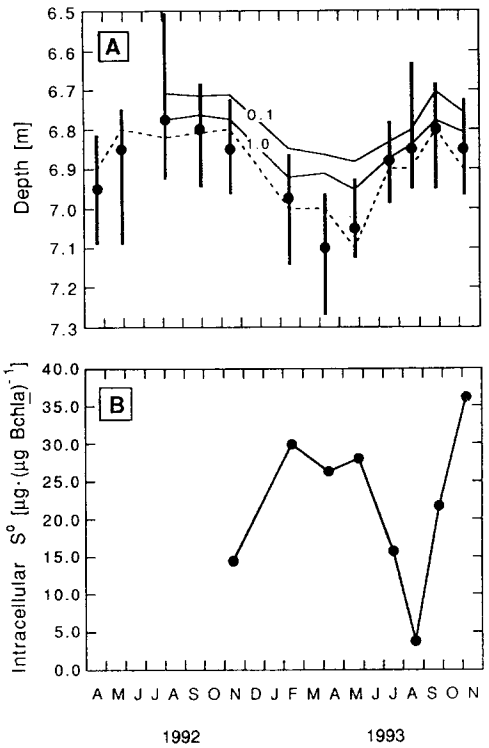


Fig. 2. (A) Vertical extension (■) and position of the bacteriochlorophyll maximum (●) of the purple sulfur bacterial layer, (- - -) vertical position of the chemocline. Isopleths for concentrations of 0.1 and 1.0 mM sulfide at sunrise are also shown. (B) Weighted intracellular sulfur content in *A. purpureus* cells sampled above the Bchl maximum.

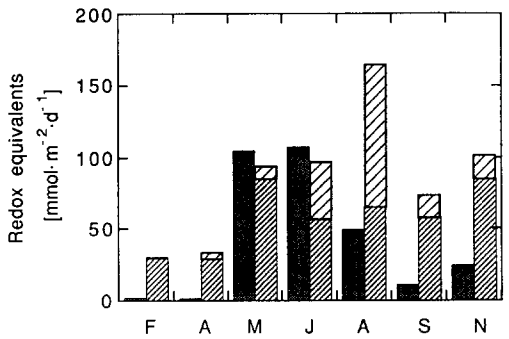


Fig. 3. Requirement of anoxygenic photosynthesis for redox equivalents (▨) compared to potentially available redox equivalents provided by diffusion of sulfide into (▩) and sulfate reduction within (▨) the bacterial plate. Calculations done for sampling dates between February and November 1993 (compare with Fig. 2).

complete oxidation of sulfide only in May and July 1993 (Fig. 3).

Values of daily photosynthetic carbon fixation were also used to calculate potential growth rates of *A. purpureus* at different times during 1993. Carbon fixation rates were converted to Bchl *a* formed using a factor of 0.01991 mg Bchl *a* (mg cellular carbon)$^{-1}$. This factor derives from the ratio of Bchl to dry cell mass given above and a carbon content of bacterial biomass of 47% (the biomass composition given above). With the exception of the time interval between August 19 and September 24 1993, our measurements of anoxygenic photosynthesis slightly underestimated the growth rates calculated from changes of Bchl *a* in the purple sulfur bacterial layer (Fig. 4). Conversely, the amounts of Bchl *a* in the layer declined sharply between August and September despite a considerable potential growth rate calculated from the photosynthetic rate measured in August.

On August 1993 the appearance of the purple sulfur bacterial layer was investigated by video camera image in a SCUBA dive. The surface of the layer showed large clumps of *A. purpureus* cells protruding out of the layer (Fig. 5A). At this time of the year, a considerable variation in the vertical distribution of Bchl was observed when four 2.5 cm interval syringe samples were obtained consecutively, reflecting this heterogeneity at the surface of the bacterial layer (data not shown).

In September numerous purple bacterial aggregates were present in the mixolimnion (Fig. 5B), changing the color of Mahoney Lake from the usual blue-green to purplish.

