respiration does not change. Periods of illumination and dark were chosen which were found experimentally to be favorable for the development of high apparent quantum yields.

Determinations 11A, 11C and 12A have been calculated by this extrapolation method as shown by the dotted lines in Fig. 1. After a period of illumination the algal suspension was kept in the dark for a period of five minutes. Oxygen concentrations were determined at the beginning of this dark period and at the end of the fifth minute. The light was turned on from the fifth to the fifteenth minute, and after five minutes in the dark the final reading was taken at the twentieth minute. The difference between this oxygen concentration and the value extrapolated from the fifth to the twentieth minute (assuming constant respiration), was used in calculating the amount of oxygen evolved during the ten-minute exposure to light. In 11A and 12A high values were obtained by this method of calculation. In 11C where the extrapolation sequence was started only after the algae had come to equilibrium with the light, as defined by a uniform rate of oxygen change (straight line), the calculations by the extrapolation method and the differential rate method are the same, and both are low.

The authors are glad to acknowledge the support of the Wisconsin Alumni Research Foundation in this investigation.

Summary

Quantum yields in photosynthesis by algae have been measured under a variety of conditions, making use of the dropping mercury electrode for the rapid measurement of dissolved oxygen. The results show that at 25°, 0.04 to 0.1 molecule of oxygen is evolved in photosynthesis by chlorella per quantum of red light absorbed. This efficiency is much lower than the efficiency 0.25 which has been hitherto accepted.

MADISON, WISCONSIN RECEIVED OCTOBER 13, 1939

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF WISCONSIN]

A Photocalorimeter. The Quantum Efficiency of Photosynthesis in Algae

BY JOHN L. MAGEE, THOMAS W. DEWITT, ELIZABETH COOLIDGE SMITH AND FARRINGTON DANIELS

The calorimeter described in this paper is designed to measure heat changes accompanying photochemical reactions. It was developed originally for determining thermally the quantum efficiency of photosynthesis by *Chlorella*. The hitherto accepted value of 0.25 molecule of carbon dioxide converted per quantum absorbed was obtained by Warburg and Negelein[1] with a differential manometer. Work originally undertaken in this Laboratory to extend the investigation of Warburg led to a much lower quantum yield, of the order of 0.05 molecule per quantum.[2] It was thus desirable to measure the quantum yield by other methods. Warburg's high efficiency demands that a large fraction of the absorbed radiation be converted into chemical energy while the lower efficiencies of Manning, Stauffer, Duggar and Daniels require that most of the radiation be dissipated as heat. A calorimetric method was suggested as an independent check on the work. The calorimeter consists of a small thin-walled quartz cell mounted in a cylindrical aluminum container. A multijunction thermocouple measures the temperature difference between the cell and container. A double thermostat keeps the latter at constant temperature. A thermopile placed behind the cell measures the amount of radiation transmitted. The calorimeter is calibrated at different light intensities by filling it with a solution of india ink or other chemically-inert, opaque liquid, and plotting galvanometer deflections against time until a steady state is reached. The heat evolved or absorbed in a chemical reaction can then be obtained by comparing the galvanometer–time curve with the curve for a chemically inert material under similar conditions.[2a]

Experimental Procedure

Apparatus.—The construction of the photocalorimeter is shown in Fig. 1. The cylindrical quartz cell with pol-

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

(2) W. M. Manning, J. F. Stauffer, B. M. Duggar, and F. Daniels. THIS JOURNAL, **60**, 266 (1938).

(2a) Dr. William A. Arnold of the Hopkins Marine Station of Stanford University at Pacific Grove, Calif., has developed independently a microcalorimeter which in August, 1938, was giving quantum yields with algae slightly greater than those obtained in this investigation.

ished ends, 2.3 cm. long and 1.3 cm. in diameter with two glass-stoppered openings in the top, has a capacity of 2.9 cc. It is supported by four fiber points in a hollow, cylindrical aluminum case 6.3 cm. long and 7.5 cm. in diameter having a central hole 4.5 cm. in diameter. The top of the case is removable for filling and emptying the reaction cell. Except for the front and rear faces, the quartz cell is silvered on the outside and covered with glyptal lacquer. The fifty copper–constantan thermocouple junctions, coated with glyptal lacquer, are fastened to the cell with de Khotinsky cement and the cold ends are attached in the same manner to the lacquered aluminum casing. The copper wire is no. 40 and the constantan wire no. 36, giving a total resistance of 34 ohms. The thermopile placed immediately behind the photocalorimeter has a surface larger than that of the calorimeter. It absorbs and measures all the transmitted light. It has twenty-eight junctions attached to the single, large, circular receiver and is enclosed in an aluminum block with a quartz window. The galvanometer (10 mm. per microvolt) is connected by a double pole switch to either the calorimeter or the thermopile.

