Optical efficiency factors of free-living marine bacteria: Influence of bacterioplankton upon the optical properties and particulate organic carbon in oceanic waters

by André Morel and Yu-Hwan Ahn

ABSTRACT

Free-living coccolith bacteria have been repeatedly isolated from the Mediterranean Sea and then cultured in detritus- and predator-free seawater, either unenriched or slightly nutrient-supplemented. The modal diameter of these cultured cells was in the 0.5 to 1 µm range. The spectral values (within the 400–750 nm λ-range) of absorption, total scattering and backscattering have been determined, together with cell enumeration, size distribution and C–N content.

Because of their smallness, bacteria are amenable to simplified theoretical expressions providing their optical efficiency factors. Experimental data are in remarkable quantitative agreement with theoretical predictions. As expected, scattering is wavelength (λ⁻²) dependent and varies according to the fourth power of the size, whereas backscattering is neutral and depends upon the square of the size. Albeit weak, absorption has been detected and distinctly exhibits a maximum around λ = 413 nm. As deduced from the optical properties, the refractive index (about 1.05 with respect to that of water) agrees with the cell material composition, namely with the intracellular carbon concentration (mean value 228 kgC m⁻³).

The chemical composition and optical properties of bacterial cells, when combined with the cell number concentrations as related to the chlorophyll concentration (Cole et al., 1988), allow the role of bacterioplankton to be estimated in oceanic waters of various trophic situations. The bacterial compartment appears to form a significant contribution to the light scattering within the upper part of the ocean as well as in forming a sizeable fraction of the particulate organic carbon pool.

1. Introduction

Free living bacteria have been identified as ubiquitous organisms in the open ocean (Ferguson and Rublee, 1976; Sieburth et al., 1978) with biogeochemical implications concerning the fluxes in the ocean’s interior (Cho and Azam, 1988). With sizes essentially in the range 0.2 to 1 µm, generally peaking in the 0.4–0.7 µm fraction (see e.g. Ducklow, 1986) and with numerical abundances approximately in the range of 10¹¹ to 10¹³ cell m⁻³, bacterioplankton can form a sizeable part of the particulate organic carbon (living and detrital), especially in oligotrophic areas where they seem to

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decrease in number less abruptly than phytoplankton. In the photic zone, in effect, bacterial numbers and chlorophyll \( a \) concentration are significantly, but nonlinearly, correlated (Bird and Kalff, 1984). This has been confirmed by Cole et al. (1988) who submitted a wide data set to regression analysis and proposed an empirical relationship which can be written as

\[
N/V = 0.91 \times 10^{12} \langle \text{Chl} \rangle^{0.52}
\]  

(1)

where \( N/V \) (in cell m\(^{-3}\)) is the bacterial cell number per unit of volume and \( \langle \text{Chl} \rangle \) is the chlorophyll concentration (in mg m\(^{-3}\)). The exponent in this expression, close to \( \frac{1}{2} \) implies that the bacterial cell number density varies less than the algal material; whereas the chlorophyll concentration can vary within 3 or even 4 orders of magnitude in the open ocean, the bacterial number would only span over 1.5 or 2 orders. Very recently Dortch and Packard (1989), using chlorophyll-to-protein ratios, have demonstrated that the relative plant to nonplant biomass decreases from eutrophic to oligotrophic situations leading to an inverted biomass pyramid in the latter situation. Such a finding also supports the validity of an expression such as Eq. (1).

This observation is to be compared with other nonlinear trends already demonstrated in the case of open ocean waters by the variations of the POC\(_{\text{tot}}\) - to - Chl ratio (\( \langle \text{POC} \rangle_{\text{tot}} \); total particulate organic carbon), or in the optical properties when related to the Chl concentration. A regression analysis restricted to Case 1 waters (Morel, 1988) has led to a statistical relationship

\[
\langle \text{POC} \rangle_{\text{tot}} = 90 \langle \text{Chl} \rangle^{0.57}.
\]  

(2)

(\( \langle \text{POC} \rangle_{\text{tot}} \) like \( \langle \text{Chl} \rangle \) is in mg m\(^{-3}\)), close to that which can be deduced from the data analyzed by Malone (1982). The relative increasing importance of nonpigmented, carbon-bearing particles while the Chl-bearing cells abundance diminishes is very likely at the origin of the nonlinear bio-optical effect (Smith and Baker, 1978). This is particularly clear when considering the total light scattering coefficient \( b_{\text{tot}} \) (at \( \lambda = 550 \) nm), which reflects the particulate matter abundance, regardless of its nature. Simultaneous measurements of \( b_{\text{tot}} \) (in m\(^{-1}\)) and \( \langle \text{Chl} \rangle \) (varying over 3 decades) have been statistically analyzed (Gordon and Morel, 1983) leading to the empirical relationship

\[
b_{\text{tot}} = 0.30 ( \pm 0.15 ) \langle \text{Chl} \rangle^{0.62}
\]  

(3)

an expression with an exponent fully consistent with the preceding one (see discussion in Morel, 1988). For the open ocean Case 1 waters, far from terrigenous influences, the primary source of variation in particulate matter abundance, and therefore in the related optical properties, is the variation in phytoplankton concentration and their breakdown products. The nonlinearities with respect to \( \langle \text{Chl} \rangle \) likely originate from a rather regular change in the relative proportions between in one hand, the algal
compartment and, on the other hand, the detrital and nonalgal compartment. More precisely, with almost identical exponents appearing in the three above equations, quasi-linear relationships between $N/V$, $\langle \text{POC} \rangle_{\text{tot}}$ and $b_{\text{tot}}$ implicitly must exist. However, a question emerges; does the bacterial compartment form an important, moderate or negligible contribution to $b_{\text{tot}}$ and to $\langle \text{POC} \rangle_{\text{tot}}$?

Answering this question requires a determination of the optical properties (namely the light scattering capacity) of natural free-living bacterial cells, as well as of their carbon content. Since the contributions of microbes to light scattering and to the particulate organic carbon pool have to be compatible, the problem is tightly constrained. An additional constraint also results from the direct link which can be established between the light scattering properties of individual cells and their carbon content. In effect, the scattering capabilities are directly governed by the bulk index of the refraction of the scattering body and, in turn, this index depends on the water and organic matter contents. The aim of this paper is thus:

(i) to examine what can be theoretically anticipated concerning the “optical object” consisting of a bacterium in suspension in water,

(ii) to experimentally determine its optical properties and interpret them in the frame of the theory,

(iii) to determine its carbon and nitrogen content, and finally

(iv) from these properties combined with information about the bacterial abundance, to infer the impact of this trophic compartment upon the optical properties of oceanic waters, as well as upon the particulate carbon pool.

2. Theory

The theoretical framework already developed for algal cells, including picoplanktonic cells (Morel and Bricaud, 1986) remains valid for heterotrophic bacteria. Only the indispensable definitions and equations are recalled.

The dimensionless efficiency factor for absorption, $Q_a$, and for scattering, $Q_b$, are defined as the ratios, respectively, of the radiative energy absorbed within, or scattered by the particle to the energy impinging onto its geometrical cross section, $s$; their sum is the efficiency factor for attenuation, $Q_c$

$$Q_c = Q_a + Q_b.$$  \hspace{1cm} (4)

The products $sQ_i$ (with $i = a$, $b$, or $c$) are the cross sections of the particle for absorption, scattering or attenuation. Theories are available to predict the values of $Q_a$ and $Q_b$, if the absorption coefficient, $a_{\text{cm}}$, the refractive index, $n_{\text{cm}}$, of the cellular material forming the particle, and also the particle size and the wavelength are known (see in reference above).

