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# Photosynthetic Enhancement in the Diatom Phaeodactylum tricornutum[1]

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*Abstract.* Enhancement phenomena in photosynthesis of the diatom *Phaeodactylum tricornutum* Lewin were studied by means of a Haxo oxygen electrode and 2 monochromatic light beams. It was necessary to correct for a minor non-linearity in rate oxygen evolution *vs.* intensity similar to that reported for *Chlorella*. Action spectra on complementary backgrounds and derived enhancement spectra were compared to *in vivo* absorption spectra in identifying the character of pigment systems 1 and 2. Fucoxanthin, chlorophyll *c*, and chlorophyll *a*-670 are clearly assignable to pigment system 2 which absorbs in excess at wavelengths 400 to 678 mμ. Chlorophyll *a*-1 (and a portion of the fucoxanthin) are assignable to pigment system 1 which absorbs in excess at wavelengths 678 to 750 mμ. Enhancement values were generally lower than those observed in *Chlorella* or *Anacystis* and lead to conclusion that diatom pigmentation provides an effective light harvesting apparatus.

Diatoms make a major contribution to the world's carbon dioxide fixation. In addition to chlorophyll *a* and β-carotene their pigment complement includes chlorophyll *c* and the special carotenoid, fucoxanthin. As compared to green, blue-green, and red algae they show least dependence of quantum yield on wavelength in the spectral region 400 to 680 mμ (3, 6, 7, 9, 19). We have, therefore, undertaken a reexamination of the pigments (14) and spectral characteristics of photosynthesis in the marine diatom *Phaeodactylum tricornutum*. We report herein a detailed study of Emerson enhancement as a method describing characteristics of the functional pigment systems.

## Materials and Methods

*Phaeodactylum tricornutum* Lewin (*Nitzschia closterium forma minutissima*, No. 646, Indiana Culture Collection) was grown in continuous culture (16) in a modified ASP-2 medium (14, 18) at 18° and aerated with 2 % $CO_2$ in air. Illumination was provided by tungsten lamps to give "W-cells" growing at 20 hr doubling time or by red (>650 mμ) BCJ photographic safe lamps to give "R-cells" growing at 24 hr doubling time.

Net rate of oxygen evolution of an algal cell layer on a Haxo stationary cathode was measured in the arrangement described in past reports from this laboratory (5, 13, 17). The 6 mm diameter cathode was recessed 0.25 mm below a cellophane membrane. A suspension at cell concentration 16 to 20 μl/ml placed in the electrode chamber should have given a settled cell layer of 4 to 5 μ. Microscopic examination showed some overlap of cells and the cell layer appeared to average closer to 2 cells thick (cell width about 5 μ). A constant flow of ASP-2 medium minus microelements aerated with 2 % $CO_2$ in air passed over the membrane.

The cathode was held at −0.5 V *vs.* a monitoring saturated calomel electrode. A potentiostat circuit (5) was used with large area platinum anodes or a simpler circuit (17) was used with silver anodes. There was no evidence of silver toxicity and no difference in results obtained with the 2 arrangements. The background current ranged from 6 to 8 μamps whereas the measured current change due to photosynthetic oxygen evolution never exceeded 2 μamps. We arbitrarily defined a change in polarographic current of 0.01 μamps as a photosynthetic rate unit (approx. $2.6 \times 10^{-14}$ moles $O_2$/sec).

The optical system (5) provided 2 light beams from grating monochromators at 4.2 and 4.4 mμ half-band width guarded by short wave cut-off filters and a 5.7 cm water filter. Cells were maintained on the electrode for periods of up to 5 days using an irradiance of 80 μwatts/cm² at 680 mμ between experiments.

## Results

*Intensity Effects.* Although conventional plots of current change *vs.* light intensity were apparently linear (fig 1A), at low intensities we were troubled by the same kind of minor non-linearity observed in *Chlorella* (5, 17). The effect was clearly observed by measuring response to a small standard light signal superimposed upon varied intensity of the

