ON THE EFFICIENCY OF CHLORELLA GROWTH *

BY

B. KOK

T. N. O. Solar Energy Research Project, Laboratory for Plant physiological Research, Agricultural University, Wageningen

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CONTENTS

INTRODUCTION**

One of the most intensively studied aspects of photosynthesis is the efficiency of the conversion of light into chemical energy. Under optimal conditions, in steady state experiments of short duration, about 8 quanta are used per molecule of oxygen evolved (1) which means an efficiency of at least 25 % of the light energy when white

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** Investigations along the lines of thought pointed out here were initiated by Prof. WASSINK in this laboratory. A research program under his direction is sponsored by the Dutch Organisation for Applied Scientific Research (T. N. O.). The author is much indebted to Prof. WASSINK for many discussions and kind aids.

light is used. There is a striking difference between this efficiency and the efficiency of the photosynthesizable solar radiation observed in agricultural crops which is at most 2 % (2, 3). Until now, the factors responsible for this discrepancy hardly received the attention they certainly deserve. Or, to state it in another way, basic knowledge of plant photosynthesis up to the present, has not been incorporated into agricultural conceptions to the same extent as many other fields of science. This seems to be changing rapidly now. Moreover, it is remarkable that along with these considerations the type organisms of photosynthesis: green algae, are introduced into growth experiments. They are easy to study and to control, show great versatility, and algal cultures are likely to be handled more easily in such a way as to avoid limitations impeding the efficiency of the light conversion in higher plant.

Aiming at mass-culture of algae as another way for converting solar energy into organic matter, it is clear that the soundest basis for comparison with agriculture is the efficiency of light conversion, $\frac{\text{energy fixed}}{\text{incident energy}} \times 100$. Until now, only quantum yield estimations based upon oxygen evolution in experiments of short duration under laboratory conditions are available and we are in want for an analysis of overall efficiency of green plant growth and its governing factors.

In the experiments, reported in this paper the efficiency of growth of *Chlorella* has been estimated under various conditions. Essentially this has been made by measuring the amount of light absorbed by a culture of algae and estimating the amount of energy fixed in this culture during growth in determining the heat of combustion of the harvested cells. Besides this, the use of large Warburg-manometer vessels as culture flasks made possible continuous checks on the rates of photosynthesis and respiration. In this way a sufficient number of data was obtained for a complete balance of the energy transformations occurring in a suspension of growing algae.

In Chapter I, the experimental procedure is described. Chapter II deals with the efficiencies observed, attention being focussed on the optimal values. Various observations on algal growth in relation to the efficiency are presented in Chapter III.

Chapter I

EXPERIMENTAL PROCEDURE

1. Apparatus

The culture device was a Warburg apparatus. A thermostat (100 × 30 × 25 cm) with a glass bottom and a rigid shaking frame (circular motion 8 mm, 250 rev./min.), was used. A vessel is shown in fig. 1. Its total volume was about 250 ml in which up to 125 ml of suspension could adequately be used with smooth circular shaking. Three ml of diethanol amine solution were applied in the upper

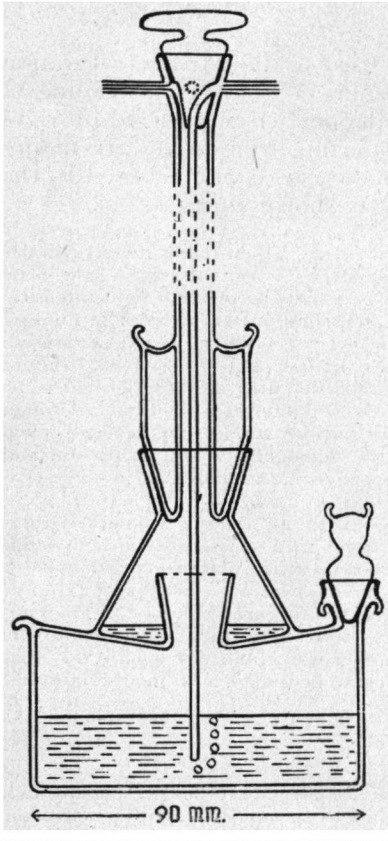


Fig. 1. Type of vessel used. Manometer is attached to stopcock, perpendicular to drawing (dotted circle drawn in the center). Turning the stopcock 90° clockwise closes the gas in- and outlets, and connects vessel to manometer.

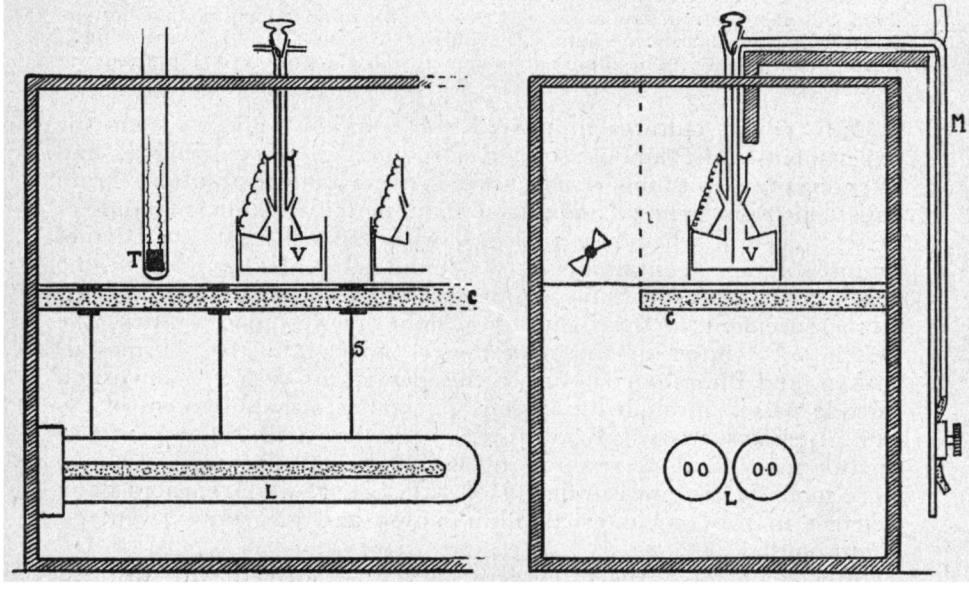


Fig. 2. The arrangement for illumination in the thermostat of the manometer vessels containing the algal cultures. L: sodium lamps, S: screens, C: glass box containing copper sulphate solution, V: vessel, T: thermophile, M: manometer.

compartment. A special stopcock was used to allow either saturation of the suspension with gas (air containing 3 % CO_2) or reading of the manometer. The vessels and the manometers were fixed on rigid manometer blocks provided with supporting iron strips. Six manometers and a thermo-barometer of smaller size could be used in the thermostat. A diagram of the set up is shown in fig. 2.

In an experiment, each vessel was surrounded by a blackened metal cylinder extending about 2 cm upwards and downwards, to screen off stray light. The cylinders could be provided with a glass filter to adjust the incident light intensity. Sodium light, filtered through 1 cm 6 % $CuSO_4$ solution was used. The number of lamps and their distance from the reaction vessels could be varied as desired. When all the vessels were illuminated with the highest light intensity used (about 2×10^4 ergs cm^{-2} $sec.^{-1}$), four Philips lamps SO 1000 and one SO 250 had to be used. Small screens and strips were used to restrict obliquely incident light. Though a higher light intensity would be desirable for various studies, this set-up proved to be satisfactory especially with respect to the homogeneity of illumination and the angle of incidence for these large vessels (ϕ 9 cm), and allowed a reliable estimation of the efficiency.

The incident light intensity was measured with an especially adapted thermopile with a large angle of incidence, enclosed in a glass tube with a hemispherical bottom. It could be placed in the thermostat at any place and depth desired.

