CONVERSION OF LIGHT ENERGY IN ALGAL CULTURE

J. L. P. VAN OORSCHOT

Dit proefschrift met stellingen van

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De Rector Magnificus der Landbouwhogeschool,

Dr J. H. Becking .

Wageningen, 14 november 1955

CONVERSION OF LIGHT ENERGY IN ALGAL CULTURE

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD

VAN DOCTOR IN DE LANDBOUWKUNDE

OP GEZAG VAN DE RECTOR MAGNIFICUS DR. J. H. BECKING,

HOOGLERAAR IN DE HOUTMEETKUNDE, DE BOSBEDRIJFSECONOMIE,

DE BOSBEDRIJFSREGELING EN DE HOUTTEELT

EN BOSBESCHERMING IN DE TROPEN,

TE VERDEDIGEN TEGEN DE BEDENKINGEN

VAN EEN COMMISSIE UIT DE SENAAT

DER LANDBOUWHOGESCHOOL TE WAGENINGEN

OP VRIJDAG 16 DECEMBER 1955 TE 16 UUR

DOOR

J. L. P. VAN OORSCHOT

H. VEENMAN & ZONEN – WAGENINGEN – 1955

STELLINGEN

I

Culturen van *Chlorella* kunnen bij lage lichtintensiteiten 20 tot 30 procent van het photosynthetisch werkzame deel van de geabsorbeerde lichtenergie vastleggen in organisch materiaal; bij groei in vol zonlicht gedurende de zomermaanden is dit 6 tot 8 procent.

Dit proefschrift

II

De opbrengsten van algenculturen kunnen bij benadering worden berekend uit gegevens van de photosynthese in dunne suspensies.

Dit proefschrift

III

De productie van algen met hoog proteïne, koolhydraat of vetgehalte geschiedt in deze volgorde met afnemend rendement van de lichtenergie.

Dit proefschrift

IV

De door TAMIYA *c.s.* waargenomen variatie van het quantenrendement gedurende de dagelijkse cyclus kan ook op andere wijze verklaard worden dan door de genoemde auteurs is aangegeven.

H. TAMIYA *c.s.*, Biochim. Biophys. Acta **12**, 23–40 (1953)

V

Door een gunstige keuze van uitwendige omstandigheden moet het mogelijk zijn om de generatieduur van de ui tot een jaar te verkorten.

M. HOLDSWORTH en O. V. S. HEATH, J. Exp. Bot. **1**, 353–375 (1950)

VI

Bij de veredeling van Nederlandse uienrassen is onvoldoende geselecteerd op resistentie tegen ziekten.

VII

Serologische reacties en premuniteitsverschijnselen hebben slechts een beperkte waarde voor de bepaling van verwantschappen tussen plantenviren.

VIII

Het optreden van erfelijke veranderingen in tomaten-enten onder invloed van de onderstam is onvoldoende aangetoond.

IX

Het onderzoek naar het gebruik van algen ter aanvulling van een eenzijdig dieet dient te worden gestimuleerd.

Proefschrift J. L. P. van Oorschot
Wageningen 1955

Aan mijn Ouders

Aan mijn Vrouw

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CONTENTS

[1]) *Laboratory for Plant Physiological Research, Agricultural University, Wageningen, Netherlands, 138th Communication; 48th Communication on Photosynthesis.*

CHAPTER I

INTRODUCTION AND LITERATURE

1. INTRODUCTION AND OUTLINE OF THE INVESTIGATION

Fairly all organic material in nature ultimately has originated from photosynthesis. Solar energy is stored in plants, which are used as food or as fuel energy, the latter especially after the occurrence of further reduction processes. The need of more food by the increasing world population emphasizes the importance of light energy conversion problems in plants (*cf.*, *e.g.*, [12], [75], [80]). Most of our food is produced by agriculture. Whatever the methods to increase the food production are, 'the principle synthetic force remains photosynthesis' (*cf.* SPOEHR [80]), and the production is ultimately limited by the efficiency of this process. About 40–50 per cent of the solar radiation is between 0.4 and 0.7 μ (*cf.* [69], [78]), and thus usable in photosynthesis. The intensity of solar radiation varies extremely, owing to climatic and weather conditions. In the Netherlands the average intensity of the total radiation on a horizontal surface at noon varies between 0.15 and 0.75 cal/cm^2.min during the year (*cf.* [71]).

For considerations about utilisation of solar energy it should, of course, be remembered that photosynthesis, under various conditions can become light saturated at a relatively low light intensity. Various authors (12, 49, 69, 80) have estimated that only 1–2 per cent of the solar radiation as usable in photosynthesis, is converted into organic matter by agricultural crops. The calculations of WASSINK (96) which were based upon optimal yields of some crops in the Netherlands, resulted in figures from 0.5 till 2.2 per cent, including all parts of the plants.

From several sides (12, 49, 80, 97) attention has been drawn to algal culture for solar energy conversion. Unicellular algae seemed to offer some advantages in light energy conversion over agricultural plants. Such algae lack a transport system, which involves that no energy is required for the construction and maintainance of such a system as in higher plants. Thus, essentially, the whole plant may be used as food. In most climates, the cultivation area in agriculture can be used only during part of the year. In algal culture, a practically fully effective light absorbing system throughout the year can be obtained by appropriate density and depth of the culture, provided that the temperature is high enough to ensure growth. As has been pointed out earlier (100), external limiting factors may be overcome more easily with cultures of algae than with higher plants. The temperature, and the carbon dioxide content of the medium can be increased quite easily, humidity is no problem, while the mineral nutrition does not depend upon complex relations as may obtain in the soil. These possibilities enable an analysis of the reasons for the low yield of energy conversion in nature.

Furthermore, algae offer attractive possibilities owing to their versatility in protein, carbohydrate and lipid content.

This study is part of a research program in this laboratory (*cf.*, *e.g.*, Comm. 76, 98, 99, 113, 126), aiming at the elucidation of discrepancies between efficiencies of light energy conversion as observed in short term measurements of photosynthesis, and those found during growth of plants, especially under natural conditions. For this purpose we have quantitatively investigated the yield, and the efficiency of light energy conversion in *Chlorella* and *Scenedesmus* cultures under natural conditions. Besides this, a comparison had to be made with cultures grown under various conditions in the laboratory. Special attention has been drawn to the versatility of the algae with regard to chemical composition, and to the efficiency of light energy conversion under various conditions.

2. SURVEY AND PRELIMINARY DISCUSSION OF LITERATURE

The interest in algae for mass-culturing comes from photosynthesis research, in which unicellular green algae have been used to a large extent. The quantum efficiency of photosynthesis as obtained in experiments of brief duration were encouraging in this respect. Because both the yield in agriculture and that in algal culture primarily depend on the efficiency of the photosynthesis process, some of these results may be mentioned briefly here.

Using the manometric method, the quantum efficiency (mol O_2 evolved per $h\nu$) was first determined by WARBURG and NEGELEIN (91, 92), and a maximum efficiency of 0.25 was obtained in dense suspensions of *Chlorella*, absorbing practically fully the incident light. This would correspond to an efficiency of light energy conversion of 50 per cent at a wave length of 525 mμ. The results were computed from experiments at low light intensities with short periods of light and darkness. EMERSON and LEWIS (17, 18) criticised the method. They reproduced the quantum efficiency of 0.25 in experiments performed under the same conditions, and assuming $\gamma \left(= \frac{CO_2}{O_2}\right)$ to be unity. In the first minutes of illumination, however, a liberation of CO_2 strongly affecting γ, was observed. When steady state rates had been established, quantum efficiency values of about 0.10 were found with various green algae. This would correspond to an efficiency of 21 per cent at a wave length of 525 mμ. Also by others (*cf.*, *e.g.*, [72]) the results of WARBURG and NEGELEIN could be reproduced, but steady state experiments gave values of about 0.10. In more recent experiments of WARBURG *et al.* (*e.g.*, [93], [94]), no fluctuations due to induction phenomena were found, and the earlier results were confirmed. Again, EMERSON and NISHIMURA (19), and WITTINGHAM, NISHIMURA and EMERSON (101) could reproduce the same results by strict adherence to the same experimental conditions. They concluded, however, that these results were affected by a systematic error, which was due to the two vessel method, and the time schedules used. KOK (33, 34) using dilute suspension of *Chlorella*, and thus diminishing the influence of the correction for respiration, measured the absorption of the incident light in a white sphere. He observed somewhat higher values ($\pm$ 0.13) than those found by EMERSON *et al.* Below the compensation point, however, efficiencies were found to be twice as high, from which KOK concluded that the quantum efficiency of true photosynthesis is lower than that of photo-respiration, which becomes light saturated near the compensation point. RIEKE (72) using similar methods, observed values of 0.08 with *Chlorella*, and 0.09–0.11 with *Scenedesmus*.

In a number of investigations various other methods (chemical, polarographic and calorimetric) were used. The results were in accordance with the lower values obtained by manometry (0.10 $\pm$ 0.02). From the results obtained with the calorimetric method some may be mentioned. MAGEE *et al.* (46), using a photo-calorimeter, found efficiency values of 10–23 per cent. ARNOLD (4), measuring the difference in heat production of an algal suspension, photosynthesizing or not, found efficiencies of 17–28 per cent with *Chl. vulgaris*, *Chl. pyrenoidosa* and *Scenedesmus*. TONNELAT (88), using an adiabatic micro-calorimeter, and correcting for respiration, observed an efficiency of 30 per cent, corresponding to a quantum efficiency of 0.14.

Values of the same order of magnitude were reported with other photosynthetic micro-organisms (*e.g.*, Chromatium [99], green sulfur bacteria [41]). Photosynthesis of higher plants too, appears to be carried out with the same quantum efficiency. WASSINK (95), using leaf discs of various horticultural plants, floating on buffer solutions in WARBURG vessels, mostly observed values from 0.07 to 0.10. Working with a gas-analytical method, GABRIELSEN (25) found values of 0.10 ± 0.02 with leaves of *Sinapis*, *Corylus* and *Fraxinus*.

We may probably consider a value of 0.10 ± 0.02 O_2/hν as the most reliable one for steady rates of photosynthesis in photosynthetic bacteria, algae, and higher plants. This would correspond to an efficiency of light energy conversion of about 21 ± 4 per cent, if an energy content of 54 kcal per mol hν (at 525 mμ) is assumed. Thus, the efficiencies obtained in agriculture are at most 10 per cent of the maximum efficiency of photosynthesis.

