

OPTICAL PROPERTIES OF DENSE ALGAL CULTURES OUTDOORS AND THEIR APPLICATION TO REMOTE ESTIMATION OF BIOMASS AND PIGMENT CONCENTRATION IN *SPIRULINA PLATENSIS* (CYANOBACTERIA)¹

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ABSTRACT

Reflectance and vertical attenuation coefficient spectra from 400 to 1100 nm were investigated in detail on dense algal cultures of *Spirulina* in order to create algorithms for remote estimation of pigment and biomass concentration. Reflectance and the vertical attenuation coefficients were compared with biomass and pigment concentration in outdoor algal cultures. For assessing biomass concentration, the sum of reflectance above the base line from 670 to 950 nm was used. This allows the estimation of biomass with an error of less than $0.06 \text{ g} \cdot \text{L}^{-1}$. For chlorophyll *a* and phycocyanin estimation, vertical attenuation coefficients at the wavelengths 440 nm (or 676 nm) and 624 nm, respectively, were employed. The developed algorithms were tested by using independent data sets in the range of chlorophyll *a* from 0.2 to $20 \text{ mg} \cdot \text{L}^{-1}$ and biomass from 0.15 to $1.1 \text{ g} \cdot \text{L}^{-1}$. An error of pigment estimation of less than $0.80 \text{ mg} \cdot \text{L}^{-1}$ was achieved. The potential use of the algorithms in algal biotechnology is further discussed.

Key index words: attenuation coefficient; biomass; chlorophyll; Cyanobacteria; dense algal cultures; phycocyanin; remote sensing; *Spirulina platensis*

The recent developments in large-scale application of algal biotechnology for the production of high-value products are making this new agrobiotechnology an example in which on-line monitoring and control are of unique importance. In conventional agriculture, decisions on when to fertilize,

harvest, or apply weed control treatments can be made on a time scale of days. In algal cultures, we are dealing with a much faster growth process with a time scale of hours. Therefore, decisions like when to harvest or when to add nutrients must be taken instantaneously. Any mistake may lead to a significant reduction in productivity or in a total loss of all the culture. In the case of high-tech agriculture, when cost of production determines the commercial viability of a production site, on-line monitoring of growth parameters may provide the grower with high-value information that will help optimize the production process and thus help reduce cost of production.

Recently, the value of remote sensing techniques as an alternative method of algal biomass estimation has been recognized. Remote sensing studies normally involve the mapping of pigment concentrations in water bodies using radiance collected by a sensor placed above the water surface. Quantification of algal biomass concentrations usually requires the development of empirical or semi-empirical models that correlate between radiance (or reflectance) measured by a remote sensor and "ground truth" chlorophyll concentrations. The spectral features of reflectance of productive waters with chlorophyll concentrations in the range of 5 – $100 \text{ mg} \cdot \text{m}^{-3}$ have been investigated in detail (e.g. for inland waters: Gitelson et al. 1986, 1993a, b, Dekker et al. 1992, Quibell 1992, Dekker 1993; for artificial ponds: Matthews and Boxall 1994, Rundquist et al. 1994a, b).

Methods for quantitative assessment of chlorophyll in such waters have been developed (e.g. Gitelson et al. 1986, 1993a, b, 1994a, b, Dekker 1993,

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Gitelson 1993). The results strongly suggest that the green to nearby infrared spectral region is suitable for retrieval of information. This is in direct contrast to the oceans where the blue to green spectral region is considered to be the most appropriate for remote sensing (Gordon and Morel 1983).

To the best of our knowledge, the optical properties of high-density productive algal ponds (HDPPs) have not been investigated. Water bodies with maximal values of chlorophyll in the range of 100–500 $\mu\text{g}\cdot\text{L}^{-1}$ have been studied (Quibell 1992, Gitelson et al. 1994a). Even for such low chlorophyll *a* concentrations (compared to typical HDPP values), scattering by phytoplankton cells (but not absorption by pigments) plays a major role in optical properties. Therefore, we could not expect that the algorithms developed for productive inland waters would be appropriate for HDPP. Optical properties of waters with extremely high chlorophyll concentrations (more than 10–15 $\text{mg}\cdot\text{L}^{-1}$) could be very different from all other kinds of waters studied before. Therefore, development of remote sensing algorithms for such waters requires the study of the spectral behavior of the optical properties of HDPPs, such as reflectance and vertical attenuation coefficients.

