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Buoyant density changes due to intracellular content of sulfur in *Chromatium warmingii* and *Chromatium vinosum*

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Abstract. Average specific density of individual cells of pure cultures of *Chromatium warmingii* and *Chromatium vinosum* were measured by isopicnic gradient centrifugation with Percoll during growth at constant illumination as a function of the increasing content of intracellular sulfur. Cell number and volume, bacteriochlorophyll *a*, sulfide, and sulfur were followed in the cultures along with cellular buoyant density. Poly-β-hydroxybutyrate was monitored at several points during growth of the cultures. The density of *C. warmingii* changed from 1.071 to 1.108 g cm^{-3} (sulfur content per cell varied from 0 to 1.71 pg). *C. vinosum* changed its density from 1.096 to 1.160 g cm^{-3} (sulfur content per cell varied from 0 to 0.43 pg). Maximum sulfur content in pg of sulfur per μm^3 of cell volume were 0.178 for *C. warmingii* and 0.294 for *C. vinosum*. Measurement of the differences in buoyant density, volume and sulfur content before and after ethanol extraction of cells with and without intracellular sulfur, allowed tentatively to estimate the density of sulfur inside the cells as 1.219 g cm^{-3}. Isolation of sulfur globules and centrifugation in density gradients gave a density higher than 1.143 g cm^{-3} for these intracellular inclusions.

Key words: Buoyant density – Percoll – Intracellular sulfur – *Chromatium warmingii* – *Chromatium vinosum*

The possible regulation of cell division by buoyant density (Poole 1977; Woldringh et al. 1981) has stimulated research on the latter for *Escherichia coli*. Values of buoyant density varying between 1.05 and 1.11 g cm^{-3} have been published for this organism (Marr et al. 1966; Koch and Blumberg 1976; Poole 1977; Martínez-Salas et al. 1981; Woldringh et al. 1981). The differences in values reported are most probably due to the different techniques used (Woldringh et al. 1981). *Salmonella typhimurium* has also been analyzed and densities between 1.076 and 1.092 g cm^{-3} have been found (Herrero et al. 1982). Measurements of buoyant densities of some other bacteria have been done in cesium chloride (Faegri et al. 1977; Van Veen and Paul 1979), and renografin (Martin et al. 1981), giving values of 1.175 to 1.190 g cm^{-3} for thiobacilli, 1.2 g cm^{-3} for *Enterobacter aerogenes* and 1.3 g cm^{-3} for *Arthrobacter globiformis*. Unfortunately measurements with cesium chloride give artificially high values, probably due to osmotic effects or to salt penetration into the cells (Bakken and Olsen 1983). Renografin also gives values higher than Percoll (Tisa et al. 1982). Using Percoll, Bakken and Olsen (1983) found values between 1.035 and 1.093 for several soil bacteria (average 1.09 g cm^{-3}). But no detailed studies exist showing reliably wide ranges in density within the same bacterial species coupled with analysis of the physiological basis underlying the variations of such a physical parameter.

This kind of information would be important for several reasons. First, detailed studies with a particular group of bacteria, namely *E. coli* and related organisms, are not enough to find the whole range of variation in buoyant density of prokaryotes, which is of great interest in itself. Second, most work has been carried under balanced growth conditions, but some microorganisms accumulate large quantities of reserve materials in the form of cell inclusions when limited by some essential nutrient (Shively 1974). Therefore, it would be important to know if these inclusions have a substantial influence on the buoyant density of the cell. Third, densities of 1.025 to 1.070 g cm^{-3} are commonly used in ecological studies to compute biomass from biovolume (Doetsch and Cook 1973; Watson et al. 1977; Guerrero et al. 1980; Pedrós-Alió and Brock 1982). This seems justified in the case of small heterotrophic bacteria which do not accumulate reserve materials, but organisms such as *Beggiatoa* (Strohl et al. 1982), *Caulobacter* (Poindexter and Eley 1983), the purple sulfur bacteria, and others, frequently accumulate large quantities of sulfur, poly-β-hydroxybutyrate (PHB), glycogen or polyphosphate in nature. Thus, if these inclusions have an effect on density, biomass calculations should be adjusted accordingly (Bakken and Olsen 1983). Finally, changes in buoyant density can significantly affect the rate of sinking in the case of planktonic bacteria, especially those forming blooms or plates at given depths.

