Optical properties of photosynthetic picoplankton in different physiological states as affected by growth irradiance

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Abstract—A phycocyanin-rich cyanobacterium belonging to the genus Synechocystis has been adapted and grown under differing irradiances (PAR), ranging from 16 to 1450 μE m⁻² s⁻¹, and differing spectral compositions ("white", "blue" and "green"). Chlorophyll-specific as well as carbon-specific spectral absorption and scattering coefficients were determined for all conditions. Due to drastic changes in chlorophyll and phycocyanin content per cell in response to the radiative level imposed to the culture, these coefficients undergo extreme variations, in a range wider than the inter-specific range already reported for eucaryotic algae. The optical dimensionless efficiency factors have been computed and used to calculate the bulk refractive index (in the range 1.05–1.06 with respect to the index of water). The optical properties of this picoplanktonic species is typical of "small" optical particles, with a scattering efficiency increasing towards the blue, and a backscattering efficiency increasing towards the red end of the spectrum. Superimposed on this pattern are features associated with the presence of pigments, including the phycocyanin signature. Although the cellular pigment concentrations are high (particularly at low irradiances), the package effect remains negligible. Thus Synechocystis is well suited for harvesting light, even if the presence of biliprotein appears to be useless in regards to the spectral quality of the light available in the deep layers of the euphotic zone.

INTRODUCTION

Recent development of remote sensing techniques and flow cytometric applications, as well as fundamental photosynthesis research, result in an increasing need for studying the optical properties of phytoplankton. Following pioneering works by Latimer (1959), Yentsch (1960) and Duntley et al. (1974), numerous studies have recently been conducted on optical properties of pure algal cultures, which indicate wide variations of these properties between species (Privoznik et al., 1978; Kiefer et al., 1979; Brbicard et al., 1983; Davies-Colley et al., 1986; Osborne and Raven, 1986; Brbicard et al., 1988). It is of importance to extend this work to minute unicellular photosynthetic "picoplankton" which are now recognized as being an important component of marine phytoplankton, especially in the oligotrophic ocean (Johnson and Sieburth, 1979; Li et al., 1983; Platt et al., 1983; Takahashi and Bienfang, 1983; Glover et al., 1985). To date there has been little research on optics of these tiny cells even if some data already exist (Morel and Brbicard, 1986; Kana and Gibert, 1987). Furthermore, previous studies, such as those of Osborne and Raven (1986), Soohoo et al. (1986) and Morel et al. (1987), focused on physiological response of algae to growth irradiance, do not provide a complete description of both absorption and scattering (but see Berner et al., 1989).

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The present study deals with a phycocyanin-rich cyanobacterium belonging to the genus *Synechocystis* (blue-green alga or Cyanophyceae), which is known to exhibit drastic change in pigment content and composition when available irradiance is varied. Our objective is therefore to examine and interpret the optical properties of these cells and their variations when grown under different light culturing conditions.

For each growth irradiance to which the cyanobacterium culture has been aclimated, several properties were repeatedly measured, including (i) biophysical–biochemical properties, namely cell density, cell-size distribution, carbon and chlorophyll *a* (Chl *a*) concentration and (ii) optical properties, namely the spectral values of absorption and beam attenuation. In addition absorption spectra of the pigments extractable by methanol were also obtained. These different parameters are combined and theoretically interpreted with the aim of understanding the adaptive modifications that *Synechocystis* undergoes in response to changes in the radiative conditions. Such an improved understanding of the optical properties of very small phytoplankters is particularly required for a comprehensive use of techniques like flow cytometry.

**MATERIAL, METHODS AND COMPUTATION SCHEMES**

**Culturing procedure**

*Synechocystis* (isolated by J. Neveux from water sampled off Banyuls-sur-Mer, Mediterranean Sea) was grown continuously in filtered seawater enriched with f/2 nutrients (Guillard and Ryther, 1962). The culturing apparatus consisted of a water bath in which 1000–1200 ml cultures, contained in a 2000 ml Fernbach flask, were maintained at 20 ± 1°C, and irradiated continuously with diffuse light of constant (to within ±10%) intensity provided by metal halide lamps (OSRAM 150 W or 250 W). The cultures were grown under steady lighting conditions differing by the radiant energy levels and spectral composition (Table 1). Measurements of photosynthetic available radiant energy (PAR), also referred to as growth irradiance, were made with a calibrated 4π quantum scalar irradiance meter (Biospherical Instruments QSL 100) immersed in the culture vessel. The normalized spectra of incident irradiance, referred to as “white”, “blue” and “green”, measured by using a spectro-irradiance meter, are shown in Fig. 1. PAR was varied by interposing neutral density screens, and modifying the distance between the light source and the culture. A commercial, colored Plexiglas, and a Kodak Wratten gelatin filter (no. 61) were used to produce the blue and green light fields, respectively. In addition, a Plexiglas diffusing plate with a 1-cm thick glass was placed in the light path to ensure a quasi-uniform diffuse light within the growth chamber, and to eliminate ultraviolet radiation from the lamp.

Prior to sampling, the cultures were allowed to adapt to each growth irradiance, for at least 1 week. Thereafter and under each particular irradiance, the working stocks were

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maintained in the exponential phase of growth for 2–3 weeks by daily dilution with fresh medium, so that the biomass concentration at the beginning of every 24-h cycle was about the same. The dilution rate was dictated by the actual growth rate. The samples for all subsequent measurements were taken just before dilution. All analyses were repeated on different days. The cell densities were adjusted to keep cultures optically thin, in order to minimize shading. Depending on the growth irradiance, the optimum final cell density (i.e. just before daily sampling) ranged from 15 to 45 × 10^6 cells ml⁻¹, with Chl a concentration from about 20 to 120 mg m⁻³. Such concentrations ensure reliable spectrophotometric measurements of in vivo absorption and scattering by the cells in suspension.