An evaluation of this difference seems to be complicated by the comparison of growth values with those obtained in photosynthesis experiments of short duration in which no growth was supposed to take place. Photosynthesis involves primarily, as has been accepted already for long, the production of carbohydrates. During growth also reactions are performed by which proteins, fats, and other plant substances are produced, and these intra-cellular transformation processes may consume energy which has been previously stored in photosynthates. On the other hand STEWART and THOMPSON (82) from data by CALVIN *et al.* (see [82]) have suggested that in photosynthesis the ratio between carbohydrate and protein production depends on the competition of carbon dioxide and nitrogen compounds for components of the photosynthetic process. Then, at least in algae, a distinction between photosynthesis and growth would become arbitrary, and in the results of experiments on quantum efficiency the effects of other metabolic processes which occur during growth are included. (Russian data recently discussed at the Geneva conference on the peaceful use of atomic energy emphasize this viewpoint ([59]).

Thus, growth does not per se yield a lower efficiency as compared with photosynthesis, although in experiments on quantum efficiency a correction for respiration is included and in growth experiments it is not. This, however, cannot account for the large discrepancy, respiration usually being low as compared to photosynthesis. Up to now, few data are available concerning maximum efficiency of light energy conversion during growth. KOK (35), growing *Chlorella* cultures in sodium light, measured the light energy absorbed, and the chemical energy fixed during growth. He found efficiencies as high as 20–25 per cent under optimal conditions, thus corroborating preliminary results obtained in this laboratory (see [97), [100]). GLAS and GAASTRA (see [100]) determined the efficiency of beets, grown under constant conditions and found values of 12–19 per cent. WASSINK, KOK and VAN OORSCHOT (100) found somewhat lower values: 11–15 per cent. Thus, the maximum efficiency of light energy conversion during growth is not essentially different from that obtained in photosynthesis experiments of short duration, although generally the values seemed to be somewhat lower, which probably is mainly due to respiration losses.

The maximum efficiency of photosynthesis is not obtainable at all light intensities. At a certain light intensity saturation of the photosynthesis process is reached, as is well known, and a decrease in efficiency is found if the light intensity surpasses this saturation intensity. The saturation intensity depends on the species, and on the conditions of the experiment (*cf.* RABINOWITCH [70]). Values varying from 0.1 sunlight intensity up to full sunlight are found. This wide variation is due to differences in optical density of leaves and cultures, in

temperature, in CO_2-supply, in internal factors such as age of plant or culture, and in adaption to strong or weak light. The maximum rate of photosynthesis of various plants was found to be in the order of 20 mg CO_2/100 cm².hr (70), which corresponds to 4 per cent efficiency at 40 klux (± 0.25 cal/cm².min), if 80 per cent of wave lengths between 400 and 700 mμ are considered to be absorbed (see [70]).

Contradictory information is available as to the maximum rate of photosynthesis under natural conditions (*cf.* RABINOWITCH [70]), even under the same climatic conditions. Excluding the values reported by KOSTYCHEV (see [70]), and correcting for respiration which causes 10–15 per cent increase, RABINOWITCH concludes that the same values as mentioned above can be reached by plants in natural habitats, and under favourable conditions. An evaluation of the efficiency from available measurements of photosynthesis under natural conditions, however, is difficult. Intensity of solar radiation, temperature, and CO_2-content of the air may vary, which affects the saturation level. Mutual shading of leaves or cells may also affect the efficiency. The results depend upon the incident light intensity, and the absorption coefficient of the leaves or cells. In weak light a high absorption coefficient will exert an unfavourable effect upon the efficiency of the whole plant or culture, whereas in strong light a high absorption coefficient is favourable. Cultures of algae seem to be most suitable for determination of the yield and efficiency of light energy conversion in nature as well as under laboratory conditions. In the latter type of experiments, effects of light intensity and temperature may be investigated more easily than with higher plants.

A remarkable character of various unicellular algae is their versatility in chemical composition, in relation to culture conditions. Fresh water algae may be grown to produce mainly proteins, carbohydrates or lipids, by applying different environmental conditions (1, 50, 81). In this way *Chlorella pyrenoidosa*, *e.g.*, can be grown with various protein (8–88%), carbohydrate (6–38%) or fat contents (5–86%) (*cf.* SPOEHR and MILNER [81]). Different species of green algae, however, had roughly the same composition if they had been grown under the same environmental conditions, leading to a high protein content (31). Because of the shortage of protein in the world, high protein algae seem especially important, while also a high lipid content may be of importance. Although the chemical composition of higher plants may be entirely different for different species and organs, they generally have a high carbohydrate content (51).

From the environmental conditions, especially the nitrogen supply seems important. BEIJERINCK (7) observed a fat accumulation in old algal cultures, which were low in nitrogen content. An increase in fat content, accompanied with lower growth rates, was found when cultures of algae and diatoms grew old, and the nitrogen content of the medium was reduced (*e.g.*, 1, 24, 29, 35, 81, 103). These lower growth rates suggest a lower conversion of light energy, although cells of higher energy content are formed. So it seems that the energy conversion in algal culture is related to the nature of organic matter produced.

For a detailed discussion of algal components and the nutritional and industrial value of algae one is referred to the surveys of MILNER (51), FISHER and BURLEW (23), FISHER (22), and KRAUSS and MCALEER (40).

3. SCOPE OF THE PRESENT INVESTIGATION

Data on actually determined conversion of light energy under culture conditions are scarce. The methods used for determining light energy conversion in

growing cultures of algae, and for the estimation of the chemical composition of the cell material are described in Chapter II.

Data on the efficiency of light energy conversion under natural conditions have been collected in the years 1951, 1952, and 1953. These data are presented in Chapters III and IV. Chapter III deals with typical mass cultures. The effects of light intensity and temperature upon light saturation have been studied. For comparison similar cultures have been grown under constant conditions of light and temperature in the laboratory. Some of these results have already been reported earlier (*cf.* [100]). With small cultures of various strains of algae under natural conditions, higher yield values were obtained (*cf.* also [38]). Some of these results will be presented in greater detail, and discussed in Chapter IV.

Although effects of light intensity and temperature upon photosynthesis have been studied extensively, such effects on growing cultures are not well known; under these conditions the situation is more complicated. In dense cultures mutual shading of the cells will affect the yield. In Chapter V yield and efficiency values are calculated for certain accepted values of the incident intensity, the saturation intensity, the maximum efficiency of light energy conversion, and the fractional absorption of the light. Three different functions which describe the relation between the rate of photosynthesis and the light intensity are compared. Further, experiments are described in which effects of light intensity and temperature were quantitatively studied, and compared with values as obtained by theoretical computation. Moreover, influences of previous adaptation of the cell material with respect to light intensity and temperature upon photosynthesis, and yield of the cultures have been studied (*cf.* also [38]).

These algae show a broad versatility in chemical composition, which is governed mainly by the nitrogen content of the culture medium. Although effects of nitrogen supply upon growth rates of algae have been investigated by a number of workers, their results are rarely related both to changes in chemical composition and to efficiency of light energy conversion. Data on such relation are presented in Chapter VI. Two nitrogen sources, different in reduction level, *viz.*, nitrate and ammonium, are compared, and the relations of chemical composition and conversion of light energy at different stages of nitrogen deficiency are determined. Part of the results of these studies have already been briefly communicated (62).

In Chapter VII the relation between the results of the various types of experiments, and their significance for the problem of light energy conversion are discussed.

CHAPTER II

MATERIAL AND METHODS

1. STRAINS OF ALGAE AND THEIR PRE-CULTIVATION

Chlorella, strain A, isolated in this laboratory (35), *Chlorella*, strain 14-10, *Chlorella vulgaris viridis*, and *Scenedesmus 3*, obtained from elsewhere[1]) were used in the various experiments.

[1]) We wish to thank Drs APPLEMAN, MEFFERT, MEYERS, and PRINGSHEIM for kindly supplying these and other strains.

In mass culture experiments (Chapter III) *Chlorella*, strain A was used. During the summer of 1953, all strains mentioned above, and others, or combinations of strains were cultivated as small cultures in sunlight (Chapter IV). *Chlorella*, strain A, and *Chlorella*, strain 14–10 were mainly used in the experiments, dealing with the effects of light intensity and temperature (Chapter V), and those of nitrogen (Chapter VI) upon light energy conversion in algal cultures.

Usually the strains were maintained in small tubes, on agar media (2 per cent) with 3.67×10^{-3}m NH_4NO_3, 1.47×10^{-3}m KH_2PO_4, 0.81×10^{-3}m $MgSO_4.7H_2O$. These tubes were placed in a light cabinet (27° ± 3 °C) and illuminated continuously with 'daylight' fluorescent tubes, yielding an intensity ($\lambda < 700$ mμ) of about 0.02 cal/cm^2.min. Further cultivation of these algae was as follows. Erlenmeyer flasks, containing 200 ml culture medium with 5.0×10^{-3}m KNO_3, 1.0×10^{-3}m KH_2PO_4, 2.0×10^{-3} $MgSO_4.7H_2O$, 0.072×10^{-3}m $FeSO_4.7H_2O$, 0.155×10^{-3}m Na-citrate, and with A_4 and B_7 solution (*cf.* [5]), were inoculated with the algae content of a tube. The cultures were supplied with air containing 5 per cent carbon dioxide by way of small glass tubes, and continuously shaken (amplitude 2 cm, 90 times/minute) on a frame with a glass bottom. The cultures were continuously illuminated by fluorescent tubes ('day light' and 'warm white') mounted below the glass bottom, and yielding an intensity of 0.03 cal/cm^2.min at the bottom of the flasks. The temperature was kept at 25° ± 1.5° C.

In another arangement, the strains were maintained on liquid media in the same type of tubes as mentioned above (*cf.* [38]). The media contained 25×10^{-3}m KNO_3, 1.0×10^{-3}m KH_2PO_4, 2.0×10^{-3}m $MgSO_4.7H_2O$, 0.072×10^{-3}m $FeSO_4.7H_2O$, 0.155×10^{-3}m Na-citrate and the micro-nutrient solutions A_4 and B_7 (*cf.* [5]). These cultures (10 ml) were supplied with air containing 5 per cent carbon dioxide by small tubes, passing through the cotton plugs. This continuous air stream in the cultures prevented settling of the algae. The tubes were placed in glass-walled water tanks in which different 'day' and 'night' temperatures were achieved by using two thermo-regulators in each bath and switching from one to the other every 12 hours. Three thermostats with 'day' temperatures of 20°, 30°, an 40 °C respectively and corresponding 'night' temperatures of 15°, 20° and 25 °C were available. The cultures were illuminated during 12 hours per day by 500 Watt incandescent lamps with internal reflectors (Philips Altrilux), yielding an intensity of 0.25 cal/cm^2.min ($\lambda < 700$ mμ, corresponding to about 40 klux) at the place of the tubes. Because of fast growth at this intensity, every day or every other day, part of the culture was discarded and replaced by fresh medium. For experiments, larger amounts of algae usually were required. Then the strains were precultivated in the same media in 'Kolle' dishes (*cf.* [38]), containing 260 ml culture medium, or in large tubes (60 ml culture medium), under the same conditions as those mentioned above.