For these reasons we undertook to measure the effect of different cytoplasmic inclusions on buoyant density of bacteria. We performed the experiments with a small and a large *Chromatium* (*C. vinosum* and *C. warmingii*, respectively) because: (a) Chromatiaceae are able to accumulate several of the aforementioned inclusions simultaneously (Stanier et al. 1976); (b) they constitute the dominant population in many anoxic water bodies where light and sulfide are present (Guerrero et al. 1980); (c) they frequently form bacterial plates seeking the optimal light and sulfide conditions (Parkin and Brock 1980) and sedimentation is a very important loss factor in this situation; and (d) both large and small *Chromatium* coexist in nature having complementary strategies for survival.

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Non-common abbreviations: Bchl, Bacteriochlorophyll; DMB, Density Marker Beads; PHB, poly-β-hydroxybutyrate

In relation to terminology, the widely used expression "cell density" should not be employed to mean optical density of a culture or a population, but as a synonym of specific cell density (i. e., cell weight divided by cell volume). Buoyant density refers to specific cell density measured by buoyancy in a given medium capable of forming density gradients. Here we report the effect that changes in intracellular sulfur have on buoyant density of these two organisms.

Materials and methods

Bacterial strains. The strain of *Chromatium vinosum* was isolated from Lake Cisó (Banyoles, Spain) by H. van Gemerden from the University of Groningen (The Netherland). *C. warmingii* was provided by H. G. Trüper from the University of Bonn (Federal Republic of Germany).

Growth conditions. Cultures of *Chromatium* were grown in Pfennig's medium (Pfennig and Trüper 1981) under nitrogen gas pressure. Initial inocula were taken from stationary cultures and were seeded into 1 or 5 l volume flasks stirred with a magnetic bar. For *C. warmingii,* the initial sulfide concentration was 0.89 mM and cell concentration was 2.71×10^6 cells ml^{-1}. For *C. vinosum,* the initial sulfide concentration was 0.37 mM and cell concentration was 2.80×10^6 cells ml^{-1}. Cultures were incubated in a 27° C chamber with stirring and continuous illumination from fluorescent lights at an intensity of 60 $\mu E\, s^{-1}\, m^{-2}$. Light was measured with a Crump quanta meter model 550 (Crump Scientific Products Ltd.). Specific bacteriochlorophyll (Bchl) content per cell did not change significantly through the experiments. Growth of the cultures was followed by light scattering at 650 nm in a Philips Pye Unicam SP1700 spectrophotometer.

Chemical and biological parameters. Elemental sulfur and Bchl *a* were measured spectrophotometrically in 96% ethanol extracts of whole cells according to Van Gemerden (1968), a molar extinction coefficient of 68 $mM^{-1}\, cm^{-1}$ was used to calculate concentration of Bchl *a* (Van Gemerden 1980).

A Coulter counter model ZBI with a 30 µm aperture tube connected to an impulse Channelyzer 1000 (Coulter Electronics), was used for counting and sizing bacterial cells. Samples were diluted with dust-free saline electrolyte (Isoton II). Results were corrected automatically in the Channelyzer for coincidence and non axial flow losses. Counts were recorded with a plotter at small size intervals (channels). The mean of the volume distribution was taken as the cell volume. The conditions used for *C. vinosum* were base channel ratio 4, window width 50, amplification 1, and aperture current 1/2, and those for *C. warmingii* were base channel ratio 5, window width 40, amplification 2, and aperture current 1. Latex beads (Coulter Electronics) of 4.31 μm^3 and 15.15 μm^3 of volume were used for calibration.

Hydrogen sulfide was measured by the methylene blue method of Pachmayr as modified by Parkin and Brock (1980). Monitoring of PHB was done at a few points during the experiments because its concentration was expected to be low under the experimental conditions used. PHB was followed by examination of Sudan black stained preparations (Herbert et al. 1971).