Free-living bacterial cells in the open sea are mainly of coccolid form, well approximated by spheres, with diameters, $d$, in the range 0.2 to 1 $\mu$m. This rather small size and
the quasi-absence of pigments (hence of significant absorption) lead to some specific properties and also possible simplifications, as explained below. The required dimensionless parameters are \( \alpha \), the relative size defined with respect to the wavelength (\( \lambda \)) and \( m \) the relative index of refraction of the cell, defined with respect to that of the surrounding medium (water)

\[
\alpha = \frac{\pi d}{\lambda} n_w
\]  
\[\text{(5a)}\]

and

\[
m = \frac{n_{cm}}{n_w}
\]
\[\text{(5b)}\]

where \( n_w \) is the refractive index of water (\( = 1.34 \)). In general, \( m \) is a complex number written as

\[
m = n - in'
\]

where the imaginary part is related to the absorption coefficient through

\[
n' = \frac{a_{cm} \lambda}{4\pi n_w}.
\]
\[\text{(6)}\]

By combining these parameters as

\[
\rho = 2 \alpha (n - 1)
\]
\[\text{(7a)}\]

\[
\rho' = 4\alpha n' ( = a_{cm} d)
\]
\[\text{(7b)}\]

are defined the dimensionless quantities \( \rho \) and \( \rho' \) on which \( Q_b \) and \( Q_a \) depend respectively.

If in a first approximation, bacteria are considered as nonabsorbing bodies, \( (n' = 0) \), it follows that \( Q_a = 0 \) and thence \( Q_b = Q_c \). If, in addition, bacteria are not strongly refringent particles (\( n \) not far from 1), then the anomalous diffraction approximation (Van de Hulst, 1957) can apply and \( Q_b \) is accordingly expressed as

\[
Q_c(\rho) = Q_b(\rho) = 2 - \frac{4}{\rho} \sin \rho + \frac{4}{\rho^2} (1 - \cos \rho).
\]
\[\text{(8)}\]

By letting for instance \( n = 1.05 \), and \( \lambda \) varying from 0.7 to 0.4\( \mu m \), it follows that \( \rho \) varies from 0.6\( d \) to 1.05\( d \). If \( d \) is now given the value 1\( \mu m \), a kind of upper limit for free living bacterial cells, \( \rho = 0.6 \) or 1.05. By developing Eq. (8), \( Q_b \) is expressed as

\[
Q_b(\rho) = \frac{1}{2} \rho^2 - \frac{1}{16} \rho^4 + \cdots
\]

Even for \( \rho \) as high as 1, the biquadratic term remains negligible (to within 5%) and \( Q_b \)
can be safely computed for bacteria according to

\[ Q_b = \frac{1}{2} \rho^2 = 2 (n - 1)^2 \alpha^2 \]

or

\[ Q_b(\lambda) = 2\pi^2 n_w^2 (n - 1)^2 d^2 \lambda^{-2}. \tag{9} \]

Note that this approximation rests on the validity of Eq. (8). For extremely small particles Eq. (8) does not conform to Rayleigh theory which predicts a \( \alpha^4 \) (or \( d^4 \)) dependency. The transition toward the Rayleigh domain takes place for \( \rho \) values smaller than 0.1 (see discussion in Morel, 1973), i.e. for particles having sizes below the typical size range of bacteria. Therefore, Eq. (9) can apply and this very simply expression allows some important consequences to be readily derived as follows:

(i) at a given \( \lambda \), the scattering efficiency factor \( Q_b \) is proportional to the square of the size (\( n \) being fixed). The partial scattering coefficient resulting from the presence of equally sized bacteria with a number density \( N/V \) is

\[ b = (N/V) sQ_b. \tag{10a} \]

Therefore \( b \) varies according to the 4th power of the size of these bacteria.

(ii) for a given size, when scanning the spectrum, \( Q_b(\lambda) \) and also \( b(\lambda) \) will vary like \( \lambda^{-2} \);

(iii) these conclusions have to be slightly modulated if the refraction increment \( (n - 1) \) cannot be regarded as constant along the spectrum or if it changes with the cell size;

(iv) it has been shown (Morel and Bricaud, 1986) that for the small particles the backscattering ratio \( \tilde{b}_b \) (the ratio of the backward scattering to the total scattering) is practically independent from the refractive index and, for sufficiently small \( \alpha \) values (say, when \( \alpha < 30 \)), \( \tilde{b}_b \) is varying as \( \alpha^{-2} \) (either as \( d^{-2} \) or as \( \lambda^2 \)). By combining this result with that in (i), the backscattering coefficient due to the bacterial cells, \( b_b \), at a given wavelength

\[ b_b = b \tilde{b}_b \tag{11a} \]

will be proportional to \( d^2 \) and by combining this result with that in (ii), it follows that \( b_b \) is “neutral,” i.e. does not exhibit any spectral dependency. The efficiency factor \( Q_{bb} \), defined as

\[ Q_{bb} = Q_b \tilde{b}_b \tag{11b} \]

is also neutral.

As shown later, bacterial absorption, albeit weak, still exists, particularly in the blue part of the spectrum. With respect to the general predictions above, minute nuances
are to be expected, when $Q_a$ departs from zero. The efficiency factor for absorption, $Q_a$, is a monotonic function of the dimensionless number $\rho'$, increasing from 0 to 1 when $\rho'$ goes from 0 to $\infty$ (Morel and Bricaud, 1981). When $\rho'$ is small enough in such a way that

$$Q_a = \frac{2}{3} \rho' - \frac{1}{4} \rho'^2 + \cdots$$

(12)

can reduce to its first term only, $Q_a$ becomes linearly related to $\rho'$. In this case the discreteness (or package) effect vanishes. With small sizes and weak absorption, bacteria very likely are relevant to this approximation. As for scattering (Eq. 10a), the partial absorption coefficient due to equally sized bacteria at the concentration $N/V$ is

$$a = (N/V) s Q_a.$$  

(10b)

Under the above approximation and by recalling that $\rho'$ is linearly linked to the size $d$ (Eq. 7b), $a$ will vary as $d^3$ for a given absorbing material (for a constant $a_{\text{cm}}$ value).

3. Material and methods

a. Preparation and culture of marine bacteria. Natural marine bacteria were repeatedly isolated from seawater, collected off the Bay of Villefranche-Sur-Mer at a depth of 10 meters. Sampling extended over a period of 10 months (June 1988–March 1989). Free living bacteria were separated from other larger organisms, grazers and particulate materials, by gravity filtration onto Nuclepore membranes (pore size 0.6 $\mu$m). Besides small debris, the filtrate contains bacteria in the size range 0.4–0.6 $\mu$m, at a concentration of about $0.5 \times 10^6$ cell ml$^{-1}$. This filtrate is used as inoculum for cultures in either unenriched or supplemented media. In both cases, cultures were grown in the dark at ca. 18/20°C.