Cell enumeration and size distribution

Epifluorescence technique (Hobbie et al., 1977; Davies and Sieburth, 1982) was used to estimate total numbers of cyanobacteria cells per unit volume, N/V. Cyanobacteria were characterized by red autofluorescence when using a green band-pass exciter filter. In general, 40–60 randomly chosen microscope fields, and more than 800 cells were counted per filter. Counts were made on duplicate samples and averaged. Typically, the two counts agreed to within ±10%. This uncertainty in the cells count, however, remains the most important source of experimental error in calculated characteristics. Microscopic inspection of the cultures with a DAPI staining method for induced fluorescence (Porter and Feig, 1980) showed that the cultures were contaminated by low and insignificant concentrations of heterotrophic bacteria.

Cell size distribution, F_R(D) (where D is the diameter of the equivalent sphere), was measured in relative values by using a 256-channel Coulter Multisizer with a 30 μm aperture tube. The relative distribution function is transformed into an absolute function F(D) by introducing the absolute cumulative count N/V

\[ F(D) = F_R(D) \left( \frac{N}{V} \int_0^D F_R(D) dD \right)^{-1}. \]  

Carbon concentration

Cells were harvested by gentle filtration onto a precombusted (550°C) Whatman GF/F glass fiber filter for determination of the concentration of particulate organic carbon
within the suspension, $C$ (in mg m$^{-3}$). Epifluorescence examination of the filtrate showed that this filter is effective in retaining all cells. Determination of carbon was made on dried filter (2–4 replicates) with a Perkin–Elmer 240 elemental analyzer, standardized with cyclohexanone.

**Chlorophyll a concentration**

A spectrophotometric method based on a single optical density reading at $\lambda = 665$ nm in methanol extract proved satisfactory to determine Chl $a$ concentration (mg m$^{-3}$) for this culture. Following (GF/F) filtration and grinding the filter in absolute methanol, the pigments were extracted in complete darkness at 4°C for 30 min, centrifuged and determined using a Perkin–Elmer 571 spectrophotometer in a 1 cm cuvette with a 95% methanol blank allowing for the residual water remaining in the filter. Control experiments showed that a turbidity correction from the 750 nm reading was unnecessary. The validity of such a single-wavelength determination is supported by the facts that the extracted pigments other than Chl $a$ contribute negligibly to light absorption in the red part of the spectrum as the species under study contains neither Chl $b$ nor Chl $c$, and that the (water-soluble) phycocyanin is non-extractable with organic solvent. Absolute methanol was most suitable for rapid and nearly complete extraction of the Chl $a$ pigment from the cyanobacteria cells. It also was found that the pigments are readily extracted with 100% dimethyl sulfoxide (DMSO) or 100% dimethyl formamide. In contrast, a reduced extraction efficiency (about 80% as compared to methanol or DMSO treatment) was systematically obtained with the traditional acetone technique (Fig. 2). A special study was carried out since the specific absorption coefficient of Chl $a$ in methanol is poorly documented (see Appendix).

Carbon and Chl $a$ contents per cell as well as intracellular concentrations were determined. The intracellular Chl $a$ concentration, $c_{i,\text{Chl}}$ was computed as the ratio of Chl $a$ concentration in the suspension to the volume concentration of the suspended cells (e.g., Morel and Bricaud, 1981b):

$$ c_{i,\text{Chl}} = (\text{Chl } a) \left[ \frac{\pi}{6} \int_0^\infty F(D) \, D^3 \, dD \right]^{-1}, $$

(2)

![Optical density spectra of pigments extracted in different solvents from Synechocystis cells grown under low light intensity. Comparison of the Chl $a$ red maxima allowing for differences of specific absorption in different solvents shows that extraction efficiency in acetone is about 80% of that obtained when methanol or DMSO are used.](image)
where the bracketed term represents the cell volume concentration of the polydispersed population of spherical cells, and is a formal modification of the expression \((v \cdot N/V)\) for equally sized cells. Analogously, by replacing \((\text{Chl} \ a)\) by \((\text{C})\) in equation (2), the intracellular carbon concentration, \(c_{IC}\) was also calculated.

**Optical properties measurements**

Two optical parameters were measured directly with a double-beam recording spectrophotometer Perkin–Elmer 571, the spectral values of absorption and beam attenuation of the cell suspension. All of the other optical characteristics desired were derived from these experimental values combined with other data. Scans were made in the wavelength range 400–720 nm (resolution 1 nm). Cuvettes of 1 cm pathlength were used and the reference cuvette contained filtered culture medium (Whatman GF/F). The special geometrical configurations and methods described by **Bricaud et al.** (1983, 1988) were adopted. When measuring absorption, the optical density at 750 nm was very close to zero, and less than 0.002 in worst cases. The baseline was adjusted accordingly, by assigning zero absorption at 750 nm.

To ensure that the measured optical coefficients are inherent rather than “hybrid” optical properties (Preisendorfer, 1961), the cell suspension was sufficiently dilute to minimize effects of self-shading and multiple scattering. The maximum optical thickness \(\tau(\lambda) = c(\lambda) \ x\), where \(x\) is the pathlength, at 400 nm, was kept well below 0.3, a threshold consistent with a criterion given by **Van de Hulst** (1957).

**Normalized optical properties**

The measured spectra in terms of optical density were digitized at 2.5 nm intervals and converted into absorption and attenuation coefficients, \(a(\lambda)\) and \(c(\lambda)\), expressed in \(\text{m}^{-1}\); the scattering coefficient, \(b(\lambda)\), is computed as the difference \(c(\lambda) - a(\lambda)\). The \(b(\lambda)\) values are likely underestimated by a few percent due chiefly to imperfect attenuation measurement (see discussion in **Bricaud et al.**, 1983).

The chlorophyll-specific or carbon-specific absorption, scattering and attenuation coefficients are obtained by normalizing to a unit Chl \(a\) concentration or to a unit carbon concentration as follows:

\[
j^{*}_{\text{Chl}}(\lambda) = j(\lambda) \cdot (\text{Chl} \ a)^{-1},
\]

\[
j^{*}_{c}(\lambda) = j(\lambda) \cdot (\text{C})^{-1},
\]

where the symbol \(j\) replaces either \(a\) or \(b\) or \(c\).

