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RESEARCH ARTICLE

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Light absorption by phototrophic bacteria: effects of scattering, cell concentration and size of the culture vessel

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Summary This article analyzes how absorption of light by suspensions of phototrophic bacteria is modulated by changes in the biomass of the culture, the size of the culture vessel and by the presence of refractile structures within the cells. Increases in biomass and culture size result in higher rates of light absorption but in the decrease of the amount of energy available per cell. The presence of refractile structures has different consequences depending on the biomass concentration. In dense cultures, the accumulation of refractile structures increases the reflection of light, and also reduces specific light absorption. In diluted cultures, however, the effect is the opposite, and refractile structures seem to increase light absorption.

Key words Light absorption · Phototrophic bacteria · Light scattering · Bacterial biomass · Culture size

Introduction

The physiological state of phototrophic bacteria is strongly conditioned by their light supply. Variations in this supply induce changes in cellular composition, in the degree of development of the photosynthetic apparatus as well as in several cellular activities. Thus, laboratory experiments attempting to analyze the physiology of phototrophic bacteria or algae must pay special attention to the light climate to which the cells are exposed. In general, experiments are set in such a way that a light source provides controlled and reproducible illumination to the culture vessel. However, researchers usually overlook the fact that light conditions inside a culture vessel are influenced significantly by factors other than the incident irradiance. The occurrence of self shading, and variations in factors affecting its magnitude, such as population density and the size and shape of the culture vessel, are often ignored.

In an attempt to circumvent the problems arising from the existence of a heterogeneous light field inside the culture, several authors have suggested solutions based on the assessment of the mean light intensity [6] either through the measurement of light irradiance at the front and back of the culture vessel [13], or after averaging the intensity of the light field at different positions inside the culture vessel [11]. The first approach may result in inaccuracies in cultures with a high scattering, in which light might exit the vessel at directions other than opposite to the light source. The second approach, although accurate, is somewhat cumbersome, since it requires custom-built equipment for the analysis of the light field and, also, because it interferes with the regular operation of the culture. In general, mean light intensity represents an average value for the irradiance which the cells receive when traveling within the stirred culture. Further improvements in this area have been introduced with the determination of the specific light energy uptake rate [1, 5, 12]. This variable, calculated as the total amount of light absorbed by the culture divided by the total biomass provides an indication of the average instantaneous specific rate at which light is absorbed by the organisms. The resulting value can be related directly to biological activity through a yield coefficient [1, 5, 12] and is a much better predictor of the level of energy supply. A direct procedure for the determination of light absorption and the specific light uptake rate in cultures of phototrophic bacteria, has been developed [7]. Using this procedure, we have carried out a study which analyzes the contribution of several factors to both, the characteristics of the light field exiting the vessel and the magnitude of the absorption. The aim of the work described in this paper is not to make an exhaustive study of all factors determining the inherent optical properties of a culture but rather to provide some insight into the phenomenon of light absorption by suspensions of phototrophic bacteria. Thus, major

factors such as cell size and shape, the specific content of photosynthetic pigments, and the formation of multicellular filaments or aggregates have not been considered. The observations focus on three factors: biomass concentration, dimensions of the culture vessel, and the presence of intracellular refractile inclusions likely to affect scattering. As a model organism, we chose the purple sulfur bacterium *Allochromatium vinosum*. This organism can be grown at high cell densities and does not form aggregates which might interfere with the measurements. Besides, it can accumulate large amounts of sulfur (as much as 40% of its dry weight) deposited intracellularly as highly refractile inclusions.

Materials and methods

Organism and growth conditions Experiments were carried out with *Allochromatium vinosum* DSM 185 grown at 25°C on the mineral medium described by Mas and Van Gemerden [3]. Illumination was provided by incandescent light bulbs placed at one side of the culture vessel. Irradiance was measured with a Quanta Meter (No. Li-185B; LiCor Inc.).