Between February and August 1993, only traces of Bchl *a* were measured in the mixolimnion (0–0.118 mg m$^{-2}$), and sedimentation rates out of the mixolimnion (0.0423–0.0632 mg m$^{-2}$ day$^{-1}$) and out of the plate (0.0619–0.1547 mg m$^{-2}$ day$^{-1}$) were also small. Although these values increased significantly between August and September 1993 (Table 1), a net loss of 1091.5 mg Bchl *a* m$^{-2}$ from the purple sulfur bacterial layer cannot be explained by the presence of *A. purpureus* cells in the mixolimnion or their sedimentation towards the lake bottom. During this inter-

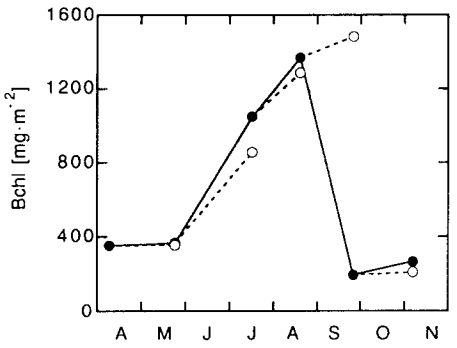


Fig. 4. Development of purple sulfur bacterial biomass (●, compare Fig. 1) in 1993 and expected growth of purple sulfur bacteria as calculated from photosynthetic rates and assuming exponential growth during the following time intervals (○).

val a high amount of Bchl sedimented out of the mixolimnion (Table 1). In September the littoral sediment of Mahoney Lake was densely covered with a thick layer of purple sulfur bacteria.

The yearly primary productivity of purple sulfur bacteria in Mahoney Lake between November 1992 and November 1993 was 33.5 gC $m^{-2}$ $year^{-1}$. Table 1 indicates that a major portion of this carbon rose into the mixolimnion of the lake between August and September and was consumed either directly or after its deposition in the littoral zones of the lake.

*Physiology of A. purpureus*

In order to investigate the possibility of low light adaption of *A. purpureus* cells, photosynthetic rates were normalized to Bchl concentrations (assimilation number P, Fig. 6). Considerable changes in the dependence of assimilation numbers on the in situ light intensities were observed between different sampling dates. Between autumn and spring, assimilation numbers reached values up to 0.432 mgC · (mg Bchl*a*)$^{-1}$ at light intensities below 20 $\mu$mol $m^{-2}$ $^{-1}$. Although much higher light intensities were available for anoxygenic photosynthesis during the summer, only slightly higher assimilation numbers were measured. The maximum assimilation number calculated for the top of the bacterial layer was 0.952 mgC (mg Bchl*a*)$^{-1}$ (on August 24 1993) which is 14% of the light-saturated value determined in a pure culture of *A. purpureus* (dotted line in Fig. 6).

Respiration rates in the dark of samples from the bacterial plate increased tenfold after addition of sulfide (Fig. 7). In the presence of sulfide a slight inhibition of respiration was observed during illumination whereas the addition of KCN decreased the rate to the value determined in the absence of substrates. With thiosulfate and sulfite as electron donor the respiration rate reached only 30 and 24% of the values observed with sulfide.

A vertical profile of respiration rates was measured on September 26 1992 (Fig. 8). In the presence of sulfide, maximum rates were observed at a depth 5 cm below the position of the maximum Bchl concentration. The highest counts of heterotrophic bacteria were found 2.5 to 5 cm below the peak of Bchl *a* (Overmann et al., in preparation).

The weighted mean of the cellular gas-vesicle volume of *A. purpureus* in the chemocline was relatively constant between April and November 1993 (Table 2). The weighted mean determined for *A. purpureus* aggregates collected at the lake surface in winter and spring was much higher. These aggregates remained buoyant in Mahoney Lake surface water after incubation at room temperature for 24 h. In September 1993 when a high concentration of aggregates was present in the mixolimnion, 77% of the surface aggregates sampled remained buoyant but, in contrast to other times of the year, had a specific gas vesicle content comparable to that of *A. purpureus* cells in the chemocline.

After collapse of the gas vesicles in the compression tube, surface aggregates on all sampling dates settled when incubated in Mahoney Lake water.