The main goal of this study was to investigate the spectral properties of the reflectance and vertical attenuation coefficient of HDPPs in the visible and near infrared region of the spectrum in as wide a range of biomass and pigment concentrations as possible. The specific objective was to create the indices sensitive to pigment and biomass concentration that may serve as indicators for the physiological state of outdoor algal cultures. The findings may serve as a basis for remote real-time monitoring of phytoplankton quality in HDPPs.

MATERIALS AND METHODS

Spirulina platensis (Norst.) Geitler strain M-2 of the culture collection of the Centro di Studio dei Microrganismi Autotrofi of Florence was used. It was grown in Zarrouk's medium, containing 200 mM sodium bicarbonate (Vonshak et al. 1982). Outdoor cultures were grown in 2.5-m² ponds. The depth of the culture was 12 cm; stirring was provided by a paddle-wheel at 15 rpm. All nutritional requirements were supplied in excess in order to prevent nutrient deficiency. For radiometric measurements, ponds were inoculated with laboratory-grown cultures at different biomass concentrations. Growth was monitored by measuring the increase in optical density using a Klett Sumerson instrument.

Concentrations of chlorophyll *a*, biomass, and phycocyanin were performed as previously described (Vonshak 1986).

Radiometric measurements. Measurements were performed on the different ponds three times per day at 0900, 1300, and 1500, so that different outdoor light conditions were tested in the range of 500–2000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. A high spectral resolution radiometer Licor LI-1800 was used to measure reflectance and vertical attenuation coefficient spectra. The spectral range of the instrument is from 350 to 1100 nm. For this study, data from 400 to 1100 nm were used with a spectral resolution of 2 nm. A micro-computer was used for initiated spectroradiometer scanning and data storing.

For reflectance measurements, the radiometer was attached to

a telescope with a 15° field of view, which was positioned over the pond at a height of 50 cm. The 15° optic resulted in an instantaneous field of view of about 15 by 15 cm on the water surface.

Upward radiance (L_w) above the water surface was measured three times. Then a reference panel (BaSO_4) was placed just above the water surface, and its upward radiance (L_{ref}) was measured. One spectrum measurement took about 25 s; therefore, one set of reflectance measurements took 2 min. Each observed upward radiance spectrum of the water was divided by the appropriate upward radiance spectrum of the reference plate to give a reflectance value as $R = L_w/L_{ref}$. The mean of three reflectance spectra was used in the analysis.

To determine the vertical attenuation coefficient, downwelling irradiance was measured just above the water surface (E_0) and at a depth (Z) of 2.7 cm (E_z). The vertical attenuation coefficient was calculated as $K_d = \text{Log}(E_0/E_z)/z$.

Statistical analyses. To determine the spectral ranges with maximal sensitivity of vertical attenuation coefficient, K_d , to pigment concentrations, K_d was normalized to that at 750 nm, $K_d(750)$, and the standard deviation of ratio $K_d/K_d(750)$ was calculated. From these data, specific wavelengths that showed the highest sensitivity to pigment concentration were chosen. The attenuation coefficients at specific wavelengths 438, 624, and 670 nm were derived from spectra and compared to analytically measured concentrations.

To find the spectral bands at reflectance spectra more sensitive to variation of biomass and pigment concentrations, the coefficient of variation of reflectance was calculated as a ratio of standard deviation to average reflectance value. To estimate biomass of phytoplankton, an artificial base line was constructed between 670 and 950 nm, and the sum of reflectance above this base line (SUM) was calculated. Then, it was compared to biomass measured analytically.

Relationships "SUM versus biomass" as well as "attenuation coefficient at specific wavelengths versus pigment concentration" were determined using the linear regression technique.