2. DETERMINATION OF THE LIGHT ENERGY CONVERSION IN GROWING CULTURES

In order to compute the conversion of light energy in growing cultures, the light energy absorbed, and the part herefrom which is fixed as cellular energy, must be determined. The efficiency of light energy conversion in per cent is then given by:

$$\frac{\text{energy fixed}}{\text{energy absorbed}} \times 100$$

The efficiency of photosynthesis mostly is expressed as quantum efficiency: molecules O_2 per $h\nu$. From this we can calculate the efficiency of light energy conversion by assuming as the overall equation for photosynthesis:

$$CO_2 + O_2 \xrightarrow{\text{light}} (CH_2O) + O_2 - 112 \text{ kcal}$$

A correction for respiration is included in the efficiency calculated in this way. The efficiency of light energy conversion during growth which is given above, has not been corrected for respiration, since only actual fixation of energy has been taken into consideration.

The amount of light energy absorbed during growth is represented by $I.t.O.\alpha$ cals, in which I is the incident light intensity in cal/cm^2.min at the surface of the cultures, t the culture period in minutes, O the irradiated surface in cm^2, and α

the absorbed fraction of the light, which depends on cell density, and thickness of the culture layer.

The light intensity at the surface of the culture has been measured with a thermopile, successively used with and without the RG8 filter (Schott and Gen.) in order to subtract the energy of wave lengths > 700 mμ. In thermostats we used a thermopile in an especially adapted mounting, and having a large angle of incidence (*cf.* [35]), in a glass tube. In outdoor experiments the light intensity is not constant which makes a continuous record necessary. In outdoor mass-culture experiments during 1951 and 1952 radiation values obtained from the Physics Department of the Agricultural University, Wageningen, were used, assuming 50 per cent of the radiation to be in the region between 400 and 700 mμ. In outdoor experiments during the summer of 1953, the light intensity at the surface of the cultures was continually recorded. For that purpose an especially adapted thermopile, with a large angle of incidence was used. It was mounted into a glass hemisphere. The thermopile was connected to a mirror galvanometer, which acted as a zero indicator in a potentiometer circuit with the aid of a photo-electric feedback amplifier.

A great part of the experiments was carried out under conditions of nearly complete light absorption in order to render absorption measurements superfluous, since these are difficult in large cultures (*e.g.* mass-culture experiments) or if non-monochromatic light is used. In experiments with sodium light (Chapter V, VI), however, thin suspensions with lower absorption were employed. The absorption measurements were made daily or twice a day, and carried out in a white sphere, as described by KOK (35). Unfortunately, high light intensities could not be obtained with sodium lamps (nor with other monochromatic light sources), so that for this purpose white light and practically fully absorbing cultures had to be used.

The amount of energy fixed during growth is given by the difference in energy content of the culture before and after the growth period. The energy content of a sample of cells is represented by: ash-free dry weight $\times$ energy content per gram. The small amount of organic material in the medium was neglected (*cf.* [81]). Dry weight was determined by taking samples from the suspension before and after the growth period. The samples were centrifuged, washed with distilled water, and dried in vacuo at 105°C, or at room temperature[1]) until constant weights were attained. A micro-analysis of the carbon, hydrogen, nitrogen and ash content was carried out [2]). The amount of organic material has been computed as: (100-ash) per cent $\times$ grams dry weight of cells. The energy content per gram organic (ash-free) material was calculated as follows. Complete combustion to carbon dioxide and water of 1 gram ash-free material, consisting of c per cent C, h per cent H, n per cent N, and having an R-value R (*cf.* [81]), requires:

$$\frac{(2.664c + 7.936h\text{-}o)}{100} \text{ gram } O_2, \text{ or}$$

$$0.125 \times 10^{-2} \times R \text{ mol } O_2 .$$

Assuming 112 kcal/mol O_2 (*cf.* KOK [35]) as the chemical energy present in the material, we obtain:

[1]) These methods yielded the same results with respect to both dry weight and chemical composition.

[2]) We wish to tender our sincere thanks to Dr VAN DER KERK, Dept. of Organic Chemistry T.N.O., Utrecht, for kindly carrying out the micro-elementary analysis.

$$0.14 \times R \text{ kcal}$$

as the energy content of 1 gram (ash-free) material.

In experiments, in which changes in chemical composition of the cells were induced (*e.g.*, Chapter VI), a micro-elementary analysis was carried out in most cases, in other ones some determinations were made, and the R-values obtained from these determinations (being fairly constant) were used for the computation of the fixed energy, also in other cases.

3. CULTIVATION METHODS

In mass culture experiments (Chapter III) tanks of 1 m^2 area with 300 liter suspension were used (see Plate 1). The culture medium contained: 5.0×10^{-3}m KNO_3, 1.0×10^{-3}m KH_2PO_4, 2.0×10^{-3}m $MgSO_4.7H_2O$, 0.072×10^{-3}m $FeSO_4.7H_2O$, 0.155×10^{-3}m Na-citrate, and the micro-nutrient solutions A_4 and B_7 (*cf.*[. [5]). The inoculation, either with pure cultures, grown under fluorescent tubes (*cf.* p. 231) or with cells from a preceding mass culture, was such as to yield practically complete absorption of the light. The cultures were mostly covered with a glass plate, which (notwithstanding regular cleaning) absorbed about 16 per cent of the incident light. The cultures regularly received pure carbon dioxide from a cylinder ($\pm$ 15 l/hr), and were stirred by a motor. At the start and at the end of the experiments, which commonly lasted 3–7 days, dry weight samples were taken, and elementary analysis was often carried out. Duplicate experiments yielded results, identical within 8 per cent.

The methods used with small cultures under natural conditions have been described recently (38). Tubes (height 20 cm, diameter 2.5 cm) and 'Kolle' dishes (circular flat surface diameter 12 cm, thickness 2.5 cm) were used as culture vessels (see Plate 2). The cultures with 60 and 260 ml nutrient solution respectively (containing 50×10^{-3}m KNO_3, further as above) were inoculated with algae grown under fluorescent tubes (*cf.* p. 231) or incandescent lamps (*cf.* p. 231), so that an absorption of 90–100 per cent of the light resulted. The suspensions were aerated intensely with air containing 1–5 per cent carbon dioxide. The samples for dry weight estimation were taken daily or at longer intervals, depending upon the growth rate of the cultures. At the same time half of the culture was discarded, and replaced by sterilized medium. Duplicate experiments fitted within 6 per cent.

Part of the experiments described in Chapter V and VI have been carried out with large WARBURG vessels (*cf.* KOK [35]) with a total volume of about 250 ml. They were filled with 100 ml nutrient solution and a certain amount of algae. The composition of the nutrient solutions will be presented in these chapters. The vessels were provided with an upper compartment for a carbon dioxide buffer and attached to manometers. A special stopcock allowed either a continuous stream of air with 3 per cent carbon dioxide or manometer readings. The vessels were shaken (circular motion 8 mm, 250 rev./min) in a thermostat. Sodium lamps were mounted underneath the bath, the light was filtered through 1 cm 6 per cent $CuSO_4$ solution; intensities up to 0.07 cal/cm^2.min were attained. By frequent measurement of the absorption in a white sphere (*cf.* [35]) the absorbed light energy during the growth period could be computed. The energy fixed during growth has been determined by dry weight estimation and micro-elementary analysis before and after growth (*cf.* p. 232). Duplicate experiments yielded results, fitting within 6 per cent.

In order to study effects of high light intensity, the sodium lamps were replaced by a set of six 24 Volt, 150 Watt incandescent lamps with internal reflectors (Philips Attralux). These lamps yielded intensities up to 0.7 cal/cm^2.min ($\lambda < 700$ mμ) over an area of about 35 cm^2. Wire screens served to obtain a series of light intensities. Dense suspensions were used in order to avoid the necessity of absorption measurements. Because of rapid exhaustion of the carbon dioxide buffer, the manometric measurement of oxygen production was not allowed under these conditions. Since we thus had to refrain from gas exchange measurements, Erlenmeyer flasks (diameter 6 cm) instead of WARBURG vessels were attached to the manometer blocks. They were filled with 30 ml of algal suspension, mostly obtained from cultures grown under incandescent lamps, at different temperatures (*cf.* p. 231). The nutrient solution was the same as that given above, but with 50×10^{-3}m KNO_3. The initial density of the experimental cultures was adjusted on the basis of a chlorophyll determination in the pre-culture (*cf.* p. 234). Generally, experiments started with the same amount of chlorophyll per culture, yielding practically complete absorption in red light. The results of duplicate experiments fitted within 3 per cent.

4. MEASUREMENTS OF GAS EXCHANGE IN GROWING CULTURES

As has been mentioned in the preceding section, large WARBURG vessels have been used. During the measurements of gas exchange, the carbon dioxide content of the gas phase was kept constant (at 3 per cent) with 3 ml diethanolamine in the upper compartment. So oxygen evolution could be measured, while γ (CO_2/O_2) was computed from the elementary composition (*cf.* [11], [35]). Constant rates were attained only after one hour, owing to the slow equilibration between carbon dioxide and the amine. In the growth experiments which are of long duration, however, this of no importance. The corrections, necessary by the use of small amounts of amine, were computed as described by KOK (35). The quantum efficiency was computed from the oxygen evolution in light and darkness, taking into account the data of light intensity and absorption measurements.

5. ESTIMATION OF THE CHEMICAL COMPOSITION OF THE ALGAE

The protein, carbohydrate, and lipid contents were estimated approximatively by the methods described by SPOEHR and MILNER (81). After the determination by micro-analysis of the carbon, hydrogen and ash contents[1]), and transference of these figures to an ash-free dry weight basis, the following computations (according to [81]) were adopted.

$$R = 0.668\ c + 1.989\ h - 0.250\ o$$
$$\text{Proteins} = 6.25 \times n$$
$$\text{Proteins} \times 42 + \text{Carbohydrates} \times 28 + \text{Lipids} \times 67.5 = 100 \times R$$
$$\text{Proteins} + \text{Carbohydrates} + \text{Lipids} = 100$$

In these equations, R means the R-value (*cf.* [81]), while the others are the percentages (on an ash-free basis) of carbon (c), hydrogen (h), oxygen (o), and nitrogen (n).

MILNER (50) showed that there is a good agreement between the calculated fat content, and that obtained by direct determination. Some doubt remains about the value of the figures for the protein and carbohydrate fractions. The high nitrogen values sometimes found by SPOEHR and MILNER (81) and during this work gave a suspicion as to accumulation of nitrate in the cells, notwithstanding they were washed with distilled water. Some samples, therefore, were analized elsewhere[2]) for total nitrogen content, and KJELDAHL nitrogen; the difference between these values was considered to be nitrate nitrogen. The following percentages of total nitrogen were found in three samples: 7.8, 8.8 and 9.04, while the corresponding figures for KJELDAHL nitrogen were: 7.8, 8.6, and 9.11. Thus no appreciable amounts of nitrate appeared to be accumulated in the cells. GEOGHEGAN (26) found that 'true protein' accounted for 90 per cent of the 'crude protein'.