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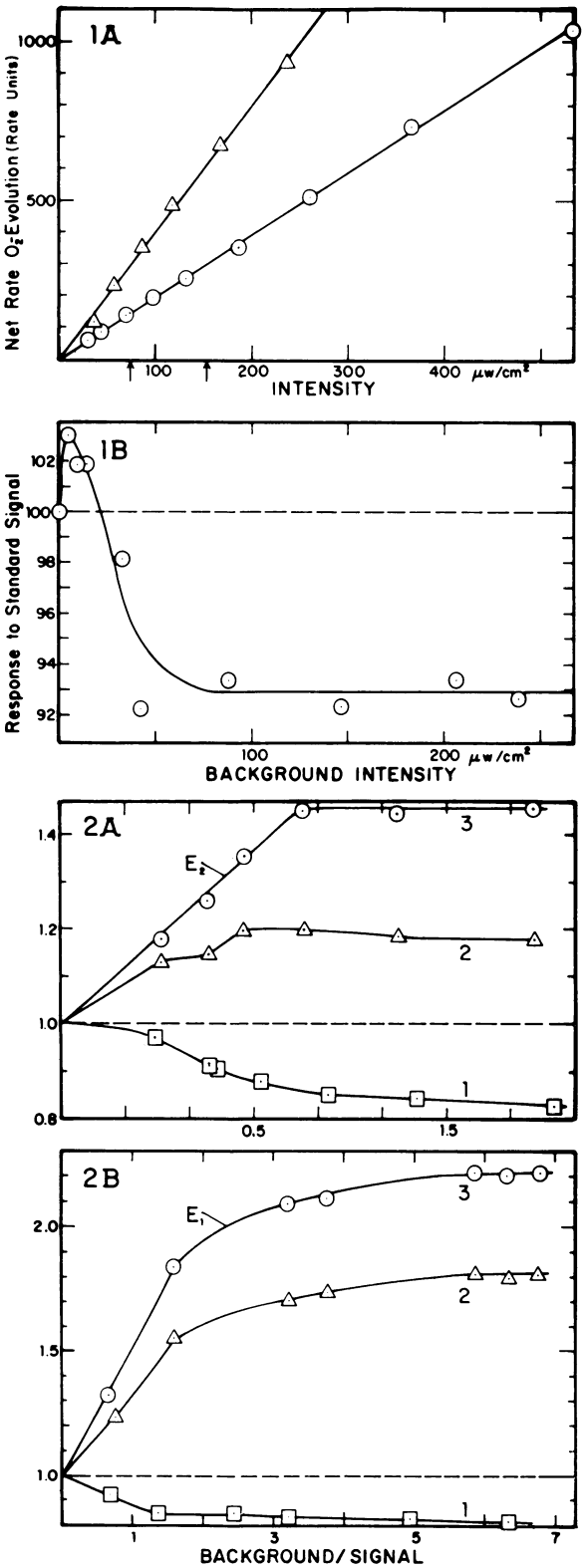



FIG. 1. Intensity effects. A) Net rate of oxygen evolution of W-cells *vs.* intensity at 680 (△) and 550 mμ (○). Compensation points shown by vertical arrows were estimated from parallel experiments with the same cells using a Clark-type concentration-measuring electrode and the same wavelengths of illumination. B) Relative response to a standard signal of 32 μwatts/cm² of 550 mμ superimposed on various backgrounds of 550 mμ. The initial rise below 25 μwatts/cm² was not seen in all preparations.

same wavelength (fig 1B). Repetitions of the experiment of figure 1B showed that the non-linearity was seen in all cell preparations (though not always in the same detail), was not notably sensitive to wavelength, occurred at intensities below the compensation point, and was far smaller than the major non-linearity observed in the blue-green algae *Anacystis* and *Anabaena* (13).

*Quantitative Characteristics of Enhancement.* We follow past notations (1, 5, 13, 15). Let $p_1$ be the rate of oxygen evolution in a beam of $\lambda_1$ (as 700 mμ), $p_2$ the rate observed in a beam of $\lambda_2$ (as 540 mμ), and $p_{12}$ the rate observed when the 2 beams are presented together. Then enhancement of $\lambda_1$ is $E_1 = (p_{12}-p_2)/p_1$; and enhancement of $\lambda_2$ is $E_2 = (p_{12}-p_1)/p_2$. An experimental advantage of these definitions is that the terms $(p_{12}-p_1)$ and $(p_{12}-p_2)$ are directly measurable. Unfortunately, the non-linearity effect is such as to reduce the enhancement effect. We therefore corrected for the non-linearity by measuring responses to a standard light signal when added to both enhancing and non-enhancing backgrounds. For example, we measured change in rate produced by a signal of 700 mμ when added to various backgrounds of 550 mμ $(p_{12}-p_2)$. We also measured the rate change produced by the same standard signal of 700 mμ when added to various backgrounds of 700 mμ $(p_{11}-p_1)$. The ratio $E_1 = (p_{12}-p_2)/(p_{11}-p_1)$ is an operational definition designed to measure enhancement. Likewise $E_2 = (p_{12}-p_1)/(p_{22}-p_2)$. A similar procedure was used in enhancement studies with *Chlorella* and *Anacystis* (13, 17).