RESULTS

Algal growth. To follow the changes in the optical properties of outdoors *Spirulina* cultures at a wide range of cell concentrations, three ponds were inoculated at three different biomass concentrations ranging from 0.2 to 0.4 $\text{g}\cdot\text{L}^{-1}$ of dry weight biomass. Growth of the cultures was monitored for 9 days as an increase in biomass and chlorophyll *a* concentrations. After the initial intensive growth in the first 4 days, the rate of increase leveled off and biomass concentration reached a level of more than 1 $\text{g}\cdot\text{L}^{-1}$ (Fig. 1), which is about twice the steady-state concentration recommended for the maximal output rate in outdoor *Spirulina* cultures (Vonshak et al. 1982). The three cultures reached the same final concentration regardless of the initial inoculation concentration; this may indicate that nutrients were not limiting and that growth slowed down mainly due to self-shading and light limitation.

Attenuation coefficient spectra. The spectra of vertical attenuation coefficients included several notable spectral features (Fig. 2). From 400 to 430 nm a slightly increasing slope with a low attenuation was observed. For the samples with a higher attenuation, this slope increased to 438 nm. The peak at 438 nm was the blue or first chlorophyll *a* absorption maximum. Beyond the peak at 438 nm a strong decrease

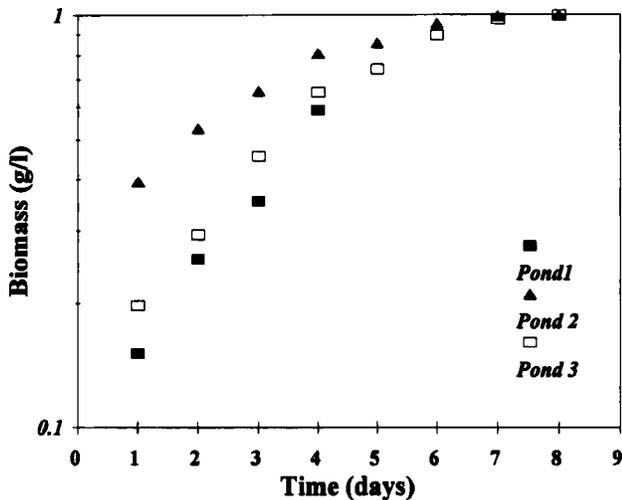


FIG. 1. Increase in biomass concentration in three *Spirulina* ponds grown under outdoor conditions.

until a shoulder at 480–500 nm occurred. This attenuation feature was due to the absorption of carotene (Davis 1976). Beyond 500 nm the attenuation decreased to a minimum at near 550 nm, where the absorption of all photosynthetic pigments reached its minimum. Then attenuation increased to a peak at 624 nm. This peak was caused most likely by phycocyanin absorption (Malinsky-Rushansky and Berman 1991). Then a further decrease in attenuation was observed from 624 nm to a relative low at 650 nm followed by an increase to a maximum at 675 nm. This was the red, or second, chlorophyll *a* absorption peak. Attenuation decreased very sharply beyond 675 nm.

For low chlorophyll *a* concentrations (up to $1 \text{ mg} \cdot \text{L}^{-1}$), a minimum near 720 nm occurred (the lowest curve in Fig. 2). Another minimum was observed at near 800 nm. For chlorophyll concentrations higher than $1 \text{ mg} \cdot \text{L}^{-1}$, a wide plateau between 700 and 950 nm occurred. For all ranges of chlorophyll concentration, attenuation reached its lowest value at near 800 nm. A high peak of attenuation occurred near 980 nm.

The distinctive property of HDPP vertical attenuation coefficient spectra was the extremely high attenuation in the entire visible spectrum including the green region near 550 nm. Very strong absorption by chlorophyll *a* and carotene at shorter wavelengths and by phycocyanin at longer wavelengths were most likely the cause for these high values of attenuation in the green region of the spectrum.

When biomass and chlorophyll concentration were increasing in the ponds, the attenuation coefficient increased, as may be expected. The slope of increase was much higher at wavelengths corresponding to maximal absorption, but in the green and near infrared regions attenuation also increased.