Light absorption by the contents of the vessels was measured with a large integrating "sphere" (a cube — sides 150 cm — with corners cut off). The construction of the sphere and the measurements were performed according to the principles described earlier. (1). The object-holder was installed in such a way that during the absorption mesurement the vessels could be shaken as in the thermostat.

2. Procedure

Chlorella was cultivated in Erlenmeyer flasks, small glass tubes passing through the cotton plugs, which tubes allow for continuous supply of the cultures with air containing 5 % of carbon dioxide. The cultures were continuously shaken (amplitude 2 cm, 90 times per min.) on a frame (120 × 60 cm) provided with a glass bottom, 12 cm below which a set of 4 fluorescent tubes (2 "day light" and 2 "warm white") burned continuously. The illumination intensity at the bottom of the flasks was $\sim$ 4000 lux.

Only young cultures in pure Knop solution were used for the experiments. The amount of algae required was centrifuged, and after eventual washing resuspended in fresh culture solution. From this suspension definite amounts were pipetted into the manometer vessels each of which was provided with 3 ml of a 40 % diethanol amine solution (containing 0.1 % thiourea, *cf.* Krebs [4]) in the central compartment. Some samples were placed in the dark at 0° C. A measurement of the light absorption (a) was made. Then, the vessels were fitted to the manometers, placed in the thermostat, shaken, and illuminated. During this period, air with 3 % of carbon dioxide passed through the vessels. Thereafter a measurement of the rate of photosynthesis (P), during 1—2 hours, and subsequently a measurement of dark respiration (R), during the next 1—2 hours, were made. These measurements of a, P, and R were repeated daily whereas in the long intervals illumination and gas supply went on continuously.

After some days, the cells were harvested, washed with distilled

water, dried for some hours at 90° C, and finally at 105° C in vacuo. The control samples of cells, stored at 0°C, were treated in the same way. No measurable loss of weight occurred during storage in these samples. For each vessel the difference in dry weight between the experimental culture and the control represented the amount of organic material formed during the experiment. The greater part of the samples collected were analysed for determination of the percentages of C, H, N, and ash [1].

Some experimental details and some additional experiments made in these respects are discussed in the next chapter.

3. On the use of monochromatic light

The use of monochromatic light simplifies or even only enables the accurate estimation of the total amount of absorbed energy. It is possible, however, that cellular processes correlated with growth are selectively influenced by light of some other part of the spectrum than the one used. The following indication was obtained that this was not the case in our experiments. Parallel cultures of *Chlorella* were irradiated during 10 days in various spectral ranges (5) with about the same quantum intensity, representing $\sim$ 1200 ergs cm $^{-2}$ sec.$^{-1}$ for red light. The result is given in fig. 3. Growth rate, measured by cell count and cell volume roughly

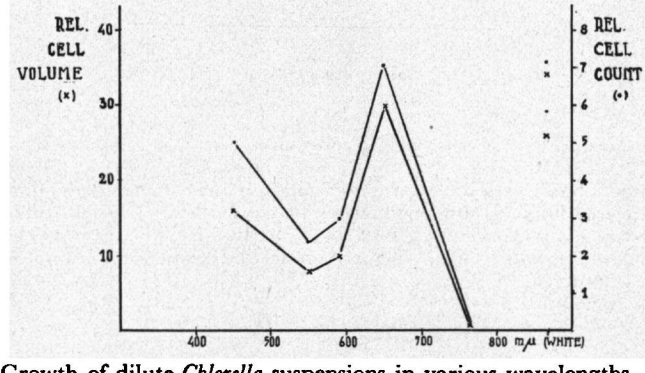


Fig. 3. Growth of dilute *Chlorella* suspensions in various wavelengths. Cell count (.) and cell volume (×) both in relative units.

follows the absorption spectrum of the photosynthetically active pigments. A more detailed analysis of this problem is difficult, owing to changes in the absorption spectrum as the cultures become denser. In another experiment one of the cultures in the Warburg thermostat received a small amount of incandescent light in addition to sodium light during growth. This did not improve the yield in any unexpected way. Thus, there are no indications that the quality of the light that we used was in any way suboptimal.

4. The measurement of gas exchanges

The use of diethanol amine as a CO_2-buffer is advantageous and is allowed since γ (CO_2/O_2) may be computed from the elementary composition of the cellular material formed. The rather low reaction rate between CO_2 and the amine in this set up is of no importance for these long time experiments; constant rates

[1] Thanks are due to Dr G. J. M. van der Kerk, head of the Dept. of Organic Chemistry T. N. O., Utrecht for carrying out these series of micro-analysis.

proved to be attained in about one hour. However, in view of the small amount of buffer, which could be used in the vessels described (3 ml of a 40 % solution), corrections were necessary. From the equilibrium data published by MASON and DODGE (6), the curve represented in fig. 4 was computed. In this curve, the amount of CO_2 (P, in μl) evolved or absorbed from 0.012 mol. (3 ml 40 %) diethanol amine for each percent change in CO_2 concentration of the gasphase, is plotted as a function of the initial percentage of CO_2 in the gas phase. In fact this curve holds only for small exchanges.

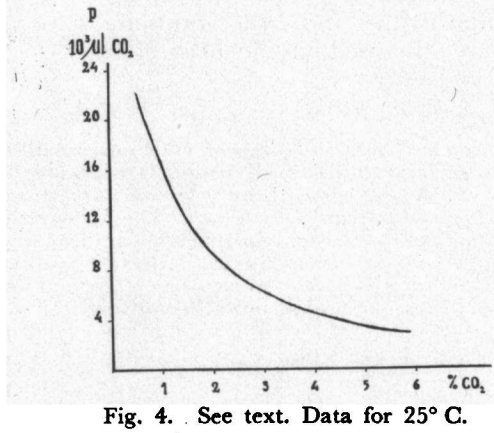


Fig. 4. See text. Data for 25° C.

For each percent decrease in CO_2 tension in the gas phase of a manometer vessel, P μl CO_2 are delivered by the buffer and 0.01 $(V_g + \alpha V_f)$ μl CO_2 by the gas and liquid phases. The latter fraction causes a manometer deflection and consequently the readings do not only represent oxygen exchange. Representing the photosynthetic quotient by $\gamma = -CO_2/O_2$, γ μl CO_2 is taken up per μl O_2 evolved. The fraction y originating from the gas phase and suspension liquid of a CO_2-uptake γx amounts to

$$y = \gamma x \frac{0.01\ (V_g + \alpha V_f)}{P + 0.01\ (V_g + \alpha V_f)}\ \mu l.$$

This amount of gas leads to a manometer deflection:

$$h_{CO_2} = \frac{y}{k_{CO_2}}\ mm.$$

The resulting manometer reading is: $h_{O_2} - h_{CO_2} = x/k_{O_2} - y/k_{CO_2}$. We now can derive the true O_2 evolution according to the formula:

$$x_{O_2} = h \frac{k_{O_2}}{1 - \frac{\gamma\, k_{O_2}}{0.01\, P + k_{CO_2}}}$$

in which, as usually, h represents the observed manometer deflection in mm and k_{O_2} and k_{CO_2} the vessel constants.

Dependent on the composition of the gas mixture used, on the vessel constants and on γ, this correction amounted to 8 – 20 %.

From the elementary composition of the cells, γ could be computed for photosynthesis only; for the corrections of the measured respiration rates, an average value $\gamma = 1.2$ (12) was used.

5. The measurement of the absorbed light

The illumination of the manometer vessels was homogeneous within about 5 %. The mean intensity (I) was obtained by averaging over various areas. The sensitive surface of the thermopile was put at the same height as the bottom of the vessel, so that the intensity of the light entering the vessel was measured. Both in the thermostat and in the sphere the area of the light beam was larger than the area of a vessel.

