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Change in Size of *Chromatium minus* Cells in Relation to Growth Rate, Sulfur Content, and Photosynthetic Activity: A Comparison of Pure Cultures and Field Populations

EMILIO MONTESINOS

Department of Agriculture, Polytechnic School of Girona, Polytechnic University of Catalunya, 17002 Girona, Spain

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The size frequency distribution of planktonic cells of purple sulfur phototrophic bacteria was measured at several depths in a bacterial layer of Lake Cisó (Spain). The bacterioplankton was dominated by *Chromatium minus* (87 to 94% of the total biomass). The largest cells of *C. minus* were found in the top part of the bacterial layer. In addition, the in situ and potential specific photosynthetic activity (CO_2 fixation and acetate uptake) and specific pigment content were measured in relation to several key environmental parameters that determine the activity of cells. Potential growth rates were estimated from production rates and biomass. A maximal specific growth rate of 0.074 h^{-1} was found for the top part of the bacterial layer. Photosynthesis versus light and versus sulfide curves among field samples indicated that light was the main limiting factor controlling the activity of *C. minus* in Lake Cisó. The specific bacteriochlorophyll *a* content was very high in all samples (0.27 to 0.36 μg μg of C^{-1}). Results of laboratory experiments performed with pure cultures indicated that the average cell volume changes from 5.9 to 20.0 μm^3 and that differences in growth rate, breakdown, or synthesis of sulfur and glycogen and degradation of the photosynthetic apparatus are the main factors accounting for the observed changes in cell volume across the bacterial layer.

It is well known that resting cells collected during the stationary phase of growth in batch cultures are smaller than exponentially growing cells (13). The scattered data from the studies performed under unrestricted balanced growth conditions or in chemostat culture indicate that cell mass (mainly RNA content) increases as a function of growth rate in many procaryotes (12, 13, 19; L. Van Liere, Ph.D. thesis, University of Amsterdam, The Netherlands, 1979) and eucaryotes (4, 5). On the other hand, under restricted growth conditions, that is, availability of sufficient energy or carbon source but strong limitation or starvation of nitrogenous compounds (or some essential micronutrients) that are necessary for growth, cells can synthesize storage materials such as glycogen, poly-β-hydroxybutyrate, or polyphosphate (7, 13, 14, 20). The synthesis of these storage materials can take place independently of growth, resulting in an increase in cell volume. In addition, degradation of such polymers under certain conditions may decrease cell volume.

Some information has been obtained about the size of bacteria in aquatic ecosystems, indicating that they are larger in eutrophic freshwater lakes than in oligotrophic or marine systems (2, 8, 24, 29, 32, 44, 45). Also, cell size is larger in bacteria that are attached to particles than it is in free-living bacteria (25). It seems that the larger sizes of bacteria in nature are associated with the richness of the environment and, in general, with the cell growth rate or metabolic activity, as occurs in laboratory cultures with synthetic media. Changes in species composition, pleomorphism, filamentation, etc., would be important factors, however, in determining size changes of the overall community, therefore providing additional difficulties in carrying out these kinds of studies. Furthermore, natural environments are more complicated than simple laboratory experiments, and often a combination of several environmental factors influences the physiological state of a given microorganism, thus affecting its size and growth rate. Environments in which sharp physicochemical gradients are formed are useful for studying factors that affect size changes in bacteria under natural conditions.

Phototrophic bacteria are suitable microorganisms for this purpose because they form blooms or plates under very sharp gradients of light, sulfide, temperature, etc. (26, 39, 42). Under many of these situations, in the bacterial layer light decreases and sulfide increases with depth to levels that result in strong limitation or inhibition of growth, depending on the species. For this reason separated layers of members of the family *Chromatiaceae* often are found underneath members of the family *Chlorobiaceae* in the vertical profile (22), and in some lakes several layers composed of a dominating species have been observed (3, 17). This situation occurs in lakes of the Banyoles karstic area, and this is extremely useful for the purposes of this study because a broad range of physiological states of a single species may be observed, depending on the sampling depth.

In this study I focus mainly on the analysis of changes in cell size of natural populations and pure cultures of phototrophic bacteria related to changes in the physiological state. The field results obtained with layers of the genus *Chromatium* are compared with results of laboratory experiments performed with pure cultures.

MATERIALS AND METHODS

Field samples. Samples were collected from Lake Cisó (Spain) located in the Banyoles karstic area (42° 08′ N, 2° 45′ W) (10, 22). Samples containing phototrophic bacteria were taken from the bacterial layer with a peristaltic pump (WAB LPA-2; W. A. Bachofen, Basel, Switzerland) connected to a sampling device that minimized water turbulence. For electronic particle analysis, 22-ml tubes were filled anaerobically, and cells were fixed in situ with formaldehyde (final concentration, 1% [wt/vol]).