## Discussion

Low correlations were found between the biomass of *A. purpureus* and light or temperature in the bacterial layer. In contrast, integral phototrophic bacterial biomass was correlated with light intensity in holomictic Lake Cisó [17]. This

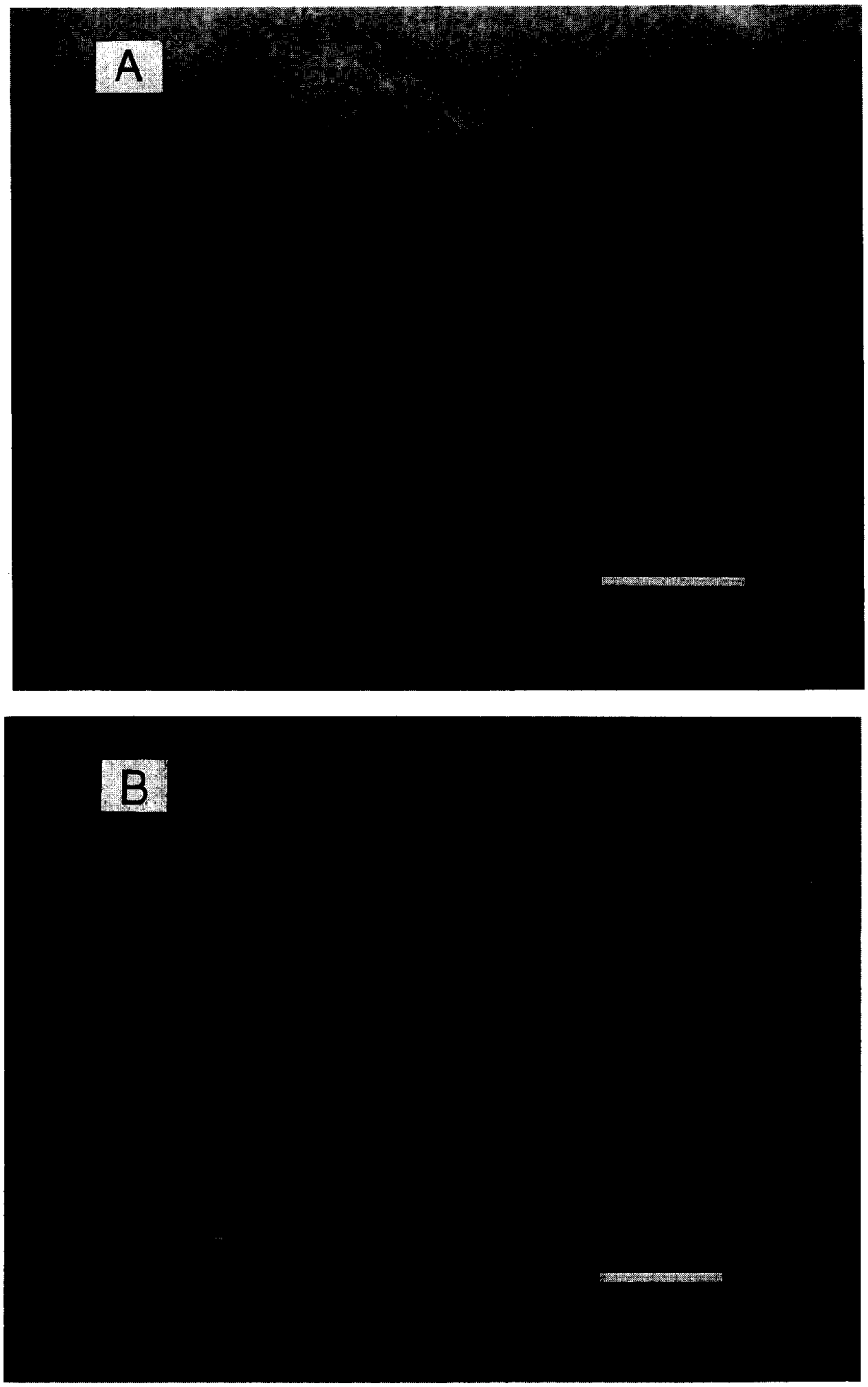


Fig. 5. **(A)** Surface of the bacterial layer at 6.7 m depth viewed at an angle of 45° from above with an underwater video camera in Mahoney Lake on August 19 1993. Bar = 10 cm. **(B)** Macroscopic aggregates of *A. purpureus* floating at the surface of Mahoney Lake (September 26 1993). Bar = 5 cm.