Buoyant cell density. Buoyant cell density determinations were performed by isopicnic centrifugation in gradients of Percoll (Sigma Chemical Co., St. Louis, MO, USA) calibrated with density marker beads (DMB) (Pharmacia Fine Chemicals, Piscataway, NJ, USA). Percoll consists of colloidal silica particles (15 to 30 nm in diameter) coated with polyvinylpyrrolidone and it offers the advantage of low osmolality and viscosity compared to other media. Gradients of Percoll diluted in 1.5 M NaCl and distilled water (6.5:1:2.5) were preformed by centrifugation at room temperature for 55 min at 15,000 rpm ($27{,}000 \times g$) in a Sorvall RC2-B centrifuge using a fixed angle rotor SS-34. DMB were placed on top of Percoll right before centrifuging. For density determinations 0.2 ml of a cell suspension containing about 10^8 cells ml^{-1} were placed on top of the preformed gradient and centrifuged under the same conditions as before for 10 min. The tubes with DMB and banded cells were photographed. The slides were projected for maximum magnification and the relative distance from the meniscus was recorded for each band. Density of the cells was obtained by plotting the density of the DMB vs. distance to the meniscus for each individual gradient and locating the cell band in this graph.

Determination of volume and density changes due to the content of sulfur. 100 ml of a culture of *C. vinosum* in the stationary phase (approx. 10^8 cells ml^{-1}) was divided into two subcultures. One of them was fed with H_2S (final concentration 1 mM), kept in the dark for about 1 h and then incubated in the light until the culture took a milky appearance indicating that the cells were loaded with sulfur. The other subculture was kept in the dark without sulfide. Samples of 10 ml were then taken from each subculture and centrifuged at 8000 rpm for 10 min and resuspended in 5 ml of 96% ethanol. After a few minutes to allow for complete extraction of sulfur and pigments, the samples were pelleted again in the same manner. The pellets were resuspended in 10 ml of Isoton II, and the ethanol supernatants were used to obtain absorption spectra. Parallel controls were run in the following fashion: 10 ml samples from each subculture were processed in the same way except that Isoton II was used instead of ethanol for all resuspensions. From all the samples, unfixed 2 ml aliquots were immediately analyzed with the Coulter counter for cell number and volume. The remaining 8 ml of each sample were centrifuged again, resuspended in 0.4 ml of Isoton II, and placed on top of preformed Percoll gradients for buoyant density measurements (see above).

Extraction of sulfur globules. One liter of a stationary phase culture of *C. warmingii* was fed with sulfide (final concentration 1 mM) and incubated until loaded with sulfur as before. The whole culture was centrifuged in 250 ml bottles in a GSA rotor at 8,000 rpm for 10 min. The pellets were suspended in 30 ml of lysozyme solution (pH 8) and incubated overnight in an ice-water bath. The lysozyme solution had the following composition: 2.5 ml of 1 M glucose, 5 ml of 0.1 M sodium EDTA (pH 8), 1.25 ml of 1 M Tris HCl (pH 8) and 100 mg of lysozyme (Sigma Chemical Co.) and distilled water up to 50 ml of solution (Birnboim and Doly 1979). The next morning the suspension was gently sonicated with a Branson sonifier B-12, at 30 W, during three periods of 1 min with two resting periods of 1 min in between, in an ice bath. The homogenate was then centrifuged at 8,000 rpm

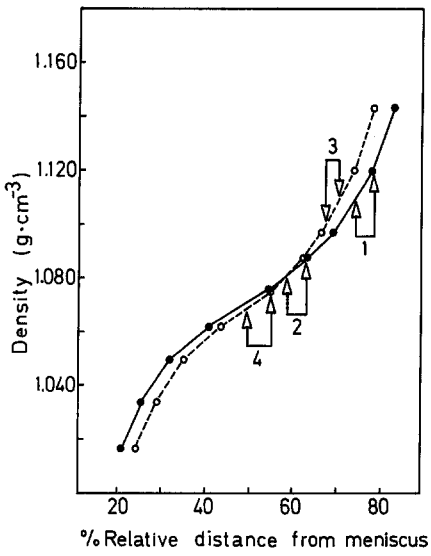

Fig. 1. Density distributions of Percoll gradients and position of cell bands as determined from measurements on projected slides. Lines correspond to two self-generating gradients (10 ml) centrifuged for 55 min at 25,000 × *g* at 20° C. The differences between the two illustrate the necessity for including DMB in every tube. Arrows 1 and 2 indicate cell bands corresponding to the highest and lowest densities found in *Chromatium vinosum*. Arrows 3 and 4 indicate cell bands corresponding to the highest and lowest densities found in *Chromatium warmingii*. Distance from the meniscus was measured on enlargements of the photographed tubes. The distance from the meniscus to the bottom of the tube was considered as 100% and the positions of bands were expressed as relative distances