Electronic particle sizing. Fractions of 0.1 or 0.5 ml were added to a counting vial containing 20 ml of Isoton (Coulter

Electronics Ltd.) and measured with a counter (model ZB_I; Coulter) connected to a multichannel analyzer (C-1000; Coulter) and recorder (X-Y; Coulter). An aperture tube (diameter, 30 μm) and appropriate windows were used for counting and sizing particles with volumes ranging from about 2 to 40 μm^3. Care was taken to minimize external interferences by following the suggestions of the manufacturer. Calibration was performed with latex spheres (average diameter, 2.02 and 3.07 μm; Coulter). Isoton was isosmolar with *Chromatium minus* cells because no changes in cell volume were detected on several hours of incubation. In addition, no significant changes in the size frequency distribution were detected between a live and a preserved sample of *C. minus*. The Coulter counter was useful for sizing these bacteria because the species that were analyzed did not remain attached after they completed division and did not form clumps under the experimental or field conditions under which they were examined.

Optical microscopy and biomass analysis. The total bacterial concentration was measured by the acridine orange direct counting (AODC) method (45). Samples were filtered through filters (pore diameter, 0.2 μm; Nuclepore Corp., Pleasanton, Calif.) stained with acridine orange, and bacterial cells were counted with an epifluorescence microscope (BHB; Olympus). The biovolume was calculated from cell concentration, as determined by the AODC method, and from the individual size frequency distribution for each morphotype obtained by either phase-contrast microscopy or measurement with a Coulter counter. This was because significant shrinkage (up to 40% of initial volume) of the *Chromatium* cells was observed when the AODC method was used. No significant differences were detected in average cell volume when the Coulter counter and phase-contrast microscopic methods of determination were compared. In the case of phase-contrast microscopy, the size of rod-shaped cells was obtained from measurements of length and width and by assuming that the cells were cylinders with hemispherical caps. Biomass was calculated by assuming an average specific density for *Chromatium* spp. of 1.10 g ml^{-1} (9) and an average specific density for the rest of the bacteria of 1.07 g ml^{-1} (6).

Chemical analysis. Sulfide was determined by the methylene blue method (33). Sulfur was assayed colorimetrically at 460 nm by the cyanolysis method (30) or by measuring the A_{260} of ethanolic extracts (96%) (H. Van Gemerden, Ph.D. thesis, University of Leiden, Leiden, The Netherlands, 1967).

Pigment analysis. Bacteriochlorophylls (Bchl) were assessed by visible absorption spectroscopy of acetonic extracts, as described previously (22).

Carbon dioxide and acetate photoassimilation. Photosynthetic activity was measured by determining CO_2 or acetate uptake. To each 25-ml bottle containing lake water, 100 μl of a stock solution of either [^{14}C]bicarbonate or [^{3}H]acetate (100 μCi ml^{-1}; Radiochemical Centre, Amersham, England) was added. Light and dark bottles were incubated at the original depth or under laboratory conditions for 30 min. Incubation was stopped by placing the bottles at 4°C in the dark, and their contents were filtered through membrane filters (pore diameter, 0.45 μm; Sartorius GmbH, Göttingen, Federal Republic of Germany). For removal of residual [^{14}C]bicarbonate, the filters were placed into scintillation vials containing 0.2 ml of 2 N HCl and degassed. For removal of residual [^{3}H]acetate, the filters were rinsed three times into the filtration unit with 10 ml of acetate buffer (0.2 M, pH 7.5). Afterward, 8 ml of Handifluor (Mallinckrodt, Inc., St. Louis, Mo.) was added to each scintillation vial, and radioactivity was measured in a liquid scintillation counter (Rackbeta 1211; LKB-Wallac, Turku, Finland). Results were corrected for quenching by the external standard channel ratio method. The parameters P_{max} and I_k for the photosynthesis-light curves were obtained by fitting data to the generalized hyperbolic inhibition equation described by Iwakuma and Yasuno (16) by using a derivative-free, nonlinear regression procedure (BMDPAR) (28).

Calculation of growth rate from production rates and biomass. Because most of the biomass detected in Lake Cisó on 31 August 1981 was due to *C. minus* (see below), photosynthetic production was mainly due to the result of light-dependent CO_2 uptake by this bacterium. Estimates of growth rates can be obtained from production rates and biomass, on the condition that both parameters are given in the same units. Assuming an exponential growth model and according to Tilzer (34), the rate of biomass increment in the absence of losses is $\mu = \ln (1 + P/B)$, where P is the photosynthetic production, in micrograms of carbon per liter per hour, and B is the biomass, in micrograms of carbon per liter.