Table 1

Balance of bacteriochlorophyll *a* (mg $m^{-2}$) of the bacterial layer in Mahoney Lake for the time period August 19 to September 24 1993.

| Layer | August 19 | September 24 | Differences |
|---|---|---|---|
| Plate | 1366.3 | 196.2 | −1170.1 |
| Mixolimnion * (0–6.8 m) | 0.01 | 40.5 | 40.5 |
| Sedimentation out of plate (8 m sediment trap) | | | 38.0 |
| Net loss of Bchl *a* | | | −1091.6 |
| Sedimentation out of mixolimnion (5.5 m sediment trap) | | | 276.2 |

* Value corrected for the dilution of *A. purpureus* cells in the volume of upper water layers.

indicates that additional processes are significant for the development of purple sulfur bacteria in Mahoney Lake. These factors might include: (1) adaptation of cells to low light intensities during winter [2]; (2) changes in the percentage of cells which are photosynthetically active; and (3) loss processes which vary during the year [18].

The assimilation numbers (P) determined in the bacterial layer were significantly lower than those in an exponentially growing culture of *A. purpureus* at saturating light intensities (Fig. 6). If changes in light adaptation of anoxygenic photosynthesis were the reason for the low assimilation numbers obtained, P would be expected to vary only under limiting light intensities (i.e. $< 18$ $\mu$mol $m^{-2}$ $s^{-1}$ in Fig. 6). Lawrence et al. [2] postulated low light adaptation of *Chlorobium* in

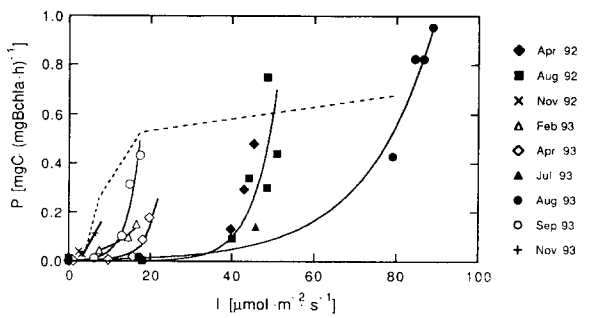


Fig. 6. Assimilation numbers P at various underwater light intensities measured at different times during the sampling period. (- - -) indicates values calculated from the growth rates of a pure culture of *A. purpureus* ML1 at 20°C [4] and multiplied by a factor of 0.1.

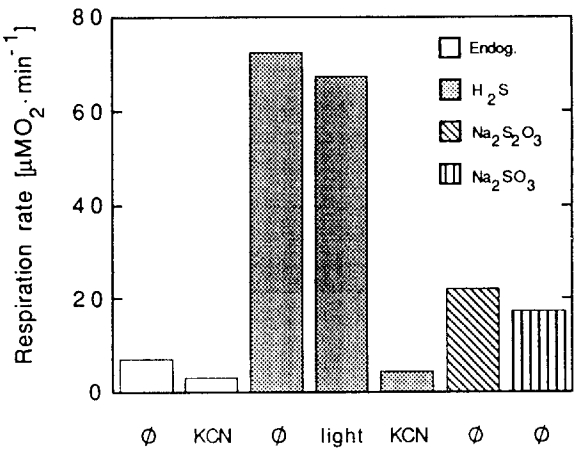


Fig. 7. Respiration rates measured with samples from the bacterial plate (August 3 1992; 6.75 m depth). Values corrected for abiotic oxidation of sulfide. Endog., endogenous rate; ($\phi$) no illumination or addition of KCN. Bchl *a* concentration 21.4 mg $l^{-1}$, T = 30°C, substrate concentrations (sulfide, thiosulfate, sulfite) = 50 $\mu$M. Concentration of KCN = 100 $\mu$M. Light intensity = 1000 W $m^{-2}$ of a tungsten lamp bulb.

meromictic Waldsea Lake to explain the persistence of the phototrophic bacterial layer under a 1 m thick ice cover during winter. However, our data provide no evidence for a low light adaptation of *A. purpureus* in Mahoney Lake.