6. CHLOROPHYLL AND NITRATE DETERMINATION

Chlorophyll determinations were made in the following way. From the suspension 1 ml or less was diluted to 10 ml by the addition of 5 per cent potassium hydroxide in methanol. This mixture was placed at 63 °C during exactly 3 minutes, and then centrifuged. The optical density of the clear green liquid (containing chlorophyll derivates) was measured at $\pm$ 650 mμ in a Lumetron colorimeter, using 5 per cent potassium hydroxide in methanol as a blank. Duplicate measurements fitted within 2 per cent. The calibration curve was obtained with definite amounts of a chlorophyll preparation, treated in the same way. By means of a simultanuous dry weight determination of the suspension, the chlorophyll content of the algae could be computed. For comparison, the chlorophyll content obtained with some samples was compared with that determined by the method of ARNON (6), by which an aceton extract is measured at 663, 645 and 652 mμ, and both chlorophyll a and b are determined. This method yielded the same results (within 3 per cent) as those obtained with the faster one mentioned above.

For determing nitrate in the medium we used the method of NOLL (61), modified according to DEYS and BOSMAN (15). One ml of the medium (nitrate content $<$0.4 mg) was diluted to 10 ml with distilled water, and slowly mixed with 20 ml of a 0.5 per cent brucine solution in sulfuric acid. After 30 sec stirring, the liquid was decanted into an Erlenmeyer flask, containing 700 ml of distilled water. After thorough mixing of the contents, the flask was placed in darkness during one hour to develop the yellow colour. Finally, the optical density was measured at 420 mμ in the Lumetron colorimeter, using brucine sulfuric acid as a blank. A calibration curve was made by treating media containing definite concentrations of KNO_3 in the same way. Duplicate measurements fitted within 5 per cent.

[1]) See note on page 232.

[2]) Thanks are due to Dr. VAN KOLMESCHATE for carrying out these determinations.

CHAPTER III

LIGHT ENERGY CONVERSION OF MASS CULTURES GROWN IN SUNLIGHT AND IN ARTIFICIAL LIGHT

1. INTRODUCTION

Although problems connected with mass cultivation of diatoms and red algae have also been studied (14, 32, 103), most attention has been given to unicellular green algae, and especially to *Chlorella* and *Scenedesmus*. Growth rates of such cultures have been expressed in various ways. The increase in cellular material is usually given in dry weight, cell volume or number of cells. The growth rate of a culture may then be computed by transferring the values to those per unit area or per unit volume. One of the most informative ways of expressing growth rates seems to be the indication of the increase in dry weight per unit area (*e.g.*, g/m^2). Transfer to efficiency values gives additional valuable information. The light intensity and the temperature must be mentioned for comparing the results with those obtained elsewhere.

In many computations, so far, growth has not been related to area, while light intensity and temperature are not well known. Some of the best yields (in g total dry weight/m^2. day) obtained by different authors are: 8.2 (*cf.* [13], p. 108), 11.7 (*cf.* [13], p. 113), 15.7[1]) (*cf.* [13], p. 143,) 11.0 (*cf.* [42]), 3.5[2]) (*cf.* [52]). The average efficiency of light energy conversion was estimated at 2 per cent (13). In our group, a yield of 7.2 g (ash-free) dry weight/m^2.day. and an efficiency of 2.6 per cent both averaged over het period from May till October (1951) were found (100). These results, and others obtained in 1952 will be given here in more detail. The cultivation methods are described on p. 233. (See also Plate 1).

2. MASS CULTURES GROWN IN SUNLIGHT

During the season light intensity and temperature vary considerably, which variation may effect the yield and efficiency values.

In fig. 1 data on several cultures of *Chlorella*, strain A, grown under natural conditions during 1951 and 1952 are summarized. The values of radiation, maximum temperature, yield, and efficiency of solar energy conversion (ordinates) are averaged over the experimental periods, the length of which is given by the width of the columns. For 1952, only the yield and efficiency values are plotted, for comparison with those of 1951. Generally, culture periods of 3–7 days were maintained. Extension of these periods to about 3 weeks (*cf.*, *e.g.*, May 1951 and July 1952) resulted in a lower overall yield and efficiency.

Fig. 1 shows that the yield and efficiency values vary considerably during the season. The yield per m^2.day varies between 1.0 and 10.2 gram during 1951, and between 2.6 and 12.8 gram during 1952. Maximum yield values are only obtained during a relatively short period of 1–2 month (June-July). Both earlier and later in the season, lower yields were found. In Arpil (1952), *e.g.*, and September (1951 and 1952) only about 50 per cent of the maxmium value is found, while in October (1951) the yield dropped to 10–20 per cent of its maximum value.

[1]) Figure calculated with the cross section of the cylindrical tubes used as irradiated area. A correction with $2/\pi$ (*cf.* [26], [38]) gives 10.0 gram.

[2]) At low temperatures (13–16 °C maximum).

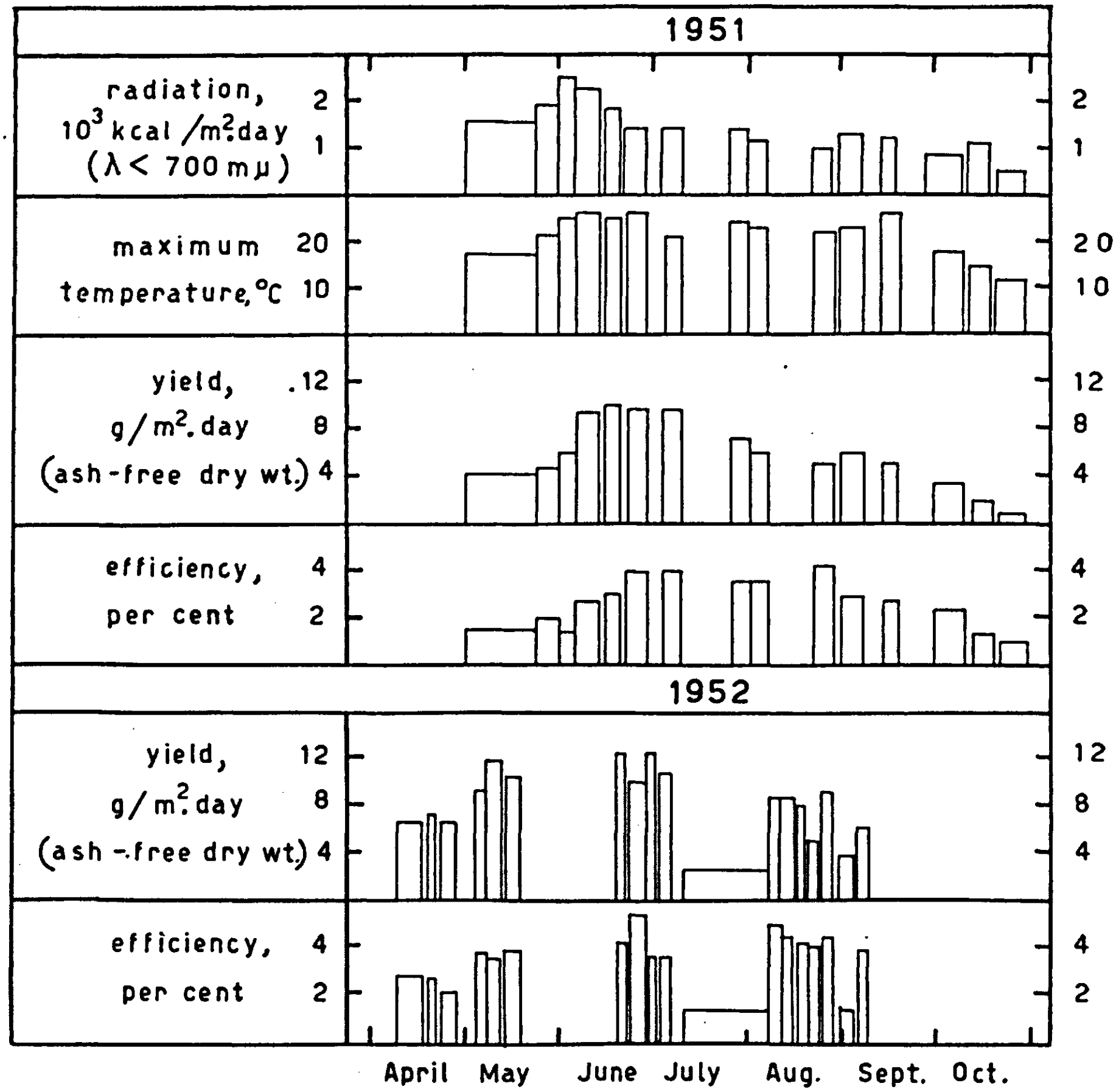

FIG. 1. Radiation, temperature, yield, and efficiency of light energy conversion in mass cultures (300 l, 1 m²) of *Chlorella*, strain A, under natural conditions during 1951 and 1952. Radiation values computed from data supplied by the Physics Department of the Agricultural University (*cf.* p. 232), and corrected for light absorption by the glass cover (*cf.* p. 233). Efficiency calculated assuming 100 per cent absorption of the incident light (*cf.* p. 232). Yield and efficiency during 1952 calculated on the basis of 10 per cent ash and an R value of 45.

The efficiency of solar energy conversion varies between 1.1 and 4.3 per cent during 1951, and between 1.3 and 5.4 per cent during 1952. The highest efficiency values are maintained during a somewhat longer period (2–3 months) than are the yield values. Then, the efficiency of solar energy conversion was about 4 per cent. Early and late in the season the efficiency values, much like the yield values, declined to 25–50 per cent of the highest values.

The course of the yield during the season shows a relationship with that of the radiation, but seems also affected by the temperature. The efficiency of light energy conversion during the season has a broad maximum, which is similar to that of temperature. The lower efficiencies found earlier and later in the season are remarkable, because the lower radiation values should favour higher efficiencies. This probably is due to the lower temperatures during those periods. A

separate computation of the effects of light intensity and temperature upon the yield, and the efficiency of light energy conversion cannot be obtained from these observations.

An average composition of the cells representing about 74 per cent proteins, 10 per cent carbohydrates, and 16 per cent lipids was found. The protein fraction ranged – independent of the season and thus of light and temperature (*cf.* also V, 3) – from 65 to 85 per cent, while the carbohydrate and the lipid contents constantly were low. In experiments with partial shading of the cells (*cf.* [100] important differences in chemical composition were neither found.