Because cell carbon was not measured in this study, transformation of the biomass data into the amount of living carbon (originally expressed as wet weight [ww]) is necessary. Dry weight (dw) is an average of 20% of the ww of microbial cells (15, 23), and living carbon is 55% of the dw in *Chromatium* spp. (Van Niel, cited in reference 18). Therefore, living carbon is about 11% of the ww. In phototrophic bacteria, however, not all of the carbon that is assimilated is used for the synthesis of structural cell material (growth); a certain portion is derived for the synthesis of storage carbohydrates or is excreted. The ratio between the specific rate of glycogen synthesis and the specific growth rate in *Chromatium vinosum* at dilution rates below 0.1 h^{-1} in chemostat cultures is fairly constant. Approximately 5% of the reducing power obtained from the electron donor oxidation is channeled into storage carbohydrate synthesis (1). Furthermore, a part of the $^{14}CO_2$ that is assimilated may be leaked or excreted into the medium. Average values of 20% of the total carbon assimilated have been reported for this process (27). Therefore, it may be expected that 25% of the assimilated carbon does not result in growth. Then, a correction factor of 0.75 should be applied to the production rates to have a more realistic estimation of the specific growth rate of *Chromatium* spp. in the field. According to these transformations, the estimated growth rate (μ) is: $\mu = \ln [1 + (0.75P/0.11W)]$, where W is the biomass, in micrograms (ww) per liter.

Pure culture experiments. Pure culture experiments were carried out with *C. minus* UA6001 grown in a modified Pfennig medium (42) of the following composition, in micrograms per liter: NH_4Cl, 300; K_2HPO_4, 300; $CaCl_2 \cdot 2H_2O$, 200; $MgCl_2 \cdot 6H_2O$, 200; KCl, 200; Na_2CO_3, 2,100; $Na_2S \cdot 9H_2O$, 150. In addition, 10 ml of trace elements solution (SL7) and 20 μg of vitamin B_{12} per liter were included. The pH was adjusted to 7.1 with hydrochloric acid. Illumination was provided by a bank of fluorescent lamps in an incubation chamber at 27°C. Particular growth rates were achieved by incubating the cultures at given light energies, which were obtained by changing the distance from the light source. Light energy was measured with a quantum meter (model 550; Crump Scientific Products Ltd.).

Statistical analysis. The $2 \times N$ chi-square test of independence was used for evaluating the differences between size frequency distributions (31). One of the advantages of this

method is that it includes evaluation of the differences in shape of the size distributions.

RESULTS

Species composition in field samples. In Lake Cisó during August 1979, 1980, and 1981, water samples were pink-purple in color, and phase-contrast microscopy revealed at least three main morphological groups of microorganisms. Motile cells with intracellular sulfur globules with widths of 2.2 to 3.0 μm and lengths of 3.3 to 6 μm were observed as the dominant microorganisms (Fig. 1). Also, rod-shaped cells without intracellular sulfur, sometimes forming chains of not more than three or four cells with an average size of 0.6 by 1.5 μm, and very few aggregates of the gas-vacuolated, unidentified phototrophic bacterium strain M3 were detected (11).

Most of these cells showed yellow-green fluorescence in vivo when illuminated with blue-UV light under the epifluorescence microscope, indicating their phototrophic nature. In vivo absorption spectra and pigment analysis of thin-layer chromatography (data not shown) confirmed the presence of dominant populations of purple sulfur bacteria, which contained okenone as the major carotenoid and Bchl *a*, and less abundant green sulfur bacteria, which contained chlorobactene-isorenieratene and Bchl *d* or *e*.

Afterward, all species of phototrophic bacteria were isolated in pure culture. The dominant purple sulfur bacterium was identified as *C. minus*, and the dominant green sulfur bacteria were identified as *Chlorobium limicola* and *Chlorobium phaeobacteroides*. In the samples obtained in August 1979, 1980, and 1981, *C. minus* accounted for 87 to 94% of the total biomass in the bacterial layer, as determined by the AODC method.

The Coulter counter method revealed three main size classes, with an average volume of 13 to 16 μm^3 (volume range, 5 to 30 μm^3) corresponding to *C. minus* cells, an average volume of 0.3 μm^3 (up to 1 μm^3) corresponding to *Chlorobium* spp. or other small nonphototrophic bacteria, and an average volume of 50 to 150 μm^3 corresponding to strain M3 aggregates. These results were in accordance with those obtained by phase-contrast microscopy.

