Optical efficiency factors of some phytoplankters

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Abstract

Absorption and total scattering coefficients of four phytoplankton species grown in batch cultures were measured simultaneously. Backscattering coefficients were obtained by using an integrating sphere. These coefficients are transformed into specific coefficients, i.e., related to a unit of concentration in chlorophyll a, and also into dimensionless efficiency factors characteristic of the cells. The specific coefficients differ noticeably from one species to another. Total scattering and backscattering coefficients are clearly depressed near and inside the absorption bands. These minima can be interpreted by combining the theory of anomalous dispersion with Mie-Lorentz theory applied to polydisperse suspensions. The backscattering efficiency (ratio of backscattering to total scattering) of algal cells appears to be very low (typically <0.1%). These different results must be taken into consideration when interpreting and modeling the optical properties of seawater, particularly ocean color. They also must be considered when modeling photosynthesis, since the variations in the light-harvesting ability of the cells intervene directly in the quantum yield estimate.

The optical properties of natural waters depart from those of ideally pure water because of the presence, in variable amounts, of diverse dissolved and particulate substances. From an optical point of view, these can be considered as belonging to four groups: live algal cells; biogenous detritus associated with and deriving from these algae; terrigenous particles and resuspended sediment; and dissolved organic (yellow) substances. Of these, phytoplankton, as the only primary producer in the pelagic environment, is of particular interest. In addition, the algal cells and their debris predominantly determine the optical properties of the water in the open sea, away from land and bottom influence.

Depending on the physiological state of the algae and on environmental conditions, primary production is effected through photosynthesis, with a varying yield. Such a yield, estimated on a quantum basis, can be computed if—besides the amount of CO₂ fixed—the amount of radiation (quanta) actually absorbed is known. This means that the efficiency factor for absorption, Qₐ, has to be determined (Qₐ is the ratio of radiant energy absorbed within this cell to the energy impinging on its geometrical cross section).

The study of the influence of algae on the optical properties of the sea implies knowledge of both their absorption and scattering properties. This knowledge is also needed for better understanding of the inverse problem: the interpretation, in terms of algal concentration, of the “color” of the sea (i.e., its spectral reflectance) which can now be remotely sensed.

The present research has been motivated by these converging needs. It is restricted to only a few algal species, but is intended to give more general information on experimental methods and a theoretical framework concerning the “optics” of living algal cells. The symbols and definitions used follow the standard terminology adopted by the International Association for Physical Sciences of the Ocean, IAPSO (see Morel and Smith 1982).

According to Preisendorfer (1961, 1976), the absorption, scattering, and attenua-

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tion coefficients, $a$, $b$, and $c$ (with $c = a + b$), and the volume-scattering function, $\beta (\theta)$, form the so-called inherent optical properties ("IOP"). Because the additivity principle applies strictly to these properties, the IOP of seawater result from addition of the IOP of the components listed above. The IOP of seawater are needed to interpret the apparent optical properties ("AOP": Preisendorfer 1961, 1976), such as reflectance of the sea, which are involved in remote sensing applications. Relations between AOP and IOP have been established through different radiative transfer models (Duntley 1942; Kozlyaninov and Pevelin 1965; Plass and Kattawar 1969, 1972; Prieur 1976). In particular, the diffuse reflectance, $R$, appears to be linked to IOP by a relation of the form: $R(\lambda) = F(b_\beta(\lambda)/a(\lambda))$ where $\lambda$ represents the wavelength, and $b_\beta$ is the backscattering coefficient. The coefficient $F$ is a function of the incident radiant energy (Gordon et al. 1975; Kirk 1981) and also of the volume-scattering function within the water (Prieur and Morel 1975).

The magnitude and spectral behavior of $a$, $b$, and $b_\beta$ of phytoplankton are not well known. "Specific" values for absorption, $a^*$ (i.e. values normalized to a unit concentration in Chl $a$) reported in the literature show large discrepancies (Morel and Bricaud 1981a). The absolute values of specific scattering by algal cells, $b^*$, are poorly documented, and most studies are concerned only with the shape of the spectra. Colored particles such as algal cells or chloroplasts must exhibit selective scattering according to theoretical predictions (Bryant et al. 1969; Mueller 1973; Gordon 1974; Morel and Bricaud 1981b). For some phytoplankters, the influence of absorptive properties on scattering has been shown (e.g. Latimer 1959, 1963; Charney and Braddock 1961; Pivoznik et al. 1978). The almost featureless spectral attenuation, $c(\lambda)$ (with $c = a + b$), as observed by Takematsu et al. (1979) and Haardt et al. (1979), implies that the spectral variations in $a$ must be compensated by those in $b$.

Measurements of diffuse backscattering (as defined by Duntley 1942; this coefficient, influenced by multiple scattering, differs from $b_\beta$) demonstrate that this property is wavelength-dependent and affected by absorption (Duntley et al. 1974; Kiefer et al. 1979). So far as we know, the specific backscattering coefficient of phytoplankton has not been measured, and a comparison between back- and total scattering ($b_\beta/b$) not attempted. Such a comparison is needed, since theory shows that these coefficients may exhibit nonidentical spectral variations (Morel and Bricaud 1981b).

Our study concerns four phytoplankters grown in batch culture. It is a first attempt to determine experimentally the efficiency factors for absorption, scattering, and backscattering, $Q_a$, $Q_s$, $Q_{bb}$ (or, equivalently, the corresponding specific coefficients $a^*$, $b^*$, $b_{\beta}^*$), to identify the links between these efficiency factors—or coefficients—and finally to explain these links in the light of theoretical considerations.