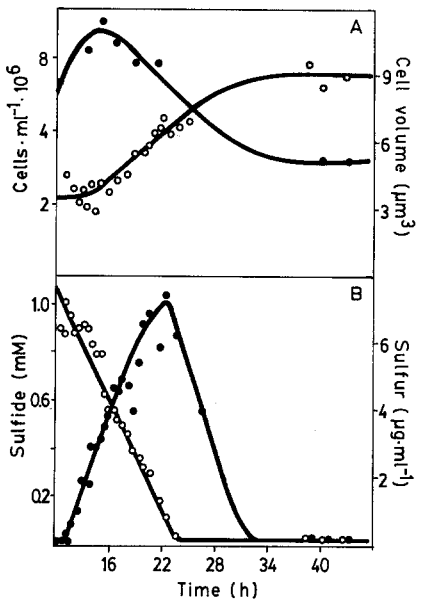

Fig. 2A, B. Time course of culture parameters during growth of *Chromatium warmingii*. **A** Changes in cell numbers (○) and mean cell volume (●). **B** Changes in total sulfide (○) and intracellular sulfur (●) in the culture. Note that cells have their maximum volume 15 h after the start of the experiment, while the maximum sulfur content is reached 7 h later

for 10 min. Chromatophores remained mostly in the supernatant. The pellet containing sulfur and cell remains was suspended in 0.3 ml of a 0.15 M NaCl solution and placed on top of a Percoll gradient for measurement of buoyant density (see above).

Results

Buoyant density measurements

Figure 1 shows the shape of typical gradients as determined by the relative distances from the meniscus to the DMB. The purple colored bands of cells were easily distinguished against the transparent background both directly in the tubes and in color pictures taken for precise measurements. The location of a few representative cell bands is also indicated. Minor differences in the tubes and the volume of Percoll necessary to balance them in the centrifuge changes the shape of the curves, thereby emphasizing the necessity of having the DMB in every tube.

Culture parameters

The increase in numbers of *Chromatium warmingii* cells with time is shown in Fig. 2A and the concentrations of sulfide and intracellular sulfur during growth in Fig. 2B. Sulfide was added to the culture, then it was kept in the dark overnight at room temperature, placed in the light and growth was monitored.

Figure 3A, B show the same parameters for a *C. vinosum* culture. In this case, after feeding with sulfide, the culture

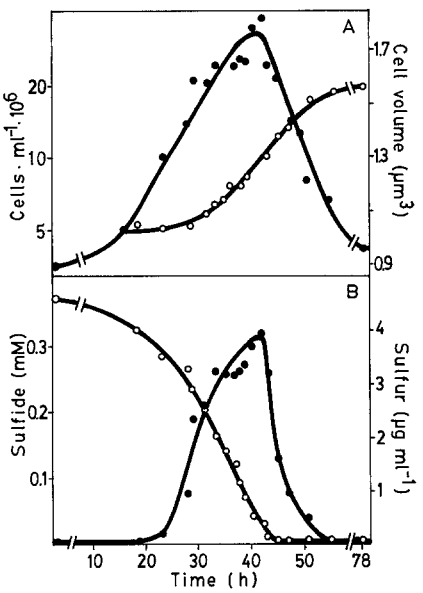

Fig. 3A, B. Time course of culture parameters during growth of *Chromatium vinosum*. **A** Changes in cell numbers (○) and mean cell volume (●). **B** Changes in total sulfide (○) and intracellular sulfur (●) in the culture. Note that both maximum volume and maximum sulfur content co-occur 42 h after the start of the experiment

was kept for one hour in the dark, transferred to the light and growth then followed.