Sampling and analyses Protein, bacteriochlorophyll *a* (BChl *a*) and elemental sulfur were analyzed in 10 ml aliquots from the culture. The samples were centrifuged (4400× *g*, 10 min) and after discarding the supernatants, the pellets were frozen and kept at –20°C. BChl *a* and elemental sulfur were measured in methanol extracts of the samples using the procedure described by Stal et al. [9]. The protein content of the pellets remaining from the previous extraction was determined according to Lowry et al. [2] after extraction at 100°C with 1 M NaOH. Sulfide was measured using the method of Pachmayr [4] in samples taken directly from the culture, as described by Trüper and Schlegel [10].

Light absorption and specific rate of light uptake The rate at which light was absorbed by the culture (µE h^{-1}) was determined according to the procedure described by Sánchez and Mas [7] as the difference between the rate of light output from a culture containing cells and the rate of light output from a culture containing only medium. Outputs were measured taking into account the photon fluxes exiting the culture vessel at different angles, an approach which also allowed the characterization of the light field. Calculation of the specific rate of light uptake (q_e) was performed dividing the rate of light absorption (Q_e) by the total biomass in the culture [7].

Light distribution To analyze how variations in the different factors studied affected the orientation of the exiting light field, we defined three different quantities which somehow summarize this distribution. The first quantity (TL) is the fraction of transmitted light, calculated as the integrated light output between 135° and 225°. This value provides an indication of the fraction of light neither absorbed nor scattered and has a maximum value when the culture contains only medium. The second quantity (FS) is the amount of forward scattered light, which is calculated as the integrated light output between 90° and 135° plus the light output between 225° and 270°. It gives an indication of the light which, although crossing the vessel, does not leave exactly opposite to the light source. The third quantity (BS) is the amount of backward scattered light which provides a measure of the light reflected by the culture. It is determined as the integrated light output between 270° and 90°.

Results and Discussion

Effect of sulfur accumulation on light absorption To assess how the presence of highly scattering structures such as sulfur inclusions affects light absorption, we designed an experiment in which we progressively forced a culture of *A. vinosum* to accumulate sulfur. The organism was grown at 25°C in an all-glass cylindrical culture vessel (1.4 l, 10.4 cm in diameter, and 20 cm high). The culture vessel was continuously illuminated from one side by two incandescent light bulbs (60 W), supplying an incident irradiance of 220 µE m^{-2} s^{-1}. The organism was fed neutralized hydrogen sulfide at the beginning of the experiment up to a concentration of 1.25 mM. When sulfide was depleted a second addition was performed (1.5 mM). Sulfur, which initially was present at a very low concentration (0.03 mM), increased over the experiment, attaining a maximum value of 2.4 mM approximately four hours later. The concentration of protein remained virtually constant over the same period (around 116 mg l^{-1}). The concentration of BChl *a* did not change over the experiment, thus resulting in a constant specific content of 49.7 µg BChl *a* mg^{-1} protein. The specific content of sulfur, on the contrary, increased steadily until reaching 21.4 µmol S° mg^{-1} protein, approximately 25.5% of the dry weight.

Accumulation of sulfur gave rise to a gradual change in the optical characteristics of the culture. The color of the culture, which initially was a deep shade of red progressively acquired a milky appearance indicating a higher reflection of the incident light. Measurements over time of the light output at different angles confirmed this observation. Some of the results (corresponding to sulfur contents of 0.2, 7.9, and 21.4 µmol S° mg^{-1} protein) have been represented in Fig. 1 as both, X-Y graphs and polar plots.

At the beginning of the experiment, when cells were virtually depleted of sulfur, light escaped from the culture through the back at 135° and 225°, probably as a consequence of forward scattering (Fig. 1A). As sulfur accumulated, back scattering increased and light output shifted progressively from the back to the front of the culture vessel (Figs. 1B, and C). Overall, the total amount of light exiting the culture

