**Summary**

Using monochromatic and polychromatic light of relatively low intensity, measurements have been made of the quantum efficiency of photosynthesis in *Chlorella pyrenoidosa*. These measurements indicate a probable value of approximately 0.05 molecule per quantum, which is smaller than the value which is now generally accepted.

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[CONTRIBUTIONS FROM THE LIMNOLOGICAL LABORATORY OF THE WISCONSIN GEOLOGICAL AND NATURAL HISTORY SURVEY, NOTES AND REPORTS NO. 66]

# Photosynthesis in Chlorella. Quantum Efficiency and Rate Measurements in Sunlight

BY WINSTON M. MANNING, C. JUDAY AND MICHAEL WOLF

Warburg and Negelein,[1] using monochromatic light at intensities of about $10^3$ ergs/sq. cm./second, found a value of 0.25 (molecules of carbon dioxide assimilated per quantum absorbed) for the quantum efficiency of photosynthesis in Chlorella. Recent experiments with Chlorella at the University of Wisconsin, in which low intensity monochromatic light was used, have yielded very much lower values for the quantum efficiency, not higher than 0.06.[2] Because of the large discrepancy between these values, it seemed desirable to make additional quantum efficiency determinations using an entirely different technique. Accordingly, the experiments described in this paper were carried out at Trout Lake, Wisconsin, using the sun as a light source and varying the intensity of the light by placing samples of Chlorella at various depths in the lake.

These experiments were carried out in July and August, 1936, and constitute one phase of the photosynthesis investigations which are a part of the general limnological program of the Wisconsin Geological and Natural History Survey.[3]

## Experimental

The *Chlorella pyrenoidosa* used in this work was the same strain as used in the experiments with monochromatic light.[2] The average cell diameter was about four microns. For each experiment, material was taken from a liquid culture suspension,[4] centrifuged, and then suspended in filtered Trout Lake water. This suspension was then transferred to round or square calibrated bottles (average volume approximately 140 cc.). The sides of the square bottles, while not optically perfect, were plane enough to permit a fairly accurate measurement of the fraction of light absorbed by suspensions of Chlorella. The concentration of the suspension, in cells per cc., was determined by counting under a microscope the number of cells per unit volume. For each depth during a run, four of the bottles were placed in a small wire basket, the two lower bottles in each basket being painted black and covered with black cloth bags to keep out light. The baskets were suspended simultaneously in the lake for a definite time (usually one to four hours), at depths varying from the surface to fourteen meters, and then analyzed immediately for dissolved oxygen, using a modified form of the Winkler method. In addition, from two to six bottles of the cell suspension had been analyzed for oxygen at the beginning of each run. The change in oxygen content of the black bottles gave a measure of the respiratory rate, while the difference in final oxygen content of the black and clear bottles gave a measure of photosynthesis. This of course involves the assumption that respiration is the same in light and darkness.

Oxygen analyses on duplicate bottles usually agreed to better than 0.2 mg. of oxygen per liter except when the amount of photosynthesis was sufficient to raise the concentration of dissolved oxygen far above the usual saturation value.

The carbon dioxide concentration of Trout Lake water is approximately 38 mg./liter. This figure includes free, half-bound and bound carbon dioxide. The carbonate–bicarbonate concentration is high enough to give a moderate buffering action.

The water temperature at the surface of Trout Lake varied from 24 to 21° during the period of this work. Down to about eight meters (top of the thermocline) the temperature dropped only slightly, usually one degree or less, and the rate of photosynthesis at eight meters in Trout Lake is in all cases so far below the maximum rate at high light intensities that the sharp temperature drop in the theromocline probably has little effect on observed rates.

The amount of solar radiation reaching the surface of the lake during the experiments was measured with a self-recording solarimeter; the percentage of transmission and the spectral composition of the radiation at various depths

(1) Warburg and Negelein, *Z. physik. Chem.*, **106**, 191 (1923).

(2) Manning, Stauffer, Duggar and Daniels, THIS JOURNAL, **60**, 266 (1938).