C. warmingii increased from 2.7×10^6 cells ml^{-1} to 7.4×10^6 cells ml^{-1}. Maximum sulfur concentration was 7.46 µg ml^{-1} 24 h after the start of the experiment. *C. vinosum* increased from 2.8×10^6 cells ml^{-1} to 1.97×10^7 cells ml^{-1} at the end of the experiment. Maximum sulfur concentration was 3.99 µg ml^{-1} 41 h after initial exposure

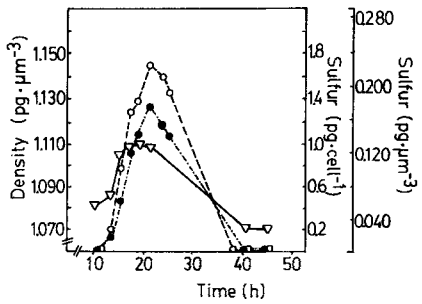

Fig. 4. Time course of cellular parameters during growth of *Chromatium warmingii*. Changes in buoyant density of the cells (▽——▽), and two measures of sulfur specific content: pg S° per cell (○---○), and pg S° per µm³ of cell volume (●-·-·-●)

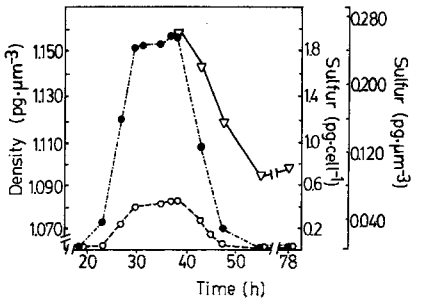

Fig. 5. Time course of cellular parameters during growth of *Chromatium vinosum*. Changes in buoyant density of the cells (▽——▽), and two measures of sulfur specific content: pg S° per cell (○----○), and pg S° per µm³ of cell volume (●-·-·-●)

Table 1. Changes in cell volume, density, sulfur, pigments and weight before and after ethanol extraction of *Chromatium vinosum* cultures with and without intracellular sulfur

Sample[a]	Cells (per ml)	Volume (µm³)	Density (g cm⁻³)	Sulfur (pg µm⁻³)	Bchl *a* (pg µm⁻³)	Weight[b] (pg)
1	1.07×10^8	1.262	1.117	0.08	0.015	1.410
2	1.10×10^8	0.467	1.093	0	0	0.510
3	1.82×10^8	0.917	1.085	0	0.011	0.995
4	1.45×10^8	0.409	1.089	0	0	0.445

[a] Samples: 1 and 2 from a culture containing cells loaded with intracellular sulfur; 3 and 4 from a culture of cells not containing sulfur. 2 and 4, cells extracted with ethanol; 1 and 3, cells treated only with Isoton II

[b] Wet weight calculated by multiplying volume (column 3) times density (column 4)

to light. Although final cell numbers were different, yields were comparable in terms of biovolume, since *C. warmingii* is about six times larger than *C. vinosum* (see next section). Specific Bchl *a* content per cell did not change significantly during any of the experiments (data not shown).

Cell parameters

Coulter counter sizing showed that cell volume changed with time in both *Chromatium* species (Figs. 2A and 3A). *C. warmingii* changed from 5.17 to 11.45 µm³ while *C. vinosum* changed from 0.89 to 1.83 µm³. However, when percent change was considered, both species showed a maximum variation around 50% of their largest volumes. *C. vinosum* had largest volume coincidentally with maximum sulfur content (Figs. 3A and 3B), while *C. warmingii* presented its largest cell volume at the beginning of the exponential phase of growth, when sulfur content was 30% of the maximum (Figs. 2A and 2B).

Changes in buoyant density and specific sulfur content of the cells throughout the experiments are presented in Fig. 4 for *C. warmingii* and in Fig. 5 for *C. vinosum*. Specific sulfur content is represented both as pg S° per cell and as pg S° per µm³ of cell volume. This second form permits comparisons between the two species correcting for volume differences. It is apparent that buoyant density changed in parallel with sulfur content in both species. *C. warmingii* varied its density between 1.071 and 1.108 g cm^{-3} with an increment in sulfur specific content from 0 to 0.178 pg S° µm^{-3}. *C. vinosum* density varied between 1.160 and 1.096 g cm^{-3} while sulfur content decreased from 0.260 to 0 pg S° µm^{-3}.

Changes in volume and buoyant density due to the content of sulfur

Results from the ethanol extraction experiment with *C. vinosum* cells are shown in Table 1. In the experiments with cells not containing sulfur, extraction with ethanol removed Bchl *a* (Table 1) and carotenoids (data not shown). Volume halved from 0.917 to 0.409 µm³, but buoyant density changed very little. In the experiments with cells containing sulfur, on the other hand, extraction with ethanol not only removed pigments but also sulfur, resulting in a threefold reduction in cell volume (from 1.262 to 0.467 µm³) and a change in buoyant density from 1.117 to 1.093 g cm^{-3}. Obviously, while pigments and other cell materials possibly extracted by ethanol had a negligible influence on density and a substantial one on volume, sulfur had a major impact on buoyant density and a large one on volume.

