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Photic Volume in Photobioreactors Supporting Ultrahigh Population Densities of the Photoautotroph *Spirulina platensis*

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Characterization of the photic zone and light penetration depth in cultures with ultrahigh cell densities represents a major issue in mass cultures of phytoautotrophic microorganisms grown in enclosed photobioreactors. In a study of the effect of underwater optical properties on the penetration depth of photosynthetically active radiation, the inherent optical properties of algal suspensions, i.e., absorption and scattering coefficients, as well as their apparent optical properties, i.e., the reflectance and the vertical attenuation coefficient of downwelling irradiance, were determined by using high-spectral-resolution radiometric measurements. The vertical attenuation coefficient was used to estimate quantitatively the depth of light penetration into a reactor containing an ultrahigh cell density (chlorophyll concentration, up to 300,000 mg m^{-3}). For such a high cell density, the photic volume in the reactor was found to be extremely small; nevertheless, it differed between the blue and red light (less than 0.06 mm) and the green light (about 0.5 mm). This suggests a singular role for green light under the unique circumstances existing in ultrahigh-cell-density cultures of photoautotrophs.

The conventional culture system for mass cultivation of species of commercial interest is at present the "open raceway," i.e., a shallow pan in which culture volume is spread over a large area, providing as large a volume-to-area ratio as is practically feasible. In these systems, temperature is not controlled and mixing, usually carried out by a paddle wheel, cannot provide high rates of turbulent flow. Most important, the output rate per reactor volume is very low, creating a heavy burden on the cost of production. This is the background for the growing interest in enclosed photoreactors, the main advantage of which rests in facilitating the maintenance of high cell densities due to a narrow light path, a high rate of turbulent streaming, and a significant measure of temperature control. A flat-panel photobioreactor designed recently (7) exhibits a particularly narrow light path coupled with high turbulence and may be set at variable orientations and tilt angles for maximal exposure to solar energy. Ultrahigh population densities are thus feasible, resulting in record output rates per reactor volume.

Mutual shading (12), however, becomes very severe at ultrahigh population densities; at a chlorophyll (Chl) content of 100,000 to 300,000 mg m^{-3}, light limitation to growth is most extreme. The light regimen of the individual cells therefore becomes the predominant factor in affecting productivity and represents the principal parameter for efficient utilization of the light source. The light regimen is governed by several factors: the intensity of irradiance at the reactor surface, the duration of light exposure of the individual cells in the photic and dark volumes of the reactor, and the frequency of fluctuation between these volumes. Characterization of the photic zone and light penetration depth in cultures with ultrahigh cell densities therefore represents a basic issue in mass microagriculture.

Much of the information on the optical properties of algae originates from experiments with ocean and inland waters (2–4, 8). The main difference between these and the optical properties of extremely dense algal suspensions lies in the magnitude of the absorption and scattering quantities and in the spectral distribution of these quantities. In addition to absorption by algal pigments in the blue range of the spectrum, scattering by water molecules, and Raman scattering, the following components become important in such ultradense algal cultures: scattering caused by cells, which becomes the dominant scattering factor, and absorption by algal pigments in the red range of the spectrum, which becomes comparable to absorption by pure water.

The vertical attenuation coefficient of downwelling irradiance is the most appropriate characteristic of the underwater light field for determining the light penetration depth. It depends on the inherent optical properties of algae, i.e., spectral absorption and scattering, but is independent of the intensity of the light source and only slightly dependent on atmospheric conditions and solar elevation. Thus, to understand the factors affecting the depth of light penetration into dense algal suspensions, the spectral absorption and scattering coefficients must be investigated. This information is lacking for ultrahigh densities of algal suspensions as reported in this work.