Prefiltered and sterilized seawater was triply filtered through Millipore disks (GS, 0.22 $\mu$m) to prepare a particle-free, unenriched, natural medium. The inoculum of 5 ml was diluted into 5 l of this medium. After 2 or 3 days of exponential growth, the cell abundance reaches 1 to $5 \times 10^6$ cell ml$^{-1}$ (growth rate with respect to the cell number, from 0.1 h$^{-1}$ to 0.2 h$^{-1}$). The optical measurements not being feasible when the cell number density is at such a level, a concentration by a factor 100, at least, is required and is effected by filtration followed by resuspension. Four 0.2 $\mu$m Nuclepore filters are necessary to process the 5 l culture. They are placed in a tube with 10 ml of triply filtered seawater and vigorously shaken by hand. The dense suspension is again filtered onto 1.0 $\mu$m Nuclepore to remove those picoflagellates which possibly have grown in the bacterial culture (Rassoulzadegan and Sheldon, 1986). As a result of this resuspension, densities exceeding $10^8$ cell ml$^{-1}$ are obtained and the bacterial cells, free from detritus, are believed to be taxonomically and morphologically very close to those living in the natural environment (Lee and Fuhrman, 1987).
The above method being somewhat laborious, supplemented cultures were grown in parallel to obtain manageable cell number densities without post-concentration. The enriched medium (F/4, Guillard and Ryther, 1962, with glucose at a concentration of 50 mg l⁻¹ and no silicate) is made particle-free as above (triple filtration, 0.22 μm). The 5 ml inoculum is diluted into 250 ml of this medium. In these cultures, exponential growth (with respect to the cell number), is between 0.3 and 0.5 h⁻¹ and then a leveling off occurs after 1 or 2 days. Cell number densities higher than 0.5 \(10^8\) cell ml⁻¹ were obtained which allow direct optical determinations. These cultures were in general maintained in a stationary phase during 4 or 5 days, (exceptionally up to 15 days) and daily subsamples were taken. Before optical measurements and counting, they were filtered (1.0 μm Nuclepore), in view of eliminating larger heterotrophic organisms occasionally present, and then the cells were killed or disabled by buffered Formalin with a final concentration of 0.2% (vol per vol). This treatment is absolutely necessary to prevent the bacteria from grouping and adhering onto walls of the vessel (the optical cuvette, for instance). The suspensions were then immediately transferred to the Coulter Counter (model ZBI) and the Perkin Elmer 571 spectrophotometer for subsequent analyses.

The fact that, as a result of the enrichment, these cultured bacteria may differ from the "natural" bacteria was expected, and this was accepted. With the aim of understanding the optical properties, the casual change of the optical object under scrutiny can constitute an advantage when confronting experience with theory.

b. Optical measurement. The spectral values of absorption and attenuation by bacterial suspensions, \(a(\lambda)\) and \(c(\lambda)\) respectively, were measured (see Table 1) according to a procedure developed for algal suspensions by Bricaud et al. (1983); the supplementary diffusing plate was slightly moved and placed directly in contact with the photomultiplier entrance window.

The small size of bacteria compared to that of algal cells, and also their low absorption induce some peculiarities when estimating \(a\) and \(c\). In effect, no perfect \(a\)-meter or \(c\)-meter exist, meaning that \(a\) and \(c\) have to be derived, after corrections, from an experimentally determined coefficient, denoted \(x\) (as in the above reference). This optical coefficient, measured by using a spectrophotometer is always a combination of \(a\) and \(b\) according to

\[
x = a + (1 - \epsilon) b
\]  

(13)

where \(b\) is the scattering coefficient and \(\epsilon\) represents this fraction of scattered radiation which enters the detector because its acceptance angle is not null; the value of \(\epsilon\) depends on (i) the geometrical configuration adopted when measuring \(x\) and (ii) the volume scattering function (VSF) of the medium (the suspension) to be studied.

When the sample is relatively far from the detector (i.e. in the conventional configuration for a spectrophotometer, with \(x = x_1\)) and if field stoppers are disposed to
Table 1. Relevant information concerning the bacterial culture experiments and the optical or chemical determinations.

<table>
<thead>
<tr>
<th>Experiments</th>
<th>$d_1$ [μm]</th>
<th>$\Delta d$ [μm]</th>
<th>$d_2$ [μm]</th>
<th>$d_3$ [μm]</th>
<th>$c$, $a$†</th>
<th>$b_5$†</th>
<th>$c_{i,c}$† [kg m$^{-3}$]</th>
<th>C/N†</th>
<th>$a_f$†</th>
</tr>
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<tbody>
<tr>
<td>UE1</td>
<td>0.64</td>
<td>0.31</td>
<td>0.75</td>
<td>0.77</td>
<td>x</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>x</td>
</tr>
<tr>
<td>2</td>
<td>0.59</td>
<td>0.35</td>
<td>0.73</td>
<td>0.74</td>
<td>x</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>0.64</td>
<td>0.34</td>
<td>0.80</td>
<td>0.82</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>0.53:0.76</td>
<td>0.44</td>
<td>0.74</td>
<td>0.76</td>
<td>x</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>x</td>
</tr>
<tr>
<td>E1.D1</td>
<td>0.69</td>
<td>0.44</td>
<td>0.74</td>
<td>0.74</td>
<td>x</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>E2.D2</td>
<td>0.73</td>
<td>0.24</td>
<td>0.79</td>
<td>0.80</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>x</td>
</tr>
<tr>
<td>E3.D1</td>
<td>1.00</td>
<td>0.31</td>
<td>1.07</td>
<td>1.08</td>
<td>x</td>
<td>x</td>
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<td>x</td>
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<tr>
<td>D2</td>
<td>0.89</td>
<td>0.30</td>
<td>0.97</td>
<td>0.98</td>
<td>x</td>
<td>x</td>
<td>191</td>
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<td>—</td>
</tr>
<tr>
<td>D3</td>
<td>0.86</td>
<td>0.32</td>
<td>0.96</td>
<td>0.97</td>
<td>x</td>
<td>x</td>
<td>194</td>
<td>4.27</td>
<td>—</td>
</tr>
<tr>
<td>D4</td>
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<td>0.32</td>
<td>0.94</td>
<td>0.95</td>
<td>x</td>
<td>x</td>
<td>219</td>
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<td>—</td>
</tr>
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<td>1.07</td>
<td>1.08</td>
<td>x</td>
<td>x</td>
<td>180</td>
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<td>—</td>
</tr>
<tr>
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<td>0.25</td>
<td>0.98</td>
<td>0.99</td>
<td>x</td>
<td>—</td>
<td>269</td>
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<td>—</td>
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<tr>
<td>D3</td>
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<td>0.25</td>
<td>0.92</td>
<td>0.93</td>
<td>x</td>
<td>x</td>
<td>266</td>
<td>4.78</td>
<td>x</td>
</tr>
<tr>
<td>D4</td>
<td>0.84</td>
<td>0.29</td>
<td>0.92</td>
<td>0.94</td>
<td>x</td>
<td>x</td>
<td>207</td>
<td>4.78</td>
<td>—</td>
</tr>
<tr>
<td>E5.D1</td>
<td>1.00</td>
<td>0.27</td>
<td>1.03</td>
<td>1.04</td>
<td>x</td>
<td>—</td>
<td>216</td>
<td>4.98</td>
<td>—</td>
</tr>
<tr>
<td>D2</td>
<td>0.86</td>
<td>0.26</td>
<td>0.94</td>
<td>0.95</td>
<td>x</td>
<td>—</td>
<td>295</td>
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<td>0.92</td>
<td>0.93</td>
<td>x</td>
<td>—</td>
<td>270</td>
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<tr>
<td>E6D1</td>
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<td>0.32</td>
<td>0.96</td>
<td>0.97</td>
<td>x</td>
<td>x</td>
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<tr>
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<td>0.90</td>
<td>0.91</td>
<td>x</td>
<td>x</td>
<td>220</td>
<td>2.88</td>
<td>—</td>
</tr>
<tr>
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<td>0.23</td>
<td>0.82</td>
<td>0.83</td>
<td>x</td>
<td>x</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>E7.D1</td>
<td>0.84</td>
<td>0.40</td>
<td>0.93</td>
<td>0.95</td>
<td>x</td>
<td>x</td>
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<td>—</td>
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<tr>
<td>D2</td>
<td>0.78</td>
<td>0.26</td>
<td>0.85</td>
<td>0.86</td>
<td>x</td>
<td>x</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>D3</td>
<td>0.74</td>
<td>0.26</td>
<td>0.82</td>
<td>0.83</td>
<td>x</td>
<td>x</td>
<td>—</td>
<td>—</td>
<td>x</td>
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<tr>
<td>D15</td>
<td>0.62</td>
<td>0.23</td>
<td>0.68</td>
<td>0.69</td>
<td>x</td>
<td>x</td>
<td>183</td>
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<tr>
<td>E8.D1</td>
<td>0.62:0.89</td>
<td>0.53</td>
<td>0.89</td>
<td>0.91</td>
<td>x</td>
<td>—</td>
<td>277</td>
<td>4.88</td>
<td>—</td>
</tr>
<tr>
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<td>0.62:0.86</td>
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<td>0.88</td>
<td>0.86</td>
<td>x</td>
<td>—</td>
<td>260</td>
<td>5.39</td>
<td>x</td>
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<tr>
<td>D3</td>
<td>0.62:0.91</td>
<td>0.61</td>
<td>0.88</td>
<td>0.90</td>
<td>x</td>
<td>—</td>
<td>228</td>
<td>4.40</td>
<td>x</td>
</tr>
<tr>
<td>D4</td>
<td>0.62:0.81</td>
<td>0.44</td>
<td>0.84</td>
<td>0.85</td>
<td>x</td>
<td>—</td>
<td>210</td>
<td>4.37</td>
<td>—</td>
</tr>
<tr>
<td>D5</td>
<td>0.59:0.81</td>
<td>0.42</td>
<td>0.84</td>
<td>0.85</td>
<td>x</td>
<td>x</td>
<td>216</td>
<td>4.10</td>
<td>—</td>
</tr>
<tr>
<td>E9.D0§</td>
<td>0.59:0.71</td>
<td>0.37</td>
<td>0.77</td>
<td>0.78</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>x</td>
</tr>
<tr>
<td>D0&amp;</td>
<td>0.65:0.73</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>D1</td>
<td>0.65:0.76</td>
<td>0.33</td>
<td>0.79</td>
<td>0.80</td>
<td>x</td>
<td>—</td>
<td>285</td>
<td>3.79</td>
<td>x</td>
</tr>
</tbody>
</table>