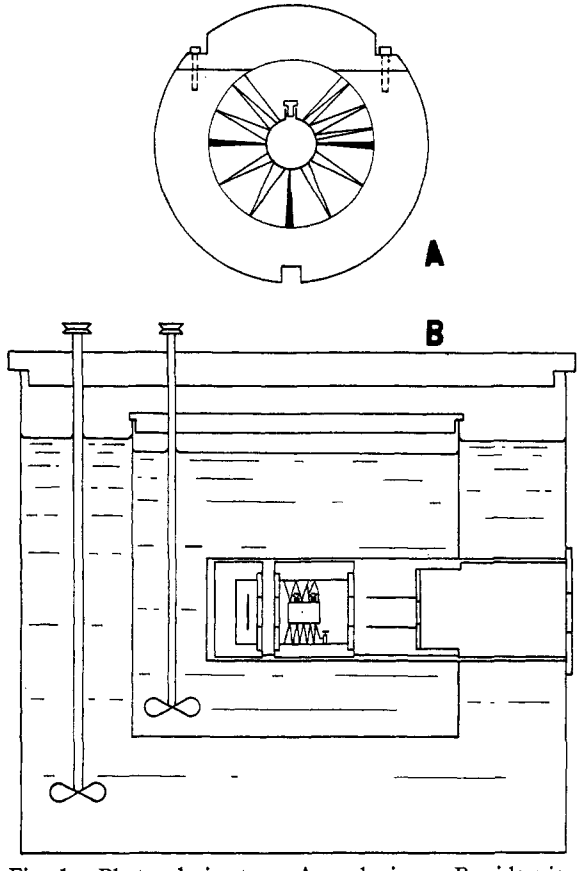

Fig. 1.—Photocalorimeter: A, end view; B, side view in thermostat.

A deflection of 1 cm. in the scale corresponds to a difference of 0.0003° in the calorimeter and it is essential therefore to have the cold junctions maintained at a very constant temperature. The problem was solved only after using a double thermostat, an outer one of water with a mercury regulator and stirrer, constant to 0.003°, and an inner one of oil supplied with a slow stirrer but no heater. The bottoms of the thermopile and calorimeter are slotted and run into the center of the inner thermostat along a track in a horizontal submarine tube as shown in Fig. 1B. In this way the cell is readily removed, filled and replaced in an exact position. After the thermopile and calorimeter are in position, a cylinder with quartz windows is telescoped inside of the thermostated tube and pushed flush with the outside of the thermostat. In this way circulation of cooler air is prevented. Thermal equilibrium is reached in about two hours after inserting the calorimeter and closing the tube.

For the measurements on *Chlorella*, a 500-watt projection lamp was used with a lens and three filters—a 10 cm. filter solution of 0.5% $CuSO_4$, 5 cm. of water, and a Corning signal red #243 filter. The parallel beam of light is diaphragmed down until it barely fills the whole calorimeter cell. The calorimeter is filled and emptied with micropipets having capillary outlets. The top of the aluminum block is removed and a piece of filter paper is placed over the top of the quartz calorimeter cell. The stoppers of the cell are allowed to protrude through holes in the filter paper and when they are replaced, the rims are dried and covered with a trace of glycerol. A slight variation in the calibration value from day to day was traced to different humidity of the laboratory air. Occasionally the galvanometer deflection in the dark was abnormal due to evaporation of traces of moisture, but this difficulty was corrected by sweeping through a current of dry air.