The daily procedure of absorption measurement was as follows: Galvanometer deflections were read after successively placing the following vessels into the object holder of the sphere: vessels 1 to 6, containing *Chlorella* suspensions (U_1—U_6), another vessel containing water (blank reading U_B) and a "black glass" (zero reading U_Z). In the last measurement, the opening in the objectholder (surface = 66 cm²) was completely shut off by the glass, so that only stray light was responsible for the galvanometer deflection. From these data, a was computed as $1-\frac{U-U_Z}{U_B-U_Z}$, U being U_1 to U_6 respectively. Incident light intensities were measured in ergs cm^{-2} $sec.^{-1}$. The total amount of light absorbed by the suspension during the time of illumination t (in sec.) was I. a. 66. t ergs. The mean light absorption ($\bar{a}$) and the total oxygen evolution during the whole growth experiment could be computed graphically. In this way the total amount of light, taken up during the growth period, I. $\bar{a}$. 66. t ergs, was found.

From the rates of photosynthesis and respiration, quantum numbers were obtained daily.

6. Estimation of the energy fixed

Micro-elementary analysis was advantageous, since it could be carried out with milligram quantities, and since its results could be used to obtain some insight into the composition of the cells by computation of the relative amounts of protein, carbohydrate and fat. The amount of energy fixed in a sample showing an increase in dry weight of a mg (on an ash free basis) and a composition of c %C, h % H, n % N and o % O was computed as follows: Complete oxidation would use

2.5×10^{-6} a $(0.33c-h-0.125o)$ = M mol. of oxygen;

if per mol. O_2 used, H kcal. are evolved, the material formed represents M × H kcal.

A direct check of this computation appeared to be very important. Therefore, we are much indebted to Prof. Coops (Org. Chem. Lab. V.U. Amsterdam) for carrying out some estimations of the heat of combustion with the bomb calorimeter.

From three parallel samples of dried algal material, either analysed or combusted (a, b, c), the following data were obtained:

a: Elementary composition 50.44 % C, 7.07 % H, 9.58 % N, 8.48 % ash.

b: 4.7 % ash, heat of combustion 5.7924 kcal./gram
c: 5.5 % ash, „ „ „ 5.7473 kcal./gram
} mean: 5.77 kcal./gr.

Unfortunately, the ash estimations showed variations. From these data a value of H = 112 kcal./mol. O_2 results for the *Chlorella* samples discussed. This value was generally used in our calculations. Probably, the errors introduced by neglecting the energy content of the ash and those introduced by the artificial drying, are of minor importance. An estimation of the overall accuracy of the final efficiency values is rather difficult, since so many data were to be collected. Generally, a good agreement between duplicates was found and the absolute values reported are likely to be significant well within about 10 %.

CHAPTER II

STUDIES AIMING AT THE OPTIMUM EFFICIENCY

1. DESCRIPTION OF A TYPICAL EXPERIMENT

About 10 experiments lasting 3—8 days, and a few covering shorter periods were made.

Fig. 5*a—f* contains data about an experiment with cells of low efficiency. Cells from a 4 days old culture (2.5 μl cells/ml), were resuspended in regular KNOP medium so as to yield a cell density in the vessels of 1.5 μl/cm^2, $\alpha_0 \simeq 0.36$. One vessel was kept in darkness and 4 vessels were illuminated with various light intensities. Fig. 5*a* shows the rate of photosynthesis (P), measured first, and the rate of respiration (R), measured after an illumination period, as ordinates plotted against the amount of light absorbed in each vessel (α I) as abscissae. The straight line indicates equal quantum numbers (ca. 9 quanta/mol. O_2) for all samples. The stimulation of respiration by a few hours illumination is remarkable, and is practically proportional to the intensity. Figs. 5*b*, *c*, *d* show similar observations for the following 3 days. Owing to cell multiplication, α is increased the second day (fig. 5*b*). In the highest light intensities, however, the rate of photosynthesis did not increase proportionally. A further decline in yield and ad ecrease of P/P—R is found the following two days (fig. 5*c—d*). Fig. 5*e* shows the increase of α and of the quantum-number and the decrease of P/P—R during the period of exposure, for one vessel. Fig. 5*f* shows the ultimate increase in dry weight of the samples, dependent on the irradiation intensity. The results of two similar experiments are given as dotted curves. Obviously, the experiment discussed led to rather low overall efficiencies, varying from 6 to 11 % fixation of the absorbed light energy.

2. GENERAL CONSIDERATIONS

The "efficiency" we were aiming at in these experiments needs some further discussion. Most measurements of photosynthetic efficiency have been made manometrically. The results thus obtained, however, only have a definite meaning if the overall reaction occurring in the cellular suspension is well known.

Even in steady state experiments considerable variation in the type

of reactions carried out by *Chlorella* cells is possible. For this reason, the measurement of the rate of CO_2 uptake is less desirable and also the determination of O_2 evolution is not completely unambiguous. The following example will demonstrate that only the knowledge of the composition of the material and the conditions under which it is formed allow the computation of definite efficiency values. We can take, *e.g.*, the sample described on page 451, with a molecular composition $C_{6\cdot14}\ H_{10\cdot3}\ O_{2\cdot24}\ N$ ("mol. wt". 134). The formation of this material puring photosynthesis in a NO_3-containing medium can be represented according to equation (1), γ being —0.69.

$$6.14\ CO_2 + 4.7\ H_2O + HNO_3 \rightarrow C_{6\cdot14}\ H_{10\cdot3}\ O_{2\cdot24}\ N + 8.9\ O_2 - 822\ \text{kcal.} \quad (1).$$

As far as energy conversion is concerned, essentially the reverse of reaction (1) takes place in the bomb calorimeter. Neglecting the heat content of the ash, we may calculate 822 kcal. ($134 \times 5.77 \times \times 1.062$) as the amount of energy fixed. Neglecting respiration losses and assuming a quantum number of 8 $h\nu$/mol. O_2, the efficiency of conversion at $\lambda = 6800$ Å would amount to $\frac{822}{8.9 \times 8 \times 41.5} = 27.8\ \%$. In this case, 92.4 kcal. would have been fixed per mol. O_2 evolved in photosynthesis.

In separate experiments (8), we measured equal quantum numbers for O_2 production in media containing either NO_3^- or NH_4^+. Now, if *Chlorella* cells, similar to those discussed above, should have been grown with ammonium as the source of nitrogen, synthesis would have occurred according to equation (2), γ being —0.89, and taking into consideration that reduction of nitrate to ammonia consumes 81 kcal./mol.

$$6.14\ CO_2 + 3.7\ H_2O + NH_3 \rightarrow \rightarrow C_{6\cdot14}\ H_{10\cdot3}\ O_{2\cdot24}\ N + 6.9\ O_2 - 741\ \text{kcal.} \quad (2)$$

In this case a quantum number 8 would mean an overall yield of $\frac{741}{6.9 \times 8 \times 41.5} = 32.4\ \%$. Per mol. O_2, evolved in photosynthesis, now 107.4 kcal. are fixed, and a 17 % better efficiency is obtained. Apparently, NO_3^- reduction in the light runs with a low efficiency $\frac{81}{(8.9 - 6.9) \times 8 \times 41.5} = 12\ \%$. From a merely economical point of view it is of interest that, theoretically, by ammonium feeding higher efficiences than with nitrate might be expected, as much as 30 % $\left(\frac{8.9}{6.9}\right)$ in the case discussed.