**Materials and methods**

**Cultures**—The strains were supplied by Station Marine de Villefranche-sur-Mer [Hymenomonas elongata (Droop) Parke and Green], by Station Marine d'Endoume, Marseille [Platyynos sp. and Coccolithus huxleyi (Lohmann) Kamptner], and by CERBOM, Nice (Tetraselmis maculata Butcher).

The culture medium was deep-sea water taken off Villefranche Bay, aged and filtered under sterile conditions through a Sartorius membrane filter (0.2-µm pore size), enriched with nutrients E.S. to a concentration of 20 ml·liter$^{-1}$ (Provasoli 1966) and with a 2 ml·liter$^{-1}$ of a silicate solution (1 g·liter$^{-1}$), then refiltered on a Sartorius filter of the same pore size. Algal batch cultures were grown by inoculating axenic strains into sterilized conical flasks which were kept at 18°C ($\pm$1°C) under continuous irradiance ($2 \times 10^{18}$ quanta·s$^{-1}$·cm$^{-2}$), supplied by fluorescent tubes, for 4–19 days according to the species. Measurements were performed on healthy cultures in active growth, sufficiently rich in chlorophyll but contain-
ing no significant amount of pheopigments. Therefore, they can be considered prac-
tically free from the influence of detritus which would certainly modify the bulk optical properties of algal sus-
ensions in an unknown manner.

Absorption measurements—Spectral variations of absorption by phytoplank-
ton in vivo cannot be obtained directly by measuring the beam transmittance 
with a conventional spectrophotometer, since a cell suspension is a highly scatter-
ing medium. For such a medium, the coefficient actually measured, \( x \), is inter-
mediate between the attenuation coefficient, \( c \), and the absorption coefficient, \( a \).
It can be expressed by \( x = a + (1 - \varepsilon)b \), where \( \varepsilon \) depends on the fraction of scattered 
light entering the detector (see e.g. Latimer 1975; Bricaud et al. 1981). If all 
the scattered light were rejected by the detector, the apparatus would be a per-
fet \( c \) meter (\( \varepsilon = 0, x = c \)). Conversely, if 
the scattered light were totally included, it would be a perfect \( a \) meter (\( \varepsilon = 1, x = a \)).

Several methods have been proposed for measuring absorption by phytoplank-
ton in vivo (Doucet and Kubin 1976). We measured absorption by setting the algal 
suspension just in front of the detector (Fig. 1B). In this arrangement, \( \varepsilon \) is very 
close to 1. In order to increase further the efficiency of this method—which depends on both the geometrical configu-
ration of the spectrophotometer and the scattering properties of particles—we intro-
duced a diffusing plate between the sample and the detector (cf. Shibata et al. 
1954; Amesz et al. 1960).

The experiments were performed by using the so-called scattered transmis-
sion accessory available for the Perkin-
Elmer 571 spectrophotometer, imple-
mented with a second diffusing plate. 
Such an optical arrangement allows the 
scattered light to be received within a 
half-angle of about 43°. Theoretical com-
putations of volume-scattering functions 
(VSF) for spherical particles (through Mie 
theory), made for mean sizes (\( \bar{d} \)) and re-
fractive indices (\( n \)) assumed to be re-
representative of algal suspensions (\( 2 \leq \bar{d} \leq 20 
\mu \text{m}, 1.02 \leq n \leq 1.05 \)), showed that \( \varepsilon \) was 
always >0.995. Consequently, in this 
configuration, \( x \) is not significantly differ-
ent from \( a \).

Total scattering measurements—The scattering coefficient can be obtained in 
an indirect way by successively measur-
ing \( a \) and \( c \). The ideal condition of mea-
surement of \( c \) would be when \( \varepsilon = 0 \). Un-
fortunately, in the case of algal cells, the 
forward peak of the VSF is so pro-
nounced that \( \varepsilon \) cannot be negligible, un-
less the acceptance angle of the detector 
is extremely reduced. In the normal con-
figuration (Fig. 1A), the half-angle is equal to about 1°. Theoretical computa-
tions as above show that \( \varepsilon \) cannot then be neglect-
ed, except for particles of very small size. 
Consequently, the half-angle was re-
duced to about 0.25° by using additional 
field stoppers (Fig. 1C). In this case, \( \varepsilon \) is 
considerably lowered (typically <0.05: 
Fig. 2) even for large algal cells, and the 
measured coefficient \( x = a + (1 - \varepsilon)b \) is 
very close to \( c \). The actual value of \( \varepsilon \) re-

Fig. 1. Relative positions of cells and detector in the spectrophotometer for measuring absorption and attenuation on a suspension. The coefficient measured in the conventional arrangement (A) is \( x(\lambda) \), intermediate between absorption and attenuation coefficients (see text). In positions B and C, it becomes respectively close to the absorption \( a(\lambda) \) and attenuation \( c(\lambda) \) coefficients.
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Fig. 2. Theoretical variations vs. relative size of particles, \( a = \pi d \lambda \) (\( d \)—diameter of particles; \( \lambda \)—wavelength in surrounding medium) of the coefficient \( \epsilon_{0.25} \) and \( \eta. \) \( \epsilon_{0.25} \) represents the ratio of flux scattered between 0° and 0.25° (computed with 0.01° increment) to total scattered flux for two values of index assumed to be representative of algal cells (dotted lines). The coefficient actually measured in position C (Fig. 1) is \( a + (1 - \epsilon_{0.25})b \) (see text); since \( \epsilon_{0.25} < 0.05 \) for large cells, and much weaker for small ones, this coefficient is close to \( c = a + b. \) \( \eta \) represents the ratio of flux scattered between 133° and 180° to total backscattered flux (90°–180°) for the same values of index (continuous lines). This ratio represents approximately the proportion of backscattered flux entering the integrating sphere and was used to estimate the absolute values of backscattering (see text). In the upper part of the figure are indicated the ranges of variation of \( \eta \) between 350 nm (V) and 700 nm (R) for the species studied.

mains uncertain due to lack of precise knowledge of the refractive index. No correction was made, and \( b \) was calculated at each wavelength by simply subtracting \( a \) from \( x. \) Consequently, \( b \) is probably underestimated by a factor of 5% at the most.