Meaning of symbols:

UEx: unenriched experiments # x. Ey: enriched experiments # y.
Dz: z refers to days, with z = 1 just at the end of the exponential phase. (note that this phase extends over 1 or 2 days after inoculation).

$d_1$ is the modal diameter (2 values are given when the size distribution is doubly peaked).

$\Delta d$: width of size distribution above where $F_c(d)$ is 0.5.

$d_2$ and $d_3$ diameters computed according to Eq. (17a) and (17b).

†: $c$, $a$ and $b_5$: respectively attenuation, absorption and backscattering measurements on bacteria in suspension.

$a_f$: absorption measurement on bacteria collected onto GF/F filter.

(x) and (—): determinations made or not made.

§ and &: 12 h and 24 h, respectively, after inoculation and thus during the exponential phase of growth. (Note that the UE4 and E9 cultures were obtained from the same strain.)

Averages($\pm \sigma$) 228.3 ($\pm 35.9$) 4.46 ($\pm 0.71$)
reduce the acceptance angle (actually 0.25°), \( \epsilon \) is small. Typically \( \epsilon \) is lower than 0.05, even for large cells considered by Bricaud et al. (1983). It becomes fully negligible when the forward lobe of the VSF is less pronounced, as is the case for small sized particles. Therefore the actually measured coefficient \( x_1 \) can safely be adopted as the attenuation coefficient of the bacterial suspension, without any correction

\[
x_1 = c = a + b.
\]  

(14)

Now by placing the optical cuvette very close to the detector (scattered transmission accessory configuration, with \( x = x_2 \)) the acceptance angle is intentionally increased to collect the scattered flux travelling around the beam as much as possible. With an actual angle of about 45°, the fraction \( \epsilon \) (ratio of the scattered flux between 0 and 45° to the total, 0–180°, scattered flux) is close to 1. For algal cells it always exceed 99.5%. The tiny bacteria cells exhibit less dissymmetrical VSF and the \( \epsilon \) fraction slightly decreases from 99 to 94% when the cell size diminishes from 1 to 0.6 \( \mu \)m. This change could have been regarded as a minor effect, if \( b \) was comparable to \( a \) in Eq. (13). This is not the case when dealing with almost unpigmented bacteria for which \( b \) values largely exceed \( a \). A careful correction has to be established in each case, which requires the computation of the actual VSF through Mie theory, in order to access \( \epsilon \) (note that this computation implies that the refractive index is known; \( n \) is given the initial value 1.05 and an iterative scheme is then operated to adjust more precisely \( \epsilon \)). In principle, when \( \epsilon \) is known, \( a \) is obtained from

\[
a = x_2 - (1 - \epsilon) b
\]

with the restriction that \( b \) is still an unknown at this stage; but by using Eq. (14) it follows

\[
a = [x_2 - (1 - \epsilon) c] \epsilon^{-1}
\]  

(15)

allowing \( a \) to be computed and then \( b \) as

\[
b = c - a.
\]

The spectral values of the backscattering coefficient, \( b_k(\lambda) \), are measured by using an integrating sphere associated with a spectroradiometer (LICOR 1800 UW). With respect to a similar arrangement previously used (Bricaud et al., 1983), two main changes have been introduced. The cuvette containing the suspension is designed to constitute a light trap, except for backward travelling radiations. This is achieved owing to the black glass walls, and to the dissymmetrical triangular section adopted for the optical cuvette (Fig. 1). The backscattering signals are recorded in reference to those obtained when a \( \text{BaSO}_4 \) diffusing plate replaces the cuvette, (the spectral reflectance of this plate is accurately known). The effect of the cuvette itself, i.e. the stray light due to reflection, as well as the elastic and inelastic (Raman) backscattering by water molecules are simultaneously measured by filling the cuvette with triply
filtered (0.22 μm) seawater. These spectral values form the base line above which the backscattered radiation by bacteria is thereafter assessed.

According to the geometrical configuration and also because of the refraction, the whole backscattered flux (θ from 90 to 180°) cannot be totally returned toward the integrating sphere. The volume of suspension involved in the scattering process is also varying with the direction θ considered. These angular effects can be computed and used as a weighting function to be convolved with the VSF. A Mie routine is operated to generate all the needed VSF (see Morel and Bricaud, 1986). On average, the fraction of backscattered flux entering into the integrating sphere, therefore the fraction of $b_b$ which is actually determined, is only of the order of 25% and varies slightly along the spectrum. Accordingly the measured values are transformed into the backscattering coefficient by applying this geometrical factor. It is acknowledged that, despite care, the uncertainties on $b_b(\lambda)$ remain of the order of ± 30%.

c. Cell enumeration and size distribution. The cell number density ($N/V$) was determined (10 replicates of 0.05 ml) by using a Coulter counter ZBI in conjunction with a 20 μm aperture tube. Regular calibration in reference to microscopic enumeration of standard particles (coultronics, 2.0 μm) shows that the coulter counting was
often slightly higher than the visual counting (by 0 to 5%). This somewhat erratic difference has been ignored. The cell size distribution function was determined in relative values, \( F_r(d) \), by using a 100-channel analyzer (model C1000); the absolute values of this function

\[
F(d) = \left( \frac{1}{V} \right) \frac{d(N)}{d(d)}
\]  

are obtained by equalizing the integral of the relative function, \( F_r(d) \) (between the size \( d = 0 \) and \( \infty \)) to the absolute cumulative count, \( N/V \), previously measured.