(3) (a) Schomer and Juday, *Trans. Wis. Acad. Sci.*, **29**, 173 (1935); (b) Curtis and Juday, *Intern. Rev. ges. Hydrobiol. Hydrog.*, **35**, 122 (1937); (c) Manning, Juday and Wolf, submitted to *Trans. Wis. Acad. Sci.*

(4) Details concerning the culture methods and other parts of the procedure may be found in refs. 2 and 3.

were determined with a pyrlimnometer.[5] Table I shows the wave length distribution of visible light at various depths in Trout Lake.

TABLE I

COLOR DISTRIBUTION IN TROUT LAKE

| Depth, meters | Red, % | Orange, % | Yellow, % | Green, % | Blue, % | Violet, % | Ave. wave length, Å. |
|---|---|---|---|---|---|---|---|
| 0 | 28 | 16 | 15 | 13 | 18 | 16 | 5590 |
| 1 | 18 | 20 | 21 | 12 | 15 | 14 | 5530 |
| 3 | 9 | 17 | 27 | 19 | 16 | 12 | 5360 |
| 5 | 5.5 | 17.5 | 36 | 22 | 12.5 | 6.5 | 5430 |
| 7 | 0 | 13 | 46 | 27 | 10 | 4 | 5380 |
| 9 | 0 | 6 | 52 | 29 | 10 | 3 | 5350 |

Measurements of light absorption by Chlorella were made with suspensions in the square bottles. One whole side of the bottle containing the algal suspension was illuminated with the light from a tungsten filament lamp. A copper sulfate solution was used to remove infrared radiation. A large area thermopile (receiver area about 2 sq. cm.) was used to measure light intensities. The thermopile was moved from place to place behind each of the three sides of the bottle which were not facing the light. In this way, the total light escaping from each of the three sides was determined. Intensity of light (per unit area) emerging from top and bottom of the bottle was assumed to be equal to that of light emerging from the two lateral faces. This assumption should be nearly correct, since the whole front of the bottle was illuminated. The difference between the amount of incident light and the total emergent light should give directly the amount absorbed, thus eliminating the scattering factor. The light scattered out the side of the bottle facing the source was neglected, but the error so introduced is small, since for particles of the size of Chlorella the scattering of light in the direction of the source is small compared to that scattered in other directions.

The results of absorption measurements made in this way are given in Table II. In these measurements, the incident light intensity was taken as that passing through the bottle when filled with filtered lake water.

TABLE II

LIGHT ABSORPTION BY SUSPENSIONS OF CHLORELLA

The figures are given in terms of incident intensity as 100. Path length, 4.0 cm.

| Algal concn., cells/cc. | Incident light—light passing through back window | Light passing out sides, top and bottom | Net light absorbed |
|---|---|---|---|
| 14,800,000 | 73.4 | 7.4 | 66.0 |
| 7,400,000 | 52.5 | 5.2 | 47.3 |
| 3,700,000 | 33.5 | 3.9 | 29.6 |

In Fig. 1 the curve marked OA is drawn from the data of Table II. The energy distribution of sunlight is somewhat different from that of the light source used in the absorption measurements. Moreover, this distribution changes continuously with depth in the lake. The change in absorption with depth is shown in the remaining curves in Fig. 1. To obtain these values, the data of Tables I and II were used, together with Zscheile's determinations of the absorption spectra of pure chlorophylls a and b.[6] A value of three for the chlorophyll a/chlorophyll b ratio in Chlorella was assumed in these calculations.