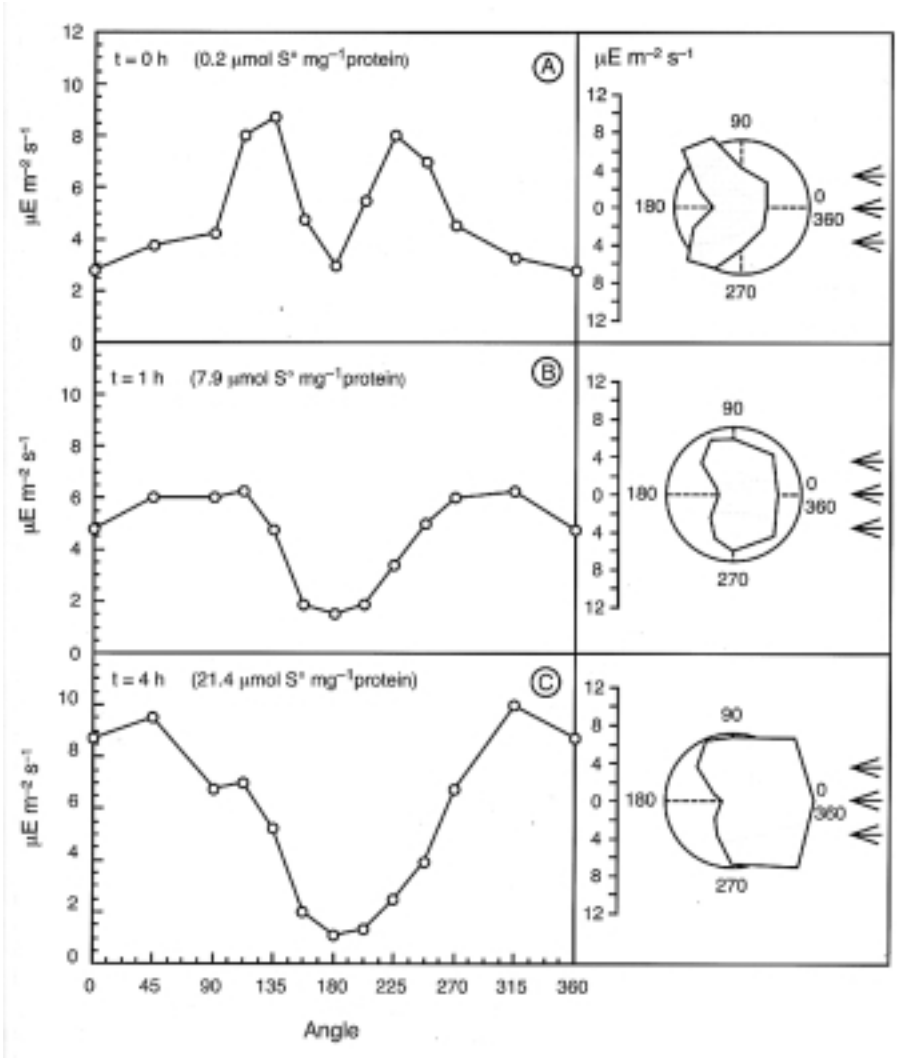

Fig. 1 Light output as a function of the angle in cultures of *Allochromatium vinosum* with different sulfur contents. The results have been represented as X-Y graphs and polar plots. The distance to the center of the circle represents the magnitude of the light output. Arrows indicate the position of the light source

(dotted area in the polar plots) hardly changed, and it increased slightly as sulfur accumulated. The consequence of this increase in light output was a small decline in light absorption (Fig. 2A), which resulted in a parallel reduction in the specific rate of light uptake (q_e).

The results described above correspond to a dense culture with a high biomass. In dense cultures, the presence of refractile structures, which increase scattering, seems to have little effect on the rate at which light is harvested by the organisms However, it has a dramatic effect on the distribution of light exiting the culture. As we will show later on in this paper, in diluted cultures, sulfur accumulation appears to intensify light uptake by the organisms.

Effect of culture size on light absorption The effect of culture size on light absorption was studied using culture vessels with different diameters. To standardize the results, we used 20 cm heigh vessels in all the experiments. For each case, we determined the spatial distribution of the light output from a blank containing medium, and from a vessel containing a dense culture (74.4 mg protein/l) of *A. vinosum* depleted of sulfur. In both cases, the vessels were illuminated by incandescent light bulbs placed in one side, supplying an incident irradiance of 92 μE m^{-2} s^{-1}. A total of eight different diameters were analyzed (3.7, 4.4, 5.2, 6.7, 7.9, 10.1, 13.8 and 18.5 cm).