The samples were diluted (by 100- or 200-fold) before measurements. With the 20 \( \mu \)m aperture, the smallest particles to be detected are of the order of 0.45 \( \mu \)m (diameter of the equivalent sphere). For the purpose of further computation, it is useful to extrapolate the \( F(d) \) distribution toward smaller sizes. For that the actual truncated distribution is fitted (mean square root method) to a log-normal distribution. The validity of this extrapolation from 0.5 or 0.45 \( \mu \)m toward 0.30 \( \mu \)m has been occasionally confirmed by measurements effected with a smaller aperture (13 \( \mu \)m), associated with a coulter multisizer (see Fig. 2a).

d. Carbon and nitrogen determination. On some occasions (see Table 1), bacterial cells were harvested by filtration onto precombusted (450\(^\circ\)C, 4 hours) GF/F filters (25 mm disk) for determination of the carbon and nitrogen content. The number of bacteria actually collected was determined by counting before filtration (\( \approx 10^8 \) cell ml\(^{-1}\)) and, after filtration, in the filtrate (\( \approx 10^6 \) cell ml\(^{-1}\)). The retention efficiency was in the range 97 to 99\%. By assuming a carbon concentration within the cellular material of 220 kg m\(^{-3}\), the volume to the filtered was adjusted in such a way that a sufficient amount of carbon (about 500 \( \mu \)g) could be retained on the filter. The samples were kept frozen (\( -20^\circ\)C) until analyses. C-N determinations were carried out with a C-H-N Analyzer Heraeus, calibrated with acetylilide. Note that these determinations were not possible in the case of the “unenriched” experiments, essentially because the number of cells (the amount of carbon) was insufficient.

With these results the carbon and nitrogen concentration within the cellular material, \( c_{i,C} \) and \( c_{i,N} \), expressed as kg m\(^{-3}\), were computed by using the number of collected bacteria and their mean diameter \( d_3 \) (see below Eq. 17b).

4. Results

a. Characteristics of bacteria. Relevant information concerning the bacteria subsequently submitted to optical measurements, is provided in Table 1. Selected examples of size distribution function are shown on Figures 2a, b, and c; the functions \( F_r(d) \) are plotted in relative units, after normalization with respect to the maximum, which occurs for \( d_1 \), the modal diameter.

The \( d_1 \) values for each culture are given in Table 1. On some occasions \( F_r(d) \) was
Figure 2. Relative size distribution functions, $F_r(d)$, normalized with respect to their maximum. The experiments are labelled as in Table 1. (a) Same population (UE 3) measured with two instruments as indicated; when the determination is made with the ZBI/C 1000 equipment, the noisy part of curve (ascending slope) has to be replaced by the extrapolated curve (from 0.5 to 0.3 μm) computed as indicated in the text. (b) Day-to-day evolution of $F_r(d)$ for the enriched culture (E4). (c) As in (b) for the doubly peaked population (E8).
bimodal (see Fig. 2c) and the diameters corresponding to these two peaks are given. The width of the size distribution is depicted by $\Delta d$, the size interval within which $F_r(d)$ is above 0.5. The average diameter, $d_2$, in Table 1, is computed as being the diameter of a sphere having a geometrical cross section equal to the average cross section of the entire population, thus

$$d_2 = \left[ \int_0^{\infty} F(d) \, d^2 \, d \, d \right]^{1/2} (N/V)^{-1} \quad (17a)$$

and similarly

$$d_3 = \left[ \int_0^{\infty} F(d) \, d^3 \, d \, d \right]^{1/3} (N/V)^{-1} \quad (17b)$$

represents the diameter of a sphere having a volume equal to the average volume of the population. With the slightly skewed or doubly peaked distributions as observed, $d_2$ and $d_3$ are significantly higher than $d_1$. Bacteria in the size range 0.53–1 $\mu$m were obtained. As a rule, bacteria grown in supplemented media were often significantly, albeit not systematically, bigger than bacteria in unenriched seawater (about twice in volume, or $\times 1.3$ in size). This is particularly true at the end of exponential growth when the first optical determinations are made. The size tends thereafter to decrease (see Fig. 2b), when the population remains in stationary phase. Perhaps “mixed” populations able to coexist during several days were cultured, as suggested by the steady doubly peaked size distribution function (Fig. 2c). No significant difference was noticed with respect to the season of sampling.
b. Optical efficiency factors. Once the spectral values of the absorption, $a(\lambda)$, scattering, $b(\lambda)$ and attenuation coefficients, $c(\lambda)$, for the bacterial suspension have been determined, as well as the size distribution function, the dimensionless efficiency factors can be derived according to (Bricaud and Morel, 1986)

$$\bar{Q}_j(\lambda) = j(\lambda) \left( \frac{\pi}{4} \int_0^\infty F(d) d^2 \, \text{d}d \right)^{-1}$$

(18)

where the symbol $j$ means either $a$, $b$ or $c$. The bracketed term is the cross sectional area of the entire bacterial population. The overbar on $Q$ means that the efficiency factor in question is that of a cell having the average diameter $d_2$ defined above. The $F(d)$ function is also used to combine the VSF computed for discrete size values in view of predicting the volume scattering function of polydispersed bacterial population.

c. Efficiency factor for absorption, $Q_a(\lambda)$. The $Q_a(\lambda)$ spectra determined for the bacteria in Table 1 are presented all together on Figure 3a. The prominent features are (i) as expected, the very low $Q_a$ values, at least one order of magnitude below the values pertinent to eukaryotic algal cells, and 5 or 10 times smaller than those of cyanobacteria (Bricaud et al., 1988; Stramski and Morel, 1989) and (ii) a general increase of $Q_a(\lambda)$ toward the shorter wavelengths which somewhat unexpectedly results in the formation of a maximum around 410–415 nm. Absorption measurements by cells in suspension below 400 nm are uncomfortably noisy and the presence of the maximum is not indubitably ascertained. Its existence, however, has been fully confirmed by separate measurements, extending down to 350 nm, easily performed when the bacteria cells are collected on a GF/F filter and by using the wet filter technique (Trüper and Yentsch, 1967), (see later Fig. 7a and Discussion).

According to Eq. (12), $Q_a$ for weakly absorbing objects, is linearly related to the size, provided that the absorption coefficient of the cellular material, $a_{cm}$, remains constant. The $Q_a$ values for $\lambda = 415$ nm are plotted as function of diameter in Figure 3b, where the straight lines corresponding to $a_{cm} = 0.03$ and $0.06 \times 10^6$ m$^{-1}$ are also drawn. The dispersion of the points in this diagram does not support the idea of a constant absorption capability of the cellular material. Nevertheless the coefficient $a_{cm}$ seems restricted to vary within a ratio of only 2, regardless of the size or the culture conditions. Careful examination of the different $Q_a$ spectra does not reveal more than subtle differences in shape, nor systematic differences between bacteria when cultured in enriched or unenriched media.

d. Efficiency factor for scattering, $Q_b(\lambda)$ and backscattering $Q_{bb}(\lambda)$. All the $Q_b(\lambda)$ spectra are presented on Figure 4a. From these values it results that such bacteria are much more efficient in scattering than in absorbing light ($Q_b/Q_a$ is about 20). The scattering efficiency, however, remains rather low, about one third of that determined for cyanobacteria with sizes of 1.1 to 1.5 $\mu$m (Bricaud et al., 1988; Stramski and Morel,
Figure 3. Efficiency factor for absorption as a function of the wavelength (a); or at a given wavelength (415 nm) and as a function of the diameter (b). The left end of the horizontal bars represent the modal diameter, the right end the average diameter $d_2$ (Eq. 17a).