3. Results

In Table I, some data of an experiment (no. 7), leading to high yields under various conditions, are collected. 550 μl *Chlorella* cells (our strain A) originating from a 2 days old culture in Knop solution

(density 1.5 μl/ml), after washing were resuspended in 210 ml nitrogen-poor medium (C after SPOEHR and MILNER [7], diluted 4 times). From this suspension, an amount of 25 ml, containing 66 μl of cells was pipetted into each vessel, while two samples of 25 ml were stored at 0° C. In vessel no. 4, another 75 ml of the same medium were added, whereas the other vessels each received 75 ml of normal KNOP solution (pH 5.0). All vessels were provided with

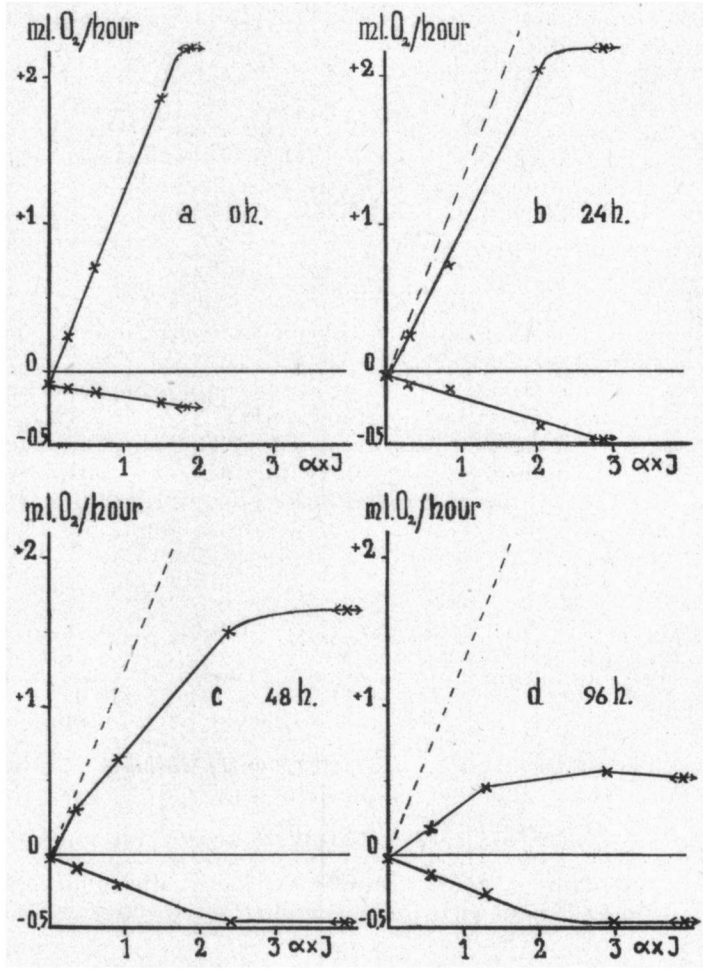


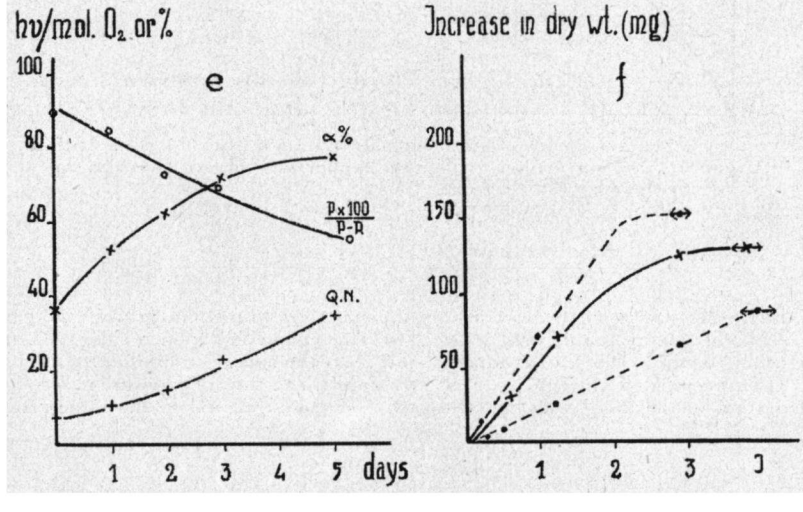
Fig. 5. Exp. No. 4. Photosynthetic and respiratory rates dependent on light intensity and time. The dotted lines in *b*—*d* indicate the initial slope shown in *a*. One vessel (indicated ←×→) was painted white outside in order to increase the mean intensity — this however prevented accurate estimations of αI. Abcissae in *a*—*d*: αI in relative units, the same for all figures, 1 relative unit is about 17×10^4 erg/sec. Fig. 5*e*: increase in α and quantum number, and decrease of P/P—R × 100 with time, for one vessel; fig. 5*f*: increase in dry weight with light intensity, I in relative units.

TABLE I

The efficiency of light energy conversion and some other data concerning growing cultures of *Chlorella vulgaris*, strain A (Exper. no. 7).

Time hours	Vessel no. . . .	2	3	4	5	6
	Inc. int. ergs cm^{-2} $sec.^{-1}$ × 10^{-3}	7.9	dark	14.8	17.9	13.3
1–3	Quant. no. . . .	10.1	—	9.0	10.2	9.1
18–21	Quant. no. . . .	8.5	—	11.1	8.7	8.9
	P/P–R	0.94	—	0.94	0.94	0.95
41–44	Quant. no. . . .	8.0	8.1	13.6	—	8.3
	P/P–R	0.95	0.93	0.95	—	0.89
65–68	Quant. no. . . .	7.5	—	19.6	10.2	9.1
90–94	Quant. no. . . .	8.1	10.0	35	—	9.8
	P/P–R	0.92	0.92	0.74	—	0.86
94	α	0.91	0.86	0.51	0.85	0.93
Total time illum. (hours) .		85.5	44.5	85.5	41	85.5
„ „ darkened („) .		8	49	8	52.5	8
Increase in dry weight mg (0° C: 13.3 mg)		101	84	82	82	176
El. analysis % C		48.51	46.90	50.91	48.44	43.53
„ % H		6.93	6.73	7.49	6.75	6.43
„ % N		8.25	9.01	2.43	10.59	5.58
„ % ash.		12.73	13.72	4.64	11.31	14.58
Efficiency (per cent). . . .		23.5	18.6	15.1	20.4	18.2
% protein		58	64	16	71	40
% carbohydrate		19	19	54	8	46
% fat		23	17	30	17	14
Heat of combustion kcal./gr (calculated)		5.75	5.63	5.38	5.76	5.90
γ computed from elementary composition		0.70	0.70	0.78	0.68	0.74

— = dark period (vessel 3 and 5)



3 ml 40 % diethanol amine and flushed with air containing 2.8 % CO_2. The temperature was 25° C, the absorption (α) in sodium light of the various samples at the start was 0.21 to 0.22 of the incident light. One place in the thermostat was darkened, which was used to put the vessels 3 and 5 alternately in dark and light, by mutually interchanging them every 24 hours. As usually, photosynthesis, respiration, and fractional absorption were measured daily, and from these data the quantum number, and P/P—R were calculated.

The final elementary analysis showed the character of the reaction which had actually occurred. Generally, we found very satisfactory agreement between the measured amounts of oxygen evolved and the amounts and compositions of cellular material built up. We will discuss in some detail the results obtained with vessels 2, 4 and 6 from this experiment:

Vessel 2: Elementary composition of the cellular material harvested: $C_{6.85}H_{11.8}O_{2.65}N$ ("mol. wt". 150).
Synthesis occurred according to:

$$6.85\ CO_2 + 6.4\ H_2O + NO_3^- \rightarrow (C_{6.85}H_{11.8}O_{2.65}N + 9.7\ O_2 + OH^- \qquad (3),$$

γ being —0.71. An increase in dry weight of 88 mg ash free was found. This corresponds to an oxygen evolution of:
$\frac{88 \times 10^{-3}}{150} \times 9.7 \times 22.4 \times 10^6 = 12.8 \times 10^4$ mm³ O_2, whereas a total O_2-evolution of 12.3×10^4 mm³ was computed from the daily manometric measurements.