Calibration.—The thermopile registered 1 cm. deflection for each 102 ergs per second, as measured with a Bureau of Standards radiation lamp, after correction for the reflection at the quartz window. The calorimeter was calibrated with a beam of light and the thermopile as shown in Table I where R is the thermopile reading with the calorimeter removed and r is the thermopile reading when the light is partially absorbed by the calorimeter cell. The energies absorbed as shown in the third column contain small corrections for reflections at the rear window back into the calorimeter. A slight cloudiness on the calorimeter windows necessitated another correction when

TABLE I

CALIBRATION OF PHOTOCALORIMETER

Absorbing material	R	r	Energy absorbed, ergs/cm./sec.	Calorimeter deflection, cm.	Energy absorbed per sec. per cm. deflection
Water	29.0	23.4	776	9.0	85
Water	68.7	56.6	1720	20.6	82
India ink	33.6	15.3	1885	21.2	85
Colloidal silver	25.5	9.5	1833	23.0	80
India ink	35.5	13.8	2491	30.0	83
India ink	33.3	10.5	2591	31.2	83
Colloidal silver	48.5	18.5	3442	40.8	84
Colloidal silver	41.3	7.3	3808	45.8	83
Colloidal silver	48.1	11.8	4093	49.0	84
Colloidal silver	46.6	7.9	4332	49.6	88
India ink	74.7	26.8	5476	62.0	88
India ink	85.0	37.5	5505	63.4	87
					Average 84.4

measuring the heat of chemical reactions. The calorimeter–galvanometer deflections are given in the fourth column for the different energy imputs. These steady-state values are reached a few minutes after the light is introduced and remain constant indefinitely, as long as the intensity of light being absorbed remains constant. Under these conditions, the rate of absorption of radiant heat is exactly offset by the rate at which heat is lost to the colder aluminum block. Different absorbing materials were used, india ink and colloidal silver, and since both gave the same calibration value, it is safe to assume that they are chemically inert.

The calorimeter–galvanometer deflections are straight line functions of the amount of energy being absorbed, at least in the range 600 to 5300 ergs per second. The averaged result shows that each cm. deflection, in the steady state, corresponds to an absorption of 84.4 ergs per second.

The calibration described here was made before and during the first eleven experiments reported in Table II. Calibration measurements were repeated from time to time and it was found that the sensitivity of the calorimeter decreased slowly with use. The de Khotinsky cement was apparently unable to keep constant the thermal contact between the thermocouples and cell. The last determinations were made nine months later and in these, 1 cm. deflection corresponds to the absorption of 96 ergs per second.

The calorimeter was tested with the inversion of cane sugar and a value in good agreement with accepted values was obtained.[3] This reaction is, of course, thermal and serves as an entirely independent check on the calibration.

Measurements on Photosynthesis.—The algae used were *Chlorella vulgaris* or *Chlorella pyrenoidosa* grown under standard conditions in Warburg nutrient solution. The Department of Botany coöperated in this work. The details of the growing conditions are given elsewhere.[2,4] The light treatment was about the same for all: namely, one week at a high light intensity and one week at a lower light intensity.

Preliminary measurements indicated that the algae use only a small fraction of the absorbed energy in photosynthesis, about 20%. These measurements were made on suspensions of algal cells (approximately 15,000,000 cells per cc.) in the Warburg nutrient solution and were complicated by the settling of the algae. The time required for the calorimeter system to reach equilibrium was so long that settling began to take place and it was impossible to obtain very accurate values of the thermal efficiency. The qualitative results, however, indicated that the low quantum yields were real.

It was found that the addition of less than 0.1% agar rendered the settling of the algae much slower. A gel was not formed. Agar was used in all of the reported work. Quantum yield measurements made with the dropping mercury electrode on these algae indicated that they behaved normally.[4]

Measurement of the steady-state deflections for the illuminated algae had to be delayed two hours for the calorimeter system to come to complete equilibrium. Enough carbon dioxide was present so that there was no danger of its exhaustion; accordingly the samples were usually illuminated while the equilibrium was being reached. In some experiments, the algae were left in the dark for the first hour and then illuminated the second hour. Photosynthesis steady-state values will be designated P.

In order to obtain the thermal efficiency of photosynthesizing algae, the rate of heat evolution due to respiration also must be known. The difference between the steady-state deflection during photosynthesis and the deflection caused by the respiration gives the net amount of absorbed radiation being dissipated as heat by the algae per unit time. The total absorption of energy is measured by means of the thermopile. The fraction of absorbed energy dissipated as heat is the ratio of these two quantities. A difficulty in determining the respiration value arises from the slowness of the calorimeter in reaching a steady-state value when illumination is started or discontinued. The algae, being in a closed cell, use up their oxygen and the respiration falls to a lower value before the proper deflection is reached. This difficulty was met by determining the rate of cooling immediately after the light is turned off.