1989). The similarity of the various spectra in terms of shape is demonstrated when a normalization (at $\lambda = 400$ nm) is adopted (Fig. 4b). The regular ascending slope toward the short wavelengths very nicely conforms to the theoretical prediction of a $\lambda^{-2}$ curvature (Eq. 9). The same equation entails a dependency of $Q_b$ upon $d^2$, when $\lambda$ is fixed. This is verified by plotting $Q_b$ at $\lambda = 550$ nm (Fig. 5) as a function of the
Figure 4. Efficiency factor for scattering as a function of the wavelength, in absolute value (a) or after normalization at λ = 400 nm (b). The theoretical λ⁻² curve is also shown (note the offset used to translate upward this curve).

Diameter. The parabole with n = 1.05 and 1.06 are also shown on this figure; the choice of these values of the relative index of refraction will be discussed later.

As theoretically predicted (Eq. 11), the \( Q_{bb}(\lambda) \) spectra on Figure 6a are essentially flat, whereas the \( \hat{b}_{bb}(\lambda) \) spectra display a parabolic increasing trend toward the red end of the spectrum (Fig. 6b). The irregular and small fluctuations, actually within the limits of detection for such difficult determinations, are presumably artifactual. The
Figure 5. Efficiency factor for scattering at 550 nm as a function of the diameter (horizontal bars with the same meaning as in Fig. 3b). The theoretical parabolae for a relative refractive index \( n = 1.05 \) or 1.06 are shown as dashed curves.

various values of \( \tilde{b}_b \) (at a given wavelength) are directly under the control of the size, according to a \( d^{-2} \) dependency. This is made clear on Figure 6c where the \( \tilde{b}_b \) values at \( \lambda = 600 \) nm are plotted vs. the modal diameter, together with the theoretical curves computed through the Mie theory and for a relative refractive index \( n \) equal to 1.05 and 1.06. As mentioned before, in this size domain, \( \tilde{b}_b \) is virtually independent from \( n \).

The smallness of bacteria cells induces \( \tilde{b}_b \) values approximately ten times higher than those typical for phytoplankton (Bricaud et al., 1983) or about twice that of Synechocystis (Stramski and Morel, 1989); they remain, however, well below 1%.

5. Interpretation of the results

a. Light absorption by bacteria. The existence of an absorption peak, centered on 412–415 nm, is indubitably confirmed by the steep descending slope from 412 toward 375 nm observed when the measurements are made on GF/F filters (Fig. 7a). These easier determination, however, present the disadvantage of being uncertain in terms of absolute units, because of the absorption amplification induced by the diffuse lighting condition, coined the "\( \beta \) effect" (Butler, 1962). Accordingly, only relative spectra (normalized by their maximum) are shown. Other reproducible features were disclosed, such as the humps at 515–520 and at 555 nm, separately or simultaneously discernible. These spectra closely resemble the absorption spectra of yeast as determined by Shibata (1958), with the typical features attributable to cytochromes (see e.g. Mehard et al., 1975 or Pettigrew, 1979), namely the prominent \( \gamma \) (Soret) band, at
Figure 6. Efficiency factor for backscattering as a function of the wavelength (a). Backscattering ratio as a function of the wavelength (b), or (c) at a given wavelength (600 nm) and as a function of the modal diameter. The theoretical curves computed for \( n = 1.05 \) and \( n = 1.06 \) are also shown.

about 415 nm, and the \( \beta \) and \( \alpha \) bands (ca. 525 and 555 nm), the intensity of which is depending on the oxydation state. Cytochromes likely are the dominant absorbing component for these heterotrophic bacteria.

Interestingly, the blue-violet absorbing substance is partly soluble in organic solvent (Fig. 7b) and HPLC analysis of the acetonic extract distinctly reveals the presence of
three major (unidentified) components which are able to absorb at 400 nm (H. Claustre, personal communication). A small amount of these substances is actually extractable, as demonstrated by applying the Kishino method (1985); after a methanol treatment of the bacteria retained on the filter, the absorption values are only slightly reduced. This is also confirmed by the fact that, for a given number of bacteria, the acetonic extract absorption is less than 10% of the intact cells absorption.

b. Scattering properties and index of refraction. The bulk refractive index of the bacteria cells can be inferred from the optical efficiency factors previously determined, according to a scheme already described in Bricaud and Morel (1986) and thereafter modified by Stramski et al. (1988). The modified scheme *a priori* relies on the applicability of the anomalous diffraction approximation (Van de Hulst, 1957), and is justified in that the bacterial cells are essentially homogenous and spherical bodies. The real part of the index of refraction is influenced by absorption owing to the anomalous dispersion effect (Ketteler-Helmoltz theory). Therefore the spectral values \(n'(\lambda)\) have to be assessed at first, before \(n(\lambda)\) can be derived. The minute size of bacteria allows the Stramski *et al.* scheme to be simplified when computing \(n'\). By combining Eq. (6) and Eq. (12), (the latter being limited to its first term according to an approximation valid for small particles), it follows that

\[
n'(\lambda) = \frac{3\bar{Q}_a \lambda}{8\pi d n_w}
\]  

(19)

where \(d\) is the average diameter \(d_2\) (Eq. 17) and \(\bar{Q}_a(\lambda)\) the experimental values in
Figure 7. Spectral values of the optical density determined after having collected bacteria on a GF/F filter. The spectra are normalized with respect to their maximum at 412–415 nm (a). The corresponding size distribution functions for these four populations are shown in insert. Absorption spectra (normalized as above) of acetonic and methanol extracts (b).

Figure 3a. The $n'(\lambda)$ spectra, similar to that of $\overline{Q}_a$ (apart from the effect of the multiplication by $\lambda$) are characterized by extremely low values, of the order of 1 to 3 $10^{-4}$ at 700 nm and 6 to 12 $10^{-4}$ at 400 nm. The variability in $n'$ (Fig. 8a) mainly reflects that of $a_{cm}$, already shown in Figure 3b.
Given that \( n'(\lambda) \) is known, the computational scheme providing \( n(\lambda) \) is operated. It rests on the use of the van de Hulst equation:

\[
Q_\varepsilon(\rho) = 2 - 4 \exp\left(-\rho \tan \xi\right) \left[ \cos \frac{\xi}{\rho} \sin (\rho - \xi) + \left( \cos \frac{\xi}{\rho} \right)^2 \cos (\rho - 2\xi) \right] + 4 \left( \cos \frac{\xi}{\rho} \right)^2 \cos 2\xi
\]  

(20)
with \( \xi = \tan^{-1} (n'/n - 1) \), which replaces Eq. (8) when absorption does exist \((n' \neq 0)\). Practically, at each wavelength \( \lambda \), \( \bar{Q}_c(\rho) \) is iteratively forced to equal the experimental \( \bar{Q}_c(\lambda) \) values; the only one free parameter is \( n \), since in \( \rho (= 2\alpha(n - 1)) \) the size is known and thus \( \alpha \) is fixed. The effect of polydispersion is accounted for by the use of averaged quantities \( \bar{\rho} \) and \( \bar{Q}_c(\bar{\rho}) \) as

\[
\bar{Q}_c(\bar{\rho}) = \int_0^{\infty} Q_c(\rho) F(\rho) \rho^2 \, d\rho / \int_0^{\infty} F(\rho) \rho^2 \, d\rho
\]

where the function \( F(\rho) \) is the experimental size distribution function, once \( d \) has been replaced by \( \rho \). When the convergence is reached, \( \rho \) and hence \( n \) are determined. The computation is repeated throughout the spectrum with a 2.5 nm increment in \( \lambda \). The \( n(\lambda) \) spectra are shown on Figure 8b. They all display a smooth ascending slope toward the short wavelengths, typical of a normal dispersion curve, with a variation in \( n \) of about +0.004 between 750 and 400 nm. Because of the weakness of absorption, the anomalous dispersion pattern, often well-marked in the case of autotrophic plankton- (see e.g. Bricaud and Morel, 1986), does not appear in the case of these bacteria. The \( n \) values, in a relatively narrow range (1.045 to 1.055), are in the middle of the typical interval found for phytoplankton (Bricaud et al., 1988).