Extraction of sulfur globules

In the sulfur globule extraction experiment from *C. warmingii* lysozyme treated cells, the cell extract was centrifuged in a preformed Percoll gradient. Four bands appeared distributed throughout the gradient. When absorption spectra were run, the three lighter bands showed low peaks at 775 nm (absorption maximum of Bchl *a*) and only background absorption at 265 nm (absorption maximum of sulfur). On the other hand, the most dense band showed no peak at 775 and a clear peak at 265 nm, indicating that most of the sulfur was in the latter band, while residual pigments were in the other three. Sulfur globules banded beyond the most dense DMB (which have

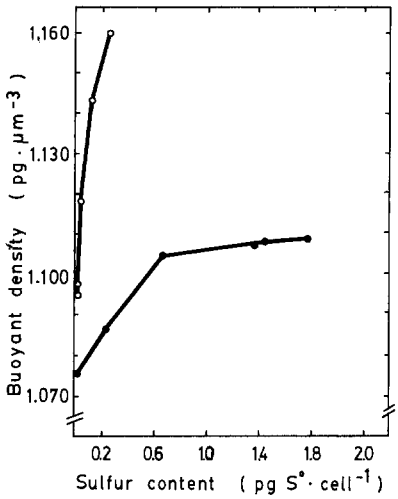

Fig. 6. Change in cell density vs. specific sulfur content for *Chromatium warmingii* (closed symbols) and *Chromatium vinosum* (open symbols). Sulfur content is represented as pg S° per cell

a density of 1.143 g cm^{-3}). Therefore, under these conditions, the density of sulfur in the extracts must be higher than 1.143 g cm^{-3}.

Figure 6 presents a plot of cell buoyant density vs. specific sulfur content. The relationship showed saturation type curves. Buoyant density changes in *C. vinosum* were much faster and larger that those in *C. warmingii*. This illustrates how different *Chromatium* species respond to the same amounts of sulfur content. It can be seen that, first, there is a substantial change within each species, and, second, the buoyant density changes are very different among different species of the same genus.

Discussion

Cell volumes were determined with a Coulter counter, since this method allows measuring a large number of cells (about 50,000 in our experiments) with minimal manipulations of the cells. This point is specially important for large Chromatiaceae which seem to shrink considerably by other methods, especially those involving dehydration with organic solvents (Montesinos et al. 1983). In accordance with this we observed a two to threefold reduction in volume when *Chromatium vinosum* cells loaded with sulfur were treated with ethanol (Table 1).

C. vinosum presented largest volume at the time of maximum sulfur content. *C. warmingii* had its maximum volume prior to the time of maximum sulfur content. *C. vinosum* has a higher sulfur content per μm^3 of cell volume and it seems that sulfur is the main determinant of cell volume in this organism. In agreement with this, in the ethanol extraction experiments, sulfur accounted for almost 30% changes in volume, while the maximum changes, including those due to the sulfur content, in the growth experiments were about 50%. Thus, sulfur was the primary factor determining cell volume in *C. vinosum*.

In *C. warmingii*, with a lower sulfur content per μm^3 of cell volume, other factors could have a similarly strong influence on volume. For example, faster growth rates are known to coincide with larger cell volumes in many bacteria (Shehata and Marr 1971; Donachie et al. 1976). Consequently, greatest cell volume did not coincide with maximum sulfur content in this organism.