Vessel 4: (low N vessel) Elementary composition: $C_{24.5}H_{43.3}O_{12.5}N$ ("mol. wt." 550).

$$24.5\ CO_2 + 20.2\ H_2O + NH_4^+ \rightarrow (C_{24.5}H_{43.3}O_{12.5}N) + 28.6\ O_2 + H^+ \qquad (4),$$

γ being — 0.86. Increase in ash free dry weight: 78.3 mg.
Corresponding O_2 evolution: 9.13×10^4 mm³.
Actually measured: 9.0×10^4 mm³.

Vessel 6: Elementary composition: $C_{9.15}H_{16.2}O_{4.96}N$ ("mol. wt." 219).

$$9.15\ CO_2 + 8.6\ H_2O + NO_3^- \rightarrow (C_{9.15}H_{16.2}O_{4.96}N) + 11.8\ O_2 + OH^- \qquad (5),$$

γ being — 0.78. Increase in ash free dry weight: 150 mg.
Corresponding O_2 evolution: 18.1×10^4 mm³.
Actually measured: 19.8×10^4 mm³.

In vessel 2, one of the highest efficiencies observed was recorded. In some shorter (24 h.) experiments the same efficiency was found.

Since the elementary composition did not differ much from the standard sample (*cf.* equation (1), and correcting 6 % for the higher ash content) we may calculate the efficiency directly as $\frac{\text{cals fixed}}{\text{cals absorbed}} \times 100$:

$$\frac{0.94 \times 101 \times 10^{-3} \times 5770 \times 100}{0.24 \times 10^{-7} \times 0.61 \times 66 \times 7.9 \times 10^3 \times 85.5 \times 3600} = 23.5\ \%.$$

in which $0.94 \times 101 \times 10^{-3}$ represents the material formed in grams, 5770 the heat content in cal./gram, 0.24×10^{-7} the conversion factor ergs/cals, 0.61 the absorption factor $\bar{a}$, 66 the irradiated surface in the sphere in cm², 7.9×10^3 the light intensity in ergs cm^{-2} sec.$^{-1}$, and 85.5×3600 the time of exposure in seconds. Calculation in the usual way, described also on page 451, led to the same result.

The distribution of all efficiencies obtained in our experiments, is given in fig. 6. The large variation shown is not due to experimental errors, but to differences in environmental conditions. Algae different from our strain A generally yielded lower efficiency values. Prolonged exposures, especially in small amounts of suspension medium per vessel (50 ml) and low nitrogen content were mainly responsible for unfavourable efficiency values (*cf.* Chapter III).

The high quantum yields and low respiration losses, shown by our best cultures, indicate that these yields approach closely the optimum for *Chlorella* and probably for all green plants. An additional improvement, theoretically can be expected from the use of ammonium instead of nitrate feeding, but no experimental data are available. Of a merely theoretical interest is the increase in efficiency by about 15 % to be expected if red light instead of sodium light were used. In our procedure, soluble organic material, eventually excreted by

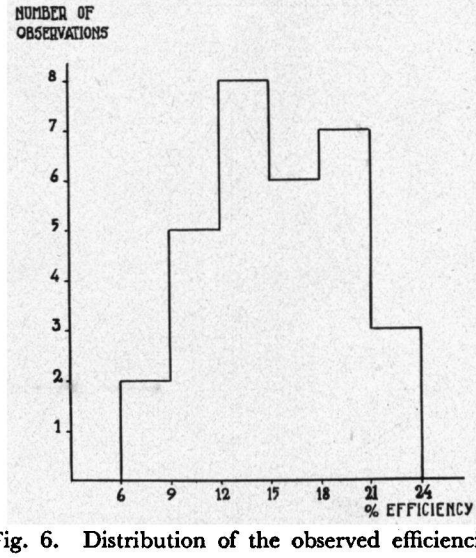


Fig. 6. Distribution of the observed efficiencies.

the algae into the culture medium was discarded. According to reports from several sides (7,9) 95—98 % of the organic material formed is retained in the cells and thus only a small error can have been introduced, neglecting the other fraction.

Chapter III

MISCELLANEOUS OBSERVATIONS ON ALGAL GROWTH IN RELATION TO THE EFFICIENCY

1. Chemical composition of the cellular material

Interest was concentrated upon compositions obtained in periods of efficient growth; this mainly restricted the duration of the exposures.

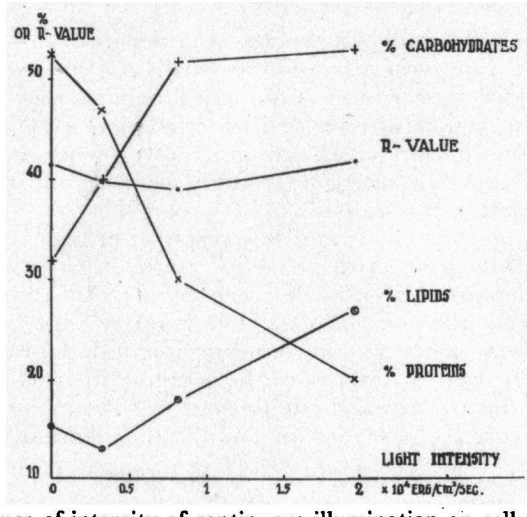


Fig. 7. Influence of intensity of continuous illumination on cellular composition.

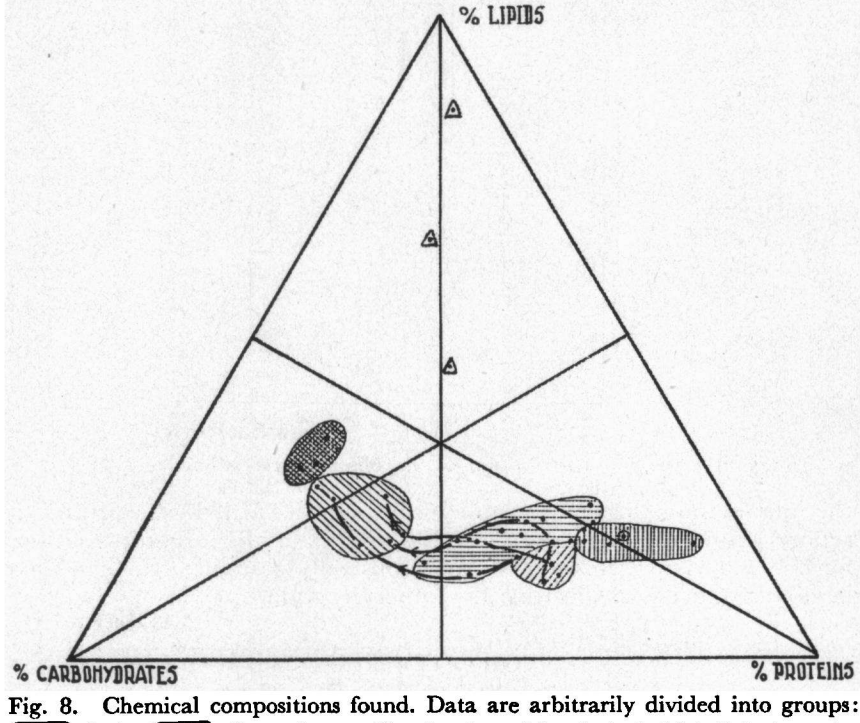


Fig. 8. Chemical compositions found. Data are arbitrarily divided into groups: dark, discontinuous illumination with relatively high light intensity (day/night). Other groups continuously illuminated: low intensity, high intensity, high intensity in N-deficient media. Arrows connect data from the same experiment with their points directed towards increasing light intensity. △ Data of SPOEHR and MILNER (7).