MATERIALS AND METHODS

Reactor. The general features of the newly designed flat inclined photobioreactor have been described previously (7). Essentially, this is a flat glass tank, 70 cm high, 90 cm long, and 2.6 cm in internal width. In the northern hemisphere, the reactor should be inclined facing south, with tilt angles of 30 and 60° for summer and winter, respectively. Mixing to effect turbulent flow is provided by 2% CO_2-enriched air supplied at 2 to 3 liters of compressed air per liter of algal suspension per min through perforated tubes which run along the bottom of the reactors as well as at ca. half the reactor height. A line of sprinklers along the top of the front panel provides water for evaporative cooling to prevent the culture temperature from rising above 35°C, the optimal temperature for the test organism.

Culture conditions. Modified Zarrouk medium was used for *Spirulina platensis* M-2 (14), and the pH was maintained at ca. 9.5 by regulating the inflow of CO_2. The culture medium was changed at intervals determined by the population density. At ultrahigh cell densities, e.g., Chl at ca. 300,000 mg m^{-3}, the entire growth medium was replaced with fresh medium every 12 h by filtering the culture suspension and dispersing the algal mass in fresh medium. Dry weight and Chl-*a* were measured by the method of Vonshak et al. (14), and growth was estimated from measurements taken at the proper time intervals.

Radiometric measurements. First, absorption was separated from scattering by spectrophotometric measurements of light attenuation carried out with sam-

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ples of algal suspensions in the laboratory. The absorption and scattering coefficients of the algal suspensions per unit concentration of Chl were calculated. Second, the apparent optical properties—reflectance and attenuation coefficient of downwelling irradiance—were measured, making it possible to determine the penetration depth of photosynthetically active radiation into the algal suspension.

(i) Attenuation coefficient. Two types of measurements were carried out to determine the absorption coefficient of the algal suspension. The spectral attenuation of the samples, c, was measured in a 1-cm-path-length cuvette with a Perkin-Elmer UV-VIS 551S spectrophotometer operating in the range from 400 to 800 nm. In this measurement, light scattered by the sample was collected over a small forward angle of 0 to 5°. Attenuation of the algal suspension was also measured in a cylindrical 6-cm-path-length cuvette placed at the opening of a LICOR LI-1800-12s external integrating sphere. In this measurement, forward-scattered light at angles of 0 to 50° was collected. Davies-Colley et al. (2) named such measured values apparent absorption (a'). Direct measurements of attenuation have been made for Chl contents up to 3,000 mg m^{-3}. Suspensions containing more than 3,000 mg of Chl m^{-3} were diluted with water to a final Chl concentration not exceeding 3,000 mg m^{-3}. The highest Chl concentration analyzed in our experiments was 270,000 mg m^{-3}, corresponding to ca. 33 g (dry weight) of algal mass per liter.

(ii) True absorption coefficient. By using the two methods for attenuation measurements, the absorption properties of the algal suspension were calculated. The method used was developed by Davies-Colley et al. (2) and was also used by Dekker (3) to determine the spectral specific absorption coefficient of Chl-*a* in inland waters. The method corrects the apparent absorption coefficient, a'_λ, measured with the integrating sphere accessory for scattered light that was not captured by the light receiver of the integrating sphere. Absorption of algal suspension was determined by using the following assumptions: (i) there is no absorption by Chl in the near-infrared range of the spectrum (beyond 750 nm), and (ii) the ratio of apparent scattering (b') to total scattering (b), b'/b, is constant along the entire photosynthetically active radiation region (400 to 750 nm).

The spectral absorption of algal suspension, a, was calculated from measurements of attenuation coefficient, c, and apparent absorption coefficient, a'_λ (3), as

$$a = [a'_\lambda - (a'_{750-800}/c_{750-800})c]/[1 - (a'_{750-800}/c_{750-800})] \quad (1)$$

where $a'_{750-800}$ is the apparent absorption coefficient as measured by the integrating sphere in the range from 750 to 800 nm and $c_{750-800}$ is the attenuation coefficient as measured by the spectrophotometer in the range 750 to 800 nm.

The assumption that b'/b is constant in the range from 400 to 700 nm for turbid inland waters was investigated (3, 10, 15). Whitlock et al. (15) estimated a deviation of 20% in the calculated scattering coefficient, which is sufficiently accurate for our purpose. Dekker (3) calculated the ratio $a'_{750-800}/c_{750-800}$ for samples of different inland water types. The spectral variations in the samples were negligible compared with variations associated with the type of water, which varied from 5 to 25%.