Table 2. Hypothetic physical state of sulfur globules in *Chromatium vinosum* cells as deduced from data in Table 1. Estimation of density and degree of hydration[a]

1. Weight and volume due to pigments[b]:
   $W_p = 0.995-0.445 = 0.550$ pg
   $V_p = 0.917-0.409 = 0.508$ μm^3
2. Weight and volume due to sulfur plus pigments[c]:
   $W_{s+p} = 1.410-0.510 = 0.900$ pg
   $V_{s+p} = 1.262-0.467 = 0.795$ μm^3
3. Weight and volume due to sulfur:
   $W_s = W_{s+p}-W_p = 0.900-0.550 = 0.350$ pg
   $V_s = V_{s+p}-V_p = 0.795-0.508 = 0.287$ μm^3
4. Estimated density of sulfur:
   $D_s = W_s/V_s = 0.350/0.287 = 1.219$ pg μm^{-3}
5. Estimated density of pigments:
   $D_p = W_p/V_p = 0.550/0.508 = 1.083$ pg μm^{-3}
6. Estimated degree of hydration of intracellular globules (percent of sulfur, PW_s, and percent of water, PW_w):

$$D_s = \frac{100}{(PW_s/2.07^{d}) + (PW_w/1.00)};\ PW_w = 65.2\%$$

[a] See text for explanation
[b] From samples 3 and 4 in Table 1
[c] From samples 1 and 2 in Table 1
[d] Density of elemental sulfur (pg μm^{-3})

Chromatiaceae are able to accumulate sulfur, glycogen and PHB in the form of intracytoplasmatic inclusions. Under the light and sulfide regime used in our experiments very little or no PHB at all should be synthesized (Van Gemerden 1968). Staining of cells with Sudan black failed to show any dark globules. Thus PHB did not appear to play any role in our experiments.

The maximum concentrations of glycogen observed in *Chromatium* amount to 14% of the dry weight, while elemental sulfur can account for more than 40% of the dry weight (Van Gemerden 1968). Parallel experiments showed that changes of glycogen content in *Escherichia coli* from 4 to 16% of the dry weight caused only a slight increase in buoyant density (unpublished results). Therefore, glycogen had little importance in determining buoyant cell density in our experiments and elemental sulfur seemed to be the main factor causing the observed changes in buoyant density.

By analyzing the data from the cell extraction experiments the physical state of sulfur in *C. vinosum* can be approximated as follows (Table 1): ethanol extraction of a culture not containing sulfur caused a reduction of 0.550 pg in cell weight and 0.508 μm^3 in cell volume (samples 3 and 4 in Table 1; point 1 in Table 2). These reductions can be attributed to the loss of pigments and any other cell materials extracted by ethanol. In the sulfur containing culture, extraction with ethanol resulted in a reduction of 0.900 pg in cell weight and 0.795 μm^3 in cell volume (samples 1 and 2 in Table 1; point 2 in Table 2). Subtraction of the reductions due to pigments and other cell material from these last differences should give reductions in weight and volume due to sulfur alone (point 3 in Table 2). These calculations give a 0.350 pg loss in cell weight and a 0.287 μm^3 reduction in cell volume due to sulfur. Dividing weight by volume would give a specific density for sulfur inside the cell of 0.350/0.287 = 1.219 pg μm^{-3} (point 4 in Table 2). This density is

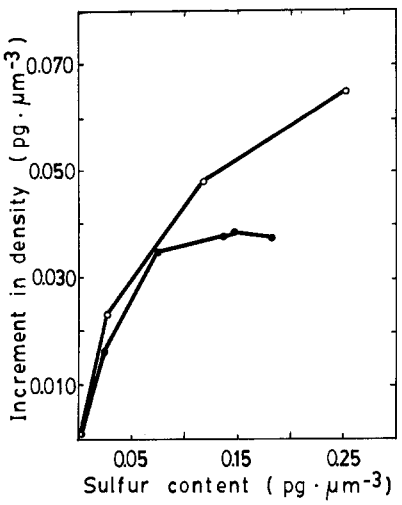

Fig. 7. Increment in cell density vs. specific sulfur content for *Chromatium warmingii* (closed symbols) and *Chromatium vinosum* (open symbols). Sulfur content is represented as pg S° per μm^3 of cell volume

much lower than the density of elemental sulfur, which is 2.07 for rhombic crystals and 1.957 for monoclinic crystals (Weast 1972). Consequently, the globules inside the cell must contain elemental sulfur mixed with some diluting substance of a density lower than 1.219 g cm^{-3}. Despite the fact that elemental sulfur is not soluble in water, we shall use the term "hydration", and assume that this diluting substance has a density of 1.00, for convenience in expressing the state of mixing of elemental sulfur necessary to form an intracellular globule. With these assumptions, the degree of hydration of the sulfur globules would have to be 65% of the wet weight of the globule (point 6 in Table 2). By the same type of analysis, the density of pigments and other materials extracted by ethanol is 1.083 pg μm^{-3} (point 5 in Table 2), which is very similar to those found (Table 1) for cells without sulfur (1.085 pg μm^{-3}) and cells from which both pigments and sulfur had been extracted (1.093 pg μm^{-3}).