40 Analyses, including duplicates, were made. From each analysis, we computed the approximate percentage protein, lipid and carbohydrate, and the heat of combustion per gram (*cf.* Table I). The "R value", introduced by SPOEHR and MILNER (7) theoretically equals 7.6 times the heat of combustion in kcal per gram. For our standard sample we found 7.8.

Some correlations between cellular components and conditions are shown in figs. 7, 8, 9 and 10. Fig. 7 illustrates the effect of light intensity under continuous illumination. As compared to cells kept in the dark, illuminated cells show a decreased protein content, while percentage carbohydrate, and in strong light percentage fat are increased. Three experiments of this type were made. In fig. 8, 9 and 10 the samples of each of these experiments are interconnected by arrows, pointing to increase of light intensity. Moreover, in the figures all points originating from samples with about the same pretreatment are enclosed in "islands" (*cf.* the legend of fig. 8). The effects, shown in fig. 7, are generally found; prolonged continuous illumination and deprival of nitrogen lead to a low protein content, discontinuous illumination induces a high one. Fig. 9 illustrates a

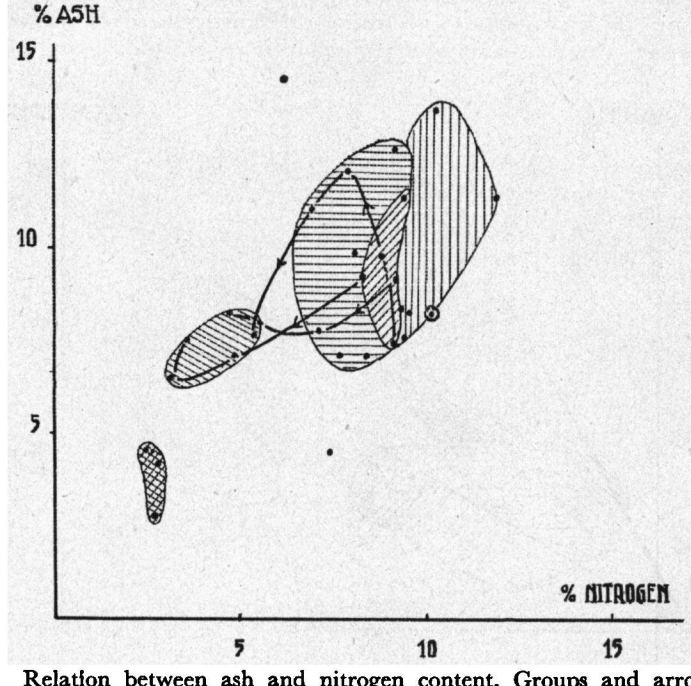


Fig. 9. Relation between ash and nitrogen content. Groups and arrows: the same meaning as in fig. 8.

correlation between nitrogen and ash content, both being highest in cells grown during day and night cycles, and lowest in nitrogen deficient cells. Small (about 20 %), but rather systematic variations

of the R value were found, the lowest values naturally occurring in carbohydrate rich cells (fig. 10).

The two extreme types may be discussed in some more detail:

1. Cells deprived of nitrogen

In relation to SPOEHR and MILNER's report on *Chlorella* cells with extremely high lipid content (some of their data are plotted in fig. 8), we tried to follow the growth efficiency of nitrogen deprived cells. Actually, in our definite experiments, we tried to transform normal cells into "fat" cells by transferring an amount of cells, previously grown in KNOP medium, into a nitrogen deficient one, in rather dense suspension.

Table I, vessel 4, shows a quick decrease in efficiency. In a dense suspension, the rate of photosynthesis declines with time in both normal KNOP medium and in a nitrogen deficient medium, as is shown in fig. 11. Refreshment of the medium, however, did not restore the initial rate in a nitrogen deficient medium as it did with cells normally grown. Smaller and more stable rates of O_2 uptake in the dark are shown in a nitrogen deficient medium. Preliminary observations did not reveal significant differences between the effect of continuous or discontinuous illumination of nitrogen deficient cultures.

Extremely high lipid and low N contents probably are not con-

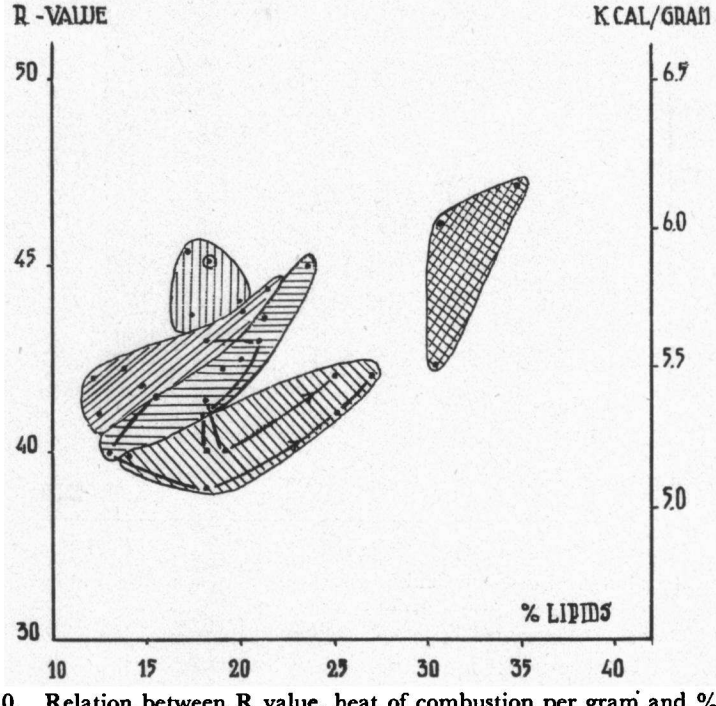


Fig. 10. Relation between R value, heat of combustion per gram and % lipids. Groups and arrows have the same meaning as in fig. 8.

sistent with favourable yields. However, the possibility is not excluded, that a short exposure in nitrogen free medium, following growth in a nitrogen containing one, leads to an optimum compromise.

2. Cells grown in discontinuous light

A considerable and more or less unexpected influence of dark periods upon cellular composition was found. In one experiment daily dark periods of 5 hours were intercalated, in order to follow the decrease of respiration after intense photosynthesis. Even these very short "nights" led to high-protein cells in nitrogen containing media. Cells grown outdoors and exposed to sunlight intensity and natural nights, showed high protein content too [1].

Based on the above and other observations (*e.g.* MYERS and CRAMER [10]), we might propose the following picture. At high

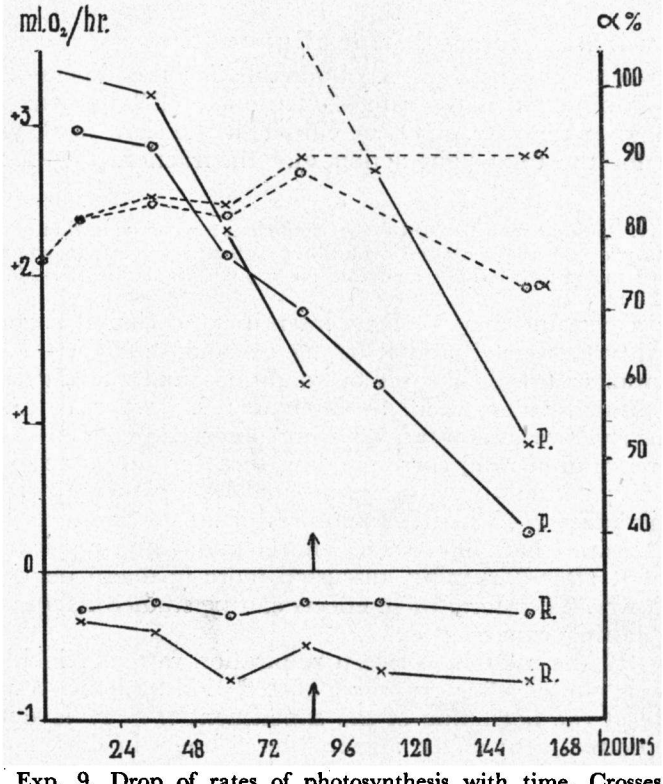


Fig. 11. Exp. 9. Drop of rates of photosynthesis with time. Crosses indicate values for cells growing in KNOP solution. Circles indicate values for a parallel culture in N-deficient medium. Arrows indicate resuspension of both cultures in fresh medium: only in the presence of nitrate the original rate is restored.