Figure 2 demonstrates the method used to find background/signal ratios needed to obtain maximum enhancement. Maximum $E_2$ requires a background/signal $(p_1/p_2)$ ratio of about 1, a higher ratio than required for *Chlorella* or *Anacystis*. Maximum $E_1$

FIG. 2. Enhancement *vs.* background/signal ratio for W-cells. A) Response to a standard light signal of 540 mμ giving 50 rate units (*vs.* dark) and its enhancement. Curve 1: relative response when presented on various backgrounds of 550 mμ; curve 2: relative response when presented on various backgrounds of 700 mμ; curve 3: enhancement, $E_2$, calculated by dividing the value of each point on curve 2 by the value beneath it on curve 1. B) Response to a standard light signal of 700 mμ giving 30 rate units (*vs.* dark) and its enhancement. Curve 1: on backgrounds of 700 mμ; curve 2: on backgrounds of 550 mμ; curve 3: enhancement, $E_1$, calculated as curve 2/curve 1. Background/signal is a ratio of effective intensities measured in terms of rate units of net oxygen evolution.

requires a background/signal ($p_2/p_1$) ratio of 5, a lower ratio than required for *Chlorella* or *Anacystis*. In subsequent work on action and enhancement spectra the background/signal ratios exceeded those needed to obtain maximum values of $E_2$ at 540 or $E_1$ at 700 mμ. We always made similar corrections for non-linearity or, as an alternate statement, we always worked upon a segment of the light intensity curve chosen to minimize effects of the non-linearity.

*Action Spectra.* Action spectra were determined with backgrounds of 550 and 700 mμ. Figure 3 presents results of a typical experiment. Solid points represent the conventional action spectrum of photosynthesis. Open points show the enhanced action spectra. Triangles approximate the action spectrum of pigment system 1, squares the action spectrum of pigment system 2.

The action spectra are compared with an estimated percent absorption spectrum normalized at 674 mμ and obtained in the following manner. Absorption was measured in the same optical system

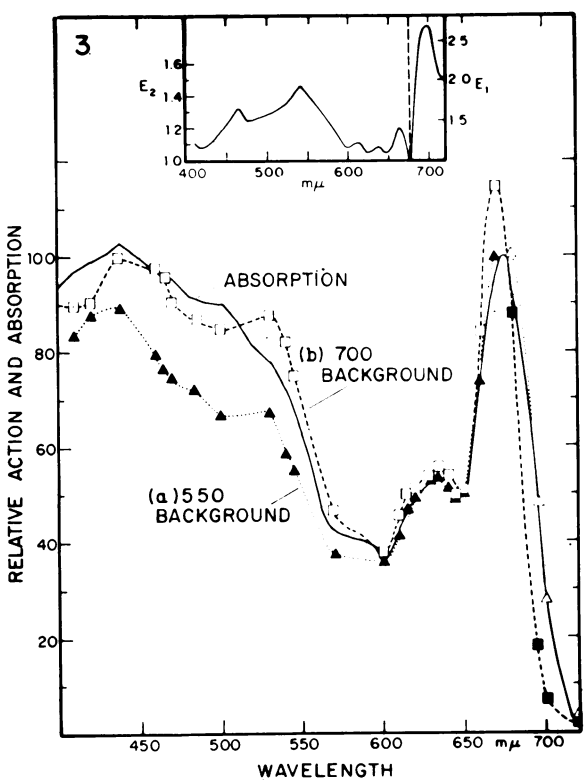



FIG. 3. Action and absorption for W-cells. Action has dimensions of relative oxygen evolved/quantum; absorption is measured in percent. Action spectra were obtained with constant background of 200 rate units of 550 mμ (△ and ▲, a) or 700 mμ (□ or ■, b). Added light signals at indicated wavelengths were adjusted in intensity to give about 50 rate units below 680 mμ and about 30 rate units above 680 mμ. Absorption was measured as described in text and normalized to 100 at 674 mμ. Inset shows an enhancement spectrum derived as the ratio of the 2 action spectra; $E_2 = b/a$ and $E_1 = a/b$.