The dimensionless efficiency factors for absorption \(Q_a\) and for scattering \(Q_s\) are the ratios of radiative energy respectively absorbed within, and scattered by, the cell to the energy impinging onto the cell’s geometrical cross section. For a (polydispersed) population of cells, these factors can be calculated according to (**Bricaud and Morel**, 1986):

\[
\bar{Q}(\lambda) = j(\lambda) \left[ \frac{\pi}{4} \int_{0}^{\infty} F(D) \ D^2 \ dD \right]^{-1},
\]

where the overbar shows that the efficiency factor is that of a “mean” cell.

The bracketed integral represents the cross-sectional area of the entire population, equal to that of an equal number of equally sized spherical particles. The efficiency factor
for attenuation $\bar{Q}_c$ is

$$\bar{Q}_c(\lambda) = \bar{Q}_d(\lambda) + \bar{Q}_b(\lambda).$$

(6)

**Derivation of the bulk refractive index**

The relative refractive index of the cells, with respect to that of water, is written $m = n - in'$. In general it depends on the wavelength. Its derivation follows a scheme, developed in Briceaud and Morel (1986), and based upon the anomalous diffraction approximation, valid when $m$ is not far from unity (van de Hulst, 1957).

The imaginary part, $n' (\lambda)$, is obtained through an iteration (repeated for each $\lambda$) which forces the theoretical $\bar{Q}_d (n', F(D), \lambda)$ values to equal the observed $\bar{Q}_d (\lambda)$ values and thus provides $n'$. The real part $n(\lambda)$ is similarly computed through another iterative process recently described (Stramski et al., 1988). It forces $\bar{Q}_c (n, n', F(D), \lambda)$ to reproduce the actual $\bar{Q}_c (\lambda)$ values. Note that this modification with respect to the Briceaud–Morel method is justified when it is possible a priori to rely on the van de Hulst approximation, i.e. when the cells are essentially homogenous and spherical.

**Computation of the VSF (volume scattering function)**

In the flow cytometry studies, the angular distribution of the light scattered by individual cells can serve as a “signature” of these organisms. In ocean color remote sensing, the light backscattered by algal colonies is of crucial interest. The VSF, denoted $\bar{\beta}(\theta)$, where $\theta$ is the scattering angle, describes the angular dependence of the scattered radiation. It can be predicted through the use of Mie theory.

In its normalized form, the VSF, defined as

$$\bar{\beta}(\theta) = \beta(\theta)/b,$$

depends on, beside $\theta$, the size and the relative refractive index of the particle according to

$$\bar{\beta}(\theta, \bar{a}, m) = \frac{1}{2\pi} \frac{\int_\alpha^\infty [i_1(\theta, a, m) + i_2(\theta, a, m)] F(a) \, da}{\int_0^\infty \bar{Q}_b(a, m) F(a) \, a^2 \, da},$$

(7)

where $i_1$ and $i_2$, the angular Mie intensity functions, depend on the relative complex index $m$ already defined, and on the relative size parameter, $a = \pi D n_w/\lambda$; $D$ is the diameter of the cell and $n_w$ the refractive index of water. $\bar{\beta}$ is thus $\lambda$-dependent explicitly through $a$ and implicitly through $m$, itself $\lambda$-dependent. $F(a)$ is a weighting function, derived from the actual size distribution $F(D)$ just by converting $D$ into $a$. The overbar on $a$ denotes the value of this parameter for the mean cell of the population (as above).

The backscattering efficiency, $\bar{b}_b$, defined as the ratio of that energy which is backscattered to the total scattered energy, is obtained by integrating over the backward hemisphere ($\pi/2 < \theta < \pi$).

$$\bar{b}_b = \int_{\pi/2}^{\pi} \bar{\beta}(\theta, \bar{a}, m) \sin \theta \, d\theta,$$

(8)

which straightforwardly can be transformed into the backscattering efficiency factor through

$$\bar{Q}_{bb} = \bar{Q}_b \bar{b}_b,$$

(8')

which is the ratio of backscattered energy to that impinging onto the geometrical cross-section.
RESULTS AND DISCUSSION

Culture characteristics

Cell size was not significantly affected by irradiance. The modal cell diameter was, on average, 1.14 μm with small variation (±5%, corresponding to ±4 channels on the 256-channel scale of the instrument). Such variations were recorded for both low-light and high-light adapted cells. In Fig. 3 are shown three typical size distributions of *Synechocystis* cultures. They essentially differ by the half-bandwidth that ranges from 0.3 to 0.65 μm, perhaps related to different states of cell division and life cycle or aggregation. Each of the curves in Fig. 3 results from averaging several (day-to-day) measurements. The narrowest distribution was only observed for cells with the highest growth rate (1.8 day⁻¹), when adapted to the highest irradiance (1450 ≈ μE m⁻² s⁻¹); the wide distribution was observed for white, moderate (170 and 310 μE m⁻² s⁻¹) irradiance and the intermediate distribution for all the remaining lighting conditions including all the low levels and the blue or green climates. It is worth noting that well-marked and systematic variations in cell size often have been recorded for many species, comprising another cyanobacterium (*Synechococcus* WH 7803 studied by Kana and Glibert, 1987).

Cellular carbon ranged from about 160 to 290 fg cell⁻¹ (Fig. 4A). The maximal content per cell was observed at white irradiances of moderate level (when the width of the size distribution is, as seen above, larger). The minimal content is associated with the narrow distribution (and the highest irradiance). In terms of intracellular carbon concentration, c_i,C, the range of variations is reduced, with values between 150 kg m⁻³ (for 16 μE m⁻² s⁻¹ blue or 1450 white irradiances) and 220 kg m⁻³ (for 50 green or 700 white irradiances).