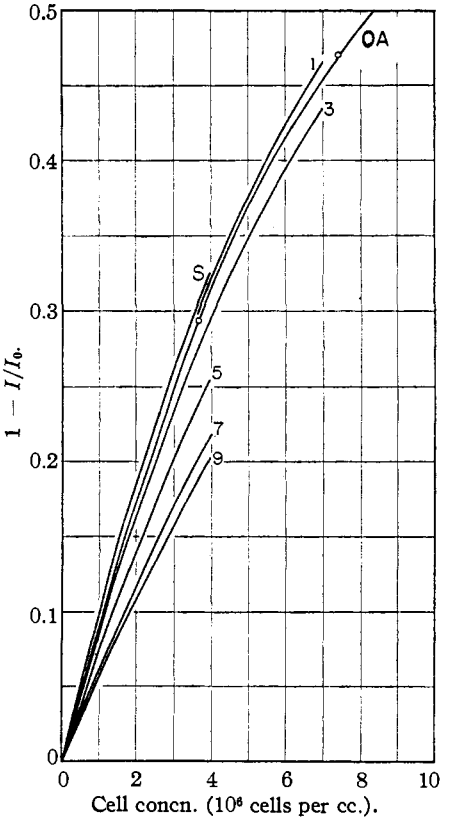


Fig. 1.—Fraction of light absorbed by Chlorella ($1 - I/I_0$) as a function of cell concentration ($10^6$ cells per cc.). Curve OA represents observed absorption; for highest point see Table II. S, 1, 3, 5, 7, 9, are calculated absorption curves for the corresponding depths, in meters, in Trout Lake.

The curves of Fig. 1 and the average wave lengths listed in Table I, together with total intensity measurements and data from oxygen analyses, were used in calculating quantum efficiencies for photosynthesis in Chlorella. The nine meter curve in Fig. 1 was used to calculate efficiencies for experiments at greater depths, but the error so introduced is not very large since further changes in wave length distribution below

(5) For details concerning these instruments, see Birge and Juday, *Trans. Wis. Acad. Sci.*, **27**, 523 (1932); Whitney, *ibid.*, in press.

(6) Zscheile, *Botan. Gaz.*, **95**, 529 (1934).

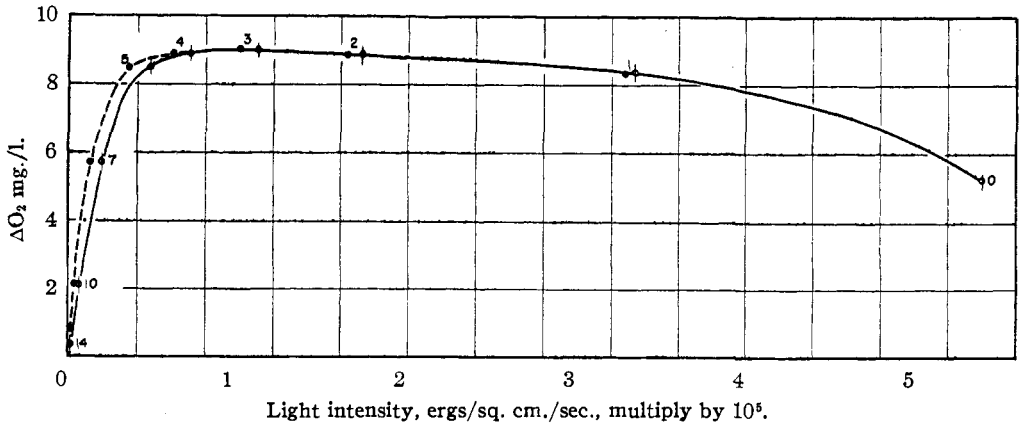


Fig. 2.—Reaction rate for Chlorella ($\Delta O_2$, mg./l.) as a function of light intensity (ergs/sq. cm./sec.). 3.17 hours. cell concentration 3,250,000 per cc.

nine meters would not alter greatly the mean absorption coefficient for the algal suspensions.

The values for quantum efficiencies reported here are subject to the criticism that the measurements were not made with monochromatic light, but this objection is not a serious one if we accept the observation of Warburg and Negelein[1] that the quantum efficiency changes only slightly with wave length throughout the visible spectrum.

The sources of error in our measurements will be discussed more fully in another report.[3c] The absolute values for the quantum efficiencies may be in error by as much as 20 or 25%, especially the efficiencies for low light intensities. However, the relative values, showing variation as a function of light intensity and algal concentration, are probably less in error.