Our results show some differences with those of MEFFERT and STRATMANN (48). They also observed a maximum yield in July, but found highest efficiencies in October. They found a higher energy content per gram later in the season, whereas our experiments showed no indications of effects of light intensity and temperature upon chemical composition (*cf.* also V, 3). These differences may possibly be ascribed to the particular species used. Moreover, the results of MEFFERT and STRATMANN may have been affected by seasonal adaptation (*cf.* [38]), because they continuously used the same culture, whereas we frequently started with pure cultures grown under fluorescent tubes.

Separate effects of light intensity and temperature can only be determined in parallel experiments, in which one of these factors is the same. An effect of light intensity upon yield and solar energy conversion was already found earlier (100). In 1952 these experiments have been extended. Black iron screens of different light transmission factors were used for weakening the solar radiation. The transmission factor of a certain screen depends upon the angle of incidence of the sun. By measuring the light intensity under different screens in the course of a number of days, this variation was recorded. The average transmission factors

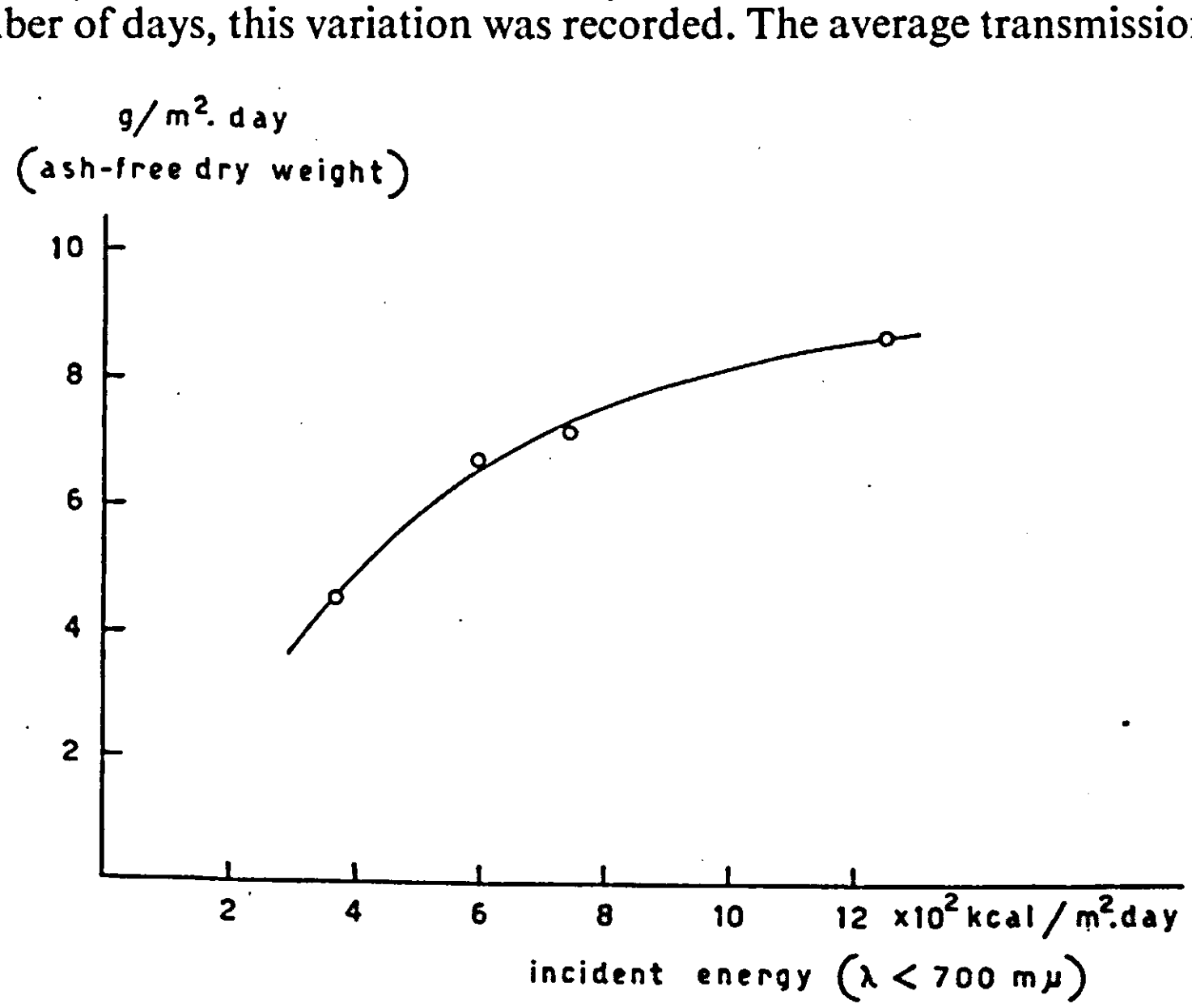

FIG. 2. Growth of mass cultures (300 l, 1 m²) of *Chlorella* (strain A) under artificially weakened daylight in August 1952. Average computed from 4 experiments of 5 days with an average unweakened radiation of 1490 ± 160 kcal/m².day (λ <700 mμ). Temperature: 20°–24 °C. Inoculum: 4 g/m² irradiated area. See also Table 1.

were calculated from the ratio between the areas under the various radiation curves obtained in this way. In August and September 1952, seven experiments with 4 relative light intensities were carried out. By means of heating coils the temperature of the cultures was kept constant (22° ± 2 °C) during the day. The results of 4 experiments with about the same radiation are given in fig. 2 and Table 1, *viz.*, the yields are represented in fig. 2, the efficiencies in Table 1.

TABLE 1

Efficiency of mass cultures (300 l, 1 m²) of *Chlorella*, strain A, in relation to intensity of solar radiation, in August 1952. Mean radiation ($\lambda < 0.7\mu$) of 4 experiments: 1490 ± 160 kcal/m². day. Duration of experiments: 4–5 days. Temperature: 22° ± 2 °C. Inoculum: 4 g/m².

Experiment	Per cent of solar radiation			
	25	40	50	84
1	8.9	8.0	6.4	5.0
2	8.7	7.6	6.4	4.3
3	6.9	–	5.9	4.8
4	6.5	5.5	5.3	3.9
Average efficiency values	7.7 ± 0.6	7.0 ± 0.8	6.0 ± 0.3	4.5 ± 0.3

Fig. 2 shows an increase in growth rate up to 1250 kcal/m².day, representing 84 per cent of full sunlight in these experiments. This is in agreement with the results reported in fig. 1, which in general showed the highest yields at the highest illumination values. As expected, and shown in Table 1, the efficiency of these cultures drops with increasing intensity of solar radiation. Thus the yield increases up to high light intensities (*cf.* also MILNER [13], p. 129), whereas the efficiency decreases. This will be discussed in greater detail in Chapter V.

From this one would expect a more clear relationship between radiation and efficiency in fig. 1. One may suggest that the results of fig. 1 also represent an effect of temperature on yield and efficiency. Therefore, late in 1951 and early in 1952, in some experiments the low temperature of outdoor cultures was raised artificially. The results of such experiments are given in Table 2. One would expect the greatest effect of a raise in temperature under conditions of high illumination (*cf.* fig. 8A), but it was found at the lowest intensity. This, probably, is due to the fact that the temperature is lower at this level of irradiation, resulting

TABLE 2

Temperature effect upon efficiency of light energy conversion in outdoor cultures (300 l, 1 m²) of *Chlorella*, strain A. Inoculum: 5–7 g/m² (± complete light absorption from the beginning). Duration of experiments: 4–6 days. Data: October, 18, 1951; April, 8, 1952; May, 12, 1952; April, 23, 1952.

Radiation, kcal/m².day ($\lambda < 700$ mμ)	Day temperature, °C		Efficiency, per cent	
	natural	raised to	at natural temperature	at raised temperature
460	8–12	18–25	1.1 [1])	3.9 [1])
1330	15–20	30–32	3.5	4.9
1390	16–22	29–31	4.7	5.6
1810	12–18	29–31	3.2	3.5

[1]) About the same chemical composition was observed (*cf.* also Chapter V, 3).

in a remarkable low value of the efficiency under natural conditions. At higher light intensities an increase in temperature from 15–20 °C to 30 °C exerted only a small effect upon the efficiency. In some cultures of low cell density, we found more pronounced effects of raising the temperature. The low fractional absorption of the incident light, which moreover changes with time by growth, renders very difficult a comparison of the effects of different temperatures in these experiments. MILNER ([13], p. 129) found maximum growth at temperatures of 25 °C with continuous illumination, while with illumination during the day only, low temperatures during the dark periods were favourable.

3. MASS-CULTURING IN ARTIFICIAL LIGHT AS COMPARED WITH THAT IN SUNLIGHT

The growth rates and efficiencies of the same type of mass cultures grown under artificial illumination have been reported earlier (100). Since some of these values unfortunately were not corrected for light losses caused by the glass cover (*cf.* p. 233), the results will be given here again. *Chlorella*, strain A, was used. The incident energy ($\lambda < 700$ mμ) was measured as described before (p. 232), using a photo-cell calibrated against a standardized thermopile, and correcting some values for light losses caused by the glass cover (16 per cent). The results are summarized in Table 3.

TABLE 3

Yield and efficiency of light energy conversion of indoor mass cultures (300 l. 1 m^2) of *Chlorella*, strain A, at constant illumination and temperature. Continuous illumination with 8 'daylight' fluorescent tubes (series A and B) or four 1000 Watt incandescent lamps (series C). Series B and C consist of parallel cultures. Inoculum: about 4 g/m^2 irradiated area. Duration of experiments 3–6 days. Maximal temperature deviation: 3 °C. (See also text).

Series	No. of expts.	Radiation, kcal/m^2.12 hrs (λ <700 mμ)	Temp., °C	Yield, g/m^2.12 hrs (ash-free)	Efficiency, per cent
A	9	184	24	3.9 ± 0.3	13.3 ± 1.1
B	3	127	32	2.6 ± 0.3	12.7 ± 2.0
C	3	1125	32	9.7 ± 0.4	5.5 ± 0.2

The results given in this Table show that the maximum efficiency of light energy conversion (occurring in the light limiting range) in this type of cultures is about 13 per cent. This efficiency is observed at low light intensities *viz.*, 184 kcal/m^2.12 hrs or 0.026 cal/cm^2.min (series A), and 127 kcal/m^2.12 hrs or 0.018 cal/cm^2.min (series B). This efficiency value is considerably lower than the maximum efficiency found with the same strain of algae in small scale experiments (*cf.* KOK [35], and Chapter V). Thus, optimal conditions for growth have not yet been achieved in the large tanks. This may also be the reason for the relatively large variation in the efficiency values (*cf.* the statistical error in Table 3), notwithstanding the experimental conditions in these cultures were kept constant as well as possible.