Chlorophyll *a* per cell, increasing regularly with decreasing irradiance, ranged from 1 to 6.5 fg cell⁻¹ with systematically (albeit slightly) higher values when blue or green lighting conditions are imposed. The pattern of intracellular Chl *a* concentration, c_i,Chl, vs growth irradiance is obviously similar to that of Chl *a* per cell content since the cell volumes are weakly variable. As an example, in Fig. 4B c_i,Chl is plotted against white irradiance. The highest c_i,Chl values, of about 5–6 kg m⁻³, were determined at 20 (white), 16, 38 (blue) and 50 μE m⁻² s⁻¹ (green irradiance), while the lowest values of about 0.9 kg m⁻³ were observed at 700–1450 μE m⁻² s⁻¹ (white irradiance).

Carbon-to-Chl *a* ratio vs growth irradiance displays a regular pattern with a well-

![Graph](image)  

Fig. 3. Typical size distributions, expressed as relative number of cells vs the equivalent spherical diameter, of *Synechocystis* cells (see text for details).
marked maximum at PAR = 700 μE m⁻² s⁻¹ which is ca 5 times as high as the minimal values observed at the lowest irradiances (Fig. 4C). These values are very close to those observed by Kana and Gilbert (1987) for WH 7803. It is necessary to note that all the above parameters showed some day-to-day variations around the averaged values presented in Fig. 4, where the error bars are omitted, for clarity. The coefficient of variation of quantities expressed on a per cell basis is of the order of 10–12%; occasionally it reached 25% for both cellular carbon and chlorophyll (at white irradiance of 40, 80 and 700 μE m⁻² s⁻¹). The coefficient of variation of the carbon-to-chlorophyll ratio was, on average, 7%, and that of intracellular chlorophyll concentration 9%.

The carotenoids and phycocyanin concentrations were not determined, apart from nine samples, for which chlorophyll and carotenoid pigments were determined by reverse-phase high-performance liquid chromatography according to the method developed by Mantoura and Llewellyn (1983). Relative variations in these pigments with changes in growth irradiance can be, however, estimated by assuming that the absorption efficiency factors, $\hat{Q}_a$, at $\lambda = 490$ nm and $\lambda = 630$ nm are, respectively, proportional to the amount of carotenoids and phycocyanin per cell (Fig. 5). Such an assumption is based
Fig. 5. Absorption efficiency factor, $\tilde{Q}_a$, at selected wavelengths, as a function of growth irradiance; shown are data for white irradiance only. Open circles and solid line are for $\lambda = 490$ nm; triangles with dotted line are for $\lambda = 680$ nm; stars with dashed line are for $\lambda = 630$ nm.

on the fact that these pigments are the major contributors to light absorption by *Synechocystis* at the wavelengths in question. The absorption efficiency factor at $\lambda = 680$ nm, which predominantly reflects the cellular Chl $a$, is also plotted in Fig. 5. If relative changes with irradiance are considered, the $\tilde{Q}_a(680)$ curve obviously mimics that presented in Fig. 4B. The phycocyanin content per cell essentially changes in a manner similar to that of Chl $a$, with almost constant values when PAR > 170 $\mu$E m$^{-2}$ s$^{-1}$ and an abrupt increase with decreasing growth irradiance. On the sole basis of absorption, the phycocyanin-to-Chl $a$ ratio increases slightly (by 20%) when irradiance diminishes (see Fig. 9). Similar changes in Chl $a$ and phycocyanin content per cell have been reported for other cyanobacteria (Raps et al., 1983; Kana and Gliebert, 1987). As shown by the $\tilde{Q}_a(490)$ curve, the carotenoid content remains nearly constant when cells adapt from low to high irradiance. Absorption spectra of carotenoids in methanol, extracted from the cells grown under different white irradiances, are shown in Fig. 6B. These spectra were calculated as a difference between the measured absorption of pigments extracted from the cells and the absorption of purified Chl $a$ in methanol (Fig. 6A). Because of the normalization with respect to Chl $a$ the rising curves directly reflect the increase in the carotenoid-to-Chl $a$ ratio with increasing irradiance. This increase (in a ratio 6 : 1), as well as the approximate constancy of the carotenoid content (predominantly zeaxanthin and $\beta$-carotene) were fully corroborated by high-performance liquid chromatography analyses (V. Dénant, personal communication).

**Chlorophyll a specific coefficients**

For bio-optical applications it is appropriate to express optical properties in terms of specific coefficients, i.e. of coefficients ideally related to a unit of phytoplankton biomass. The most common index used to quantify this biomass is the Chl $a$ concentration. A normalization of optical coefficients to a single pigment involves, however, some drawbacks associated with inevitable effects of variation in pigment composition—drawbacks particularly expected with *Synechocystis*. In Fig. 7A and B are shown the spectra of Chl $a$-specific absorption and scattering coefficients $a_{\text{Chl}}^a(\lambda)$ and $b_{\text{Chl}}^a(\lambda)$ for cells acclimatized to various white irradiances. These data are averages based on measurements made on several days under the same conditions. The day-to-day variability remained weak. As an example, a systematic study extended over a 2-week period with 20 $\mu$E m$^{-2}$ s$^{-1}$ showed that the coefficient of variation of $a_{\text{Chl}}^a(\lambda)$ was of the
order of a few percent, except in the vicinity of the absorption minima in the green and near infra-red region, where it may reach 20%. For \( b_{\text{Chl}}(\lambda) \) the coefficient of variation remains always smaller than 10%, everywhere within the whole spectrum.