Although phytoplankton pigment absorption was the cause for the most prominent features of the

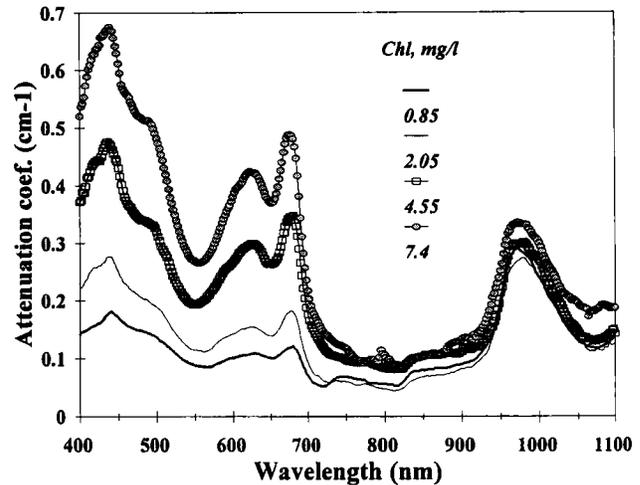


FIG. 2. Vertical attenuation coefficient spectra (ratio of downwelling irradiances at water surface to a depth of 2.7 cm) of *Spirulina* cultures at different chlorophyll concentrations.

vertical attenuation coefficient spectra, scattering by the cells also contributed to the attenuation. The “background” of the attenuation coefficient increased with the increase in phytoplankton biomass density (Fig. 2).

To determine the spectral bands with maximal sensitivity to pigment concentrations, the standard deviation of the attenuation coefficient was calculated. Taking into account that minimal absorbance by pigments exists in the near infrared range of the spectrum, the standard deviation of the attenuation coefficient was normalized to that at 750 nm. The normalization decreased to a certain degree the effect of cell scattering on attenuation coefficient. The standard deviation of $K_d/K_d(750)$ shows maxima at 438, 624, and 676 nm and a shoulder near 490 nm (Fig. 3). It reflects a strong influence of phytoplankton pigments on attenuation spectra at these wavelengths.

Reflectance spectra. At chlorophyll *a* concentrations above $2.5\text{--}3 \text{ mg} \cdot \text{L}^{-1}$, reflectance from the bottom of the pond did not play any role in the reflectance spectrum measured. This was evident when the reflectance of ponds, the bottom of which were black or white in color, were compared. The obtained reflectances were coincident. Thus, the reflectance spectra of the ponds with chlorophyll *a* concentration above $2.5 \text{ mg} \cdot \text{L}^{-1}$ will be considered.

The reflectance spectra had two notable features: extremely low reflectance (1–2%) in the visible region of the spectrum and extremely high reflectance (15–30%) in the near infrared region (Fig. 4). From 400 to 500 nm, very low reflectance (about 1%) occurred (Fig. 5). A noticeable peak, reaching 2%, occurred near 550 nm. Two other wide minima occurred: between 600 and 650 nm and at 675 nm. A peak between them occurred near 660 nm. The minimum at 675 nm was followed by a very sharp increase of reflectance toward longer wavelengths.

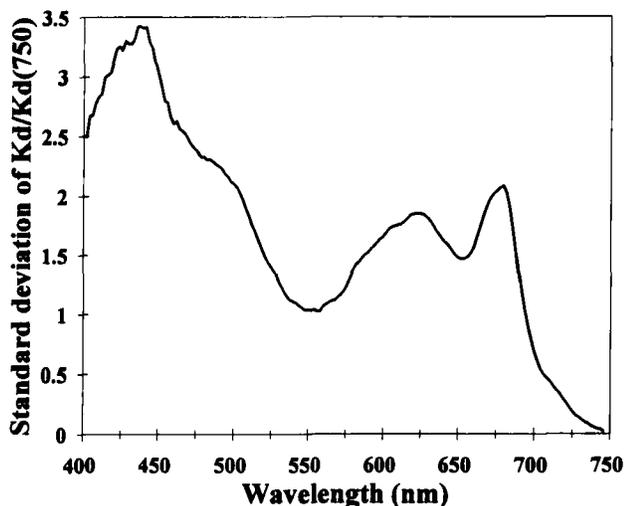


FIG. 3. Spectrum of standard deviations of the vertical attenuation coefficient, K_d , normalized to that at 750 nm, $K_d(750)$.