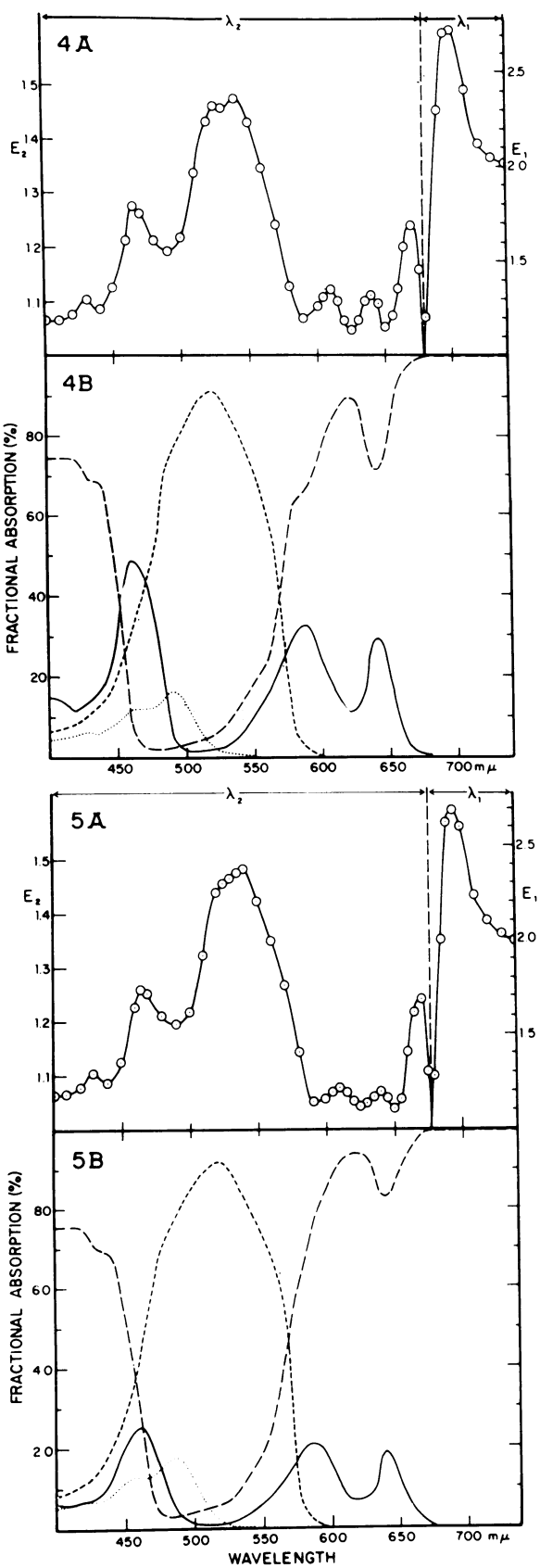
used to measure action. A cell layer of calculated thickness 9.3 μ was deposited on the bottom of a lucite chamber 1 mm deep and covered with a coverglass. Transmission was measured with a silicon barrier cell covered by a diffusing plate, as previously described in detail (17). In order to better approximate actual absorption of cells on the platinum electrode we corrected all values of absorption by the factor $1 + RT$ where $T$ is transmission and $R$ is the reflectance of platinum. Unfortunately, the relatively thick cell layer, uncertainties in exact match in cell layer thickness, and variation in percent absorption with thickness limit precision in comparison of action and absorption spectra. In general the unenhanced action spectrum shows the same deviations from absorption at 400 to 520 mμ and above 680 mμ as expected from Tanada's data (19) on quantum yield *vs.* wavelength.

*Enhancement Spectra.* An enhancement spectrum may be obtained as the variation in ratio between the enhanced and non-enhanced action spectra shown by the inset of figure 3. Figure 4A presents a more detailed enhancement spectrum representative of 6 separate experiments on W-cells. It may be compared to the fractional absorption spectra (fig 4B) estimated for *in vivo* pigment components and reported in detail elsewhere (14). The fractional absorption spectra are not notably different from those obtained from Tanada (19) for *Navicula minima* except that they resolve the chlorophyll *a* and *c* components.

The enhancement spectrum shows 2 wavelength regions designated $\lambda_2 < 678$ mμ $< \lambda_1$. The $\lambda_1$ region, as in *Chlorella* (17), has only a single far-red segment and lacks the second segment in the blue found in phycobilin-containing algae (2, 9, 13). The pronounced $\lambda_1$ peak (700 mμ) is at slightly shorter wavelength and not so high but otherwise remarkably similar to that in *Chlorella* (17) and *Anacystis* (13); the decrease at longer wavelengths remains unexplained.