The most striking feature revealed by the *Synechocystis* absorption spectra in Fig. 7A is the considerable variation in the blue region where the absorption by carotenoids is added to the Chl a Soret band. Around 440 nm, \( a_{\text{Chl}}^* \) varies by a factor of 4.5 from 0.04 to 0.18 m\(^2\) (mg Chl a\(^{-1}\)), a wider range than the inter-specific range recently reported for eucaryotic organisms (Briceaud et al., 1988). This variation obviously originates from the highly variable carotenoid-to-Chl a ratio and can be seen as an artifact due to the Chl a normalization. Although the changes in Chl a and phycocyanin are roughly parallel, some variability is detected in the phycocyanin absorption band at 630 nm. In the red absorption band (670–680 nm) the absorption is chiefly due to Chl a (superimposed on the wing of the phycocyanin band). Therefore, the variability of the Chl a-specific absorption coefficient is lower here than elsewhere. The values of \( a_{\text{Chl}}^* \) (680) varying in a restricted range, reflect subtle differences in the packaging effect (for a given size, this effect increases with increasing intracellular pigment concentration; see Kirk 1975a,b and Morel and Briceaud, 1981a). In summary, the pigment composition plays a much more important role in determining the \( a_{\text{Chl}}^* (\lambda) \) values of *Synechocystis* is response to available irradiance than does the pigment packaging.
Fig. 7. The chlorophyll-specific absorption spectra (A), and the chlorophyll-specific scattering spectra (B) of *Synechocystis* intact cells grown under different (white) irradiances (designated by numbers in units of μE m⁻² s⁻¹). The shifting effect of absorption on light scattering is shown in the inset for the culture grown at 20 μE m⁻² s⁻¹. The units for this enlargement are: abscissae, from 650 to 710 nm; ordinates, from 0 to 0.022 m² (mg Chl a)⁻¹ for *a*ₐ₅₅₅, and from 0.080 to 0.095 m² (mg Chl a)⁻¹ for *b*ₐ₅₅₅.

The *a*ₐ₅₅₅ (λ) spectra for the cells grown under blue and green light (not shown in Fig. 7A) reveal a reduced sensitivity to the irradiance level, with *a*ₐ₅₅₅ (440) values ranging from 0.038 to 0.057 m² (mg Chl a)⁻¹ when PAR (Table 1) varies between 16 and 140 μE m⁻² s⁻¹. They are smaller than those obtained under white light with PAR < 80 μE m⁻² s⁻¹, in agreement with the observation (Fig. 4B or 4C) that the Chl a content per cell is distinctly higher in green and blue light than in white light with similar PAR. In the phycocyanin band, *a*ₐ₅₅₅ (630) remains almost constant, about 0.024 m² (mg Chl a)⁻¹, for blue and green light whatever PAR, a value which matches closely that observed only for very low white irradiance (20 μE m⁻² s⁻¹).

The chlorophyll-specific scattering, *b*ₐ₅₅₅, varied in response to (white) growth irradiance to an even greater extent than *a*ₐ₅₅₅ (Fig. 7B). If the extreme cases are considered, the *b*ₐ₅₅₅ values at 700 μE m⁻² s⁻¹ exceed those at 20 μE m⁻² s⁻¹ by a factor of 7–8.5 within the whole spectral range; for instance, *b*ₐ₅₅₅ (440) varies from 0.14 to 1.18 m² (mg Chl a)⁻¹ and *b*ₐ₅₅₅ (550) from 0.12 to 0.94 m² (mg Chl a)⁻¹ when white irradiance changes from 20 to 700 μE m⁻² s⁻¹. Note that the *b*ₐ₅₅₅(λ) spectra for cells grown under blue or green light (not shown in Fig. 7B) all remain within the narrow range delimited by the spectra for white low irradiances, with PAR = 20 and 40 μE m⁻² s⁻¹.
The *Synechocystis* culture behaves as a suspension of optically small scatterers, with an increasing scattering toward the short wavelengths. Because the process of absorption affects the scattering of light, a spectral selectivity, approximately inverse to that of absorption, is superimposed on the overall pattern of scattering with wavelength. The scattering minima, when sufficiently pronounced (i.e. mainly at low irradiances), are slightly shifted to the short wavelength side of the associated absorption maxima. As an example, the variations of \( b_{\text{Chl}}^* \) within the red band of Chl \textit{a} absorption for the culture grown under 20 \( \mu \text{E m}^{-2} \text{s}^{-1} \) are shown in the inset to Fig. 7B with an appropriate expanded scale. This effect of absorption on light scattering was analysed in terms of the anomalous dispersion theory by Morel and Brizac (1981b).

The general change of \( b_{\text{Chl}}^* \) in response to irradiance conditions can be simply interpreted according to a theoretical analysis previously presented (Morel and Brizac, 1981b), which leads to

\[
b_{\text{Chl}}^* = \left( \frac{3}{2} \right) (D c_{i,\text{Chl}})^{-1} \hat{Q}_b. \tag{9}
\]

\( \hat{Q}_b \), which will be discussed later, depends on \( \bar{a} = 2 \tilde{c}(n - 1) \). Inasmuch as mean cell size (included in \( \bar{a} \)) was weakly variable from one experiment to another, \( \hat{Q}_b \), at a given wavelength, will also be weakly variable, at least if \( (n - 1) \) does not change too much (a reasonable assumption at this stage). Therefore the main effect results from the inverse relationship between \( b_{\text{Chl}}^* \) and \( c_{i,\text{Chl}} \), and the general increase of the \( b_{\text{Chl}}^* (\lambda) \) values, when PAR goes from 20 to 700 \( \mu \text{E m}^{-2} \text{s}^{-1} \), chiefly reflects the decrease in chlorophyll intracellular concentration. Refinements of this scheme will be developed later, when the behaviours of \( \hat{Q}_b \) and \( n \) are analysed.

**Carbon-specific optical coefficients**

Figure 8A and B shows the carbon-specific absorption and scattering spectra, \( a_{c}^* (\lambda) \) and \( b_{c}^* (\lambda) \) respectively, for the diverse experiments.

The \( a_{c}^* (\lambda) \) spectra display a more complex pattern than do the \( a_{\text{Chl}}^* (\lambda) \) spectra. They provide a realistic view of the actual light harvesting capability of the living carbon biomass and of its variations with ambient radiative levels. In the blue spectral region, absorption per unit of carbon is unexpectedly changing only within a rather restricted range (at the most by a factor 1.6 at 440 nm) with no well-defined trend. Much wider and regular are the variations at 630 nm (within a factor of 9) produced by the phycocyanin enhancement at low irradiance. The Chl \textit{a} red peak is also increased by a factor of about 5, which obviously reproduces the evolution of the Chl \textit{a}-to-carbon ratio.