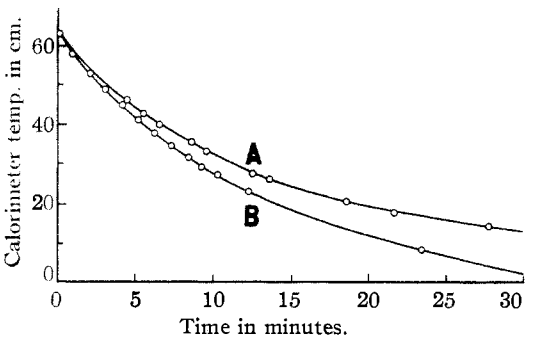

Fig. 2.—Cooling curves: A, with inert material; B, with respiring algae.

A cooling curve with inert material in the calorimeter is given at B in Fig. 2 and the cooling curve of algae after being illuminated is given at A. The slower rate of cooling of A is due to the heat added from respiration of the algae. Whereas curve B approaches zero deflection asymptotically, curve A approaches asymptotically a fixed deflection (temperature). This fixed deflection a is a measure of the respiration and is calculated from deflections shortly after turning off the light, since, at that time, the heat effect is not complicated by the settling of the algae nor the decreas-

(3) J. L. Magee and F. Daniels, forthcoming publication.

(4) H. G. Petering, B. M. Duggar and F. Daniels, *ibid.*, **61**, 3525 (1939).

TABLE II

FRACTION OF RADIATION CONVERTED INTO CHEMICAL ENERGY DURING PHOTOSYNTHESIS

1	2	3	4	5	6	7	8	9
Incident intensity, ergs/sec.	Total absorption, ergs/sec.	Absorption by algae, ergs/sec.	Photosynthesis defl. (P), cm.	Respiration defl. (a), cm.	Total heat evolved in calorimeter, ergs per sec., $(P - a) \times 85$ or $(P - a) \times 96$	Energy used in chem. reacn., ergs/sec. (2) − (6)	Energy efficiency (7)/(3) = f	Quantum yield $\Phi = 43f/112$
6740	5595	5109	63.5	10.0	4548	1047	0.205	0.079
3640	2291	2028	22.5	2.0	1743	548	.270	.104
3490	2539	2283	23.5	2.0	1981	558	.244	.094
1300	961	856	11.0	1.0	850	111	.130	.054
3830	2092	1805	24.2	4.0	1717	375	.208	.080
1450	816	707	12.5	4.0	723	93	.132	.051
8350	4857	4147	49.3	5.0	3766	1091	.263	.101
2640	1553	1354	17.8	3.5	1216	337	.249	.096
950	310	204	3.6	0.0	306	4	.019	.007
1890	948	801	9.3	.0	791	157	.196	.075
1830	1233	1099	10.8	.0	918	315	.287	.110
2825	1888	1514	15.4	.0	1478	410	.270	.104
2482	1653	1354	14.9	1.1	1325	328	.242	.094
2370	1606	1312	15.2	1.1	1354	252	.192	.075
2090	1494	1267	14.3	1.7	1210	284	.224	.086[a]
1420	1175	1047	12.0	1.3	1027	148	.141	.054
1240	1041	942	10.6	1.0	922	119	.126	.049
							Average	0.077

[a] A determination on this sample with the dropping mercury electrode gave a quantum yield of 0.073.

ing rate of respiration caused by the gradual exhaustion of oxygen.

The calorimeter deflections, x, which are proportional to temperature differences, obey approximately the cooling law

$$\frac{-dx}{dt} = k(x - a)$$

or

$$\ln \frac{x - a}{x_0 - a} = -kt$$

where x_0 is the value of x when the time t is zero.

Then in the form

$$a = \frac{x - x_0 e^{-kt}}{1 - e^{-kt}}$$

it is easy to evaluate a from a few readings near the beginning of a cooling curve with algae. Constant k is evaluated from the slope of the straight line produced by plotting log x against t when the calorimeter is filled with an inert material, as in curve B.