## Results

**Experiments at Different Concentrations.**—Emerson and Green[7] have criticized the use of closed bottles in measuring photosynthetic rates, especially for long runs with large amounts of plant material, on the ground that the carbon dioxide supply may be inadequate to maintain a constant rate for more than a few minutes. Consequently, two series of experiments were made in which the quantum efficiency was determined for several concentrations of algal cells at the same light intensity. The results indicate that for experiments of three hours or less, the efficiency is independent of concentration below a value of about $4 \times 10^6$ cells / cc. Since concentrations used in all other experiments were below this value, it may be concluded that the carbon dioxide supply was adequately maintained during our experiments.

**Experiments at Different Light Intensities.**—Figure 2 shows photosynthetic rate as a function of light intensity (visible light only) for an experiment made at a series of depths. The solid curve is in terms of incident intensity; the broken line curve is in terms of relative amounts of energy actually absorbed. The intensities for corresponding points on the two curves are different except for the surface point because the absorption coefficient decreases with increasing depth in the lake (Fig. 1). The number near each point on the graph indicates the depth in meters at which the measurement was made. The probable magnitude of uncertainty in oxygen analysis is indicated by the length of the vertical lines drawn through the experimental points.

The very distinct falling off in rate at high light intensities, shown in Fig. 2, was noted for all of the experiments with Chlorella, except for a few carried out on very cloudy days. Several other species of aquatic plants showed similar behavior. Evidence discussed elsewhere[3c] indicates that high light intensity and changing spectral distribution are both factors in causing this inhibition.

In Fig. 3, quantum efficiency is shown as a function of light intensity (logarithmic scale) for several series of experiments with Chlorella. Curve A in Fig. 3 is for the same series of experiments as shown in Fig. 2. Variations in efficiency shown by the different series of experiments may have been due in part to variations

(7) Emerson and Green, *J. Gen. Physiol.*, **17**, 817 (1934).

in active chlorophyll concentration, or to other physiological variations. The point at fourteen meters on curve A is subject to large uncertainty because the total reaction there was very small.

Table III illustrates the method of calculation used in obtaining the quantum efficiency values. The calculation is for the ten-meter point on curve A in Fig. 3 (also Fig. 2).

TABLE III

SAMPLE CALCULATION

Average surface intensity (including infrared), $1.08 \times 10^6$ ergs/sq. cm./sec. Average intensity at 7 meters, $1.94 \times 10^4$ ergs/sq. cm./sec. Approximate area of absorbing surface, 36 sq. cm. Total incident energy = $1.94 \times 10^4 \times 36 = 7.0 \times 10^5$ ergs/sec. Concentration of algae, $3.25 \times 10^6$ cells/cc. Energy fraction absorbed, $(1 - I/I_0) = 0.182$ (see Fig. 1). Time of illumination, 11,400 seconds (10:30 A.M.-1:40 P.M.). Total quanta absorbed = $0.182 \times 7.0 \times 10^5 \times 11{,}400/h\nu = 3.96 \times 10^{20}$.

$O_2$ analysis (concentration in mg./liter)

| Initial $O_2$ concn. | Final $O_2$ concn. Dark | Light |
|---|---|---|
| 8.29 | | |
| 8.26 | | |
| 8.31 | 7.91 | 13.70 |
| 8.41 | 7.90 | 13.52 |
| Mean 8.32 | 7.91 | 13.61 |

$\Delta O_2$ (photosynthesis) = 13.61 − 7.91 = 5.70 mg./liter. $\Delta O_2$ (respiration) = 7.91 − 8.32 = −0.41 mg./liter. Av. bottle vol., 0.134 liter. $O_2$ evolved = $5.70 \times 0.134$ = 0.76 mg. = $1.44 \times 10^{19}$ molecules.

$$\phi = \frac{1.44 \times 10^{19}}{3.96 \times 10^{20}} = 0.0364.$$

## Discussion

The absolute values for quantum efficiencies at high light intensities are not of very great theoretical significance, because factors other than light intensity then play an important part in controlling photosynthetic rate. Probably the most important of these factors in our experiments are carbon dioxide concentration and the speed of the Blackman or enzyme reaction which forms a part of each photosynthetic cycle. Preliminary experiments indicated that the maximum rate, under the conditions of our work, was slightly increased by increasing the carbon dioxide concentration. Probably a higher temperature for our measurements would also have increased the maximum rate.