The carbon-specific scattering spectra \( b_{c}^* (\lambda) \) vary only within a factor of about 1.5 at 440 nm and less than 1.2 at 700 nm between low-light and high-light adapted cells. The relative ordering of the spectra is governed by the product \( D c_{i,c} \), since equation (9) can be rewritten as

\[
b_{c}^* = \left( \frac{3}{2} \right) (D c_{i,c})^{-1} \hat{Q}_b. \tag{10}
\]

The shape of individual spectra, with their typical ascending slope for decreasing wavelength, results from the spectral variations of \( \hat{Q}_b \) which are examined in the next section.
Fig. 8. The carbon-specific absorption spectra (A), and the carbon-specific scattering spectra (B) of *Synechocystis* culture adapted to different white irradiances (designated by numbers in units of µE m⁻² s⁻¹).

**Efficiency factors for absorption and scattering \( \tilde{Q}_a \) and \( \tilde{Q}_b \)**

These optical properties at the cell level, computed according to equation (5), are shown in Fig. 9A and B. The absorption capabilities, when related to individual *Synechocystis* cells, now change regularly with growth irradiance (a feature not evidenced by the chlorophyll or carbon normalization). The light energy absorbed by a cell adapted to low irradiance is of the order of 20% (in the blue) or 10% (in the red) of the incident energy upon the projected surface area of this cell. These proportions, both in the blue and red regions, decrease when the cells become adapted to high available irradiance. Disregarding the particular (phycocyanin) absorption capacity, this alga does not markedly differ from eucaryotic algae examined in BRICAUD et al. (1988). In spite of the chlorophyll enhancement at low energy, *Synechocystis* can be ranked among the "weak" but efficient absorbers, as many algae often exhibit \( \tilde{Q}_a \) values exceeding 50% in the blue region.

In the short wavelength domain (400 nm), \( \tilde{Q}_b \) is about 0.8 for low-light adapted cells and close to 1 for cells adapted to moderate radiative levels (80–700 µE m⁻² s⁻¹).
Fig. 9. The spectral values of the absorption (A), scattering (B), and attenuation (C) efficiency factors for the *Synechocystis* cells adapted to different (white) growth irradiances (designated by numbers in units of \( \mu \text{E m}^{-2} \text{s}^{-1} \)).

means that such a cell can cause scattering of an amount of light equal to that which impinges on its cross-sectional area. With increasing wavelength, \( \bar{Q}_b \) decreases towards 0.35–0.45 (at \( \lambda = 720 \text{ nm} \)), with a regular descending slope on which some features are superimposed. These features, related to absorption, are obviously more remarkable for those cells, grown under low irradiance, which are the most absorbing.

The behaviour of the efficiency factors \( \bar{Q}_b \) and \( \bar{Q}_c \) as a function of size and refractive properties of a homogeneous spherical particle can be interpreted in terms of the Mie theory, or equally well of the “anomalous diffraction approximation” (Van de Hulst, 1957) which provides a simplified treatment valid under the proviso that the refractive index of the particle is very close to that of surrounding medium. Such a simplification gives a valuable approximation to algal cells for most practical applications (see
discussion in Morel and Briceaud, 1986). Both the spectral pattern and the range of experimentally derived values of $Q_c$ and $Q_b$ for the *Synechocystis* cell are well explained by the theory. For this cell, characterized by a small relative size ($\alpha = 7-13$ when $\lambda$ varies from 700 to 400 nm), the parameter $\tilde{\rho}$ remains within the approximate range 0.7–1.3, if the relative index $n$ is assumed to be of the order of 1.05 (see below). Such $\tilde{\rho}$ values correspond to the initial rising part of the theoretical $Q_c (\tilde{\rho})$ (or $Q_b (\tilde{\rho})$) function, before its first maximum (occurring at $\rho = 4$). Therefore the $Q_c (\lambda)$ or $Q_b (\lambda)$ values (smaller than 1 when $\tilde{\rho} \leq 1.5$) display a decreasing trend from the blue to the red end of the spectrum.

**Relative index of refraction**

The imaginary part of the index of refraction is derived from the $\tilde{Q}_a (\lambda)$ spectrum. Two selected examples corresponding to the most contrasted situations (cells grown under 20 and 700 $\mu$E m$^{-2}$ s$^{-1}$) are shown in Fig. 10A. The spectral shape of $n'$ is closely related to that of $\tilde{Q}_a$.

Given $n'$, $F(D)$, and experimental $\tilde{Q}_c (\lambda)$, one can calculate $n$. The spectral values of the real part of the index $n (\lambda)$ are shown in Fig. 10B. For the less absorbing cells (grown at high light) $n$ is relatively constant with values about 1.058. As expected, lower $n$ (\lambda) values are obtained for the more absorbing, low-light adapted cell, with a more complex pattern due to the anomalous dispersion effect (resulting in a typical red-shift of the $n$ maxima with respect to the $n'$ maxima).

Figure 11 shows the pooled data concerning the real part $n$ at the particular wavelength 440 nm. The rationale for this choice, discussed in detail elsewhere (Stramski et al.,

![Fig. 10. The spectral values of the imaginary (A) and real parts (B) of the relative refractive index for the cells adapted to low and high (white) irradiances as indicated (in $\mu$E m$^{-2}$ s$^{-1}$).](image-url)
Fig. 11. (A) Real part of the refractive index at the wavelength 440 nm as a function of growth irradiance. The data for white irradiance are linked by the dotted line. (B) Refractive index, $n$ (440 nm), vs intracellular carbon concentration. The linear regression is plotted as the solid line. The standard deviation around the regression line is shown as dashed lines; the typical error bars are indicated for one of the points. The different symbols used to distinguish colour of growth irradiance are as in (A).