Table 3 also shows the influence of light intensity upon the yield and efficiency values. The results appear to be similar to those found in outdoor cultures; a higher intensity causing a higher yield, but a lower efficiency of light energy conversion. The cells have a chemical composition similar to that of outdoor cultures, with a high protein content (~75%), a low carbohydrate content, and a low lipid content.

A comparison between the absolute values of the yield and of the efficiency in series C (Table 3), and those of outdoor cultures under similar conditions (*i.e.*, Table 1 and fig. 2; 84 per cent solar radiation) shows, that nearly the same results are obtained. Under artificial light these values are somewhat higher, which may be due to the more favourable temperatures, and to the regular spreading of the energy over the exposition time as compared with that in outdoor cultures. Moreover, it should not be forgotten that, in doing so, effects of discontinuous illumination are compared with those of a continuous one. Some authors (26, 47, 83) have found that discontinuous illumination is more favourable. Regarding this question we do not have many data available. Some observations, however, indicate that at low light intensities (as, *e.g.*, in series B, Table 3), no significant differences between continuous and discontinuous illumination (12 hrs light, 12 hrs dark) exist (see also Table 10). Under these conditions a difference could hardly be expected; at high light intensities, however, such a difference would seem feasible.

4. CONCLUSIONS

1) The maximum efficiency of light energy conversion in *Chlorella*, strain A, in large cultures (1 m^2 area, 30 cm depth), is about 13 per cent (average of 12 experiments).

2) Under natural conditions of light and temperature, from April to November, efficiency values ranging from 1 to 5 per cent were observed. The yield values varied between 1 and 13 g/m^2.day (ash-free dry weights).

3) Increase of light intensity, up to high intensities, results in lower efficiencies, but leads to higher yields, both under natural conditions, and at constant light and temperature.

4) Increase of temperature, up to 30°C, causes only slightly higher efficiency values in outdoor cultures.

CHAPTER IV

THE YIELD AND EFFICIENCY OF SMALL CULTURES GROWN IN SUNLIGHT

1. INTRODUCTION

During the 1953 summer various strains were cultivated in small scale culture devices. (See Plate 2). The results which were obtained with these strains and types of cultures have been reported recently by KOK and VAN OORSCHOT (38). The cultivation of the cells (*cf.* p. 231, par. 3) before use in the outdoor experiments was entirely different from that used earlier, because we aimed at specially adapting the cells so as to obtain a higher photosynthetic capacity. Although some adaptation, especially to temperature was observed (see Chapter V, 4), permanent alterations upon exposure of the cells to high light intensities, and to different temperatures did not appear to occur (38). Strains pre-cultivated in the way outlined p. 231 were grown outdoors, either in 'Kolle'dishes or in tubes (cultures of 260 and 60 ml, *cf.* p. 233), while, additionally, some strains taken directly from the light cabinet (p. 231), were cultivated in the same way.

Scenedesmus 3, and *Chlorella*, Tx1105, grew equally well if they were taken from the light cabinet or from cultures exposed to high light intensity. Other strains with high yields (*Chlorella* 14–10, *Chlorella* 7, and *Chlorella* 19) did not show much differences in growth either. In laboratory experiments at 30 °C significant differences in growth between some strains were not observed, whereas at 20 °C marked differences were found (38). On the other hand, *Chlorella vulgaris viridis* appeared to grow preferably at somewhat lower temperatures, both under constant conditions and outdoors. Unfortunately, *Chlorella*, strain A, used in mass-culture experiments before, could not be compared, which was due to the fact that cultures of this strain continually settled in outdoor experiments. Laboratory experiments (*cf.* p. 250), however, indicated that no differences in capacity of light energy conversion existed between *Chlorella*, strain A, and 'good' strains grown outdoors in 1953.

The lower depths of the cultures as compared with the large cultures (Chapter III), and the fact that the cultures were kept in vessels, having only a relatively small opening, caused much higher and faster responses of temperature to illumination (within about half an hour) than in the large cultures with a depth of 30 cm. Differences of 20 °C between the temperature of the cultures and that of the surrounding air were found sometimes, and temperatures up to 50 °C were observed in the culture medium. The yield (as g/m².day) was calculated by taking half of the area of the unilaterally illuminated tubes and dishes as irradiated surface instead of the area of the longitudinal cross-section. The fixed energy was calculated as 5.8 × g total dry weight (*cf.* [35]), corresponding to 0.14 × 45 × g ash-free dry weight and an ash percentage of 10 (*cf.* p. 233). Working with dense cultures, all the incident light (recorded at the same place with the set up described on p. 232) was absorbed.

2. RESULTS AND THEIR DISCUSSION

Although extensive results have been given recently, some of them be mentioned here in order to compare them with those of large cultures (Chapter III). For the reason mentioned in section 1, we will restrict ourselves to those obtained with one special strain, *Scenedesmus* 3. In fig. 3 some data connected with the growth of this strain are given as weekly averages from July to October. In the top section, radiation, maximum temperature, and average temperature during the day are given. A close connection between temperature and radiation exists. The yield and efficiency of light energy conversion are given in the bottom section.

During July and the first part of August high yield values of 20 g/m².day, and more, corresponding to efficiencies of about 8 per cent, were found. The yield appeared to be roughly correlated with the incident energy, while the efficiency of light energy conversion was fairly constant. The more or less linear relation between daily yield and incident energy, up to high values (implicating a fairly constant efficiency), obtained during July and August (38), may be due to the close connection between incident energy and temperature in the vessels. Later in the season, both the yield and the efficiency declined (4 g/m².day, and 4 per cent in October) with decreasing radiation and temperature.

TAMIYA (86) reported high yields of about the same order of magnitude with some temperature resistent strains (see also [79]), cultivated during the summer in flat bottles or in a special type of open trenches (see also [28]).

The decline of both yield and efficiency later in the season was also observed in large cultures (*cf.* fig. 1). At constant temperature, however, a decrease in irradiation resulted in lower yields, but higher efficiency values (*cf.* fig. 2, and Table 1). Because also in the experiments represented in fig. 3 the temperature

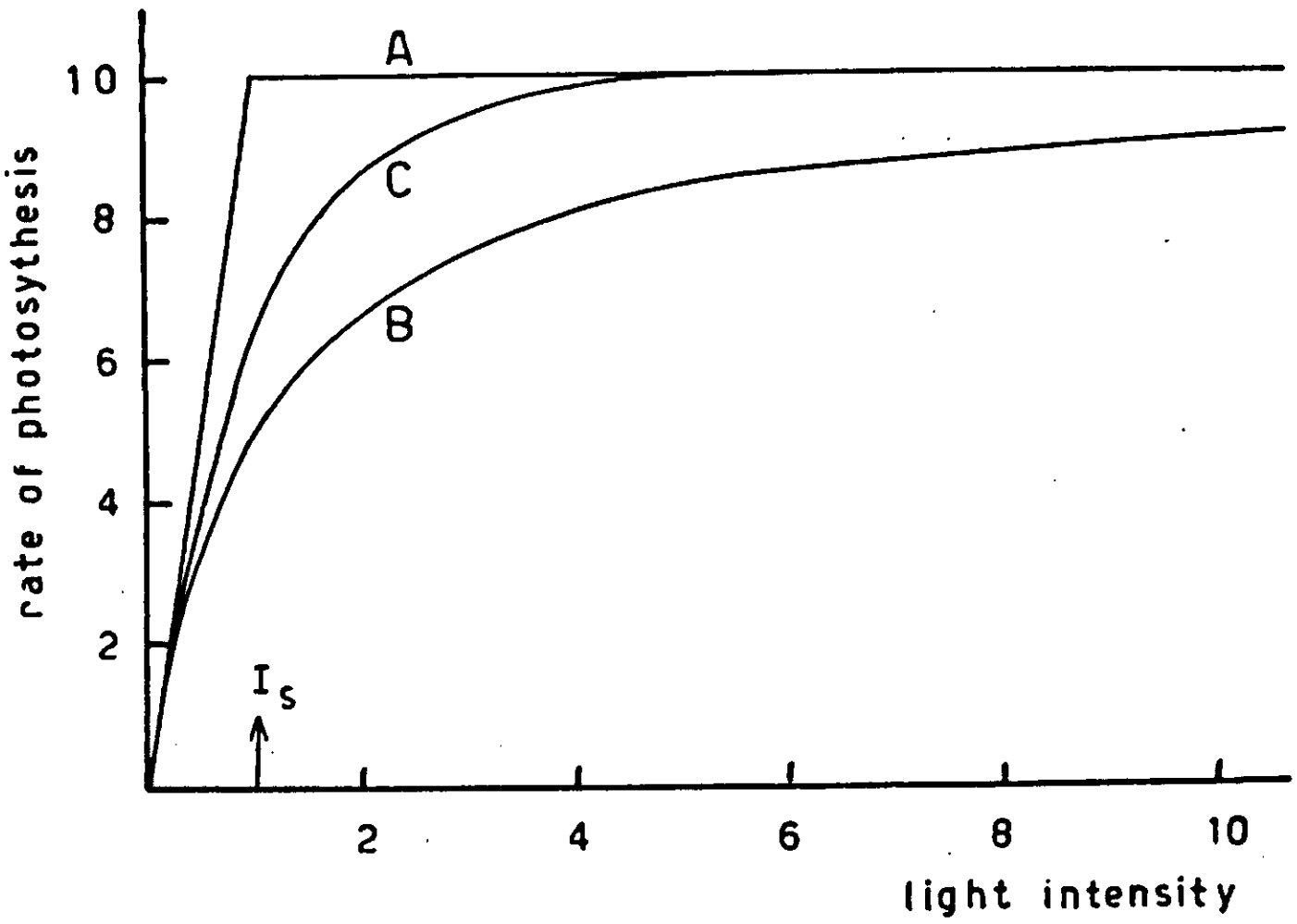

FIG. 4. Curves in which different functions for the relation between the rate of photosynthesis and light intensity are used. Curve A: $f(I) = E_m I$ and $f(I) = R_m$; curve B: $f(I) = \frac{R_m E_m I}{R_m + E_m I}$; curve C: $f(I) = R_m\left(1 - e^{-\frac{I}{I_s}}\right)$. For meaning of symbols, see text of section 2.

tensity is given bij I_o, the concentration of the light absorber by c, and the extinction coefficient by ε, the light intensity at a distance x from the irradiated surface will be:

$$I = I_o e^{-\varepsilon c x} . \tag{1}$$

The rate of increase of cell material (R) at any depth is proportional to the concentration c of the light energy absorber, and proportional to the rate of photosynthesis per unit light absorber. If $f(I)$ is the rate of photosynthesis, then in suitable units we have at depth x:

$$R(x) = cf(I) = cf(I_o e^{-\varepsilon c x}) . \tag{2}$$

Integrating over all depths, we find the total rate of increase, T (or yield per unit area and time):

$$T = \int_o^d R(x)dx = \int_o^d cf(I_o e^{-\varepsilon c x})dx . \tag{3}$$

For comparison we will consider the three different functions $f(I)$, as given in fig. 4.