In all cases, most of the light exited the vessel at 180°, opposite to the light source. The total light output decreased

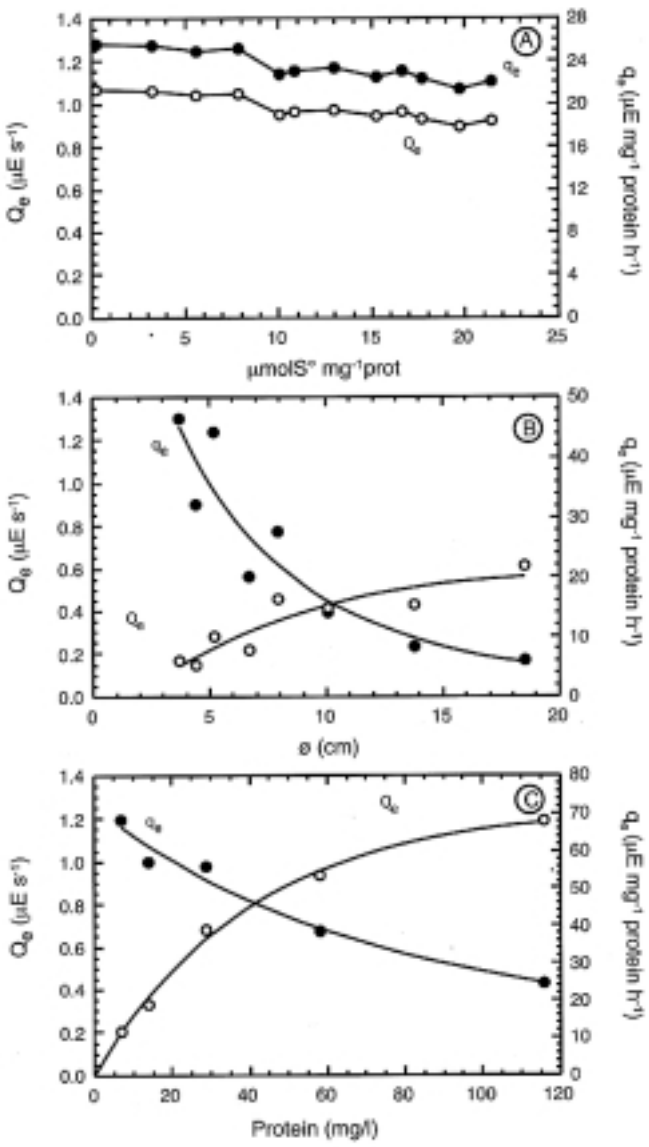

Fig. 2 (A) Rate of light absorption (Q_e) and specific rate of light uptake (q_e) as a function of the specific content of sulfur. The experiment was carried out in a culture vessel 20 cm high and 10.4 cm in diameter. Incident irradiance at the surface of the vessel was 220 µE m^{-2} s^{-1}. (B) Rate of light absorption (Q_e) and specific rate of light uptake (q_e) as a function of the diameter of the vessel in cultures of *Allochromatium vinosum.* (C) Rate of light absorption (Q_e) and specific rate of light uptake (q_e) as a function of biomass in cultures of *A. vinosum*

steadily as the diameter increased, due to a higher absorption by the culture.

The rate of light absorption (Q_e) and the specific rate of light uptake (q_e) have been represented in Fig. 2B. The rate of light absorption increases with diameter. At high diameters, the rate at which light is absorbed by the culture approaches a maximum value which depends somewhat on the distance between the light source and the culture vessel. Although light absorption increases with diameter, the specific rate of light uptake decreases dramatically when the size of the culture vessel increases. Thus, an increase of approximately five times in the diameter gives rise to a ten-fold decrease in q_e. Since photosynthetic activity is proportional to q_e, it is assumed that the size of the culture vessel will have a substantial effect on the behavior of phototrophic cultures.