In the near infrared region of the spectrum (near 750 nm), a shoulder was observed followed by a pronounced peak near 800 nm. Then the reflectance decreased. The lowest value of reflectance was recorded at near 950 nm. In the spectral region between 700 and 950 nm, the reflectance increased when an increase in biomass occurred. At longer wavelengths, a slight increase of reflectance was recorded, reaching more than 5% at 1100 nm.

To determine the spectral range maximally sensitive to phytoplankton biomass, the spectrum of the coefficient of variation was calculated and analyzed (Fig. 6). The spectrum shows very low variation of reflectance during the growth period over all the visible spectrum. An extremely high variation in reflectance was observed in the near infrared range of the spectrum between 700 and 1000 nm.

DISCUSSION

One of the aims of this study was to develop algorithms for determination of biomass and pigment concentrations in HDPPs from remotely sensed data. Therefore, spectral regions with high sensitivity of reflectance and attenuation coefficient to biomass and pigment concentration had to be determined.

At short wavelengths in the blue region of the spectrum, the first chlorophyll *a* absorption peak, and the absorption of carotenoids caused a high total absorption. This was the reason for the low reflectance observed in this spectral band. In the green region, beyond 500 nm, absorption slightly decreased, remaining, nevertheless, very high. The reflectance in this region correspondingly remained very low. Reflectance in the visible spectrum did not follow the conceptual model of algal reflectance (Gordon and Morel 1983), and higher concentrations of algae had a higher reflectance at all wavelengths. This was most likely the result of the ex-

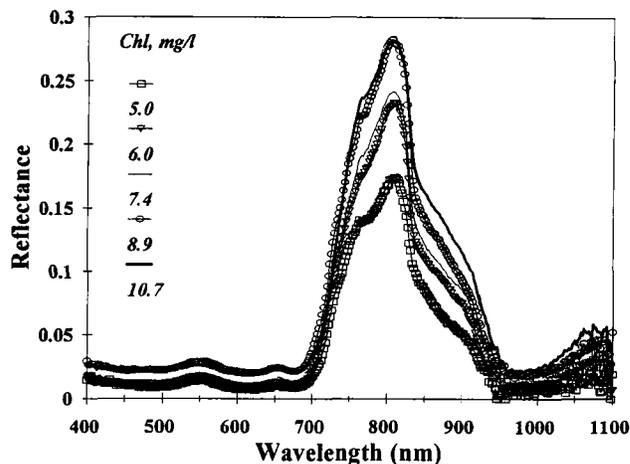


FIG. 4. Reflectance spectra of five *Spirulina* cultures in the visible and near infrared region of spectrum measured above the water surface.

tremely strong scattering caused by the algal cells at very high density.

For chlorophyll *a* concentrations less than $1 \text{ mg} \cdot \text{L}^{-1}$, the lowest attenuation over the entire visible spectrum was observed at 700–720 nm, conversely coinciding with the maximum reflectance values. This minimum of the attenuation coefficient (and maximum of reflectance) was due to a combined effect of chlorophyll *a* and pure water absorption (Gitelson et al. 1986, Vos et al. 1986, Gitelson 1992). A decrease in chlorophyll absorption and increase in pure water absorption resulted in the minimum combined absorption curve of algae and water (Fig. 2). An increase in algae density leads to enhancement of scattering and, as a consequence, to an increase in reflectance. Therefore, beyond 720 nm for relatively low algal density (chlorophyll *a* $< 1 \text{ mg} \cdot \text{L}^{-1}$), water absorption was the dominant factor in total attenuation.

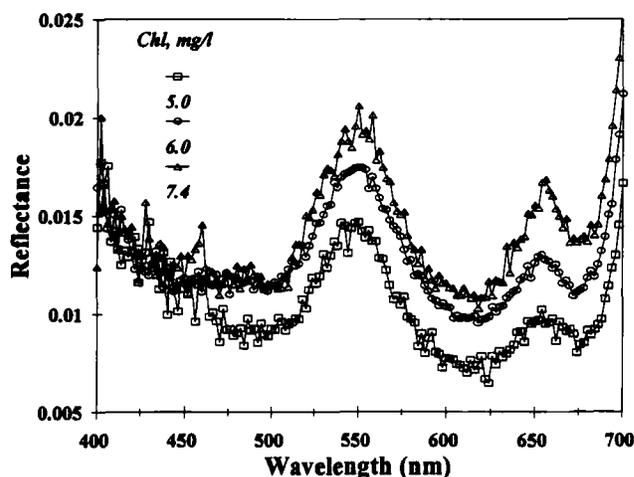


FIG. 5. Reflectance spectra of three *Spirulina* cultures in the visible region of spectrum measured above the water surface.