The relationship between sulfur and cell buoyant density showed a saturation type curve (Fig. 6). In order to check if this different response could be due to the unequal sizes of the two *Chromatium* species, density was plotted vs. sulfur content per μm^3 of cell volume, thus eliminating size differences between the two species (Fig. 7). In this case responses were similar up to about 0.1 pg S° μm^{-3}, but divergent curves were still observed at higher specific contents. *C. warmingii* did not change its density above this sulfur content, while *C. vinosum* increased its buoyant density further with higher sulfur contents.

Specific density is the result of interactions among several cell parameters. Some of them, such as ribosomal content, proteins and RNA, are carefully attuned to growth rate and are affected by specific regulation mechanisms. However, density, at least in *E. coli*, only changes by 1% (i. e., the increase of one unit in the second decimal) with growth rate (Woldringh et al. 1981; Martínez-Salas et al. 1981). Increases in volume probably compensate for higher cellular contents of RNA and protein. Reserve materials, on the other hand, appear when there is unbalanced growth and no such precise regulation of cell volume and weight exists to limit changes in density, thus permitting the observed 3.5% and 6% changes in *C. warmingii* and *C. vinosum* respectively (Fig. 7). Therefore, it is clear that specific density must increase with sulfur content, but may be a critical density exists above which cells are no longer viable.

As a consequence of the experiments presented, we conclude that specific cell density in *Chromatium* can change during growth of illuminated cultures by as much as a 6%, and that these variations are mostly due to sulfur content of the cells. The observed variations are sufficiently high to merit attention when calculating wet or dry weight estimates of bacterial biomass from cell volumes and numbers in ecological studies. Furthermore, these changes in density could have an effect on sedimentation of the cells in nature. In the absence of flagellar movement and water turbulence, *Chromatium* cells loaded with sulfur or other reserve materials should sink faster than cells without them, and thus, have a disadvantage in competing for light. Substituting our highest and lowest values of density and volume, Stokes' Law gives terminal velocities, at 20° C, of 1.3 to 3.2 cm d^{-1} for *C. warmingii* and from 0.5 to 1.4 cm d^{-1} for *C. vinosum*. Thus, almost a threefold difference in sinking rates can be found as a result of the sulfur content of the cells. This difference could be of major importance in bacterial plates where light dissappears very rapidly. For example, field data (for August 21, 1981) from our studies in Lake Cisó (Banyoles, Spain), showed that, due to the thickness of the bacterial plate, light was 59 μE s^{-1} m^{-2} at 0.25 m, 2.1 μE s^{-1} m^{-2} at 0.5 m, 0.1 μE s^{-1} m^{-2} at 0.75 m. Thus, cells living between 0.5 m and 0.75 m would pass from light limitation at 1 or 2 μE to light starvation at 0.1 μE by just sinking a few centimeters in one or two day-night cycles.

Measured swimming velocities for Chromatiaceae are between 45.9 μm s^{-1} for *Chromatium okenii* and 86.5 μm s^{-1} for *Thiospirillum jenense* (Vaituzis and Doetsch 1969). Under optimal conditions, *Chromatium* could easily compensate for the sedimentation rates given above, but in the field there is a certain depth below which severe light limitation prevents swimming because not enough energy is available for motility. Thus, after crossing a certain threshold level bacterial cells are bound to sink. Several physiological and morphological studies performed in our laboratory agree with this conclusion (Montesinos and Esteve, Verh. Internat. Verein. Limnol., in press).

Finally, the buoyant densities found widen both the range of densities in prokaryotes and the range for a single organism. This indicates that intracellular inclusions can have a determinative effect on a theoretically conservative parameter such as density (Woldringh et al. 1981). In this respect, the influence of cellular inclusions other than sulfur requires examination. Also, it points out to the need for more physiological studies on organisms different from *E. coli*, specially the more bizarre or peculiar bacteria, in order to better understand the range of structural possibilities and diversity among the prokaryotes.

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