In order to calculate the quantum yield from the thermal efficiency, an assumption regarding the chemical reaction must be made. It is generally believed that the photosynthesis reaction is

$$CO_2 + H_2O + Nh\nu \longrightarrow 1/6\ (C_6H_{12}O_2) + O_2$$
$$\Delta H = 112{,}000 \text{ cal.}$$

The energy of an einstein of the light used (approximately 6500 Å.) was roughly 43,000 cal. per mole. Thus, for the above reaction with 100% energy efficiency a maximum quantum yield of 43,000/112,000 = 0.38 is obtained. The observed energy efficiency multiplied by 0.38 gives the values listed as quantum yields.

The data are given in Table II for the seventeen experiments carried out in this way. The incident energy given in the first column is only slightly less than the incident energy per square cm. since the cell window is about 1 sq. cm.

An examination of the data of Table II shows that the conditions were varied over a considerable range, the incident light intensity ranging from 950 to 6700 ergs per second and the energy absorption by algae from 200 to 5100 ergs per second. The absorption of energy in the photocalorimeter is determined by the concentration of algae as well as by the intensity of incident light. The difference between the second and third columns is due partly to reflection and partly to a correction for energy absorbed in the imperfect face of the photocalorimeter. In some of the experiments the concentration of algae was so low that respiration was hardly detectable and the results cannot be considered accurate. In these experiments, there may have been a slight evaporation of moisture from the cell which balanced the heat

of respiration. Only the difference $P - a$ is important in determining the efficiency, however, and such errors cancel in the result.

In some cases the quantum efficiencies were determined also by the dropping mercury electrode,[4] using the same cultures of algae. The average quantum yield of the last six determinations is 0.077 by the calorimeter, and 0.063 by the dropping mercury electrode. In the third from the last, the two different measurements were made on samples of the same solution of algae at the same time. The agreement is within the experimental error.

Discussion

The average of the quantum yields obtained in Table II, namely, 0.08 molecule of carbon dioxide consumed per quantum absorbed, is in good agreement with other investigations in this Laboratory[2,4] and gives a much lower value than the generally accepted value of 0.25.[1]

The respiration corrections work in the direction to increase the efficiency and most of the uncertainty in the calculations of the quantum yield is connected with the measurements of this quantity. When the oxygen is partially depleted by respiration, the respiration is slower and subject to larger variations. Under these conditions, the rate of respiration may be affected by factors which do not matter when the oxygen supply is larger. After sufficient photosynthesis, there is, of course, an ample supply of oxygen, corresponding to saturation of oxygen.

The quantum yields apparently do not depend greatly upon the factors which change respiration rates.[5] The rate of respiration is not proportional to oxygen concentration or rate of photosynthesis, although at very low concentrations it tends to increase with both. The concentration of oxidizable intermediates is, of course, increased with increasing rate of photosynthesis, and so the general tendency is for respiration to increase with light intensity.

This work corroborates the low quantum yields in photosynthesis obtained in the Wisconsin laboratory and indicates that the algae are much less efficient than expected on the basis of Warburg's results. These efficiencies based on thermal measurements appear to run slightly higher than those based on measurements of oxygen. If such a difference should be definitely established, it might indicate the existence of intermediate steps involving less heat absorption than that which is involved in combining carbon dioxide and water to give oxygen and carbohydrate.

The authors wish to express their appreciation to Professor B. M. Duggar of the Department of Botany for advice; and to Dr. W. M. Manning, Dr. H. G. Petering, and Mr. W. E. Moore for advice and experimental assistance. The dropping mercury electrode measurements were made by Mr. Moore. They wish also to thank the Wisconsin Alumni Research Foundation for aid in carrying out this investigation.

Summary

1. A small calorimeter is described capable of measuring a rate of heat change as small as a millionth of a calorie per second. It is designed to admit light and thus give a measurement of heat changes accompanying photochemical reactions.

2. The thermal efficiency of photosynthesis by green algae has been measured. About four-fifths of the red light absorbed in living algae is converted into heat, leading to a quantum yield of about 0.08 molecule per quantum absorbed.

MADISON, WISCONSIN RECEIVED OCTOBER 13, 1939

(5) See Reference 4, Petering, Duggar and Daniels. This fact has been corroborated by the results of Mr. W. E. Moore, in more recent experiments.