c. Refractive index and carbon content. The material forming a bacterial cell can be seen as a mixture of organic compounds and water. The real refractive index of such a mixture can be derived by adding the contributions of the components, weighted according to their partial volume, \( V \) (Gladstone-Dale “rule,” 1863; see also Aas, 1981)

\[
n_{cm} = n_w V_w + n_o V_o \\
n_{cm} = n_w + (n_o - n_w) V_o.
\]

The subscripts \( w \) and \( o \) stand for water and (dry) organic matter taken as a whole. In terms of real relative index, \( n = n_{cm}/n_w \), the above expression becomes

\[
n = 1 + \Delta n \, V_o
\]  

(21)

where

\[
\Delta n = (n_o - n_w) / n_w.
\]

The partial volume \( V_o \) is linked to the intracellular organic matter concentration, \( c_{i,o} \) through:

\[
c_{i,o} = V_o \rho_o
\]

where \( \rho_o \) is the density of the dry organic matter. If the conversion factor, \( f \), from carbon to organic dry mass is introduced, Eq. (21) becomes

\[
n = 1 + \Delta n f \frac{c_{i,C}}{\rho_o}
\]  

(22a)
where \( c_{i,C} \) is the carbon concentration within the cellular material. The question now is to select the \( \rho_o, f \) and \( \Delta n \) values, typical of the cell material. The average C/N ratio (see Table 1) is 4.46; this figure leads us to estimate that the organic matter forming the cell could be a mixture of carbohydrates and proteins, (for which C/N = 3.33; Copin, 1980), with approximately the respective proportions 25 and 75%.

The \( f \) factor being 1.9 for proteins and 2.5 for carbohydrates, the weighted mean is 2.05. By adopting the \( \rho \) values, 1335 and 1530 kg m\(^{-3}\), and \( n \) values, 1.1642 and 1.1418, respectively for "mean" proteins and carbohydrates (Aas, 1981), it follows that after a 25%-75% weighting, \( \rho_o = 1384 \) kg m\(^{-3}\) and \( \Delta n = 0.158 \). By replacing in Eq. (22a), \( n \) is numerically expressed by

\[
n = 1 + 0.233 \times 10^{-3} c_{i,C}.
\]  

(22b)

The corresponding straight line is shown on Figure 9, where the \( n \) values obtained at \( \lambda = 415 \) nm, are plotted versus the measured \( c_{i,C} \) concentrations. Interestingly, the above expression is almost identical to that derived from a regression analysis of data obtained for the cyanobacteria *Synechocystis* (Stramski and Morel, 1989). The departure of the experimental points with respect to the theoretical line in Figure 9 is largely explained by cumulated uncertainties, and definitely not by an erroneous guess of the protein-to-carbohydrate ratio. In effect, it is easy to see that the slope value is insensitive to this ratio. With counteracting evolutions of the involved terms, the slope \( (\Delta n f \rho_0^{-1}) \) keeps a constant value (within \(<1\%) from "pure" protein to "pure"
carbohydrate material. In spite of the scattering of the points, there exists a general agreement between the experimental results and the prediction, meaning that the optical determinations on one side and carbon concentration on the other side are consistent.

If the mean $c_{iC}$ value, 228 kg m$^{-3}$, is introduced in Eq. (22b) the resulting $n$ value is 1.053 instead of 1.050 for the mean $n$ value (i.e. an overestimate by 7% in terms of $\Delta n = n - 1$). The high carbon contents in bacterioplankton found for instance by Lee and Fuhrman (1987) and Bratbak (1985), respectively 380 and 560 kg C m$^{-3}$, are not confirmed by the present measurements; according to the above relationship 22b and 21, they would induce surprisingly high relative indices (1.09 and 1.13, respectively) for living organisms, associated with low water content ($V_w = 43$% and 17% respectively).

6. Discussion and conclusions

The free-living coccolid bacteria are perfect testing materials for a verification of the Mie theory, if such a verification was still needed. Their scattering properties conform to the theoretical predictions that, due to the smallness of these cells, take simple expressions such as a scattering coefficient varying with the 4th power of the size, a backscattering coefficient as the square of the size, a spectral dependency of the scattering according to $\lambda^{-2}$ and a wavelength-independent backscattering. The very weak absorption capacity, which nevertheless has been detected, does not alter the above simple conclusions. In addition, the refractive index, as inferred from the optical properties, complies with the cellular carbon content to which it is linearly related. In spite of having been collected at all seasons, bacteria were not markedly different with regards to size or C-N content. It is not claimed, however, that all possible features have been explored; likely free floating bacteria in the world ocean may have more diversified complexions. The theoretical framework as developed and tested above can apply just as well to these other cases. However, the major problem is being able to obtain more simultaneous information about size, cellular composition and also about abundance as far as the influence of bacterioplankton upon the optical properties of ocean water is discussed.

For this examination it is, at first, admitted that the bacteria as sampled, grown and studied are representative of those living in natural environment with respect to the cell material bulk properties (namely $a_{cm}$, $n_{cm}$ and $c_{iC}$). This proviso will be relaxed thereafter.

A size range, wider than that observed during the present experiment has to be considered, in agreement with values reported in literature (for instance the 0.2 to 1 $\mu$m range, as in Sieburth et al., 1978). There is no particular problem, since theory has been fully verified and hence can be safely used for prediction. Size dependency of the optical properties is important; the scattering coefficient, $b$, varies like $d^4$ (Eqs. 9 and 10a) and $a$, the absorption coefficient, like $d^3$ (Eqs. 12 and 10b), under the proviso of a
constant cell material. This is made clear on Figure 10, where $b$ and $a$ are plotted against the diameter (up to 10 μm, to show the domain of applicability) and for two cell number densities, $10^{11}$ and $10^{12}$ cell m$^{-3}$. The relative index of refraction is given the mean value 1.05 and $\lambda = 0.55$ nm when computing $b$ (Eq. 8 and 10a); $a$ is computed (Eq. 12 and 10b) with $a_{cm} = 0.045 \, 10^6$ m$^{-1}$, a mean value of the absorption coefficient by the cellular material at its maximum for $\lambda = 415$ nm, (see Fig. 3a and 3b). When the approximations valid for small sized particles can apply ($d < 2 \, \mu m$ for scattering, $d$ up to 10 μm for absorption, because the discreteness effect does not occur for these weakly absorbing particles) the $d^4$ and $d^3$ dependencies, which are well observed, correspond to the following expressions

\begin{align}
    b_{bact} &= \frac{1}{2} \pi^3 n_w^2 (n - 1)^2 \lambda^{-2} d^4 (N/V) \\
    a_{bact} &= \frac{1}{6} \pi a_{cm} d^3 (N/V)
\end{align}

straightforwardly derived from Eq. (9) and (10a), or (10b) and (12) (reduced to its first term) respectively.