[1] In the summer of 1950 preliminary growth experiments in outdoor tanks (content 150—200 l, irradiated surface 1 m²) were made. Efficiencies observed did not surpass 2 % utilization of sunlight. This work has been continued during the summer of 1951 by VAN OORSCHOT (*cf.* 16).

light intensities, carbon fixation outranges nitrogen fixation, and cellular composition shifts to low protein content. After prolonged exposures cell division rate and photosynthetic efficiency may decrease. Upon darkening, during the phase of high respiration rate, protein synthesis and cell division restore cellular composition and size. Probably, natural days and nights are of primary importance for the efficient utilisation of bright light, *e.g.*, sunlight.

2. Assimilation and dissimilation, influence of dark periods on the efficiency

The respiration of algae in the dark results in a decrease in organic material. During illumination, the fraction of the light used for compensating respiration does not contribute to the formation of new material. Some remarks about both aspects may be made.

1. Respiration losses during illumination

At present, the best index obtainable for the dissimilation rate during illumination is the rate of O_2 uptake (R), immediately after darkening. If the rate of O_2 evolution in the light is P, we obtain (P—R) for the total light action, and the fraction P/P—R results in growth.

Actually, the light may be used twice as efficient for compensation of respiration (4 quanta process) as for oxygen evolution (8 quanta process) in which case the fraction of the absorbed light available for growth would be P/P—0.5 R.

Over a certain range of light intensities, we found a rough proportionality between P and R (*cf.* fig. 5*a*) and, thus, P/P—R constant and characteristic for the set of conditions and the algal material used. Optimal values were as high as 0.95, whereas under bad conditions values as low as 0.3 were observed.

The respiration rate, corresponding to a certain value of P (or of incident light intensity), reaches its final level rather slowly. Apparently this takes 2—3 hours. Exposures of 20—30 minutes to higher light intensities had only small effects upon after-respiration. The decay of R after darkening, discussed more in detail below, even is much slower. Therefore, these effects can be studied adequately only in long lasting experiments.

In fig. 12 the relation between respiration rate and incident light intensity is plotted after exposures of 0, 3, and 95 hours respectively. The rate at the beginning of the experiment ($t = 0$) is determined by the pretreatment of the algae. After 3 hours in dark and at low light intensity, the rate has decreased, whereas it has increased at high intensities. At the end of the experiment ($t = 95$h), respiration in dark was very low, but in strong light too the rate per unit cells had decreased. It was possible to estimate roughly from the course of the light absorption how the mean light intensity per cell: I mean (proportional to $\alpha I/C$, C = concentration at time 0) in the various vessels had changed, owing to the increase in concentration. Plotting the rates against I mean, instead of against the incident

intensity, closer agreement between initial and final rates is found. This might constitute an argument for the statement that it is the mean light intensity in a cell suspension which actually determines the respiration rate. An interesting phenomenon is shown by the weakly illuminated suspension. At the start of the experiment, even after 3 hours illumination, respiration was not compensated; after an adaptation period, positive pressure changes in the light and increase in dry weight occurred. A remarkably low respiration, probably representing the endogenous rate, was finally found.

2. Respiration losses during the night

The rate of O_2 uptake after a period of illumination declines slowly, until after about 20 hours a low and steady level of endogenous respiration is obtained (at 25° C we found a rate of about 1 μl O_2/hour/mg dry wt.). This decline was studied by various authors in algae (11, 12) and leaves (13). In fig. 13 some data are collected and plotted as % loss in dry weight versus time. The first steep slope of the curves is correlated with the previous illumination intensity

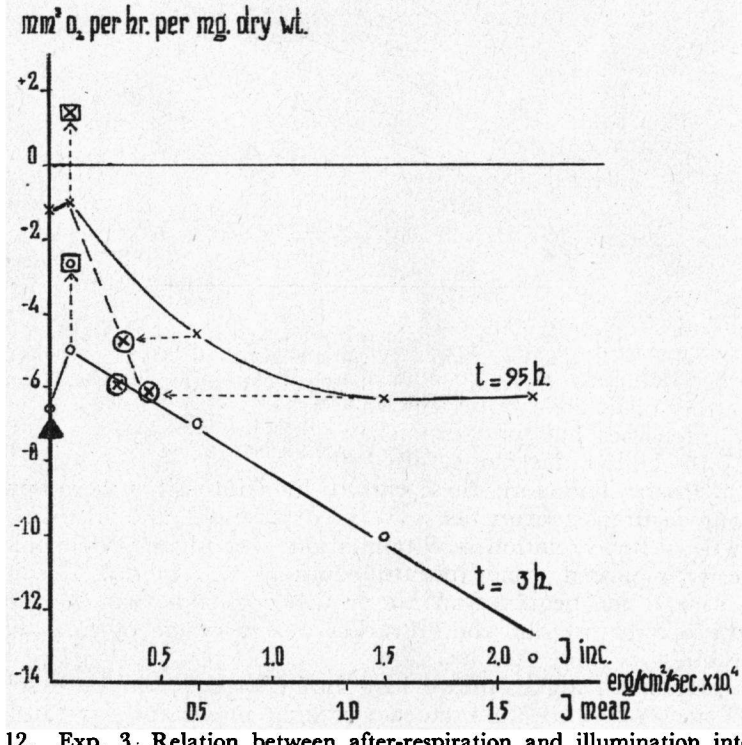


Fig. 12. Exp. 3. Relation between after-respiration and illumination intensity at the start (o) and the end (×) of a growth experiment. After applying a correction factor for the decrease of the mean intensity per cell during growth for the three strongly illuminated vessels, curve —⊗— is obtained for $t = 95$ hours. For the sample with the weakest illumination the initial and final rates of gas exchange in the light are indicated in squares. ▲ : respiration at $t = 0$ hours.

(*cf.* the previous section). So a loss as high as 10 % of the total dry weight during one night at 25° C may correspond to rates of photosynthesis, *e.g.*, doubling the dry weight during day time. In dense suspensions the relative changes probably are much smaller. The net result of natural dark periods thus may be a relatively small decrease (about 10 %) in overall efficiency of light utilisation. Very likely, the effect of these small losses during night periods is many times overcompensated by the benificial effects of the "rebalancing" of the chemical composition of the cells in the dark.