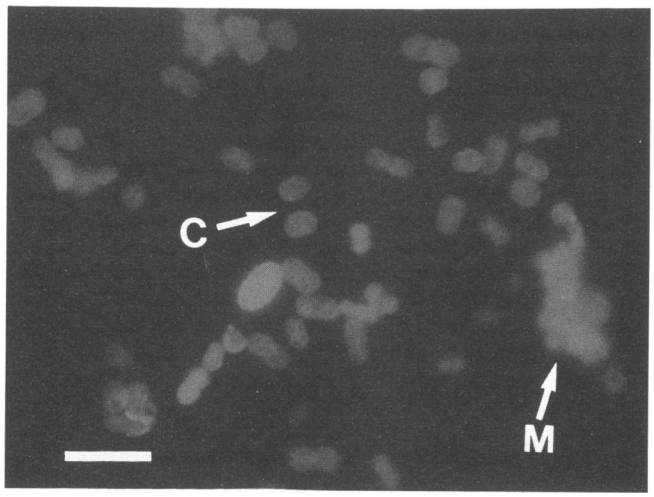

FIG. 1. Epifluorescence photomicrograph of a sample taken from the peak part of the bacterial layer of Lake Cisó on 31 August 1981. The sample was stained with acridine orange and prepared by the method of Zimmerman (45). Arrows indicate cells of *C. minus* (C) and M3 aggregates (M). Bar, 10 μm.

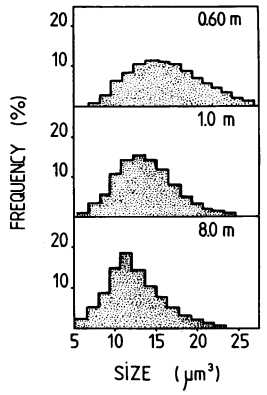

FIG. 2. Size frequency distribution of *C. minus* cells at several depths within the bacterial layer in Lake Cisó on 28 August 1981.

Biomass, cell size, and activity across the bacterial layer. At the time of this study the lake was sharply stratified. Lake Cisó showed very strong vertical gradients of temperature, light, and sulfide and oxygen concentration near the surface. The environmental conditions differed greatly every few centimeters. On 28 August 1981, statistically significant differences ($P < 0.005$) in the size of *C. minus* cells were detected when their size frequency distribution among the top (depth, 0.6 m) and bottom (depth, 3 and 8 m) parts of the bacterial layer was compared (Fig. 2). Differences between top and peak cells were borderline between being significant ($0.25 < P < 0.50$) or not. No significant differences were found between peak and bottom cells ($0.90 < P < 0.95$). In this top part the cells were larger (average volume, 16 μm^3) than in the bottom part (average volume, 13.0 μm^3).

To assess the physiological state of *C. minus* cells across the vertical profile, in situ measurements of the photosynthetic activity (CO_2 and acetate light-dependent uptake) were performed on 31 August 1981. At the same time, key physicochemical and biochemical parameters were measured (Table 1). Photosynthetic activity was strongly related to temperature and the available light energy, suggesting that the more active cells were situated in the top part of the bacterial layer. The specific growth rate estimated from photosynthetic production and biomass at each sampling depth (see above) suggested that growth occurs at a depth of between 0.25 and 0.75 m, whereas no significant growth was detected in the bottom layers (depth, 3 to 8 m). A maximum specific growth rate of 0.074 h^{-1} (doubling time, about 9 h) was calculated for the top part of the bacterial layer. When a correction was applied for the effect of temperature on photosynthetic activity or growth rate, light appeared as the key environmental factor controlling these parameters. Assuming a Q_{10} of about 2.0 and taking into account a difference of 7°C in the temperature of samples from the top and bottom parts, the values changed only slightly. In a parallel experiment, the bottom cells, which remained in situ with little or no light, were found to have significantly lower photosynthetic efficiencies than peak or top cells (Fig. 3). The sample taken from a depth of 1.75 m showed an almost linear relationship with light energy (up to 30 microeinsteins

TABLE 1. Vertical distribution of temperature, light energy, sulfide, biomass, and Bchl *a* in relation to the photosynthetic activity and estimated growth rate of *C. minus* in Lake Cisó on 31 August 1981

Depth (m)	Temp (°C)	Photon flux density ($\mu E\ m^{-2}\ s^{-1}$)	Sulfide concn (mM)	Biomass (mg ww $liter^{-1}$) of[a]:		Bchl *a* concn ($\mu g\ liter^{-1}$)	Photosynthetic activity of[b]:		Estimated specific growth rate (h^{-1})[c]
				C	NC		CO_2	Acetate	
0.25	21	850	<0.1	4.6	1.8	190	72.0	1,180	0.074
0.60	20	25	0.1	13.4	0.6	539	51.6	455	0.025
0.75	19	1.5	0.3	21.4	1.0	749	34.8	250	0.011
1.00	17	0.25	0.6	10.9	0.8	394			
3.00	14	0	1.4	5.6	0.2	195	4.8	115	0.006
8.00	14	0	1.6	4.6	0.4	143	2.4	120	0.003

[a] C, *C. minus* cells; NC, rod-shaped cells with less than 1-μm^3 volume and aggregates of M3 (50 to 150 μm^3).
[b] CO_2 uptake rates are in micrograms of C per liter per hour; acetate uptake rates are in kilodisintegrations per minute per milligram of protein per hour.
[c] Calculated from production data and total biomass (see text).