\( a \) and \( c \) were measured in 1-cm-path-long cells. The reference cell contained the culture medium after filtration, to eliminate molecular scattering and absorption, as well as absorption by possible organic substances produced by the algal cells and dissolved in the medium (cf. Yentsch and Reichert 1962).

When necessary, the suspension must be diluted to ensure that multiple scattering and absorption do not significantly affect the \( a \) or \( c \) values. A simple test con-...
the inner wall of the sphere, placed in the path of the incident beam by rotating the sphere around a vertical axis. Relative values of $b_\gamma$ were obtained by calculating the ratios $[\Phi_s(\lambda) - \Phi_r(\lambda)]/\Phi_0(\lambda)$.

The spectral variations of $\Phi_r$, $\Phi_s$, and $\Phi_0$ were measured successively (as functions of $\lambda$) between 400 and 750 nm. The need for three successive measurements imposes severe constraints on the optical-electronic stability. The weakness of the backscattered energy requires both a high gain and an efficient reduction of the stray light level. A highly stabilized power supply for the tungsten lamp was used, and the beam was chopped in order to minimize noises and drifts due to electronic components as well as to ambient stray light. The flux reflected by the exit window of the cell, and then scattered by the suspension toward the integrating sphere, was minimized by using long (110 cm) cells with a black coating on the exit window.

In the procedure described above, the fluorescence of Chl $a$ (emitted at about 685 nm) is excited by the incident beam and erroneously accounted for as backscattering. This additional light consequently distorts the spectral values of backscattering. This effect was corrected for by a method similar to that of Latimer and Rabinowitch (1959). The respective proportions of fluorescence and backscattered light can be estimated at each wavelength—outside and even within the fluorescence bands—from additional measurements with red filters in front of the detector. A detailed description of the method is given by Bricaud (1979). Figure 4 shows an example of spectral values, before and after correction. As expected, this effect is especially important in the blue part of the spectrum (within the peak of the excitation spectrum, around 436 nm—see Rabinowitch and Govindjee 1969).

The method provides only relative values of $b_\gamma$. Since molecular backscattering coefficients are known with sufficient ac-
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Fig. 4. Spectral values of backscattering obtained with *Platymonas* sp., before (1) and after (2) correction for fluorescence. The difference of the two curves (3) represents nothing else than the excitation spectrum of fluorescence of this sample, since it represents the amount of light emitted by fluorescence in the red part of the spectrum as a function of the incident (excitation) wavelength.

cally \( \pm 0.002 \) around the mean value), consequently \( \eta \) can be considered constant. This quasi-constancy justifies the principle of the measurement, which postulates the proportionality of the measured signal to \( b_b \). For lack of precise information concerning the index of cells, we have adopted a mean value of \( \eta = 0.2 \) for the species studied; absolute values have been obtained by multiplying relative values by 5. Obviously these absolute values cannot be fully ascertained, and the results must be considered as only a first approximation in estimating the backscattering by algal cells.

As with the measurements of \( a \) and \( c \), the suspension has to be diluted to minimize the influence of multiple scattering on the measurement of \( b_b \).

Other parameters—Chlorophyll \( a \) and pheophytin \( a \) concentrations were determined on acetone extracts (Lorenzen 1967). Chlorophyll \( b \) and \( c \) concentrations (including pheopigments) were calculated through SCOR-UNESCO equations, and total carotenoids with Richards' equation (see Strickland and Parsons 1968). The cell density was determined with a Fuchs-Rosenthal hemacytometer; the cell size was measured with a Coulter Counter TA and also indirectly estimated as explained below (cf. application).

Results and discussion

The spectral values of absorption, \( a \), attenuation, \( c \), and total scattering, \( b \), were determined for the four algal species. Information concerning these cultures is given in Table 1. Independently, spectral values of backscattering, \( b_b \), were determined on the same species (except for *C. huxleyi*) at the same age. Figure 5 shows the values of these coefficients, hereafter called specific coefficients, i.e. related to a unit of Chl \( a \) (+Pheo \( a \), if any); the amount of pheophytin was always low (<6%) or even not significant. The absorption curves of acetone extracts, also corresponding to unit pigment concentration, are also shown.

These specific coefficients were converted into efficiency factors for absorption \( (Q_a) \), attenuation \( (Q_c) \), scattering \( (Q_b) \),

accuracy, the absolute values were calibrated from measurements of optically pure water (Bricaud 1979). Distilled water, evaporated without boiling and then condensed on a cooled quartz wall, was used. The coefficients obtained approximately obey the power law \( \lambda^{-4.63} \) between 480 and 700 nm, in good agreement with the actual law \( (\lambda^{-4.3}) \) (Morel 1974). This agreement demonstrates the validity of the method and the reliability of the measurements.