At sufficiently low light intensities, according to the generally accepted view, the rate of photosynthesis depends only on the rate at which light is absorbed by the chlorophyll. The quantum efficiency measured under these circumstances should then be highly significant since it would show directly the limitations of the photo-process or processes. However, as pointed out in another paper,[2] it is possible that at very low light intensities the quantum efficiency may be reduced by the action of reverse reactions during the intermediate steps of the complex photoreaction. According to this view, the temperature coefficient for photosynthetic rate may be less than unity for low light intensities.

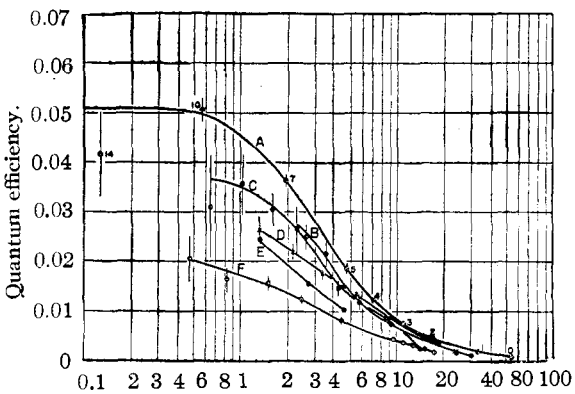


Fig. 3.—Quantum efficiencies for Chlorella as a function of light intensity (ergs/sq. cm./sec.): Curve A. 3.17 hours, cell concentration 3,250,000 per cc., B, 1.03 hours, cell concentration 718,000 per cc.; C, 4.00 hours, cell concentration 331,000 per cc.; D, 4.00 hours, cell concentration 718,000 per cc.; E, 4.05 hours, cell concentration 1,900,000 per cc.; F, 3.30 hours, cell concentration 1,210,000 per cc.

It may be seen from Fig. 3 that the maximum value found in these experiments for the quantum efficiency of photosynthesis, namely, 0.05 ±0.01, is far below the value obtained by Warburg and Negelein,[1] who used monochromatic light at intensities near $10^3$ ergs/sq.cm./second.

Even if the fourteen meter point in Fig. 3 be ignored, it appears that curve A should intersect the $10^3$ intensity ordinate at a value much lower than that found by Warburg and Negelein. Moreover, there seems no reason to doubt the validity of the fourteen meter point, within the wide range of experimental error. The value shown on the curve, 0.041, is the mean of the values 0.033 and 0.049, obtained for the two duplicate bottles. The temperature at fourteen meters for this experiment was 10.8°, as compared with 16.2° at ten meters, 21.3° at seven meters, 23.5° at five meters and 24.5° at the surface, but the low temperature at fourteen meters should have had no appreciable inhibiting effect on the rate, since experiments with Chlorella by

Warburg[8] showed that the temperature coefficient had dropped to unity at intensities as high as 6 or 7 $\times 10^3$ ergs/sq. cm./second. (Warburg's data permit only an approximate estimate of the absolute values of the light intensities used in his work.) This is in accord with the results for Hormidium obtained by Van den Honert and for Elodea obtained by Ruttner.[9]

The photosynthetic rate at fourteen meters shown in Fig. 2 was about twice the rate of respiration at the same depth. In the quantum efficiency measurements of Warburg and Negelein,[1] the respiratory rate exceeded the photosynthetic rate, doubtless because their suspensions were so concentrated that a large fraction of the cells received almost no light.

The quantum efficiency value of 0.05 is in fair agreement with the maximum of 0.04 to 0.06 found for measurements with the same species of algae made at low light intensities in the laboratory at the University of Wisconsin.[2]

(8) Warburg, *Biochem. Z.*, **100**, 230 (1919).