[μE] $m^{-2}\ s^{-1}$), and the slope was considerably higher than for the sample from 8 m.

Further laboratory experiments performed with field samples resulted in additional evidence that light energy is probably the most important environmental factor determining the activity and growth rate of *C. minus* in Lake Cisó. A sample from the peak of the bacterial layer of June 1984 with a similar microbial composition (*C. minus* being the dominant microorganism) was taken, and the rate of CO_2 photoassimilation was measured in the laboratory under light saturation conditions (100 $\mu E\ m^{-2}\ s^{-1}$) at several initial sulfide concentrations (Fig. 4). Results indicate that photosynthetic activity is fairly constant (480 to 510 μg of C mg of Bchl $a^{-1}\ h^{-1}$) within the range of sulfide concentrations of 0.1 to 1.0 mM (concentrations observed in the field samples obtained in 1981). Sulfide concentrations of about 2.5 mM resulted in a drop of 50% in the CO_2 photoassimilation rate, and no activity was detected at a 10 mM sulfide concentration. On the other hand, when the photosynthetic activity was measured under a nonlimiting sulfide concentration (0.3 mM) but at several light energies, light had a pronounced effect (Fig. 5). The activity increased linearly up to 40 $\mu E\ m^{-2}\ s^{-1}$, the P_{max} was 578 μg of C mg of Bchl $a^{-1}\ h^{-1}$, and the I_k was 40.8 $\mu E\ m^{-2}\ s^{-1}$.

Changes in size during growth in pure cultures. Figure 6 shows the results of an experiment in which *C. minus* isolated from Lake Cisó was grown in batch culture under photoautotrophic conditions, with sulfide as the electron donor. The time course of sulfide and sulfur and the number and size of the cells were monitored during the experiment. Cells utilized sulfide and accumulated intracellular sulfur, but when sulfide was depleted, a marked decrease in the sulfur content of the cells began. During the lag phase cells were the smallest (average size, 8.5 μm^3), whereas during mid- and late-exponential phases the average sizes were 16.0 and 14.3 μm^3, respectively. Differences in size were significant only between cells in the lag and exponential phases ($P < 0.005$). For assessment of the influence of sulfur content and growth rate on the size of *C. minus* cells, the time course of sulfur, Bchl, cell concentration, and size distribution were monitored in detail in batch cultures. Different growth rates were obtained by incubating each culture at a given light energy.

Results indicate that the average size of cells with similar sulfur contents (92 to 103 fg μm^{-3}) was dependent on the growth rate (Fig. 7). Cells that grew at high rates (0.035 and 0.067 h^{-1}) were significantly larger ($P < 0.005$) than their counterparts that grew at low rates (0.019 h^{-1}). The Bchl *a* content was 6,633 fg μm^{-3} (0.047 μg μg of C^{-1}) at 0.019 h^{-1} and 4,152 fg μm^{-3} (0.031 μg μg of C^{-1}) at 0.067 h^{-1}.

In cells that grew at the same growth rate (0.035 h^{-1}), size was directly related to sulfur content (Fig. 8). Statistically significant differences ($P < 0.005$) were detected between the size distribution of cells with a high sulfur content (66 and 93 fg μm^{-3}) and cells with a low sulfur content (21 fg μm^{-3}). The average size of *C. minus* cells ranged from 5.9 μm^{-3} for stationary-phase cells without sulfur to about 20.0 μm^3 for cells growing at a maximal rate (0.067 h^{-1}) with 120 fg μm^{-3}.

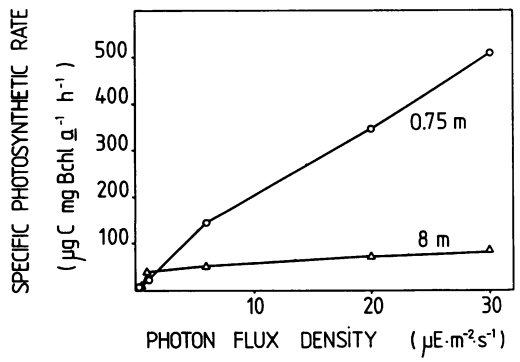

FIG. 3. Relationship between specific photosynthetic rate of CO_2 photoassimilation and light energy in samples of *C. minus* from Lake Cisó taken from the peak (0.75 m) and bottom (8 m) parts of the bacterial layer on 28 August 1979.