1988) is that $n(440)$ equals, or is very close to, the so-called "central value" (BRICAUD and MOREL, 1986), i.e. a value free from absorption effect. Such a value is fixed by the nature of the cellular material. This material can be seen as a mixture of (dry) organic compounds to which a considerable amount of water is added. The variations in $n$ are mainly ruled by the water content rather than by the chemical composition of the organic pool (see discussion in MOREL and BRICAUD, 1986). Therefore, it can be presumed that $n$ is closely related to $c_{i,C}$, the intracellular carbon concentration. This hypothesis is supported by data in Fig. 11B. The linear regression for these data, including the point $n = 1, c_{i,C} = 0$, provides (with $r^2 = 0.88$)

$$n(440) = 2.43 \times 10^{-4} c_{i,C} + 1.0051 \pm 0.0052,$$

(11)

where $c_{i,C}$ is expressed in kg m$^{-3}$. The uncertainties in the cell count and size distribution seem to be of crucial importance here. As indicated previously (STRAMSKI et al., 1988), the uncontrolled changes in the size distribution could lead to variations in the central value of refractive index to within $\pm 0.0025$. The intracellular carbon concentration varied, from day-to-day for given culturing conditions, typically by 10% (occasionally by 25%). The error bars are indicated in the figure for one of the experiments. The intracellular carbon could perhaps be used as an indicator of the bulk refractive index of algal cells. However, the validity and uniqueness of such a relationship, when diverse species and a wider range in $n$ and $c_{i,C}$ are considered, remain to be verified. In spite of
Photosynthetic picoplankton in different physiological states

Fig. 12. Normalized volume scattering functions at $\lambda = 560$ nm computed for *Synechocystis* cells adapted to low (solid line) or high (dotted line) white irradiance (logarithmic scale for the vertical axis).

This reservation, it is worth noting that extrapolation of the above relationship (equation 11) to marine bacterioplankton, for which $c_{i,c}$ could be as high as 380 or 560 kg C m$^{-3}$ (Lee and Fuhrman, 1987; Bratbak, 1985) could lead to a relative refractive index up to 1.14. If such high values are confirmed, heterotrophic bacteria would be the most refringent particles amongst the living microorganisms with a refringence similar to that of calcite debris.

**Volume scattering function and backscattering efficiency**

The angular distribution of scattered radiation, computed for $\lambda = 560$ nm, is shown in Fig. 12 for two extreme cases. The computations [according to equation (7)] were made by using the actual $F(D)$ distribution and refractive indices 1.050–0.0015 i and 1.055, respectively, for cells grown under 20 and 700 $\mu$E m$^{-2}$ s$^{-1}$. These values of indices approximate the actual ones at $\lambda = 560$ nm. The two volume scattering functions are close to each other and not very different from that previously measured (and computed) for the same species (Morel, and Bricaud, 1986). The mean size was slightly higher whereas the index was lower in this previous experiment, in spite of similar culturing conditions. (In effect a certain plasticity, confirmed by other unreported experiments, exists in this species.) A tiny object such as this cyanobacterium cell still behaves as a “large” particle and the forward scattering predominates in the scattering process. The similarity of the curves in Fig. 12 is theoretically predictable; the scattering pattern for small sized picoplankters ($\alpha < 10$) is chiefly determined by the size of the particle and is almost independent from its refringence (Morel and Bricaud, 1986).

For the same examples as above and by using equations (8) and (8'), the computed spectral values of the backscattering efficiency factors, $\bar{Q}_{bb} (\lambda)$, are shown in Fig. 13A. Contrary to $\bar{Q}_b$, they display an increasing trend towards the red end of the spectrum on which is superimposed a pattern, approximately inverse to that of absorption. The normalized backscattering coefficients (Fig. 13B) are much higher than those exhibited by bigger cells studied by Bricaud et al. (1983). They remain nevertheless within the range 0.1–0.4%. These figures showing the regular increasing trend with increasing wavelength are typical for picoplankters. With no absorption, it can be predicted (Morel and Bricaud, 1986) that $\bar{b}_b$, weakly dependent on the real part of the refractive index,
would increase as $\lambda^2$. The overall trend of the experimental curves coincides well with the parabola drawn as a dotted curve. Absorption and non-zero $n'$ values result in diminishing $\bar{b}_b$. The depressing effect of absorption on $\bar{b}_b$ (or on $\bar{Q}_{bb}$) is always expected to be more marked than it is when dealing with $\bar{Q}_b$ (Morel and Brécaud, 1981b).

**Conclusions**

With respect to other photosynthetic phytoplankers cyanobacteria exhibit specific optical properties because they are small and contain, in addition to the chlorophyll–carotenoid complexes, a variable amount of biliprotein absorbing pigments. *Synechocystis* is not, however, an exceptional optical “object”. Like many common algal cells, this cyanobacterium is more efficient in scattering than in absorbing light. Its VSF displays a strong asymmetry as do larger particles. The small size, however, results in a smaller scattering efficiency ($Q_b < 1$), which decreases toward the red end of the spectrum. This pattern, typical of very small scatterers, could tentatively be expressed as a $\lambda^{-x}$ function, with $x$ about 1.3 ($\pm 0.3$). Another attribute of such small cells is a relatively enhanced backscattering efficiency compared with that of the common larger eucaryotic cells. The backward scattering ratio, $\delta_b$, is nevertheless not very high; it ranges from 0.1 to 0.4% for $\lambda = 400$–700 nm, respectively, and as predicted varies like $\lambda^2$ within this domain (Morel and Brécaud, 1986).
The intrinsic optical coefficients expressed on a per-cell basis (the $Q$-factors) or a carbon basis (the carbon-specific coefficients) are much more preferable to the chlorophyll-specific coefficients. The normalization with respect to a single pigment, Chl $a$, becomes strongly misleading when drastic variations in the proportions of accessory pigments and Chl $a$ occur. Characterization is better when scattering is normalized vis-à-vis carbon concentration. In Fig. 14 the scattering coefficient, $b(560 \text{ nm})$, from all experiments with *Synechocystis* (white, blue and green irradiances), are plotted against carbon concentration [28 points, plus the (0, 0) point]. When subjected to a linear regression analysis, a good fit is obtained ($r^2 = 0.82$). leading to:

$$b(560) = 3.08 \times 10^{-3} C + 0.26 (\pm 1.85),$$  \hspace{1cm} (12)

where $b$ is expressed in m$^{-1}$ and $C$ in mg m$^{-3}$. The slope parameter can be regarded as the single carbon-specific scattering coefficient, $b^\ast (560)$, of the *Synechocystis* population, regardless of its physiological state and growth irradiance.