A conspicuous feature of the $\lambda_2$ region is the wide peak between 500 and 570 with maximum at about 540 mμ and clearly identifiable with fucoxanthin absorption. The 670 mμ peak is attributed to chlorophyll *a*-670. The 465, 610, and 640 mμ peaks are identifiable with chlorophyll *c* absorption although we cannot explain simply a displacement of the 610 peak from the 590 mμ absorption peak.

We grew cells in far-red light (>650 mμ, R-cells) in the hope of inducing pigment shifts of magnitude such as those observed in *Anacystis* (13). It turned out that the observed changes were not dramatic and evident only as a decrease in chlorophyll *c* to about 60 % of the concentration found in W-cells. This is observed as a decrease in fractional absorption by chlorophyll *c* (fig 5B) and a decrease in height of the 465, 610, and 640 mμ peaks of the enhancement spectrum (fig 5A). Repeated exploration over the 590 to 650 mμ region confirmed the reproducible character of the 610 and 640 mμ peaks and the reality of small differences between




W-cells and R-cells as shown by figures 4A and 5A. Furthermore, minor shifts and decrease in peak heights could be observed in W-cells after one or more days of illumination with 680 mμ light while on the electrode.

## Discussion

We have observed no distinctive features of photosynthesis in *Phaeodactylum* other than spectral characteristics attributable to its pigments. Our enhancement spectra provide greater resolution than previous spectra on the diatom *Navicula* (8, 11), show the character of both the $\lambda_1$ and $\lambda_2$ regions, and provide fractional absorption spectra for direct comparison. The earlier anomaly of "negative enhancement" (8) has been removed by correction for minor non-linearity in net rate of oxygen evolution *vs.* intensity.

It is clear that fucoxanthin, chlorophyll *c*, and chlorophyll *a*-670 constitute pigment system 2 in the operational sense proposed by Duysens *et al.* (4). At the same time it appears that fucoxanthin, which absorbs more than 90 % of all quanta absorbed at 520 mμ, must also contribute appreciably to pigment system 1 along with chlorophyll *a*-1. Fucoxanthin is the only carotenoid which is an efficient light harvesting pigment and clearly identifiable in enhancement spectra.

Chlorophyll *c* poses the problem that, though characteristic of diatom pigmentation and identifiable with pigment system 2, its low concentration and low absorption in the red do not allow functional explanation as an effective light-harvesting pigment. The 40 % variation in chlorophyll *c* concentration which we were able to induce (R *vs.* W-cells) had so little effect that we were obliged to make detailed comparison in order to confirm that there was an effect. The chlorophyll *a*-670 contribution to enhancement has been seen also in *Navicula* (11) and is more clearly evident than in *Chlorella*, because of the small contribution by chlorophyll *c*. Chlorophyll *a*-670 has been demonstrated explicitly (10) in aging cultures of *Phaeodactylum* as 1 of 2 chlorophyll components with maxima at 669 and 683 mμ.

It has been proposed by Joliot *et al.* (12) that action spectra obtained on complementary back-

FIG. 4. Enhancement and fractional absorption spectra for W-cells. A) Enhancement spectrum obtained from measurements on backgrounds of 550 and 700 mμ as described in text. In the $\lambda_2$ region enhancement $E_2$ is given by 700 mμ. In the $\lambda_1$ region $E_1$ is given by 550 mμ. B) Fractional absorption of *in vivo* pigments from (14) : Chlorophyll *a* (— — —), chlorophyll *c* (———), fucoxanthin (----) and other carotenoids, principally β-carotene (....). The ordinate has dimensions of percent of total absorbed quanta absorbed by each pigment.

FIG. 5. Enhancement and fractional absorption spectra for R-cells (*cf.* fig 4).

grounds of $\lambda_1$ and $\lambda_2$ are not entirely correct in providing the action spectra of pigment systems 1 and 2. However, their action spectra for systems 1 and 2 of chloroplasts, though obtained directly, are not far different than those expected from enhancement studies on *Chlorella* (17). Hence we consider that our action spectra on complementary backgrounds are reasonable approximations to the spectra of pigment systems 1 and 2 in *Phacodactylum*.

Comment should be made about the fucoxanthin-chlorophyll *c*-chlorophyll *a* pigment complement of diatoms as compared to the chlorophylls *a-b* or the chlorophyll *a*-phycobilin pigment complements. The diatom pigment complement provides for minimum variation in quantum yield *vs.* wavelength. It also provides for more equal capture of quanta by pigment systems 1 and 2. Largely because of the efficiency of fucoxanthin the diatoms have a pigment arrangement at least as effective as that of higher plants.

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