On the same figure, the typical values of the scattering coefficient for seawater in oligotrophic, mesotrophic and eutrophic situations are also indicated (by horizontal stripes) (see e.g. Gordon and Morel, 1983). For comparison, it must be remembered that bacterial abundance is of the order of 1 to 1.5 $10^{11}$ cell m$^{-3}$ in very oligotrophic
zones (Sargasso Sea; Neveux et al., 1989), 0.2 to 1 $10^{12}$ cell m$^{-3}$ (at McMurdo Sound; Furfman and Azam, 1980) or 1 to 2.5 $10^{12}$ cell m$^{-3}$ in a mesotrophic warm core ring (Nelson et al., 1985), and 3 to 4 $10^{12}$ cell m$^{-3}$ in coastal upwelling (McManus and Peterson, 1988). With these figures, it is clear that microbes are not responsible for scattering if their size is below 0.3 or 0.4 $\mu$m. They could begin to play a significant role, if they are in the size range 0.5–0.8 $\mu$m, even with concentrations as low as $10^{11}$ cell m$^{-3}$. For instance, with sizes ranging from 0.36 to 0.57 $\mu$m and cell abundance between 0.3 and 3 $10^{12}$ cell m$^{-3}$, bacterioplankton within, and at the periphery of, the warm core rings studied by Ducklow (1986) certainly contribute to the scattering properties of these waters in a sizeable manner. Violet-absorption by bacteria, 10 or 20 times smaller than scattering, is believed to remain negligible in most of the situations.

These preliminary and inevitably vague conclusions can be made more quantitative by returning back to Eq. (1), which establishes a link between the cell number and the trophic state, as depicted by the chlorophyll concentration. The role of bacterioplankton in forming the scattering coefficient as well as in contributing to the particulate organic carbon pool, has to be examined and conciliated.

The bacterial carbon concentration, $\langle$POC$\rangle_{bact}$, is expressed as

$$
\langle$POC$\rangle_{bact} = (N/V) \nu c_{i,C}
$$

where $\nu(= \pi d^3/6)$ is the cell volume. If $c_{i,C}$, the intracellular carbon concentration, is given the mean value 228 kg C m$^{-3}$ (Table 1) and if $N/V$ is replaced by its expression in Eq. (1), the above equation becomes

$$
\langle$POC$\rangle_{bact} = 109 d^3 \langle$Chl$\rangle^{0.52}
$$

graphically represented in Figure 11a for some selected sizes ($d$ is expressed in $\mu$m in Eq. 25) and for chlorophyll concentrations varying between 0.01 and 1 mg m$^{-3}$, (both scales are logarithmic). Also shown is the total particulate organic carbon $\langle$POC$\rangle_{tot}$ as a function of $\langle$Chl$\rangle$ according to Eq. (2). Similarly, by combining Eq. (1) and (23a) and, as above, letting $n = 1.05$, $n_w = 1.34$ and $\lambda = 0.55$ $\mu$m, it follows that

$$
b_{bact} = 0.21 d^4 \langle$Chl$\rangle^{0.52}
$$

graphically represented in Figure 11b for the same $d$ values, together with the total scattering coefficient, $b_{tot}$, also a function of $\langle$Chl$\rangle$ according to Eq. (3), when used with the lower and upper limits for the coefficient (i.e. 0.15 and 0.45). The link between these two figures, or the constraint in the problem, is ensured by the provision of compatible $n$ and $c_{i,C}$ values, (that is respected here).

The implicit quasi-linear relationships which exist between $N/V$, $\langle$POC$\rangle_{tot}$ and $b_{tot}$ (cf. Introduction) result in a quasi-parallelism of the curves (straight lines in the log-log plot) showing $\langle$POC$\rangle_{tot}$ and $b_{tot}$ and those computed for $\langle$POC$\rangle_{bact}$ and $b_{bact}$ with various discrete $d$ values. If the typical free-living bacteria keep roughly a constant size independently from the trophic state, such a parallelism means that their
Figure 11. (a) Particulate organic carbon due to bacteria $\langle POC \rangle_{bact}$ of various sizes (diameter $d$ in $\mu m$) computed as function of chlorophyll concentration according to Eq. (25) (dashed lines); the solid heavy line shows an empirical relationship (Eq. 2) between the total particulate organic carbon, $\langle POC \rangle_{tot}$, and $\langle Chl \rangle$. (b) Scattering coefficient due to bacteria $b_{bact}$ of various sizes as in 11a, computed as function of $\langle Chl \rangle$ according to Eq. (26) (dashed lines); the solid lines correspond to an empirical relationship (Eq. 3 with the extreme values 0.15 and 0.45 given to the coefficient) which links the total scattering coefficient, $b_{tot}$, to $\langle Chl \rangle$.
Figure 12. As a function of the bacterial cell diameter are shown the ratios \((\text{POC})_{\text{bact}}/\langle \text{POC} \rangle_{\text{tot}}\) (solid curves) and \(b_{\text{bact}}/b_{\text{tot}}\) (dashed curves). The two couples correspond to two values of the intracellular carbon concentration, 228 and 456 kg m\(^{-3}\), for A and B, respectively.

contributions to \(b_{\text{tot}}\) or \(\langle \text{POC} \rangle_{\text{tot}}\) also remain a constant. With the \(c_{iC}\) value, and therefore the \(n\) value adopted, these contributions appear of similar importance; for instance, bacteria with a mean diameter of 0.6 \(\mu\)m would form 29% of the total particulate organic carbon content and 23% of the light scattering. It is worth remarking that some incompatibilities arise. With a mean size of about 1 \(\mu\)m, for instance, most of the light scattering (if not all) would be due to bacterioplankton, whereas the POC they represent would exceed that corresponding to all kinds of particulates. Compatibilities are restored when \(d\) becomes smaller than about 0.9 \(\mu\)m, even if, under this hypothesis, the role of bacteria is strongly exaggerated.

The relative role of bacteria, as varying with their mean size, is evidenced by forming the ratios \((\text{POC})_{\text{bact}}/\langle \text{POC} \rangle_{\text{tot}}\) and \(b_{\text{bact}}/b_{\text{tot}}\) \((b_{\text{tot}}\) in Eq. 3 is computed with 0.15). These ratios, computed for \(\langle \text{Chl} \rangle = 0.1\) mg m\(^{-3}\), (but the results are, as said before, practically independent from the trophic state), are shown in Figure 12, which illustrates more quantitatively and for the whole size range the preceding comments. With the help of this representation, the consequence of a change in \(c_{iC}\), and subsequently in \(n\), is readily evident.

For instance, if a doubling in \(c_{iC}\) is envisaged (such high values have been reported), \(\langle \text{POC} \rangle_{\text{bact}}\) is also doubled (Eq. 24). Meanwhile the index increment \((n - 1)\) is also doubled (Eq. 22b), resulting in a four-fold increase in \(b_{\text{bact}}\) (Eq. 23a). Therefore the contributions of bacteria to POC and to \(b_{\text{tot}}\) are no longer numerically similar and are strongly diverging when the size increases (curves labelled B in Fig. 12). The unsurpassable 100% limit in \(b_{\text{tot}}\) necessarily implies cell sizes below 0.6 \(\mu\)m. These
curves (B) also show that high intracellular carbon concentrations remain reasonably compatible with optical data (20 to 50% of $b_{tot}$ due to bacterioplankton), only if such cells are in the range 0.4 to 0.5 μm.

In a paper, recently translated into English, Kopelevitch et al. (1987) arrive at a conclusion somewhat different from that exemplified by Figure 12, mainly because they considered a lower numerical abundance; they determined the bacterial concentration in near-surface waters at 4 locations in the Pacific and only at one station the concentration (of bacilliform bacteria) was high enough to render significant their contribution to light scattering. To the extent that the ubiquitous validity of Eq. (1) will be confirmed, the relative contribution of free-living bacteria to light scattering, as well as to the particulate organic carbon (varying as $d^4$ and $d^3$ respectively), are significant even with the small mean size generally observed in the open ocean. With sizes in the range 0.45 to 0.6 μm these relative contributions would amount to about 10–30% in the upper layers and would be of the same order regardless of the trophic state. The strong spectral $\lambda^{-2}$-dependence of the light scattering by bacteria is likely partly responsible for the approximate $\lambda^{-1}$-dependence observed in oceanic waters (Morel, 1973).

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