(iii) Specific absorption coefficient. The specific absorption coefficient, a^*, was determined as the ratio of calculated absorption coefficient, a (reciprocal meters), to Chl-*a* concentration (milligrams per cubic meter).

(iv) Total scattering coefficient. The total scattering coefficient, b, for all angles (except for the acceptance angle of the photomultiplier tube [0 to 5°]) was determined by subtracting the beam attenuation, c, from the true absorption coefficient, a. The specific scattering coefficient at 550 nm (b^*_{550}) was determined as a ratio of the total scattering coefficient (b) to the Chl-*a* concentration.

(v) Reflectance. A LICOR LI-1800 high-spectral-resolution radiometer was used to determine the radiance reflectance of the algal suspension in the reactor in the spectral range of 400 to 1,100 nm with a spectral resolution of 2 nm. The radiometer was attached to a fiber-optic cable (1.5 m long) and a telescope with a field of view of 15°. The upward radiance (L_w) of the 30° tilt reactor was measured from the nadir direction, and the measurement was repeated at least three times. Thereafter, a reference panel ($BaSO_4$) was placed just at the front surface of the reactor and its upward radiance (L_{ref}) was measured. Each observed upward radiance spectrum of the reactor was divided by the appropriate upward radiance spectrum of the reference plate to give a reflectance value of $R = L_w/L_{ref}$. The mean of three reflectance spectra was used in the analysis.

Reflectance spectra of the algal suspension itself were also measured. Algal suspension with a Chl content of 8,000 to 270,000 mg m^{-3} was removed from the reactor in a glass cylinder 25 cm in diameter and 30 cm high. The radiometer was attached to a fiber-optic cable and a telescope with field of view of 4°. The telescope was positioned 10 cm above the cylinder, and upward radiance was measured at least three times. The $BaSO_4$ reference plate was then placed at the front of the cylinder, and its upward radiance was measured by the same telescope. Reflectance was determined as the ratio of radiance of water to the radiance of the reference plate.

(vi) Vertical attenuation coefficient of downwelling irradiance. For determining the vertical attenuation coefficient, K_d, a sample of the algal suspension was transferred from the reactor to the glass cylinder used for measuring the reflectance spectra. The radiometer was attached to a remote cosine receptor, and downwelling irradiance was measured at two points: just above the water surface (E_0) and under the water surface at a depth z (E_z). For Chl concentrations up to 10,000 mg m^{-3}, E_z was measured at a depth of 2 cm; for higher Chl concentrations, the measurements were carried out at a depth of 1 cm. The vertical attenuation coefficient was calculated as $K_d = \log (E_0/E_z)/z$.

(vii) Light penetration depth. The light penetration depth, which gives a direct determination of the photic volume in the photoreactor, was calculated from the attenuation coefficient of downwelling irradiance as the depth in which the downwelling irradiance is decreased to 10% of the incident irradiance.

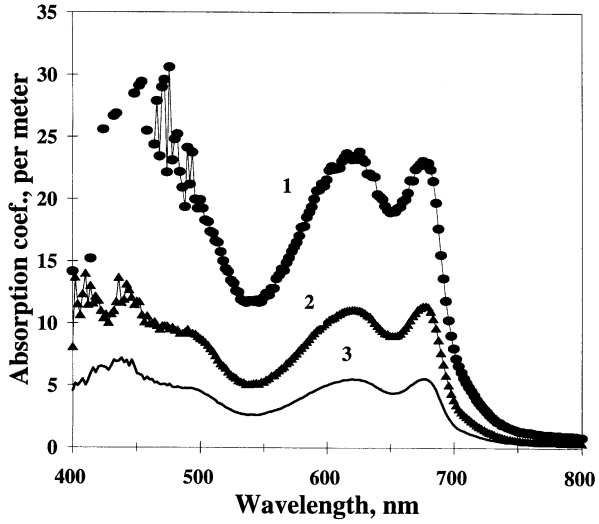

FIG. 1. True absorption spectra of *S. platensis* for various Chl concentrations. Curves: 1, 3,000 mg m^{-3}; 2, 1,500 mg m^{-3}, 3, 750 mg m^{-3}.