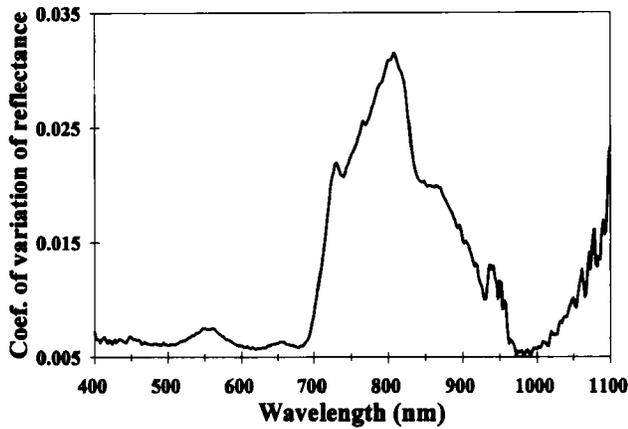


FIG. 6. Spectrum of the coefficient of variation of reflectance, calculated as a ratio of standard deviation of reflectance to average reflectance value.

With an increase in algal biomass concentration, absorption by chlorophyll *a* became much higher than absorption by pure water, and the attenuation coefficient near 700 nm increased. The minimum near 720 nm disappeared. Beyond 720 nm, the attenuation coefficient decreased, achieving minimal values near 800 nm. In this region of the spectrum, there was no absorption by any photosynthetic pigments. The only absorbent in this spectral region was pure water. Nevertheless, even very strong water absorption did not lead to a decrease in reflectance. High reflectance may be caused solely by scattering of algal cultures at extremely high densities. The absorption of the infrared light by the water no longer influenced reflectance, and the spectra of algae were more typical of those for terrestrial plants (e.g. Elachi 1987).

At algal cultures with chlorophyll concentration above $1 \text{ mg} \cdot \text{L}^{-1}$, scattering by cells is dominant in the near infrared region of the spectrum. It governs the spectral behavior of apparent optical properties, reflectance, and vertical attenuation coefficients. The same effects were dominant for quite low concentrations of green algae (Quibell 1992). These observations were significantly different even from those very productive inland waters where the region 600–720 nm is considered the most appropriate to derive information on water quality (Gitelson et al. 1986, 1993a, b, Dekker 1993).

Very strong absorption by algal pigments in all visible spectra, together with the opposing effect of an increase in scattering toward shorter wavelengths, makes this spectral region inappropriate for retrieval information from the resultant reflectance. For high phytoplankton concentrations, typical for HDPPs, reflectance in the visible region of the spectrum could hardly be used to derive information on pigment and biomass concentration.

Biomass estimation. The largest changes in reflectance associated with an increase in biomass and

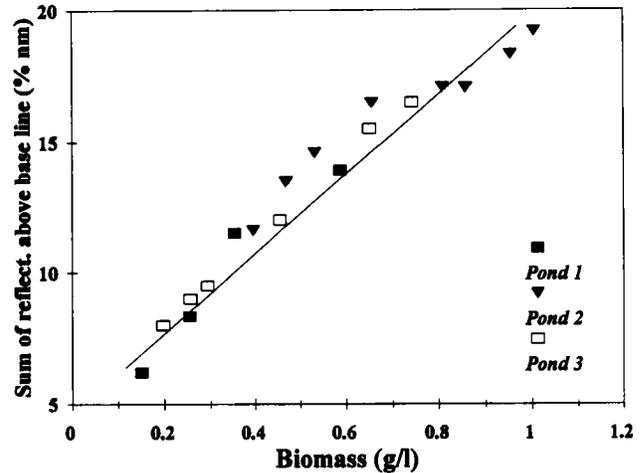


FIG. 7. Sum of reflectance above the artificial base line constructed through 700 and 950 nm versus biomass concentration for three *Spirulina* ponds.