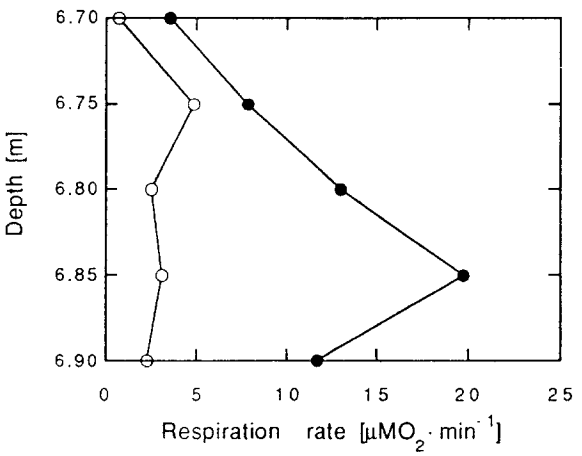


Fig. 8. Vertical profile of endogenous respiration rates (○) and maximum respiration rates (●) in the presence of 50 $\mu$M sulfide in samples taken from Mahoney Lake on September 26 1992. T = 26°C. On this day the maximum Bchl *a* concentration was found at 6.8 m.

Table 2

Bchl-specific gas vesicle volume ($\mu$l $\mu$g Bchl$a^{-1}$; weighted mean) of *A. purpureus* cells in the bacterial plate and in aggregates floating at the lake surface *

| Date ** | Plate | Surface aggregates |
|---|---|---|
| 10/02/93 | 0.0219 | 0.1034 |
| 06/04/93 | 0.0474 | 0.1315 |
| 22/05/93 | 0.0400 | - |
| 24/09/93 | 0.0366 | 0.0305 |
| 05/11/93 | 0.0419 | 0.1020 |

* Only samples from the bacterial plate with Bchl concentrations above 500 $\mu$g $l^{-1}$ were considered.

** No aggregates were found at the lake surface on May 22, July 16 and August 19, 1993

In lower parts of the bacterial layer the low values for P might in principle be due to selective absorption of photosynthetically usable wavelengths of light by *A. purpureus* cells above (self-shading). However, at maximum light intensities at the top of the purple sulfur bacterial layer in August 1993, maximum assimilation numbers reached only 14% of the values measured in a pure culture at the same temperature (20°C). This indicates that a large fraction of the cells in the chemocline were photosynthetically inactive.

Aggregation of *A. purpureus* in pure culture is observed exclusively under conditions of sulfide limitation [19]. In August, *A. purpureus* cells formed large aggregates at the top of the layer which seems to contradict the finding that at the same time less than 30% of the available sulfide was oxidized by bacterial photosynthesis. Since our measurements of photosynthetic rates underestimated the calculated growth rates only by a factor of 0.78 during summer, this discrepancy is not likely to be due to experimental error. Moreover, *A. purpureus* cells taken from the chemocline of Mahoney Lake at this time contained very small amounts of elemental sulfur. We observed that the viability of *A. purpureus* cells in pure cultures decreases during incubation of sulfur-depleted cells at 20°C in the light. Therefore the low photosynthetic activity in August can be explained by sulfide limitation of anoxygenic photosynthesis and concomitant loss of cell viability during the three previous months. A reoccurring pattern of high photosynthetic rates of phototrophic bacteria in early summer and a drop in August was observed in a meromictic lake with limnological features very similar to Mahoney Lake [2]. Taken together, these results indicate that the physiological inactivation of a large fraction of anoxygenic phototrophic bacteria in meromictic lakes during late summer months might be a common phenomenon.