(9) Van den Honert, *Rec. trav. bot. neerland.*, **27**, 149 (1930); Ruttner, *Planta*, **2**, 588 (1926).

## Summary

Measurements have been made of the rate of photosynthesis in *Chlorella pyrenoidosa* at various depths in a fresh water lake. Using light intensity measurements made at these depths, the approximate quantum efficiency of photosynthesis at various intensities has been calculated. The value approached for low light intensities is approximately 0.05.

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[CONTRIBUTION FROM THE RESEARCH LABORATORY OF ORGANIC CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY, NO. 163, AND FROM THE INSTITUTO DE FISIOLOGIA, FACULTAD DE MEDICINA, BUENOS AIRES]

# The Chemistry of the Tetrose Sugars. III. *l*-Threose and Certain of its Derivatives. *d*-Lyxose Diacetamide and *d*-Arabinose Diacetamide Tetraacetate[1]

BY R. C. HOCKETT, V. DEULOFEU, A. L. SEDOFF AND J. R. MENDIVE

The two previous papers of this series[2] described the preparation of *d*-threose by modifications of the degradation procedures of Ruff[3] and of Wohl,[4] respectively, and announced a specific rotation[5] for this sugar at equilibrium in water or in dilute sulfuric acid of $-12.5°$. This figure deviated both numerically and in sign from that reported by Freudenberg[6] for a compound thought to be *d*-threose, and from the value to be expected from measurements previously made[7] upon what was considered to be pure *l*-threose. The conclusion drawn in these two papers to the effect that the earlier figures for both *d*- and *l*-threose were erroneous, was confirmed in later work.[8] It was therefore agreed that the study of *l*-threose should be repeated as a joint project. This undertaking was facilitated by two methods for preparing *l*-xylose which were described as plans were being laid.[9] Both methods were employed with success, that of von Vargha being employed in Buenos Aires and that of Appel in Cambridge. An early communication will report further observations upon *l*-xylose and certain of its derivatives.

The results obtained in the two laboratories with degradation of *l*-xylose by the way of *l*-xylose oxime, tetraacetyl-*l*-xylonic nitrile and *l*-threose diacetamide, are in close agreement and are wholly in accord with the predictions of the two previous papers in this series. Hydrolysis of the last named substance with 0.1 *N* sulfuric acid and titration of the sugar released by Cajori's method,[10] along with polarimetric observations, permitted determination of the equilibrium specific rotation of *l*-threose in 0.1 *N* acid as $+13.2°$.

Iwadare, Fukunaga and Kubota, using precisely the procedure described in the second paper of this series,[2] also have prepared *l*-threose and have determined its specific rotation as $+13.1°$. Their recent paper[11] anticipates our own an-

(1) A report of this investigation was delivered before the Harvard-Technology Chemical Club in Cambridge, Mass., in January, 1937, and before the Organic Division of the American Chemical Society in Chapel Hill, North Carolina, April, 1937.

(2) Hockett, THIS JOURNAL, **57**, 2260, 2265 (1935).

(3) Ruff, Meusser and Kohn, *Ber.*, **34**, 1362 (1901).

(4) Wohl, *ibid.*, **32**, 3666 (1899); Maquenne, *Compt. rend.*, **130**, 1402 (1900).

(5) All the rotations stated in this paper are specific rotations for the D line of sodium at 20°.

(6) W. Freudenberg, *Ber.*, **65**, 168 (1932).

(7) Deulofeu, *J. Chem. Soc.*, 225, 2458 (1929).

(8) Deulofeu, THIS JOURNAL, **58**, 855 (1936).

(9) Von Vargha, *Ber.*, **68**, 18 (1935); Appel, *J. Chem. Soc.*, 425 (1935).

(10) Cajori, *J. Biol. Chem.*, **54**, 622 (1922).

(11) Iwadare, Fukunaga and Kubota, *Bull. Chem. Soc. Japan*, **12**, 116 (1937).