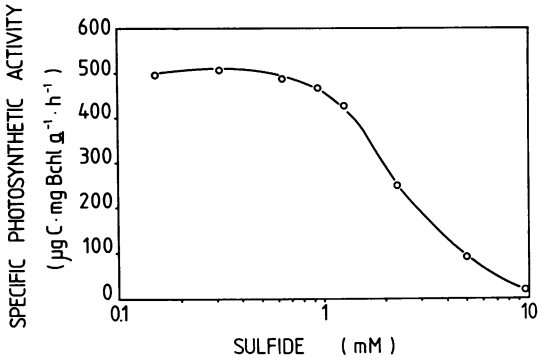

FIG. 4. Effect of sulfide concentration on the CO_2 photoassimilation rate of a sample taken from a bacterial layer of Lake Cisó in June 1984. The experiment was performed under constant illumination, at 100 $\mu E\ m^{-2}\ s^{-1}$. The microbial composition was similar to that observed during August 1981.

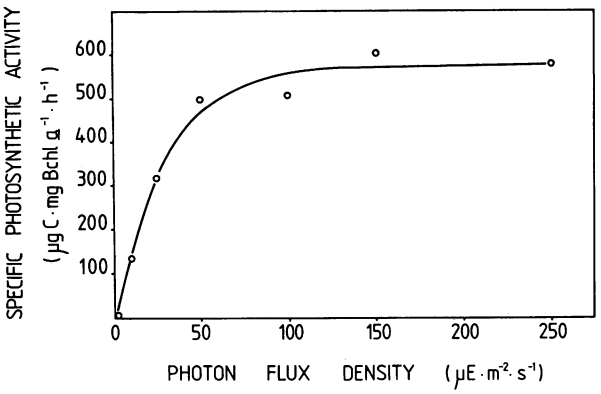

FIG. 5. Effect of light energy on the CO_2 photoassimilation rate of a sample taken from the bacterial layer of Lake Cisó in June 1984. The experiment was performed at an initial sulfide concentration of 0.3 mM.

DISCUSSION

In Lake Cisó significant changes were observed in the average size of *C. minus* cells in samples collected from the top and bottom parts of the bacterial layer during summer stratification. The decrease in cell size that I observed within the vertical profile of the lake was strongly related to the decrease in the photosynthetic activity, which followed a light-dependent pattern. Theoretically, however, there are several metabolic processes that affect cell size changes in field populations. They include changes in growth rate, accumulation or degradation of storage materials, and adaptation of the photosynthetic apparatus to changes in light energy within the vertical profile.

Changes in growth rate. The specific growth rates for *C. minus* in Lake Cisó, as calculated from production and biomass data, gave maximum values of 0.074 h^{-1} at the top of the bacterial layer. Pure cultures showed a maximum specific growth rate of 0.067 h^{-1} in Pfennig medium at 100 $\mu E\ m^{-2}\ s^{-1}$, suggesting that cells at the top of the bacterial layer grew at the maximum growth rate (μ_{max}). Below 0.75 m the estimated specific growth rate was very low (0.003 to 0.006 h^{-1}) because light energy was lower than 0.25 $\mu E\ m^{-2}\ s^{-1}$. According to this it has been reported that light energy values below 0.3 $\mu E\ m^{-2}\ s^{-1}$ do not provide sufficient energy for growth in members of the family *Chromatiaceae* (38). The growth rate in phototrophic sulfur bacteria, however, is controlled not only by light energy, but also by the concentration of electron donor (namely, sulfide), which also has influence. In purple sulfur bacteria the K_s value for growth with sulfide ranges from 7 to 10 μM (37, 39, 41). As a consequence the differences in growth rate of *C. minus* cells

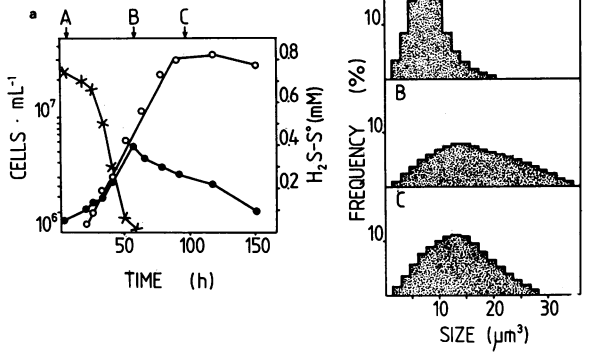

FIG. 6. Size frequency distributions of *C. minus* cells (b) in relation to sulfide (×), sulfur (●), and cell concentrations (○) (a) in a growing batch culture. The lag phase (A), mid-exponential phase (B), and early stationary phase (C) are shown.