chlorophyll concentration occurred in the near infrared wavelengths (700–950 nm). As the absorption coefficient of water in this region of the spectrum was high, reflectance can be related to the total algal biomass. Therefore, the 700–950-nm region seems more appropriate for retrieval information on phytoplankton concentration from reflectance spectra. The reflectance in the range from 700 to 950 nm increased with increase in biomass. The coefficient of variation of reflectance shows that maximal sensitivity of the reflectance to biomass and chlorophyll *a* concentration occurred in this region of the spectrum. Even for very high-density cultures, the sum of reflectance from 700 to 950 nm remained very sensitive to biomass. This is consistent with the results of Bagheri and Dios (1990), Hardisky et al. (1986), and Millie et al. (1992), where near infrared reflectance has been recognized as an important variable in correcting for particle scattering in a variety of highly turbid systems, including those with quite large phytoplankton biomass.

To derive biomass from reflectance spectra, the approaches developed for productive inland waters could be used (Gitelson et al. 1993a, b, 1994a, b). We suggest that the sum of the reflectance above the base line (SUM) through 670 and 950 nm is more suitable for estimation of biomass at a wide range of phytoplankton density.

The equation

$$\text{biomass} = -0.34 + 6.5 \cdot \text{SUM} \quad (1)$$

describes the relationship between biomass in $\text{g} \cdot \text{L}^{-1}$ and the sum of reflectance in (% nm) for pond No. 2. A determination coefficient (r^2) of more than 0.95 was achieved (Fig. 7).

Due to its empirical nature, the model has to be validated by using independent data sets. We used equation (1) to derive the biomass from the reflectance spectra of pond Nos. 1 and 3. Sums of reflectance

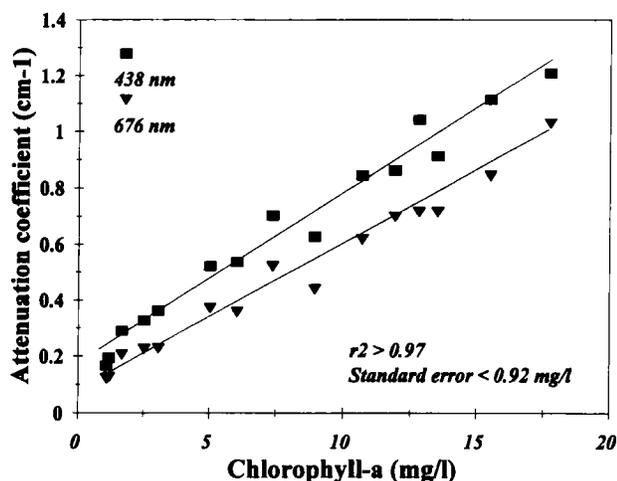


FIG. 8. Vertical attenuation coefficient at 438 and 676 nm versus chlorophyll *a* concentration for pond No. 2.

tance above the base line for pond Nos. 1 and 3 were used in model (1). Calculated biomass was compared with measured values. An estimation error of biomass assessment was found to be less than $0.06 \text{ g} \cdot \text{L}^{-1}$.

For remote determination of phytoplankton biomass, we recommend the use of sensors in spectral bands 676 and 950 nm with bandwidth 20 nm and a wide band sensor (from 700 to 950 nm).

Chlorophyll *a* and phycocyanin estimation. Attenuation coefficient spectra and the standard deviation of $K_d/K_d(750)$ revealed several spectral features that are appropriate for the remote estimation of chlorophyll *a* and phycocyanin concentration. The highest changes in total attenuation by water and algae associated with the increase of chlorophyll *a* concentration occurred at 438 and 676 nm. For pond No. 2, the following relationship between chlorophyll *a* concentration and attenuation coefficient at 438 nm, $K_d(438)$, was found:

$$\text{Chl } a = 2.45 + 0.16 \cdot K_d(438). \quad (2)$$

Chlorophyll *a* concentrations were determined in $\text{mg} \cdot \text{L}^{-1}$ using vertical attenuation coefficients in m^{-1} . A determination coefficient for the relationship of more than 0.97 was achieved (Fig. 8).