Effect of biomass on light absorption Light absorption by cultures of phototrophic organisms was also expected to change as a function of biomass. To study the effects of this variable, a dense culture of *A. vinosum* (116 mg protein/l) was diluted several fold using fresh culture medium. Each of the dilutions was placed in a culture vessel 10.4 cm in diameter and 20 cm high, and light output was measured at different angles. Determinations of light outputs were carried out also in dilutions of a culture with the same initial biomass but containing 16.6 µmol S° mg^{-1} protein. In both cases, the vessels were illuminated by incandescent light bulbs placed at one side, which provided an incident irradiance of 220 µE m^{-2} s^{-1}. Light output as a function of the angle has been represented in Fig. 3 for some of the dilutions from the sulfur depleted culture. Light output from a blank has also been included in the same graph for comparison.

At low biomass concentration (7 mg protein/l), light distribution resembles very much the light distribution of the blank. As the biomass concentration increases, the light output decreases and the distribution changes. Whereas at low biomass concentrations, light exits the culture vessel preferentially at 180°, high biomass concentrations cause a decrease in the output at 180° and a relative increase of the outputs at 135° and 225°, as a consequence of forward scattering within the culture.

The specific rate of light uptake (q_e) has been represented in Fig. 2C as a function of protein concentration together with the rate of light absorption (Q_e). Q_e increases with protein attaining a maximum value at high concentrations when all light has been absorbed by the culture. On the contrary, q_e decreases approaching zero. Both variables change following the same pattern observed in Fig. 2B, which indicates that biomass concentrations and culture size have nearly equivalent effects on Q_e and q_e.

Changes in q_e can also be observed in cultures which have accumulated sulfur. Figure 4 shows the relationship between q_e and biomass (expressed as protein) in cultures of *A. vinosum* depleted of sulfur and in cultures containing 16.6 µmol S° mg^{-1} protein. Differences in q_e were more pronounced in cultures with low cell densities. In diluted cultures, the presence of refractile structures causes scattering, increasing the light path inside the culture vessel and therefore, affecting the rate at which light is harvested by the organisms. As a consequence, when sulfur is present the overall light output decreases and q_e shows a substantial increase. In dense cultures, however, structures apt to increase scattering seem to have little effect on the specific rate of light uptake.