RESULTS AND DISCUSSION

***Spirulina* absorption spectra.** Equation 1 was applied to the spectroradiometric data obtained from samples measured either with or without the integrating sphere accessory (data not shown) to calculate the true absorption spectra (Fig. 1).

Spectral features evident in the absorption spectra (Fig. 1) include a peak close to 440 nm, which represents the first Chl-*a* absorption maximum. This peak is followed by a decrease in absorption, forming a pronounced shoulder near 490 to 500 nm, which represents carotenoids. Beyond 500 nm, absorption decreases substantially, reaching a minimum in the green range of the spectrum (near 550 nm) but nevertheless remaining rather high. The absorption spectra of photosynthetic algae in the green range of the spectrum result from overlapping spectra of individual pigments as follows: (i) absorption by carotenoids that peaks in situ near 500 nm and is significant in the green range (1); (ii) absorption by Chl-*a* that can contribute to the absorption at short wavelength (500 to 530 nm) in the green range (this is particularly true for suspensions with extremely high Chl contents, like those investigated in this study); and (iii) phycobilin absorption that contributes to the absorption in the range from 550 to 600 nm. Indeed, a peak near 625 nm, due to absorption by phycocyanin (3, 5), can be clearly seen in Fig. 1. It is worth noting that such high absorption by photosynthetic pigments in the green range of the spectrum was reported for inland waters rich in carotenoids (16). A peak near 675 nm is due to absorption by Chl-*a*. Beyond 670 nm, absorption decreases to very low values and remains low up to 750 nm.

Specific absorption of Chl-*a*. The specific absorption of Chl-*a*, a^*, was calculated from the true absorption spectra (Fig. 1). For all samples measured in the range of 700 to 10,000 mg of chlorophyll m^{-3}, the specific absorption coefficient did not

vary more than 5%. At 440 and 670 nm, it was 0.009 and 0.0075 $m^2 mg^{-1}$, respectively.

Dekker (3) calculated a_{675}^* for several freshwater phytoplanktons and proposed an average of 0.0153 $m^2 mg^{-3}$. Haardt and Maske (6) calculated a_{675}^* to be between 0.007 and 0.013 $m^2 mg^{-1}$. The specific absorption value reported in this work is in the same range.

Scattering coefficient. Once the true absorption is determined, derivation of the total scattering coefficient, b, is straightforward. Beam attenuation, c, minus true absorption, a, gives the scattering coefficient for all angles except the acceptance angle (5°) of the photomultiplier tube. Since more than 50% of the scattering, however, occurs within scattering angles smaller than 5°, b_{0-5} could represent a significant part of the total scattering (3), and it would not be correct to determine b from b_{5-180}. For our purposes, the scattering at small forward angles, b_{0-5}, is much more important than for remote reflectance measurements, in which b_{5-180} could represent backscattering.

The scattering coefficient of the cyanobacterium *S. platensis* was thus calculated only for comparison with values found for phytoplankton in inland waters. The specific scattering coefficient at 550 nm was determined as a ratio of the scattering coefficient b_{5-180} to Chl-*a* concentration. The value of b^*_{5-180} obtained was 0.09 $m^2 mg^{-1}$, a little smaller than the average b^*_{550} calculated for inland waters by Dekker (3), and is in the range of 0.044 to 0.139 $m^2 mg^{-1}$ obtained by Davies-Colley et al. (2) for freshwater algal cultures. The spectral variation of the ratio $a'_{750-800}/c_{750-800}$ was between 15 and 20%, depending on algal cell density. It should be noted that the specific scattering coefficient b^*_{5-180} of the algal suspension was much higher (ca. 10-fold) than the specific absorption coefficient of algae. This value may be even twice as high for scattering angles from 0 to 180°. Clearly, scattering plays a major role in light attenuation in ultradense algal suspensions, similar to its reported role in productive turbid inland waters (8).