From these results concerning molecular backscattering, we estimated that the light returned into the integrating sphere corresponds to light scattered between about 180° and 133°. The ratio, \( \eta \), of the entering flux (133°–180°) to the total backscattered flux (90°–180°) was then obtained from computations of VSF assumed representative of algal cells. Figure 2 shows the variations of \( \eta \) vs. the relative size of cells for reasonable values of the refractive index. It appears that the value of \( \eta \) is more dependent on the index of cells, i.e. on their structure, than on their relative size (\( \alpha \)). For a given suspension, the variations of index throughout the visible spectrum are weak (typi-
Table 1. Information concerning algal cultures studied: concentrations of the different pigments; mean equivalent diameter of the cell population, $d$, as measured with Coulter Counter TA and as estimated from $c^*$ curves (see text); equation for intracellular pigment concentration $c_i$ given in text; imaginary part of the refractive index, $n'$ (computed as described by Morel and Bricaud 1981a), for $\lambda = 435$ nm and 600 nm.

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Chl a (mg m$^{-2}$)</th>
<th>Chl b</th>
<th>Chl c</th>
<th>Carot.</th>
<th>Pheo a or Carot. (Chl a + Pheo a) (%)</th>
<th>$d$ (um)</th>
<th>$c_i$ (mg Chl a m$^{-2}$)</th>
<th>$n'$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Meas.</td>
<td>Computed</td>
<td>435 nm</td>
<td>600 nm</td>
</tr>
<tr>
<td>Hymenomonas elongata</td>
<td>19</td>
<td>408.5</td>
<td>—</td>
<td>56.5</td>
<td>523.2</td>
<td>6.0</td>
<td>120</td>
<td>2.94 x 10$^4$</td>
</tr>
<tr>
<td>Platydomonas sp.</td>
<td>10</td>
<td>624.4</td>
<td>297.8</td>
<td>—</td>
<td>661.6</td>
<td>3.9</td>
<td>102</td>
<td>7.4</td>
</tr>
<tr>
<td>Tetraselmis maculata</td>
<td>4</td>
<td>466.5</td>
<td>231.2</td>
<td>—</td>
<td>434.7</td>
<td>0</td>
<td>93</td>
<td>9.5</td>
</tr>
<tr>
<td>Coccolithus huxleyi</td>
<td>7</td>
<td>89.6</td>
<td>—</td>
<td>53.4</td>
<td>129.2</td>
<td>5.8</td>
<td>136</td>
<td>1.14 x 10$^4$</td>
</tr>
</tbody>
</table>

(where $d$ is size of this mean cell and $c_i$ is intracellular pigment concentration)

$Q_o = \frac{3\pi b^2}{l_0}$

$Q_e = \frac{3\pi b^2}{l_0} e^*$

and

$Q_{o'} = \frac{3\pi b^2}{l_0} e'$(1)

(2)
Fig. 5. Specific spectral values of absorption ($a^*$), attenuation ($c^*$), total scattering ($b^*$), and backscattering ($b_b^*$) determined on four algal species and normalized to unit (Chl $a$ + Pheo $a$) concentration ($1 \text{ mg} \cdot \text{m}^{-2}$) (the parts of the $b_b^*$ curves plotted as dashed lines correspond to a signal-to-noise ratio $<10$). The spectral values of absorption by the corresponding acetone solution of extracted pigments are also given with the same normalization (dotted lines). Right scales—corresponding values of efficiency factors for absorption ($Q_a$), attenuation ($Q_c$), total scattering ($Q_b$), and backscattering ($Q_{bb}$). Spectral values of $b_b^*$ are not shown for C. huxleyi. A previously published curve (fig. 4: Morel and Bricaud 1981b) has been discarded because later microscopic examination showed contamination of that culture. The present data are from an uncontaminated culture and deal only with $a^*$, $b^*$, and $c^*$.

unidentified. Cellular components not extractable, or destroyed, by organic solvents, presumably account for it.

The values of specific absorption by living cells vary strongly with species, especially in the blue part of the spectrum. The reasons for the dispersion of the specific values have been examined in detail
by Morel and Bricaud (1981a). In summary, these variations arise from the differences in size and pigment concentration of the various algal cells, by the variations in their accessory pigment composition, and casually by the presence of detrital particles associated with the cells.

The absorption properties of a discrete medium containing a given amount of an absorbing substance present as suspended particles vary with the type of suspension. This effect of "discreteness" of the absorbing substance (i.e. of the cellular material) is a function of the product of two characteristics of the cells: their size \(d\) and the absorption coefficient \(a_{cm}\) of the material forming the cells. For a given \(a_{cm}(\lambda)\) spectrum, the specific absorption decreases with increasing size, and simultaneously the absorption spectrum is "flattened." Conversely, for a given size, the specific absorption decreases with increasing \(a_{cm}\).

This discreteness effect can be eliminated by computing the absorption properties of the substance forming the cells, hypothetically dispersed in solution. This is achieved by extrapolating the absorption properties of the actual cells (which are assumed to be spherical and homogeneous) to a null diameter (Morel and Bricaud 1981a). The extrapolated curves so computed are shown in Fig. 6. The differences between these extrapolated spectra should in principle be due only to differences in the pigment composition. With the normalization to a unit concentration in Chl \(a\), these spectra should exhibit identical red peaks (or almost identical, because of the interfering influence of Chl \(b\) and Chl \(c\)), whereas they might differ in the blue part of the spectrum. The quasi-identity of the red peaks is not really attained, probably because of the simplifying assumptions used in the computations. Nevertheless the variability of this peak appears considerably reduced for extrapolated spectra compared to in vivo spectra: the range of variation of its height, measured above a somewhat arbitrary baseline, is 0.018–0.023 m²·mg⁻¹ instead of 0.0125–0.022 m²·mg⁻¹ (this baseline was handmade by assuming that the residual near-IR absorption increases toward shorter wavelengths). It seems also that the specific value for Chl \(a\) hypothetically dissolved in water tends to resemble the value in acetone solution (0.0206 m²·mg⁻¹ according to SCOR-UNESCO equations).