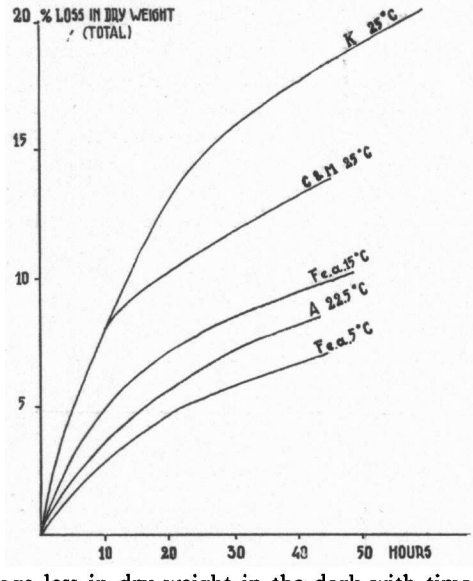


Fig. 13. Percentage loss in dry weight in the dark with time. Data for *Chlorella* after FRENCH *et al.* (F.c.a.) for 5° and 15° C, after CRAMER and MYERS (C & M) and from the present study (K) for 25° C. Data from AUDUS (A) are for Cherry Laurel leaves (22.5° C).

3. INFLUENCE OF LIGHT INTENSITY AND TIME

In the experiments in which various light intensities were applied, a linear relation between increase in dry weight and intensity was observed. Under constant illumination intensity, proportionality between dry weight and time of exposure was found. The linear relation between growth and I t, in fact indicates that the yield is independent of cellular concentration over a range of considerable extension.

Growth of an algal culture in a limited space can be described as follows: Accepting the efficiency of light conversion constant (K_1) and the respiration rate proportional to the concentration (C) of cells K_2C, growth occurs according to:
$dC/dt = \alpha I_0 K_1 - K_2 C$, $\alpha = 1 - e^{-\beta C}$, in which: I_0 = incident intensity, α = fractional absorption, β = constant.

Substituting: $y = \beta C$, $x = I_0 K_1 t$ and $z = K_2/I_0 K_1$ (so $z < K_2$ and

constant), we get: $dy/dx = 1 - e^{-\gamma} - zy$. At the beginning of an experiment, when a small inoculum is used, $zy \ll 1$ so that $dy/dx = 1 - e^{-\gamma}$, and an exponential growth curve is obtained. When y increases, $e^{-\gamma} \to 0$ and $dy/dx = 1 - zy$. Upon integration we find for $x \to \infty$, $y = 1/z$ and $I_0K_1 = K_2C$. This means that ultimately a stationary phase is reached, in which the (totally absorbed) incident light just serves to compensate respiration. Between the initial and final parts of the growth curve a zone occurs in which a nearly linear relation between C and I t exists. The maximum growth rate occurs in this zone at the inflection point of the curve, where the light absorption approaches 100 %. In fact, linear growth occurs over a far more extended range beyond the point of complete absorption than predicted by the foregoing formulae. The decrease of K_2 with time can be held mainly responsible for this.

As described in section 2, the respiration rate per unit cells, instead of being constant, appears to depend on the illumination intensity. When a suspension becomes denser, the mean intensity I_m per cell decreases, resulting in a lower value of K_2. The intensity I_m being inversely proportional to C, over a certain range K_2C might be constant and proportional to I_0, so that $K_2C = K_3I_0$. In this range, linear growth occurs according to $dC/dt = I_0 (K_1 - K_3)$. Ultimately, however, when the respiration rate approaches the endogenous level, an endpoint of growth is to be expected. Changes in chlorophyll content per cell (influencing β) and in photosynthetic efficiency (K_1), both especially important in strong light cultures, will complicate the picture. In our experiments on the relation between growth and I t, severe difficulties were encountered owing to deterioration of the medium. Increase in cellular concentration (to 2—4 g dr. wt./l) was accompanied by decrease in efficiency. Resuspension of the cells in fresh culture medium only temporarily restored the optimal efficiency (*cf.* fig. 11). Depletion of nitrogen or other major salt constituents of the medium by the cells did not occur. So, probably, either deficiency of micronutrients or auto-antibiosis phenomena have to be considered responsible for the mentioned effects. Recently, by adequate provision of micronutrients, MYERS (14) achieved linear growth of *Chlorella* up to very high densities.

DISCUSSION

Sodium light rather closely matches the mean of the visible solar radiation spectrum and has been found to be converted to cellular material with an efficiency of 15—20 %. Therefore, cultures of algae sufficiently dense to perform complete light absorption, may be expected to convert about 20 % of the incident solar radiation below 7000 Å into cellular material. This efficiency value should be considered as close to the maximum obtainable and more or less as a goal to strive after in mass culturing of algae. Dependent on geographical latitude, this figure would lead to harvests of 100—300 tons dry material per year per hectare. We may put the question

in how far there is any hope of practical approach to this efficiency.

The finding of suboptimal values in many cases even though all our experiments were made at non saturation intensities, illustrates the difficulties to maintain optimal conditions for the plant material. No fundamental limitations, however, seem to be involved here. On the other hand, the efficiency values were consistently higher than the ones found until now with algal cultures under natural conditions (16). This strongly indicates that in those cases an additional impeding factor enters the picture: the high intensity of solar radiation, surpassing factorfold the saturation intensity of photosynthesis, even under abundant supply of carbon dioxide (*cf.* also [2]). Experiments with flashing light, now under way, indicate that this limitation neither is a fundamental one (15). But it looks as if there is a long way between the fundamental possibility and its practical and economical realization.

SUMMARY

The efficiency of conversion of light into organic matter by cultures of growing *Chlorella* cells was measured under various conditions. During growth periods of 24—180 hours, we daily measured the incident light intensity, the fractional absorption, and the photosynthetic and respiratory rates. Finally, the increase in dry weight and the chemical composition of the harvested algae were determined. A correlation between chemical composition and heat of combustion was established. In this way a complete balance of the energy transformations during algal growth was obtained.

Efficiencies were expressed as $\frac{\text{calories fixed in organic matter}}{\text{calories light absorbed}} \times 100.$

Optimal efficiencies of 20—24 % for conversion of sodium light, representing about the mean of the visible solar radiation spectrum, were found in culture media, containing nitrate. Under conditions of nitrogen starvation which leads to cells of low protein and high carbohydrate and fat content, the yield dropped rather quickly.

Strong continuous illumination induced cells with low nitrogen-content, whereas cycles of light and darkness favoured the occurence of algae rich in protein. A relatively small decrease in overall efficiency is caused by natural day and night periods. The linear relation found between growth and intensity and time of exposure, which means that the yield is independent of cellular concentration, is discussed.

REFERENCES

1. KOK, B., Enzymologia *13*, 1, (1948).
2. WASSINK, E. C., Med. Dir. Tuinbouw *11*, 503 (1948).
3. SPOEHR, H. A., Proc. Am. Phil. Soc. *95*, 62 (1951).
4. KREBS, H. A., Bioch. J. *48*, 349 (1951).
5. WASSINK, E. C. and C. v. D. SCHEER, Proc. Kon. Ned. Akad. v. Wetensch. Amsterdam, *53*, 1064 (1950).
6. MASON, J. W. and B. F. DODGE, Trans. Am. Inst. Chem. Eng. 32—27 (1936).

7. SPOEHR, H. A. and H. W. MILNER, Plant Physiol. *24*, 120 (1949).
8. KOK, B., in preparation.
9. MYERS, J. and J. A. JOHNSTON, Plant Physiol. *24*, 111 (1949).
10. MYERS, J. and M. CRAMER, J. Gen. Physiol. *32*, 103 (1948).
11. FRENCH, C. S., H. J. KOHN, P. S. TANG, J. Gen. Physiol. *18*, 193 (1934).
12. CRAMER, M. and J. MYERS, Plant Physiol. *24*, 255 (1949).
13. AUDUS, L. J. Ann. Bot. N. S. *9*, 165 (1947).
14. MYERS, J. Unpublished.
15. Unpublished work at Stanford and Wageningen *cf.* note on page 15.
16. WASSINK, E. C., B. KOK and J. L. P. VAN OORSCHOT: On the yield of light energy conversion in *Chlorella* cultures as compared with higher plants. Carnegie publication (1952, in press).