Reflectance. Two distinguishing spectral features were found in the reflectance spectrum (Fig. 2): extremely low reflectance in the visible range of the spectrum (less than 3%), with minimal spectral variation, and extremely high reflectance (higher than 40%) in the near-infrared range of the spectrum (not shown). As found for relatively low phytoplankton cell density with Chl contents up to 20,000 mg m^{-3} (5), an increase in density led to a decrease in reflectance up to Chl contents of about 5,000 mg m^{-3}. For Chl contents above 6,000 mg m^{-3}, reflectance leveled off and a further increase in biomass concentration did not lead to any significant variations in reflectance. In this study, reflectance of algal suspensions with Chl concentrations above 100,000 mg m^{-3} was very similar to that measured for open raceways with a Chl-*a* content near 10,000 mg m^{-3} (5).

Vertical attenuation coefficient and depth of light penetration. The vertical attenuation coefficient of subsurface downwelling irradiance depends, in addition to the inherent optical properties (absorption by algae and scattering), on the angle of refracted incident photons and on the volume-scattering function (8, 9). Having found that specific absorption and scattering coefficients remained quite stable for *Spirulina* cultures in the studied reactor, we adopted a working hypothesis that the vertical attenuation coefficient of downwelling irradiance can be used to determine the depth of light penetration into the algal suspension in the reactor.

The vertical attenuation coefficient of subsurface downwelling irradiance (Fig. 3) has the same spectral features as those found for the absorption spectra (Fig. 1). The spectral behavior of this coefficient indicates unique light climates in different spectral bands. Strong attenuation was found along the entire visible spectrum, including the green range, in which it was at least three times lower than in the blue and red ranges but nevertheless remained very high.

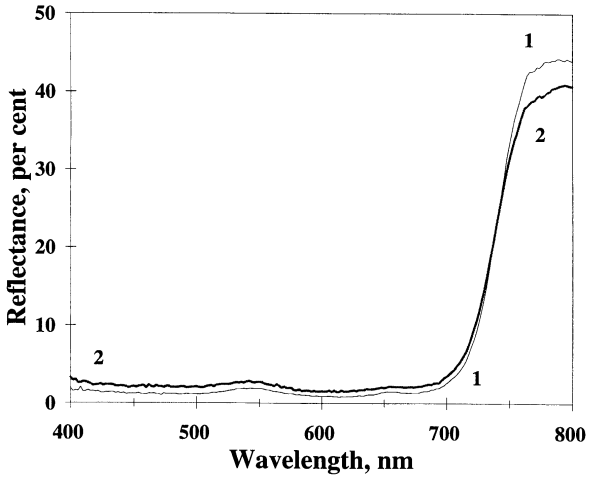

FIG. 2. Reflectance spectra of *S. platensis* measured in a cylindrical container. Chl concentrations were 107,000 mg m^{-3} (curve 1) and 53,000 mg m^{-3} (curve 2).

The presence of quite a high contrast in absorption between the green range, on the one hand, and the red and blue ranges, on the other hand, suggested large variations in the depth of light penetration into the algal suspensions. When cell density increased, the depth of light penetration in the blue and red ranges decreased from 10 mm for a Chl content of 1,000 mg m^{-3} to 8 mm for a Chl content of 10,000 mg m^{-3} (Fig. 4). In a typical algal suspension used in this study, i.e., a Chl content of 100,000 mg m^{-3}, the light penetration depth decreased drastically, and in the blue and red regions it was less than 1 mm. In a suspension containing 300,000 mg of Chl m^{-3}, the penetration depth ranged between 0.03 and 0.05 mm in the blue and red ranges, indicating that light is absorbed in ultrahigh-density algal suspensions at the surface of the reactor only. In contrast, the penetration depth was much greater in the green range at this ultrahigh population density (300,000 mg m^{-3}), being close to 1 mm. Green light is therefore seen as