The specific absorption is also influenced by the pigment composition. Carotenoids, in addition to Chl \(a\), absorb strongly in the blue. They are present in variable amounts (see Table 1), and consequently the specific absorption in the blue varies when the Chl \(a\) concentration is taken as an index of the pigment content.

Moreover, the absorption is influenced by the presence of detrital particles originating from the phytoplankton and present in variable amounts according to the age of the culture. The influence of debris on the magnitude and spectral behavior of absorption has been demonstrated by Kiefer et al. (1979). With pheopigment concentrations low or zero in our experiments (see Table 1), this disturbing effect is reduced or eliminated, and we believe the results presented here represent only living cells.

Spectral values of attenuation and total scattering—The spectral attenuation curves (Fig. 5) are almost featureless. The \(c^*(\lambda)\) values increase regularly toward shorter wavelengths (C. huxleyi), or remain about constant throughout the spectrum. The absorption bands do not significantly influence the \(c^*(\lambda)\) curve, apart from a distinct but small feature in the vicinity of the red peak.

With \(b(\lambda) = c(\lambda) - a(\lambda)\), and the \(c(\lambda)\) spectra almost flat, the scattering spectra exhibit variations approximately inverse to those of absorption. The scattering minima related to the absorption maxima occur at the same wavelengths or are slightly shifted (=3 nm) toward shorter wavelengths.

The magnitude of specific scattering, like that of absorption, varies widely from one species to another. For instance, \(b^*(550 \text{ nm})\) varies within about a factor of 8, from 0.08 to 0.62 m²·mg Chl \(a\)⁻¹. The
results of Privoznik et al. (1978) for nine random cultures of *Chlorella pyrenoidosa* also show this variability, even within the same species. From their data concerning the scattering cross sections of these suspensions, $b^*(550 \text{ nm})$ can be computed; it would vary from 0.03 to 0.09 $\text{m}^2 \cdot \text{mg Chl a}^{-1}$.

*Spectral values of backscattering*—The spectral values of $b^*_b$ are, like those of $b^*$, depressed inside absorption bands (Fig. 5). However, the spectral behavior of this coefficient is different from that of $b$, and the depressing effect of absorption is generally more pronounced.

The magnitude of the backscattering efficiency, $b^*_b (=-b_b/b)$ is strongly variable from one species to another, ranging from about $10^{-4}$ to $1.5 \times 10^{-3}$ for three of the phytoplankters we used. These values can be compared to those indirectly determined in situ in the upwelling area off Mauritania. According to irradiance data (Morel and Prieur 1975), it ranged from $2.8 \times 10^{-3}$ to $1.9 \times 10^{-2}$ (at 546 nm). The lowest values, for waters with the highest pigment concentration (and not influenced by resuspended sediment, i.e. in Case 1 waters), are greater than those for pure phytoplankton. This suggests that even highly productive waters contain significant amounts of detrital particles, which have a higher backscattering efficiency.

For lack of available data concerning direct measurements of the single backscattering coefficient of pure phytoplankton, our results can only roughly be com-
pared with those concerning the "diffuse" backscattering coefficient (Kiefer et al. 1979). Kiefer et al. measured absorption and backscattering coefficients in diffuse light in highly concentrated (centrifuged) cultures of *Thalassiosira pseudonana* and *Monochrysis lutheri*. The values of $Q_{bb}$ obtained at 540 nm are of the order of 2 to $6 \times 10^{-3}$ for "young" cultures, which is on average higher than our results: $Q_{bb}$ (540) varied from $1.3 \times 10^{-4}$ (H. elongata) to $2.8 \times 10^{-3}$ (*Platymonas* sp.). The corresponding ratios $b_{bb}/a$ are also higher, of the order of $4 \times 10^{-4}$ at 540 nm, whereas in our experiments they vary from $6 \times 10^{-4}$ to $1.3 \times 10^{-2}$ at the same wavelength. This could be due at least in part to the fact that the coefficients, when measured in diffuse light, are multiplied by $1/\mu$, where $\mu$ is the mean cosine relative to the radience field (see e.g. Morel and Smith 1982), and therefore enhanced by an unknown factor. Discrepancies may also result from differences in size and refractive index of cells, which determine the magnitude of $b_{bb}$ (see below), and from the effect of the fluorescence of cells, which apparently was not subtracted from the backscattered signal in the results reported by Kiefer et al.

Theoretical interpretation—The spectral behaviors of $c$, $b$, and $b_{bb}$ can be predicted through Mie-Lorentz theory. If the particles are assumed to be spherical and homogeneous, and if their index of refraction is close to that of the surrounding medium, the general trend of $c$ (or $Q_c$) to increase or decrease throughout the visible spectrum is ruled by the variations of $Q_c$ with the parameter $\rho = (2\pi d/\lambda)(n - 1)$ within this domain, where $d$ is the diameter of cells, $n$ is the real part of their (complex) relative index (close to 1), and $\lambda$ is the wavelength in the surrounding medium.