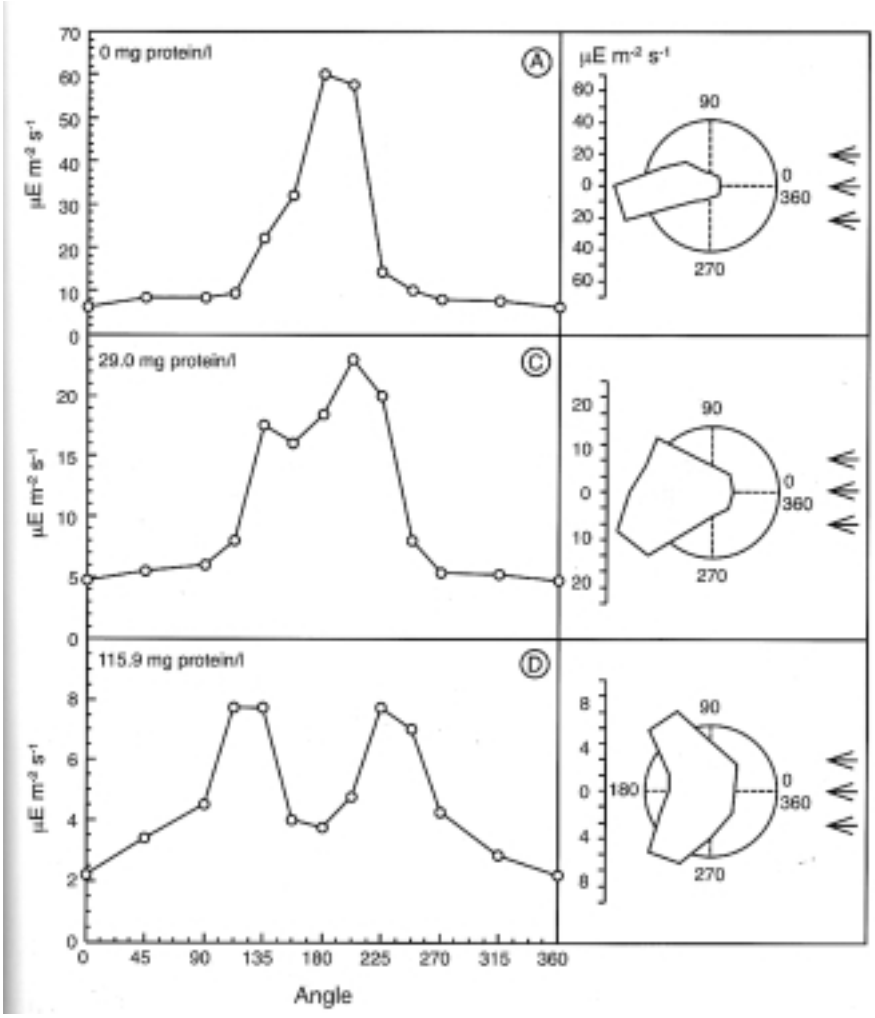

Fig. 3 Irradiance as a function of the output angle in cultures of *Allochromatium vinosum* at different biomass concentrations. The results have been represented as X-Y graphs and polar plots. Arrows indicate the position of the light source

This effect is more obvious when looking at the distribution of the light output for the two extreme situations in Fig 4A. These situations have been represented as polar plots in Figures 4B and 4C. In Fig. 4B, the distribution of the light output corresponds to two diluted cultures of *A. vinosum* with the same biomass concentration (6.95 mg protein/l), one depleted of sulfur and the other containing 16.6 µmol S° mg^{-1} protein. The presence of sulfur does not increase back scattering, but it rather decreases transmitted light and results in a reduction of the overall output, which suggests there has been an increase in light absorption. A different situation can be observed in Fig. 4C, which shows the light output of two dense cultures containing the same biomass concentration (115.9 mg protein/l) and different sulfur contents (0.2 and 16.6 µmol S° mg^{-1} protein). In this case, the presence of refractile inclusions increases dramatically the amount of back scattered light. These results in a light distribution radically different from the distribution found in the sulfur depleted culture, in which light is scattered forward and exits the culture at 135° and 225°.

Spatial distribution of the light output Light output can be divided in three fractions, which indicate the relative amount of forward scattered, backward scattered and transmitted light. These fractions have been represented for three different