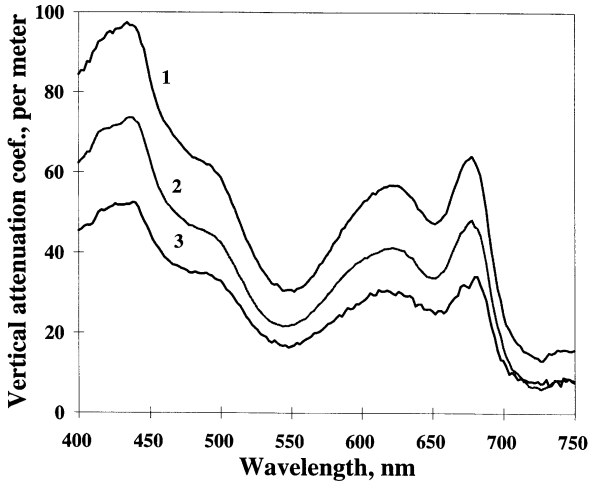

FIG. 3. Vertical attenuation coefficient of downwelling irradiance in *S. platensis*. Chl concentrations were 6,500 mg m^{-3} (curve 1), 10,000 mg m^{-3} (curve 2), and 13,000 mg m^{-3} (curve 3).

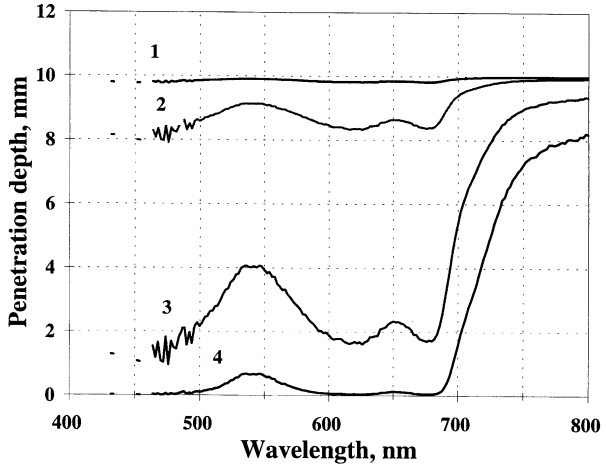

FIG. 4. Penetration depth of light in the photosynthetically active radiation range, determined as the depth at which downwelling irradiance is decreased to 10% of incident irradiance. Chl concentrations were 1,000 mg m^{-3} (curve 1), 10,000 mg m^{-3} (curve 2), 100,000 mg m^{-3} (curve 3), and 300,000 mg m^{-3} (curve 4).

an additional and essential energy source available for photosynthesis in ultrahigh cell densities, greatly expanding the very limited photic volume in such cultures and seemingly supporting photosynthesis in the absence of blue and red light a small distance underneath the surface. Since the green range of the spectrum, which represents approximately one-third to one-quarter of the entire photosynthetically active radiation range, corresponds to the maximal flux of solar energy, the intensity of the green light when mutual shading is extreme is sufficient to facilitate photosynthesis at a level corresponding to that induced by the red and blue ranges of the spectrum. Further investigation is required to determine the efficiency of green light for photosynthesis in our system.

Conclusion. The general conclusions pertaining to determination of the inherent optical properties required for calculating light penetration depth into algal suspension are as follows. (i) A similar absorption coefficient for Chl was found for *Spirulina* cultures grown in widely different reactor types (5). The absorption coefficient, however, was quite sensitive to the physiological state of algae (not shown), opening the possibility of monitoring some aspects of the physiological state of algae in real time by using nondestructive remote-sensing techniques. (ii) Absorption and scattering coefficients for Chl were quite close to those reported for algae in ocean and inland waters. (iii) The vertical attenuation coefficient of downwelling irradiance can be used to estimate the depth of light penetration into a reactor supporting ultrahigh cell densities. (iv) In ultrahigh-cell-density cultures, the photic volume, which is only a small fraction of the reactor volume, greatly differs for the blue and red light compared with the green light. A photosynthetic role for green light under these unique circumstances is suggested.

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