The reduced photosynthetic activity of purple sulfur bacteria during August did not account for the amount of sulfide oxidized in the chemocline. Unlike purple sulfur bacteria in other environments [20], respiratory oxidation of sulfide by *A. purpureus* cells seems to be insignificant in Mahoney Lake. This compares well with the very low respiration rates determined in pure cultures of *A. purpureus* [21]. Highest respiration rates were determined with samples from beneath the maximum of Bchl where total counts of nonphototrophic bacteria and sulfate reduction rates (Overmann et al., in preparation) and viable counts of sulfate-reducing bacteria (M. Rodrigo, J.T. Beatty and K.J. Hall, unpublished) reached maximum values. Therefore, the oxidation of sulfide in the chemocline of Mahoney Lake appears to be catalyzed by sulfate-reducing and/or chemoautotrophic bacteria. Respiration of sulfide by several species of sulfate-reducing bacteria was demonstrated by Dannenberg et al. [22].

The losses of *A. purpureus* from the chemocline due to sedimentation or rise to upper water layers were very small during spring and early summer 1993. The major loss of *A. purpureus* cells from the layer in Mahoney Lake occurs by upwelling into the mixolimnion in the fall, and the subsequent degradation of the bacterial biomass in littoral sediments and the mixolimnion.

Cells in pure cultures of *A. purpureus* were less dense than Mahoney Lake water only when they contained high amounts of gas vesicles and a low content of intracellular ballast compounds [19]. Aggregates floating at the lake surface after the upwelling event between August and September 1993 had a cellular gas vesicle content very similar to cells sampled in the chemocline, but were less dense than lake water from the surface. Therefore the upwelling of aggregates to the

mixolimnion cannot be explained by an increase in cellular gas vesicle content.

The buoyant density of intracellular sulfur is 1220 kg $m^{-3}$ [23]. In August 1993 *A. purpureus* cells contained small amounts of intracellular sulfur and the plate extended further into the mixolimnion water than during the rest of the year. Carbohydrate is a major determinant of the ballast mass of bacterial cells and decreases in pure cultures of *A. purpureus* from 16.3 to 4% of dry cell mass during the transition from exponential to stationary growth phase [19]. Because of the presence of a large number of chemotrophic bacteria in the chemocline, we could not determine if the cellular carbohydrate content of *A. purpureus* cells decreased in parallel to intracellular sulfur. Nevertheless, the available data indicate that such a concurrent decrease in sulfur and carbohydrate content is very likely, so that *A. purpureus* cells must have reached their minimum density values just before the upwelling event. During 1993, isothermal and isohaline conditions from the lake surface down to the bacterial layer were observed only in August and September when the stability (resistance to vertical mixing by wind) of the water column above the layer was very small (Overmann et al., in preparation). Therefore, these results indicate that the appearance of the major fraction of the purple sulfur bacterial biomass in the mixolimnion and the littoral zone of Mahoney Lake is caused by the coincidence of a low buoyant density of the cells during late summer and the low stability of the water layers above the chemocline.