For a monodisperse population of nonabsorbing particles, $Q_c$ is given by (Fig. 7A, curve labeled 0)

$$Q_c = 2 - \frac{4}{\rho} \sin \rho + \frac{4}{\rho^2}(1 - \cos \rho), \quad (3)$$

and for absorbing particles, $Q_a$, $Q_c$, and $Q_b$ are given by (Van de Hulst 1957)

![Fig. 7. A. Theoretical variations of the efficiency factor for attenuation, $Q_c$, vs. $\rho = (2\pi d/\lambda)(n - 1)$ (d mean diameter of particles, n index of refraction, $\lambda$ wavelength in the surrounding medium), for polydisperse systems of nonabsorbing particles, obeying different size distribution laws [1—log-normal law, with a probability of occurrence $P(\rho) = 0.01%$ for $\rho/2$ and $2\rho$; 2—normal law with $P(\rho) = 10%$ for $\rho/2$ and $3\rho/2$; 3—log-normal law with $P(\rho) = 10%$ for $\rho/2$ and $2\rho$; 4—log-normal law with $P(\rho) = 30%$ for $\rho/2$ and $2\rho$]. The variations of $Q_c$ vs. $\rho$ for a monodisperse system are shown as dotted curve 0. B. Theoretical variations of $Q_c$ vs. $\rho$, for particles obeying a log-normal distribution law [$P(\rho) = 0.01%$ for $\rho/2$ and $2\rho$], and of complex refractive index $m = n - i n'$. The different curves correspond to different values of the ratio $n'/(n - 1)$.](image)
\[ Q_c = 2 - 4 \exp(-\rho \tan \xi) \]
\[ \cdot \left[ \frac{\cos \xi \sin(\rho - \xi)}{\rho} + \left( \frac{\cos \xi}{\rho} \right)^2 \cos(\rho - 2\xi) \right] \]
\[ + 4 \left( \frac{\cos \xi}{\rho} \right)^2 \cos 2\xi, \]
\[ Q_a = 1 + \frac{\exp(-2\rho \tan \xi)}{(2\rho \tan \xi + 1) - 1} \]
\[ 2\rho^2 \tan^2 \xi, \]
\[ Q_b = Q_c - Q_a \]

with \( \tan \xi = n'(n - 1) \), where \( n' \) is the imaginary part of the refractive index, linked to the absorption coefficient of the cell material \( (a_{cm}) \) through \( n' = a_{cm}\lambda/4\pi \).

If these particles are polydisperse with respect to size according to a law \( F(\rho) \), the \( Q \) factors are averaged according to

\[ \bar{Q}_i(\bar{\rho}) = \frac{\int_0^\infty Q_i(\rho)F(\rho)\rho^2 \, d\rho}{\int_0^\infty F(\rho)\rho^2 \, d\rho} \]

with \( i = a, b, c, \) or \( b_b \).

According to computations for polydisperse systems of absorbing particles—which simulate algal suspensions—the oscillations of \( Q_c \) vs. \( \bar{\rho} \) are smoothed by two combined effects: the effect of the polydispersion (only the first maximum of \( Q_c \) remains well marked: Fig. 7A), and the effect of absorption, which reduces the amplitude of oscillations (Fig. 7B).

Consequently, for algal suspensions, \( Q_c \) tends to present only weak variations throughout the spectrum, except for low \( \bar{\rho} \) values below or around the value \( \bar{\rho}_M \) which corresponds to the first maximum in \( Q_c \). Such low \( \bar{\rho} \) values are typical of small cells and of cells having a low refractive index. In general, four different patterns can be observed according to the value of \( \bar{\rho} \) (Fig. 7).

1. \( Q_c \) increases with \( \bar{\rho} \) (i.e. with decreasing \( \lambda \)) when \( \bar{\rho} \) remains lower than \( \bar{\rho}_M \) throughout the spectrum. It is recalled that the variation in \( \bar{\rho} \) throughout the visible spectrum (say 350–700 nm) is within a factor of 2, and that \( \bar{\rho}_M \) is not a fixed value; it ranges between 2 and 4 and depends on the size distribution law.

2. \( Q_c \) exhibits a more or less pronounced maximum inside the visible spectrum when the corresponding range of variation in \( \bar{\rho} \) includes \( \bar{\rho}_M \).

3. \( Q_c \) decreases with increasing \( \bar{\rho} \) (i.e. with decreasing \( \lambda \)) when \( \bar{\rho} \) remains close to but always higher than \( \bar{\rho}_M \).

4. \( Q_c \) tends toward its limiting value, 2, and is approximately constant throughout the spectrum for high values of \( \bar{\rho} \).

(Note that the above description reasonably presupposes that the second, third, and other maxima in the \( Q_c \) curve have vanished by virtue of polydispersion and absorption.)

The selectivity of \( Q_c \) for a small phytoplankter such as \( C. \) huxleyi (\( Q_c \) decreases with increasing \( \lambda \)) corresponds to the first case and means that \( \bar{\rho} < \bar{\rho}_M \). The almost flat spectra observed for the other large-sized species mean that \( \bar{\rho} > \bar{\rho}_M \) (fourth case).

The attenuation values presented by Bryant et al. (1969) for \( E. coli \) cells and spinach chloroplasts (average equivalent diameters 1.2 and 3.8 \( \mu m \)) and

![Fig. 8. Schematic variations of the real and imaginary parts, \( n \) and \( n' \), of the refractive index near and inside an absorption band. The imaginary part, \( n' \), is positively linked to absorption, while variations of the real part, \( n \), obey the so-called anomalous dispersion.](image)
those of Privovnik et al. (1978) for *Chlorella pyrenoidosa* (diameters ranging from 0.8 to 4.0 μm), are also representative of the first case. Calculation of the \( Q_e \) factors from the results of Privovnik et al. is not easy, because a bimodal size distribution was observed on these cultures so that the definition of a "mean diameter" becomes questionable. This notwithstanding, by using the mean diameters given by these workers (which actually correspond to the minimum of the size distribution), the attenuation values at 550 nm would lead to \( Q_e \) values ranging from 2.24 to 3.39. These \( Q_e \) factors seem uncomfortably high for a polydispersion of absorbing particles: according to Mie theory (cf. Eq. 1 and Fig. 7A), the upper limit of \( Q_e \), for a monodisperse population of nonabsorbing particles, is 3.17 (for \( \rho = 4.09 \)) if \( n \) is close to 1 and does not exceed 3.3 for \( n \) as high as 1.05.