The characterization of an algal population through its carbon-specific coefficients (or its $Q$-factors) is relatively easy in the case of *in vitro* monospecific culture experiments. Unfortunately the transposition to natural populations is difficult or even impossible because the cross-sectional area of the living algae must be determined in the presence of a complex assemblage of particulate (detrital and bacterial) matter. The problem is particularly difficult in the case of scattering, since the coefficient $b$ for Case I waters appears, to a first approximation, to be linearly related to the total particulate organic carbon content, whatever its nature (*Morel*, 1988). It is intriguing that the relationship expressed by equation (12) is close to, or overlapped by, a general relationship deduced from equations (18) and (28) in the above reference

$$b = 2.7 \times 10^{-3} \text{ (POC)},$$

where (POC), also in mg C m$^{-3}$, describes living as well as inanimate particulate organic carbon.

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**Fig. 14.** Scattering coefficient $b$ at $\lambda = 560$ nm vs carbon concentration in the cell suspension. Included are 28 data points obtained under all lighting conditions (energy and colour) used during the present experiment. The solid line is a least-squares fit to the points, the dashed lines show the standard error of estimate.
Absorption contributes less than 20% of the total attenuation by *Synechocystis*. The highest contribution is reached only inside the absorption bands (around 440, 630 and 680 nm), and only when the cells are adapted to low light levels. In terms of theory, such low $\hat{Q}_a$ values mean that *Synechocystis* is in the “quasi-linear” domain of absorption (Morel and Brinaud, 1981a). In other words, the package or discreteness effect remains weak even for the most pigmented cells. The adaptative strategy which consists of enhancing the chlorophyll content per cell in response to low radiative levels is therefore fully efficient. This is a feature of very small sized cells (even if the smallness is not a sufficient condition; see below). *Synechocystis* appears to be remarkably well suited for absorption. The absorption cross-section per unit of carbon, restricted to the blue part of the spectrum (as in deep oceanic layers), is not far from $1 \text{ m}^2 (\text{g C}^{-1})$, at least twice that of other species discussed in Morel et al. (1987), including the *Synechococcus* studied by Kana and Glibert (1987).

It is widely believed that picoplankton, given their size, should systematically exhibit a reduced package effect and thence an increased efficiency (per unit of pigment) in capturing light. If *Synechocystis* constitutes a good illustration of such an advantage, this argument cannot be generalized. The package effect depends equally on the size and the intracellular pigment concentration (through their product, e.g. Morel and Brinaud, 1981a), therefore the advantage induced by the smallness can be thwarted if the inner pigment content becomes very high. Such ill-oriented arrangements seem to exist in the natural environment; extremely high intracellular Chl $a$ concentrations have been recently observed in tiny cells (0.2–0.8 $\mu$m, Prochlorophyta ?) living in the deep chlorophyll maximum of the Sargasso Sea (Neveux et al., 1989).

A detailed discussion of the adaptative modifications and their physiological implications is outside the scope of the present paper. It is, however, worth noting that, aside from the irradiance level, the spectral composition seems to play a specific role in the case of *Synechocystis* and of its particular pigmentation. According to the shapes of the absorption spectra, the phycocyanin synthesis and accumulation drastically decrease in response to increasing white irradiance, and in a less abrupt manner if blue or green light are used [when the cells are grown under 125 (green) or 140 (blue) $\mu$E m$^{-2}$ s$^{-1}$, the phycocyanin content, as depicted by $a^*_{	ext{chl}}$ (630), is essentially similar to that observed in white light, but for a radiative level as low as 20 $\mu$E m$^{-2}$ s$^{-1}$]. This suggests that phycocyanin can be effectively synthesized when no “red” light, amenable to absorption by this pigment, exists. As a consequence, the major role of phycocyanin would not necessarily be harvesting radiant energy. This conclusion is in any case supported by the fact that in the natural oceanic environment, low radiation levels are also synonymous with blue or blue-green ambiances.

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REFERENCES


**APPENDIX**

Determination of the specific absorption coefficient of Chl *a* dissolved in methanol and in DMSO.

Purified Chl *a* (Sigma), isolated from *Anacystis nidulans* was used for this determination. Absorption spectra were recorded for Chl *a* dissolved either in 95% methanol or in 90% acetone. The latter solvent is used as a standard one as the (Naperian) specific absorption coefficient at 663 nm, 0.0202 m²/(mg Chl *a*)⁻¹, is well ascertained and widely accepted (JEFFREY and HUMPHREY, 1975). Several comparative measurements repeatedly led to 0.0191 m²/(mg Chl *a*)⁻¹ at the maximum occurring at 665 nm, when Chl *a* is dissolved in 95% methanol; this value is approximately 10% higher than that previously reported by Lenz and Zeitschel (1968). In the same way, a value of 0.0196 m²/(mg Chl *a*)⁻¹ at 665 nm for 95% DMSO was also reported. These values were (and could be) useful when testing the efficiency of pigment extraction by various solvents.

On many occasions, the Chl *a* concentration was, in parallel, fluorometrically determined on the methanol extracts with a Turner Model 111 calibrated with pure Chl *a*. Typically the Chl *a* estimates through absorption at 665 nm or through fluorescence agreed to within a very few percent. This lends confidence to the rapid, monochromatic determination applied in the present study.