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## References

1 Biebl, H. and Pfennig, N. (1979) Anaerobic $CO_2$ uptake by phototrophic bacteria. A review. Arch. Hydrobiol. Beih. Ergeb. Limnol. 12, 48–58.
2 Lawrence, J.R., Haynes, R.C. and Hammer, U.T. (1978) Contribution of photosynthetic green sulfur bacteria to total primary production in a meromictic saline lake. Verh. Int. Verein. Limnol. 20, 201–207.
3 Northcote, T.G. and Halsey, T.G. (1969) Seasonal changes in the limnology of some meromictic lakes in southern British Columbia. J. Fish Res. Bd. Can. 26, 1763–1787.
4 Overmann, J., Beatty, J.T., Hall, K.J., Pfennig, N. and Northcote, T.G. (1991) Characterization of a dense, purple sulfur bacterial layer in a meromictic salt lake. Limnol. Oceanogr. 36, 846–859.
5 Hall, K.J. and Northcote, T.G. (1986) Conductivity-temperature standardization and dissolved solids estimation in a meromictic saline lake. Can. J. Fish Aquat. Sci. 43, 2450–2454.
6 Cline, J.D. (1969) Spectrophotometric determination of hydrogen sulfide in natural waters. Limnol. Oceanogr. 14, 454–458.
7 Luther, G.W., Giblin, A.E. and Varsolona, R. (1985) Polarographic analysis of sulfur species in marine porewaters. Limnol. Oceanogr. 30, 727–736.
8 Broecker, W.S. and Peng, T.H. (1974) Gas exchange rates between air and sea. Tellus 26, 21–35.
9 Steinmetz, M.A. and Fischer, U. (1981) Cytochromes of the non-thiosulfate-utilizing green sulfur bacterium *Chlorobium limicola*. Arch. Microbiol. 130, 31–37.
10 American Public Health Association (1985) Standard methods for the examination of water and wastewater. APHA.
11 Steenbergen, C.L.M. and Korthals, H.J. (1982) Distribution of phototrophic microorganisms in the anaerobic and microaerophilic strata of Lake Vechten (the Netherlands). Pigment analysis and role in primary production. Limnol. Oceanogr. 27, 883–895.
12 Walsby, A.E. (1982) The elastic compressibility of gas vesicles. Proc. R. Soc. Lond. (Biol.) 216, 355–368.
13 Walsby, A.E. (1973) A portable apparatus for measuring relative gas vacuolation, the strength of gas vacuoles and turgor pressure in planktonic blue-green algae and bacteria. Limnol. Oceanogr. 18, 653–658.
14 Lee, C., Hedges, J.I., Wakeham, S.G. and Zhu, N. (1992) Effectiveness of various treatments in retarding microbial activity in sediment traps material and their effects on the collection of swimmers. Limnol. Oceanogr. 37, 117–130.
15 van Gemerden, H. (1968) Growth measurements of Chromatium cultures. Arch. Mikrobiol. 64, 103–110.
16 Harder, W. and van Dijken, J.P. (1976) Theoretical considerations on the relation between energy production and growth of methane-utilizing bacteria. In: Symposium in Microbial Production and Utilization of Gases ($H_2$, $CH_4$, CO) (Schlegel, H.G., Gottschalk, G. and Pfennig, N., Eds.), pp. 403–418. E. Goltze KG, Göttingen, FRG.

17 Pedrós-Alió, C., Montesinos, E. and Guerrero, R. (1983) Factors determining annual changes in bacterial photosynthetic pigments in holomictic Lake Cisó, Spain. Appl. Environ. Microbiol. 46, 999–1006.
18 Mas, J., Pedrós-Alió, C. and Guerrero, R. (1990) In situ specific loss and growth rates of purple sulfur bacteria in Lake Cisó. FEMS Microbiol. Ecol. 73, 271–281
19 Overmann, J. and Pfennig, N. (1992) Buoyancy regulation and aggregate formation in *Amoebobacter purpureus* from Mahoney Lake. FEMS Microbiol. Ecol. 101, 67–79.
20 Seitz, A.P., Nielsen, T.H. and Overmann, J. (1993) Physiology of purple sulfur bacteria forming macroscopic aggregates in Great Sippewissett Salt Marsh, Massachusetts. FEMS Microbiol. Ecol. 12, 225–236.
21 Overmann, J. and Pfennig, N. (1992) Continuous chemotrophic growth and respiration of Chromatiaceae species at low oxygen concentrations. Arch. Microbiol. 158, 59–67.
22 Dannenberg, S., Kroder, M., Dilling, W. and Cypionka, H. (1992) Oxidation of $H_2$, organic compounds and inorganic sulfur compounds coupled to reduction of $O_2$ or nitrate by sulfate-reducing bacteria. Arch. Microbiol. 158, 93–99.
23 Guerrero, R., Mas, J. and Pedrós-Alió, C. (1984) Buoyant density changes due to intracellular content of sulfur in *Chromatium warmingii* and Chromatium vinosum. Arch. Microbiol. 137, 350–356.