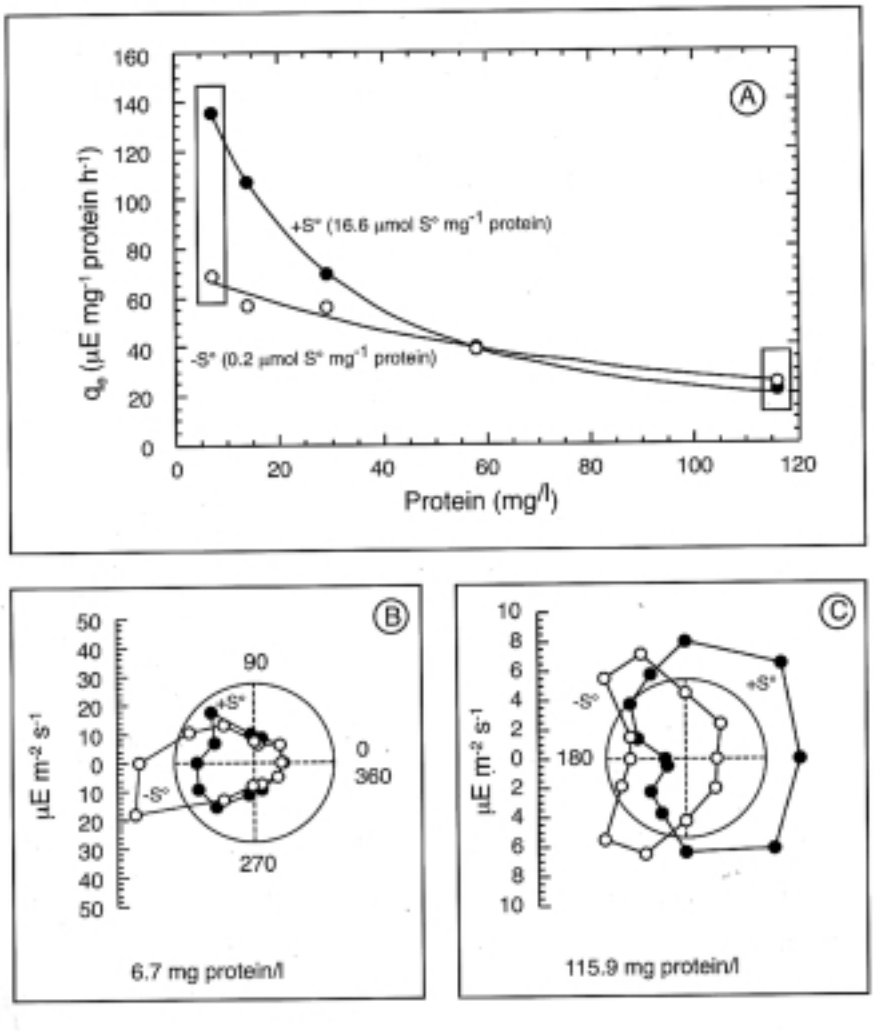

Fig. 4 (A) Specific rate of light uptake as a function of biomass (mg protein/l) in cultures of *Allochromatium vinosum* depleted of sulfur (○) and in cultures containing 16.6 µmolS° mg^{-1} protein (●). The points enclosed in rectangles at the right and left sides of the plot have been used to draw the light output distributions in Figs. 4B and 4C. (B) Distribution of the light output in diluted cultures of *A. vinosum* with a high (●) and a low (○) sulfur content. (C) Distribution of the light output in dense cultures of *A. vinosum* with a high (●) and a low (○) sulfur content. (–S°: 0.2 µmol S° mg^{-1} protein, +S°: 16.6 µmol S° mg^{-1} protein)

experiments, as a function of the sulfur content (Fig. 5A), the size of the culture vessel (Fig. 5B) and the concentration of protein (Fig. 5C).

In Fig. 5A, as the content of refractile inclusions increased, the back scattered fraction rose substantially from a value of 600 µE m^{-2} s^{-1} degree at low contents of sulfur up to 1500 µE m^{-2} s^{-1} degree at high specific contents. On the contrary, transmitted and forward scattered light tended to decrease, although to a lesser extent. When biomass concentrations remained constant and the diameter of the vessel increased (Fig. 5B), more light was absorbed. As a consequence, transmitted light decreased considerably, from 1550 to 50 µE m^{-2} s^{-1} degree. Forward and back scattered light also experienced a small decline. A similar situation was found when light output was plotted against biomass concentration (Fig. 5C) in sulfur depleted cultures. As biomass concentrations increased, more light was absorbed and consequently, the amount of transmitted light decreased. The quantities of forward and back scattered light showed a slight reduction, although at high biomass concentrations they turned out to be a major fraction of the total light output, totaling 70% of the exiting light. Summarizing, increases in biomass concentration and in the diameter of the culture vessel always result in a reduction in the amount of transmitted light, and have very little impact on the scattered fractions. Accumulation