Curve A. The rate of photosynthesis is proportional to the light intensity until a maximum rate is attained at a certain light intensity (I_s). So we have:

$$f(I) = R_m, \text{ if } I \geqslant I_s \text{ and}$$
$$f(I) = E_m I, \text{ if } I \leqslant I_s,$$

where R_m is the maximum rate of photosynthesis and constant for $I \geqslant I_s$, and E_m is $df(I)/dI$, being the maximum efficiency of light energy conversion, which is constant for $I \leqslant I_s$. In order to calculate the total yield of the culture, fig. 5 is given. At the distance s from the surface, the light intensity is decreased to I_s. The total rate of increase, T will be (3):

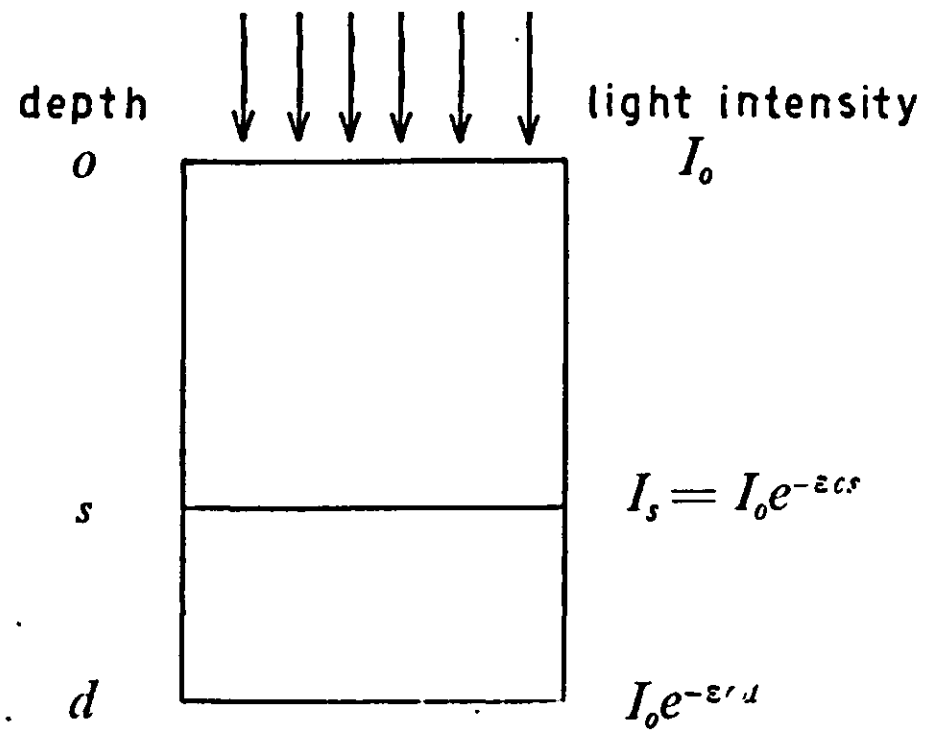

FIG. 5. Light intensities in a culture of algae, assuming the validity of curve A (fig. 4). For meaning of symbols, see text, p. 244.

$$T = \int_o^d cf(I_o e^{-\varepsilon c x})\, dx$$

$$= \int_o^s cR_m dx + \int_s^d cE_m I_o e^{-\varepsilon c x} dx$$

$$= cR_m s + cE_m I_o (\varepsilon c)^{-1}[-e^{-\varepsilon c d} + e^{-\varepsilon c s}].$$

Since $R_m = E_m I_s$ and $e^{-\varepsilon c d}$ is equal to $1 - \alpha$, in which α is the fraction of the light absorbed by the culture, we get, with s obeying $I_s = I_o e^{-\varepsilon c s}$:

$$T = cE_m I_s (\varepsilon c)^{-1} \ln \frac{I_o}{I_s} + cE_m I_o (\varepsilon c)^{-1} \left[\frac{I_s}{I_o} - (1-\alpha)\right]$$

$$= \frac{1}{\varepsilon} E_m \left\{I_s (\ln \frac{I_o}{I_s} + 1) - (1-\alpha) I_o\right\}. \qquad (4)$$

BUSH' formula (see [10]) is of a similar nature, ε and α being eliminated, while T has been related to efficiency of light energy conversion.

Curve B. The relation between the rate of photosynthesis and light intensity is described as a rectangular hyperbolic function. So:

$$f(I) = \frac{E_m R_m I}{R_m + E_m I},$$

in which R_m is the value of $f(I)$ at large values of I, and E_m is the value of $df(I)/dI$ at low values of I. From this function of $f(I)$ the total yield per unit area and time has been calculated by TAMIYA *et al.* (84). A formula of the following nature was obtained:

$$T = \frac{1}{\varepsilon} R_m \ln \frac{R_m + E_m I_o}{R_m + E_m I_o e^{-\varepsilon c d}}.$$

Bij transferring $R_m = E_m I_s$ (I_s being the intensity at which $f(I) = R_m$ and $f(I) = E_m I$ intersect), and $e^{-\varepsilon c d} = 1 - \alpha$, we get:

$$T = \frac{1}{\varepsilon} E_m I_s \ln \frac{I_s + I_o}{I_s + I_o(1-\alpha)}. \qquad (5)$$

Curve C. The relation between the rate of photosynthesis and light intensity is represented by an exponential function. So:

$$f(I) = R_m \left(1-e^{-\frac{I}{I_s}}\right).$$

Then the total production per unit area and time (3) becomes:

$$T = cR_m \int_o^d \left(1-e^{-\frac{I_o}{I_s}e^{-\varepsilon cx}}\right) dx$$

$$= cR_m d - cR_m \int_o^d e^{-\frac{I_o}{I_s}e^{-\varepsilon cx}} dx. \qquad (6)$$

By substituting $-\frac{I_o}{I_s} e^{-\varepsilon cx} = t$, and $\frac{I_o}{I_s} \varepsilon c e^{-\varepsilon cx} dx = dt$, so that $dx = \frac{dt}{-\varepsilon ct}$, we may write:

$$\int_o^d e^{-\frac{I_o}{I_s}e^{-\varepsilon cx}} dx = \frac{1}{\varepsilon c} \int_{-\frac{I_o}{I_s}}^{-\frac{I_o}{I_s}e^{-\varepsilon cd}} \frac{e^t}{-t} dt,$$

which is equal to the following Ei functions:

$$\frac{1}{\varepsilon c}\left\{-\mathrm{Ei}\left(-\frac{I_o}{I_s}e^{-\varepsilon cd}\right) + \mathrm{Ei}\left(-\frac{I_o}{I_s}\right)\right\}.$$

Thus we find for (6):

$$T = cdR_m - \frac{1}{\varepsilon} R_m \left\{\mathrm{Ei}\left(-\frac{I_o}{I_s}\right) - \mathrm{Ei}\left(-\frac{I_o}{I_s}e^{-\varepsilon cd}\right)\right\}.$$

Substituting $E_m I_s = R_m$ and $1-\alpha = e^{-\varepsilon cd}$, so $cd = \frac{1}{\varepsilon} \ln (1-\alpha)^{-1}$, we get:

$$T = \frac{1}{\varepsilon} E_m I_s \left[\ln (1-\alpha)^{-1} - \mathrm{Ei}\left(-\frac{I_o}{I_s}\right) + \mathrm{Ei}\left\{-\frac{I_o}{I_s}(1-\alpha)\right\}\right] \qquad (7)$$

The values of the Ei functions are given in 'Tables of functions' (E. JAHNKE and F. EMDE, 4th edition, Dover Publications 1945, New York), p. 1.

In order to calculate the yield per unit area and time from the equations (4), (5) and (7) for certain values of the incident light intensity I_o, the values of ε, α, E_m and I_s must be known. The extinction coefficient ε in these formulae may be omitted by putting $x' = x/\varepsilon$ (and $d' = d/\varepsilon$). In most cases the value of α approaches unity, because dense suspensions, implicating a linear growth phase, are used. The maximum efficiency, E_m, may be determined by culturing cells at low light intensities. The value of I_s (*cf.* fig. 4), which depends upon temperature, has to be determined in photosynthesis experiments. Carrying out some preliminary determinations, effects of light intensity and duration of previous growth upon I_s were found (*cf.* section 3).

In fig. 6 calculated yield curves in dependence of incident light intensity are given for equations (4), (5) and (7). By putting $I_s = 0.05$ cal/cm².min, $E_m = 0.20$