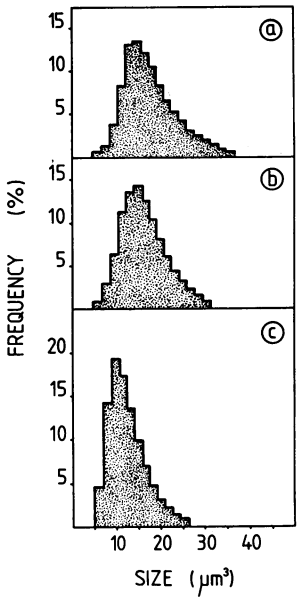

FIG. 7. Effect of growth rate on the size frequency distribution of *C. minus* cells with similar intracellular sulfur content (92 to 103 fg μm^{-3}). Specific growth rates are 0.067 (a), 0.035 (b), and 0.019 (c) h^{-1}.

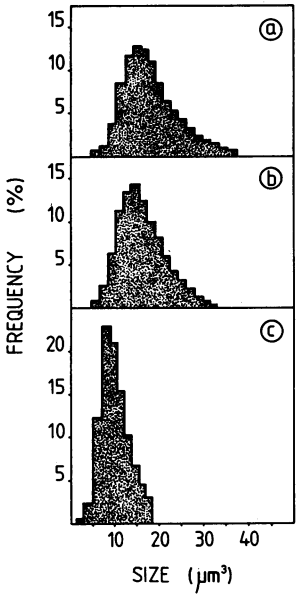

FIG. 8. Effect of sulfur contents on the size frequency distribution of *C. minus* cells at constant growth rate (0.035 h^{-1}). Sulfur contents are 93 (a), 66 (b), and 21 (c) fg μm^{-3}.

observed between samples from 0.6- and 1.0-m depths in Lake Cisó should not account for differences in sulfide concentration (100 to 600 μM) because they were 10 to 100 times higher than the K_s reported for members of the family *Chromatiaceae*. In addition, this was confirmed in laboratory experiments with field samples, because no significant changes in photosynthetic activity were observed in a sulfide concentration range from 0.1 to 1 mM.

Therefore, because there is a direct relationship between the average size of *Chromatium* spp. and growth rate in pure cultures, it is understandable that actively growing cells from the top part of the bacterial layer in the lake are larger than their nongrowing counterparts from the bottom.

Differences in sulfur content. The assumption that size is related exclusively to growth rate is valid only for certain microorganisms when growth is balanced and no accumulation of storage polymers is taking place (glycogen, sulfur, poly-β-hydroxybutyrate, polyphosphate, etc.). In purple sulfur bacteria balanced growth occurs together with synthesis or degradation of storage polymers (40; Van Gemerden, Ph.D. thesis). In *C. minus* cells at a constant growth rate, size is affected by the specific sulfur content. It has been proven that accumulation of sulfur affects size in other members of the family *Chromatiaceae*, but only to a certain extent (9, 35). Van Gemerden (35) showed that in *Chromatium* sp. strain 6412, cells were larger during the phase of maximum sulfur accumulation, whereas they were progressively smaller when sulfur was oxidized during the sulfur depletion phase. In these experiments, however, the decrease in cell volume could not be accounted for exclusively by the disappearance of intracellular sulfur. The maximum sulfur content in the *Chromatium* cells from the phase of maximum sulfur accumulation was about 7% (vol/vol), whereas the observed variation in cell volume when all sulfur was consumed accounted for 50%. Similar conclusions can be deduced from the experiments of Guerrero et al. (9) with *C. vinosum*. In *C. minus* the maximum sulfur accumulation was about 8%, whereas the observed differences in cell volume after complete sulfur consumption accounted for 46%. Glycogen, RNA, and DNA were not measured in the experiments described here. RNA and DNA, however, would not be expected to have an effect on the results presented here because during balanced growth (exponential phase) the amount per cell was relatively constant. Therefore, it can be concluded that other storage polymers such as glycogen that accumulate or that were oxidized at the same time as sulfur probably explain the difference.

In this report it has been shown that for *C. minus* in the field, the variation in size from top and bottom cells accounts for about 18%. Furthermore, a decrease of 5 to 10 times in the specific sulfur content and of 4 times in the specific glycogen content was found for bottom cells when compared with top cells in an experiment performed during July 1982 in Lake Cisó (11, 43). Then, degradation of sulfur and glycogen occurred during sinking of the cells across the dark zone of the bacterial layer. This may be the result of endogenous respiration because in *Chromatium* spp. the glycogen that accumulated in the presence of light could be oxidized in the dark with sulfur as the electron acceptor, producing sulfide (36). Nevertheless, this process did not provide sufficient energy for growth (Van Gemerden, Ph.D. thesis) but still contributed to cell survival and maintenance for long periods of time. Therefore, part of the differences in volume between top and bottom cells also could be explained by differences in the sulfur and glycogen content caused by a breakdown of storage materials used for survival purposes in the dark zone.