As shown by Eq. 4–6, the spectral behaviors of \( Q_e \) and \( Q_b \) are affected by changes in \( \lambda \), and in \( n \) and \( n' \) throughout the spectrum. Near and inside absorption bands, the variations of \( n \) and \( n' \) are ruled by Ketteler-Helmholtz’s theory of anomalous dispersion: the real part of the index, \( n \), presents a minimum and a maximum on each side of the band, while the imaginary part, \( n' \), varies with the absorption (Fig. 8). This theory, combined with Eq. 4–7, explains the behavior of \( Q_e \) and \( Q_b \) in the region of an absorption band. Van de Hulst (1957) showed that, for particles of small size or index or both (in such a way that \( \rho < 2 \) or 3), the attenuation curve, \( Q_e(\rho) \), follows the pattern of anomalous dispersion, whereas for larger (or more refractive) particles, attenuation is simply depressed inside the absorption band. The behavior of \( Q_b(\rho) \) in the vicinity of an absorption band was studied theoretically (Morel and Bricaud 1981b) for mono- and polydisperse populations. The spectral behavior of \( Q_b \) is similar to that of \( Q_e \). When the depressing effect occurs (\( \rho > 3 \) or 4), it is more marked on \( Q_b \) than on \( Q_e \) (since \( Q_b = Q_e - Q_a \)). These predictions are verified by the experimental data: for instance, within the red absorption band, the \( Q_b \) curve for *C. huxleyi* (for which \( \rho < 3 \) or 4) resembles the curve of anomalous dispersion, while the curves of the other species (for which \( \rho > 4 \)) are only depressed inside the absorption band.

In principle, complete computations of \( Q_e, Q_a, \) and \( Q_b \) as a function of \( \lambda \) can be performed (Bryant et al. 1969; Morel and Bricaud 1981b). Such modeling, theoretically possible, provides ambiguous answers in the present situation because of the inadequate knowledge of the actual size distribution which is an input parameter of critical importance. This degree of freedom has to be canceled to apply the model meaningfully.

The spectral values of \( Q_{bb} \) result from the combined variations of \( Q_b \) and \( b_b \) with \( \lambda \), since \( Q_{bb} = Q_b b_b \). Theoretical computations of VSF show that \( b_b \) is dependent on the mean relative size of particles, \( \tilde{a} = \pi d/\lambda \), and on the real and imaginary parts of their refractive index (Fig. 9). The variations of these three parameters (\( \lambda, n, n' \)) throughout the spectrum result in spectral variations of \( b_b \). Theoretical computations (Morel and Bricaud 1981b) show that the behaviors of \( Q_b \) and \( Q_{bb} \) may be quite different and that the variations of \( n \) and \( n' \) through absorption bands tend generally to decrease \( b_b \); the depressing effect of absorption on \( Q_{bb} \) is thus more marked than on \( Q_b \), as experimentally shown.

As shown by Fig. 9, the magnitude of the backscattering efficiency is mainly controlled by the value of the index of refraction. Very few measurements, to our knowledge, have been made to determine this index. Carder et al. (1972) found it to vary between 1.026 and 1.036 for the unarmored cells of *Isochrysis galbana*. It is probably higher for armored types of phytoplankters, as indicated below. For the species studied, the imaginary part of the index, \( n' \), varied from 0.003 to 0.005 at the absorption maximum (435 nm) and from 0.0004 to 0.0015 at its minimum (=600 nm) (see Table 1). If \( n \) is assumed to vary between 1.02 and 1.05, and \( n' \) between 0 and 0.006, \( b_b \) would range from about \( 4 \times 10^{-5} \) to \( 3 \times 10^{-3} \), which is consistent with the experimental values.
Fig. 9. Theoretical variations of the backscattering efficiency \( b_b(b) \) for a system polydispersed according to a narrow log-normal distribution, \( F(a) = \exp[-101.638 \log(a/\bar{a})^2] \), corresponding to \( F(a) = 0.01\% \) for \( \bar{a}/2 \) and \( 2\bar{a} \), vs. the mean relative size of particles, \( \bar{a} \), and for different values of the complex refractive index. The choice of these values and of the distribution was made so as to be representative of a monospecific population of algal cells.

Application: Size and refractive index determinations from optical characteristics

According to the above theoretical considerations, the spectral variations of the mean efficiency factor for attenuation, \( \bar{Q}_e \), can provide information about the mean size of cells, \( \bar{d} \), and the real part of their refractive index, \( n \), at least on some occasions.