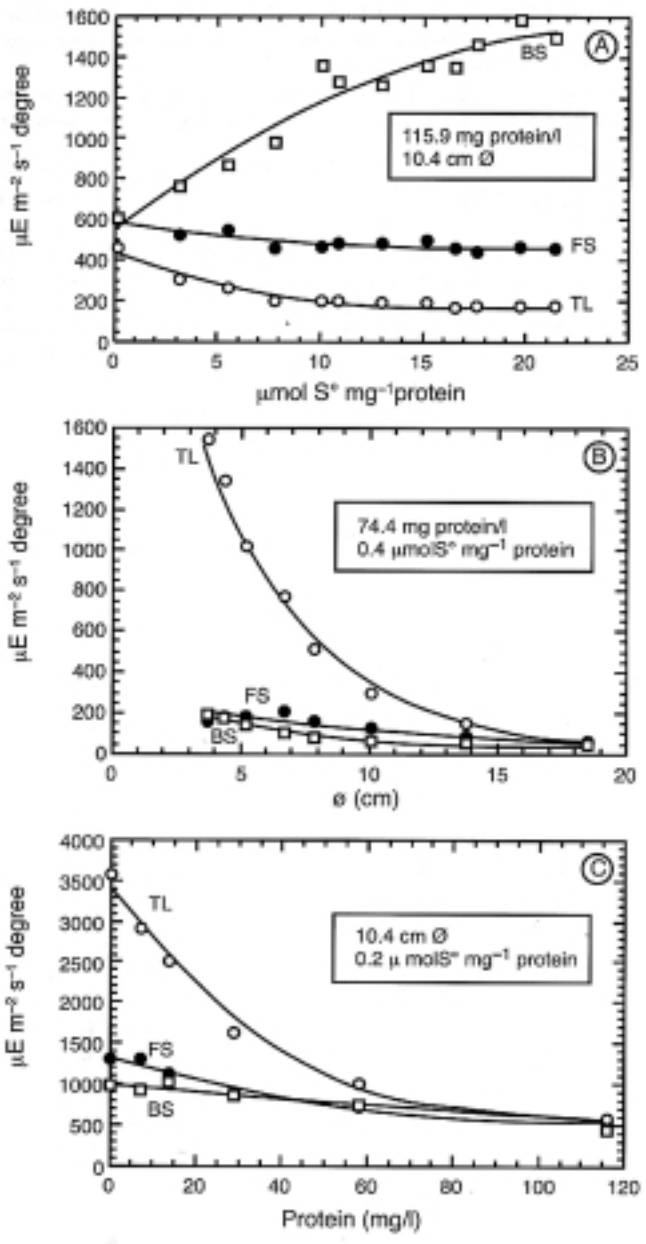

Fig. 5 Amounts of transmitted (TL) (○), forward scattered (FS) (●) and backward scattered (BS) (□) light output as a function of: (A) the specific content of sulfur, (B) the diameter of the culture vessel and (C) the protein concentration. The conditions for each set of experiments are specified in the plots

of refractile inclusions, on the contrary, seems to increase the amount of back scattered light, although this conclusion only holds for dense cultures.

Concluding remarks The results indicate that non absorbed light exits the culture vessel according to a spatial distribution which is considerably influenced by factors such as the concentration of organisms, the size of the vessel, and the presence of refractile inclusions. In diluted cultures or in cultures with a small diameter, as much as 80% of the light output is transmitted. When either the biomass concentration or the diameter of the culture increase, transmitted output decreases to between 25 and 35% of the total output, the remaining fraction being distributed in roughly equal amounts between forward scattered light and backward scattered light. As a consequence, since light exits the vessel at directions other than opposite to the light source, point measurements of light irradiance at the front and at the back of the culture vessel result inappropriate to determine the amount of light absorbed by a culture. This phenomenon is even more obvious during sulfur accumulation (Fig. 1). When sulfur accumulates, transmitted light decreases to about 8% of the total output, whereas backward scattered light (i.e. reflected light) increases up to 70% of the total output.

The substantial increase of q_e in the presence of intracellular sulfur is an unexpected finding and indicates that refractile structures affect dramatically the rate at which light can be absorbed by cells. However, this effect can only be observed in diluted cultures. In nature, where the concentrations of microorganisms are relatively low, sulfur may increase the magnitude of light absorption and this fact would be especially relevant for populations living in a light-limited environment. In turn, it has been observed in the laboratory that cells of *A. vinosum* store sulfur under conditions of light limitation [8]. Under this situation, sulfur could behave as a storage of reducing power but also as a structure apt to increase light absorption, thus constituting an adaptive mechanism to improve light absorption in light-limited environments.

Overall, the observations gathered in this paper indicate how light absorption can be maximized by increasing the culture cross section and/or the concentration of phototrophic biomass. Maximum light utilization, while of some relevance for the economy of applied processes, will necessarily result in a decrease of the specific rate of light uptake (q_e), which is the amount of energy available for growth per unit biomass. Therefore, maximization of light absorption is always associated to a decrease in the biological activity and, as a consequence, the optimization of phototrophic production in biological reactors will require a trade off between these two factors.

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