TABLE 2. Specific Bchl *a* content in several pure cultures of members of the family *Chromatiaceae* in comparison with field samples

Sample	Specific Bchl *a* content[a]		Reference or source
	μg mg ww^{-1}	μg μg C^{-1}	
Several species of the family *Chromatiaceae*[b]	0.2–5.0	0.002–0.045	22
Chromatium vinosum[b]	4.1	0.037	39
Chromatium sp.[b]	4.0	0.036	18
Chromatium minus[b]	3.5–5.4	0.031–0.049	This study
Chromatium minus under day-night cycles[c]	53.9	0.49	This study
Field samples of *Chromatium* spp.	5.1–64.9	0.046–0.59	This study; 21

[a] Values originally reported were transformed into carbon, assuming that the C/ww ratio is 0.11, the C/protein ratio is 2, and the C/dw ratio is 55%.
[b] Grown under continuous illumination conditions.
[c] Incubated at room temperature in front of a window.

Adaptation or degradation of the photosynthetic apparatus. The specific Bchl *a* content in the field populations of *C. minus* that were studied was very high (0.27 to 0.36 μg μg of C^{-1}) when compared with data reported previously with pure cultures of members of the family *Chromatiaceae* grown under conditions of continuous illumination (Table 2). At first it might be suggested that these high values are caused by high amounts of nonliving (detrital) Bchl *a* in the samples. There is no evidence, however, for such a hypothesis because the amount of bacteriophaeophytin accounted for only 10 to 25% of the total Bchl (J. M. Gassol, personal communication). Therefore, other possibilities should be thought of to explain such high values. Adaptation to light limitation conditions by increasing the bulk of light-harvesting pigments has been reported for phototrophic bacteria (38; R. Matheron, Ph.D. thesis, Université d'Aix-Marseille III, Marseille, France, 1976). Under field conditions this may be important because natural populations are exposed to diel fluctuations in light energy. Consequently, a pure culture of *C. minus* was grown at room temperature (15 to 20°C) and exposed to natural sunlight fluctuations (day-night cycles). Analysis of specific Bchl *a* content gave values of 0.49 μg μg of C^{-1}, which is in great contrast with values for cultures grown at constant light energy (0.031 to 0.047 μg μg of C^{-1}).

Another point of interest is the fact that the specific pigment content and photosynthetic efficiency decreased with depth in Lake Cisó. According to this, it has been shown that in the dark or under conditions of very low light energy, the specific content of photosynthetic pigments in the genera *Chromatium* and *Chlorobium* decreased, probably as a consequence of the degradation of the photosynthetic apparatus (22, 38). Additional evidence is given by the fact that the viability of members of the family *Chromatiaceae* in Lake Cisó during the summer of 1983 sharply decreased with depth (43). Therefore, such degradation and loss of viability when the average volume of the cells in the dark zone of the bacterial layer is decreased is expected.

A dynamic model has been proposed to explain the development of phototrophic bacterial layers in lakes. The model is substantiated by results of studies on population dynamics, diel cycle of metabolism (43), and sedimentation characteristics (9) of purple sulfur bacteria. It assumes that

during the formation of a layer of *Chromatium* spp., some cells that are loaded with elemental sulfur and glycogen because of their active photosynthesis in the top part of the bacterial layer slowly sink (about 7 cm day^{-1}) because of the increase in buoyant density due to the storage of reserve polymers. During sinking, cells thrive in a continuous gradient of decreasing light and increasing sulfide and reach the bottom layers, where light is absent.

In this report I have included additional data that support a previously proposed model (43) and that show that natural populations of members of the family *Chromatiaceae* may grow at a μ_{max} especially at noon and in the top part of the bacterial layer. Because the rate of biomass changes observed for the population of *C. minus* is only about 2% of the actual rate (estimated from production and biomass) (22; E. Montesinos, Ph.D. thesis, Autonomous University of Barcelona, Barcelona, Spain, 1982), it is expected that losses of biomass in the ecosystem (sedimentation, outflow, and predation or lysis) should be very important. Furthermore, in this study I have shown that some of the generally proposed physiologic or metabolic processes that affect cell volume in several microorganisms in pure culture also take place, with minor differences, in pure cultures and field populations of purple sulfur bacteria. Growth rate, degree of storage of reserve polymers, and adaptation or degradation of the photosynthetic apparatus appear as the most important factors that determine the size of phototrophic bacteria under field conditions. Nevertheless, the specific weight of these processes remains to be determined under field conditions.

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