Mean size of cells—As shown by Fig. 7, if \( \bar{Q}_e \) is approximately constant throughout the spectrum, whereas \( \bar{\rho} \) is forced to vary within a factor of 2, its value cannot differ significantly from 2, the limiting value. Since \( \bar{Q}_e \) is linked to the specific attenuation coefficient, \( c^* \), through (cf. Eq. 1 and 2)

\[
\bar{Q}_e = \frac{3}{2c_i d} c^* = \frac{3(N/V)}{2[Chl \, a]} \frac{\pi}{6} d^2 c^*,
\]

the mean diameter, \( d \), of the cells can be estimated from the value of \( c^* \) (considered as constant throughout the spectrum), by letting \( \bar{Q}_e = 2 \). The other parameters, \( N/V \) and the Chl \( a \) concentration, are known. The results of this computation are given in Table 1 for three species. For \( C. \) huxleyi, which presents a selective spectrum, such a calculation is not possible.

The estimation of \( \bar{d} \) with a Coulter Counter TA is very uncertain for large cells, because of the increasing width of the channels with increasing nominal size. The computed \( \bar{d} \) values are presumably more representative and precise, since the postulate \( \bar{Q}_e = 2 \) remains approximately true even if the \( \bar{Q}_e \) spectrum is not absolutely flat. It is encouraging that these computed values fall within or very close to the limits of the unique channel where all the cells were counted (see Table 1). The drawback of the Coulter Counter partly disappears for small cells; for instance, the \( C. \) huxleyi cells were all counted in a narrow channel (limits 3.0–3.8 \( \mu m \)).

Refractive index of cells—The shape of the \( \bar{Q}_e \) spectrum brings information on the values of \( \bar{\rho} \). Once the diameter is measured (or computed), a constraint is imposed on the value of the real part of the index.

For \( C. \) huxleyi, the shape of the spectrum suggests that \( \bar{\rho} \) is lower than 4 (approximately) throughout the spectrum. The range of variation of \( a \) (20.3–40.6 between 350 and 700 nm, if the value adopted for \( d \) is 3.4 \( \mu m \)) implies that \( n \) is <1.049 to maintain \( \bar{\rho} <4 \). For the other species, we can assume that \( \bar{\rho} \) is >4. With the computed values of \( \bar{d} \) (Table 1), this means that \( n \) is >1.029, 1.046, and 1.035 for \( H. \) elongata, \( P. \) platymonas sp., and \( T. \) maculata. These somewhat high values could be due to the fact that these cells have hard, highly refractive walls. Since cells have been assumed to be homogeneous, this index corresponds in fact to an average over the refractive indices of the different parts of the cell.

It must be pointed out that with a perfect knowledge of the size distribution law, the only unknown parameter is the refractive index. By using the above-mentioned modeling, and by trial and error, this index can in principle be inferred from the variations of \( \bar{Q}_e \) with \( \bar{\rho} \). However, if the \( \bar{Q}_e \) spectrum is perfectly
flat, the range of variation of $\hat{\rho}$ remains undetermined and only a lower limit of $n$ can be inferred from the inequality $\hat{\rho} > \hat{\rho}_M$.

Conclusions

Our experimental results show that specific (normalized for Chl a) absorption and scattering coefficients of living algal cells vary greatly from one species to another. This variability is explained by considering the size, the pigment concentration, and the pigment composition of the phytoplankters. The representativity of spectral "mean" values for these optical coefficients, as adopted for some applications (ocean color modeling, radiant energy uptake by the algal biomass) is questionable, although the use of these mean values is sometimes efficient, at least as a first approximation. Besides, it has been theoretically and experimentally evidenced, that spectral variations of scattering (and backscattering) resemble the inverse variations of absorption. Therefore the spectral variations of the backscattering-to-absorption ratio, $b_b/a$—which governs reflectance—are more marked than those of $1/a$. In other words, the effect of phytoplankton in modifying ocean color would be more intense than is generally expected (under the assumption of constant $b_b$). This conclusion actually is counterbalanced by the fact that $b_b$ is very low. In natural environments (as opposed to a pure culture), a significant or dominant part of the light returned upward originates from backscattering by other particles. These particles do not scatter with the same wavelength dependency as phytoplankton.

Ocean color, as remotely sensed from air- or spacecraft, is interpreted in terms of pigment (Chl a + Pheo a) concentration through application algorithms. These algorithms, as now developed, postulate constant optical properties of phytoplankton. On the contrary, variability of the specific spectral absorption seems to be the general rule. In the case of monospecific cultures, the range of variation appears wide. It would presumably be reduced for natural assemblages owing to an averaging effect induced by the simultaneous presence of different species (and covarying detritus). But even if reduced in this way, this variability generates a noise which limits the accuracy of the algorithms now in use.

For want of something better, the in situ quantum yield, $\phi$, was also estimated on the basis of constant absorptive properties (Dubinsky and Berman 1976; Morel 1978; Takematsu et al. 1981). Bannister's (1974) model of photosynthesis also rests on the same assumption. It has been shown that such an approximation fails both theoretically and practically (Platt and Jassby 1976; Taguchi 1976; Morel and Bricaud 1981a; Welschmeyer and Lorenzen 1981). The difficulty, however, which arises from the actual variations in the light-harvesting ability of cells, is not easily circumvented in field experiments dealing with natural assemblages. Ideally a correct evaluation of in situ $\phi$ would require first the determination, at different depths, not only of the Chl a concentration but also of that of the other pigments, then the assessment of the discreteness effect, and finally the computation of the radiation actually absorbed by combining the absorption spectrum of cells with the spectral distribution of the remnant light at the level considered (Morel 1978; Atlas and Bannister 1980). For in vitro experiments, insofar as the parameters are more easily monitored, the situation is more comfortable. The estimation of $\phi$ can be made independent of the variations in the energy capture capability of cells by the method of Welschmeyer and Lorenzen (1981). A proper estimate of $\phi$ can also be made by inferring the energy capture from absorption measurements by intact cells, as reported here.

References


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