The accuracy of chlorophyll *a* estimation can be further improved by determining it from the attenuation coefficient at 676 nm, $K_d(676)$:

$$\text{Chl } a = -1.46 + 0.195 \cdot K_d(676). \quad (3)$$

For this regression, the determination coefficient was more than 0.98 (Fig. 8).

Maximum of phycocyanin absorption was found at 624 nm. Such a peak may thus be used as an indication of the presence of cyanobacteria in mixed algal culture and for its quantitative assessment. The concentrations of phycocyanin were related to the magnitudes of the attenuation coefficient at 624 nm, $K_d(624)$. The regression in the form

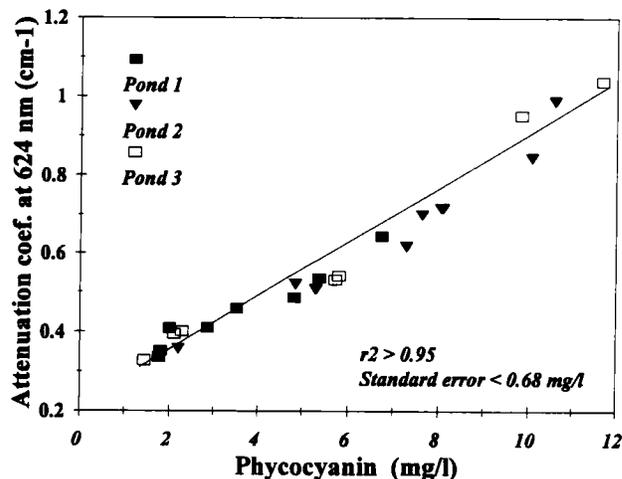


FIG. 9. Vertical attenuation coefficient at 624 nm versus phycocyanin concentration for pond No. 2.

$$\text{phycocyanin} = -2.85 + 0.145 \cdot K_d(624) \quad (4)$$

gives a determination coefficient of more than 0.95 (Fig. 9). Phycocyanin was determined in $\text{mg} \cdot \text{L}^{-1}$ and $K_d(624)$ in m^{-1} .

Algorithms (2) and (3) for assessment of chlorophyll *a* concentrations were validated by independent data sets taken from pond Nos. 1 and 3. Use of $K_d(438)$ to derive the chlorophyll *a* concentration allowed an estimate of chlorophyll *a* with an error of less than $0.92 \text{ mg} \cdot \text{L}^{-1}$. The use of the attenuation coefficient at 676 nm lowered the estimation error of chlorophyll *a* determination to $0.84 \text{ mg} \cdot \text{L}^{-1}$.

Validation of model (4) for phycocyanin assessment was carried out by independent data sets from pond Nos. 1 and 3. Phycocyanin concentrations were estimated with an error of less than $0.68 \text{ mg} \cdot \text{L}^{-1}$.

Thus, it appears that the models can accurately predict both pigment concentrations and biomass of *Spirulina*.

The significance of biomass model (1) presented here is that it is independent of highly variable chlorophyll- and phycocyanin-specific absorption coefficients. The stability of this model depends primarily on scattering by the phytoplankton cells. For this model to be successful for *Spirulina*, the variation of the scattering coefficient for *Spirulina* as a function of light history and package effects have to be investigated. Parameters of the model can be adjusted for other species. Our preliminary experiments showed that the same approach can be used successfully for assessment of *Dunaliella* biomass.

Algorithms for chlorophyll *a* and phycocyanin determinations (2)–(4) are based on diffuse attenuation coefficient measurements. The parameters to be used in these models pertain to the nature of phytoplankton cells. Stability of algal-inherent optical properties (specific absorption and scattering coefficients) is crucial to the models. They depend on numerous factors. Changes in intrinsic composition of the cells

("package effect") and the particle size distribution will all affect the measured signal. Further investigation is required in order to clarify whether the parameters of the models are constant and, therefore, to understand the potential use and limitations of the models.

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