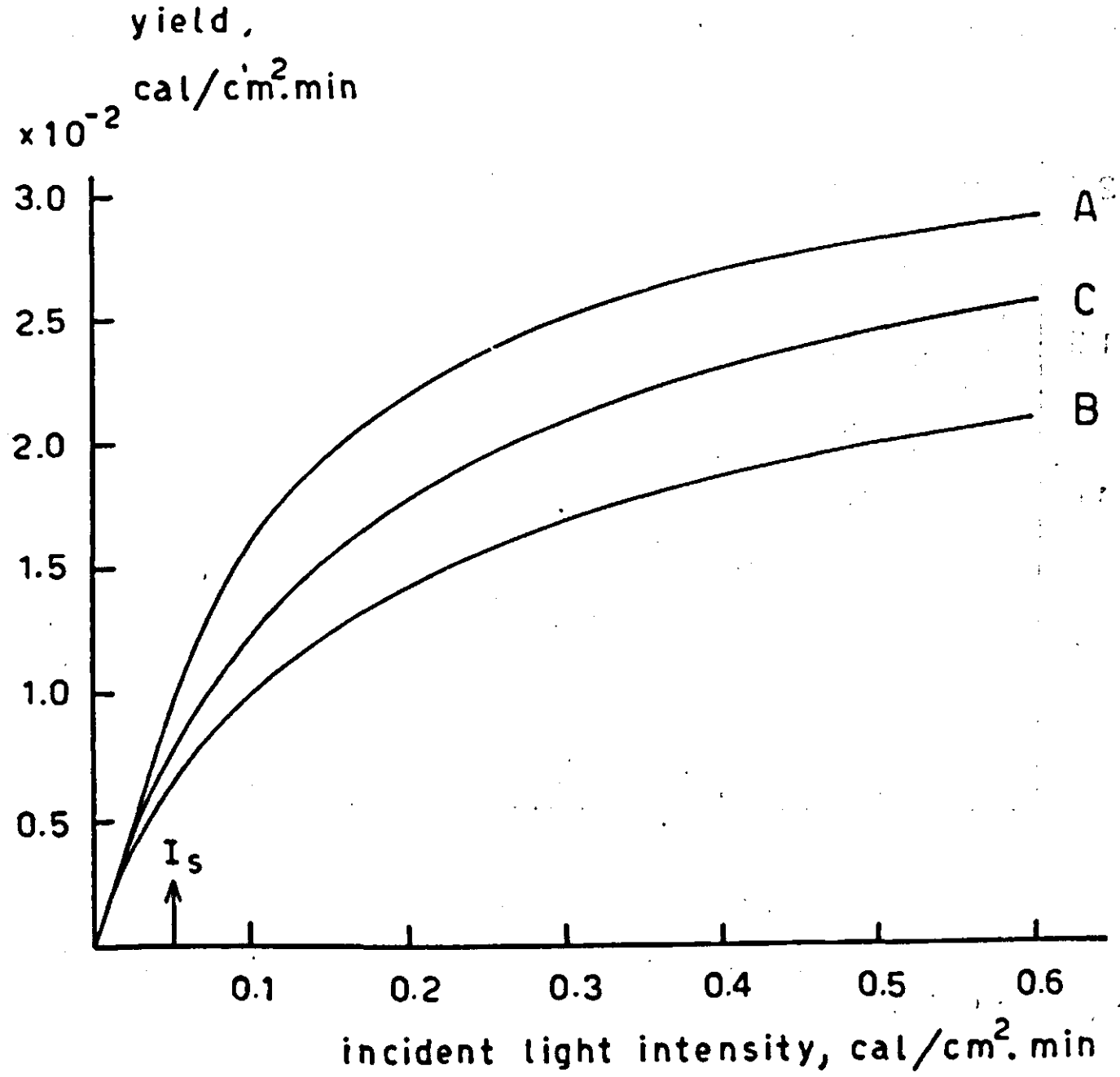

FIG. 6. Calculated curves for the relation between yield and incident light intensity. Curves A, B and C are based on the same assumptions as curves A, B, and C respectively, of fig. 4, and correspond to equations (4), (5) and (7) respectively (see text), assuming I_s = 0.05 cal/cm². min, E_m = 0.20 and α = 0.95.

and $\alpha = 0.95$, the yield has been calculated in cal/cm².min. Curves A, B and C correspond to equation (4), (5) and (7) respectively. Although different yield curves are obtained, each of them shows that the yield gradually increases with the incident light intensity, even up to $I_o = 0.6$ cal/cm².min (or $I_o = 12\ I_s$). The highest yield values are found with curve A (equation [4]), derived from the simplified photosynthesis curve represented under A in fig. 4. The yield values of curve B (equation [5]), which are based upon a rectangular hyperbolic relation between the rate of photosynthesis and light intensity (fig. 4, curve B), are lowest. Intermediate values (curve C, equation [7]) are found, if the rate of photosynthesis is considered to be an exponential function of light intensity (*cf.* fig. 4, curve C). Curve A attains light saturation at $I_o = I_s/(1-\alpha)$, thus at lower light intensities in as much as the absorption is smaller (*e.g.*, at $I_o = 1.0$ cal/cm².min with $\alpha = 0.95$, and at $I_o = 0.25$ cal/cm².min with $\alpha = 0.80$). Curves B and C will approach to the same maximum value as curve A, although only at much higher incident light intensities. Besides α, the value of I_s, which varies with temperature, strongly determines the yield value. A certain increase of I_s, however, causes a relatively smaller increase of the yield.

Efficiency values may be obtained from the yield values by dividing them by α and I_o. The efficiency curves of fig. 7 are obtained from the corresponding yield curves of fig. 6. Curve A would correspond to BUSH' formula (see [10]) if α would be taken unity. The curves show that, with a high fractional absorption, relatively high efficiency values may be expected at high incident light intensities

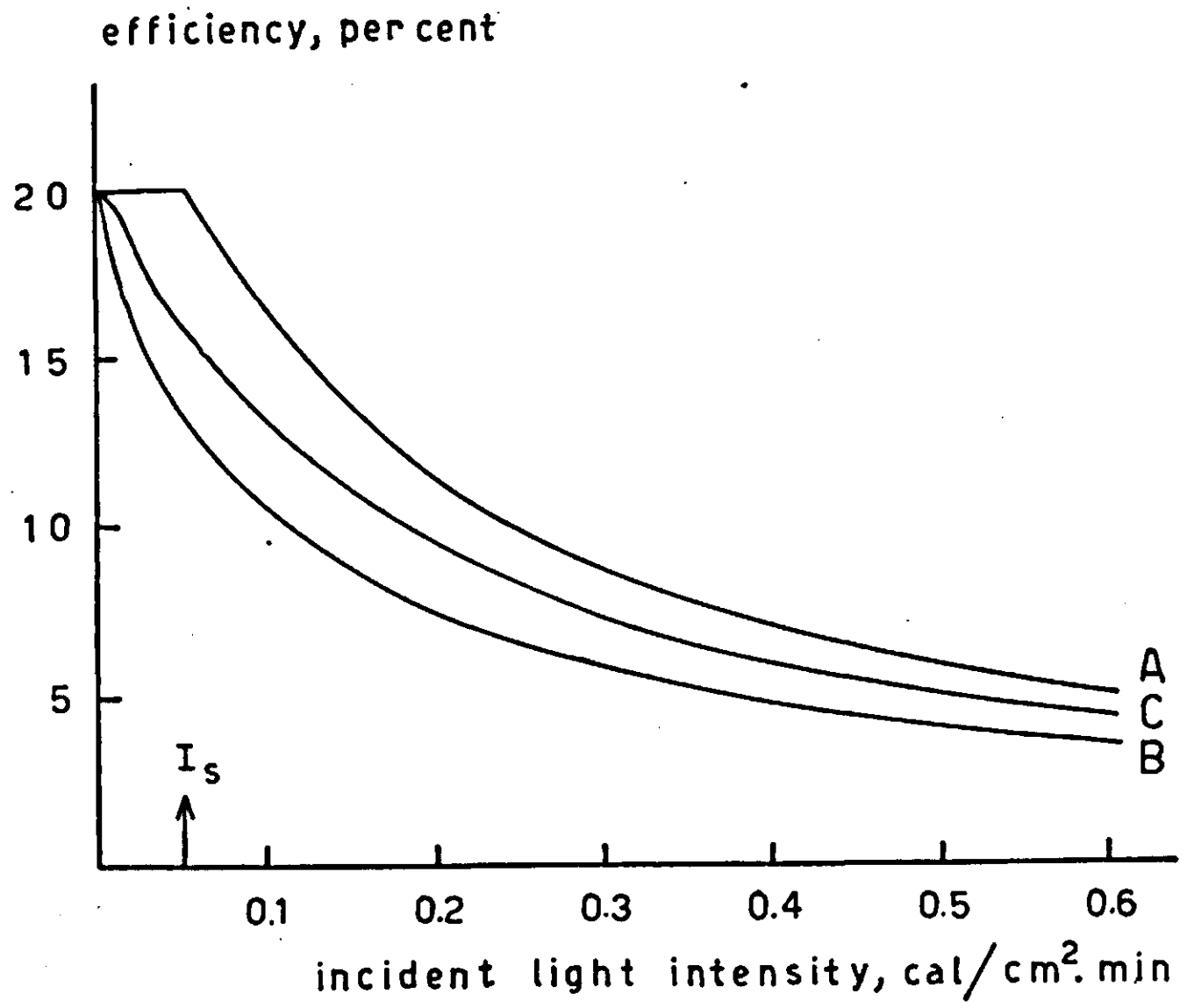

FIG. 7. Curves representing the efficiency of light energy conversion, calculated from the yield curves of fig. 6.

(*e.g.*, at $I_o = 10\ I_s$: 5.9% [A], 4.2% [B], and 5.1% [C], accepting $E_m = 0.20$ and $\alpha = 0.95$).

If we would not have taken into consideration the mutual shading of cells, supposing that all cells receive the incident light intensity, much lower values would have been expected (*e.g.*, at $I_o = 10\ I_s$: 2%).

In growing cultures α will increase with time. The calculated curves, however, represent yield and efficiency values at a certain moment. With practically complete light absorption, more or less stationary conditions will obtain, unless too long culture periods are introduced. Then an increasing part towards the bottom of the culture will be darkened, and respiration losses decrease the overall yield and efficiency.

3. EXPERIMENTS ON LIGHT SATURATION OF GROWTH AND LIGHT ENERGY CONVERSION AS AFFECTED BY LIGHT INTENSITY AND TEMPERATURE

In this section experiments on effects of light intensity and temperature upon growth are described. The yield values have been related to light energy conversion by including measurements of the absorption of the light during growth. In experiments in which sodium lamps were used, the absorption could easily be followed during growth (*cf.* p. 233). High light intensities could not be attained with these lamps, nor with any other monochromatic light sources which would permit a convenient measurement of the light absorption. High light intensities of white light (up to those of solar radiation) were attained with Philips Attralux lamps (*cf.* p. 233). Dense suspensions with about complete absorption of the incident light were then used. The latter cultures resemble those under outdoors conditions, which also nearly completely absorbed the incident light. In order to

exclude adaptation phenomena (*cf.* section 4) cells were pre-cultivated at the same temperature as that used during the experiments.

In Table 4 the effect of light intensity upon the efficiency of light energy conversion in *Chlorella*, strain A, grown in sodium light is given. The nitrogen content of the medium, affecting the efficiency (see Chapter VI), was checked several times. Always sufficient nitrogen was present, which follows also from Table 7.

TABLE 4

Efficiency of light energy conversion of *Chlorella*, strain A, grown in sodium light as dependent on light intensity. Data from 6 experiments. Cultures of 100 ml in WARBURG vessels (*cf.* p. 233), during 48 hrs at 25 °C and indicated intensities. Inoculated with 1.5 μl of cells (pre-cultivated during 3 days under fluorescent tubes, *cf.* p. 231) per cm² irradiated area (fractional absorption ~0.30). Culture medium with 20 × 10^{-3}m KNO_3,) otherwise the same as that of pre-cultivation (*cf.* p. 231). Experiments of August 1952.

Intensity, cal/cm².min	0.018	0.054	0.069	0.083
Efficiency, per cent	22.8 ± 0.7	19.9 ± 0.6	17.0 ± 0.6	15.9 ± 0.6

A maximum efficiency of about 23 per cent is found. This is in agreement with the results of KOK (35), who found maximum values of 24 per cent. The efficiency decreases with increasing light intensity. A decrease in quantum efficiency, as measured in photosynthesis experiments of short duration with the same cultures, was observed at light intensities higher than 0.05 cal/cm².min.

In experiments in white light and practically complete light absorption, *Chlorella*, strain 14–10 was used. Averages of experiments at some temperatures are given in Table 5, while fig. 8 shows results of typical experiments. All cultures were inoculated with an amount of cells equivalent to a definite amount of chlorophyll, yielding about complete light absorption. As is shown in Table 5, the chlorophyll content of the inoculum depends upon the temperature, at which the algae had been pre-cultured. Lowest contents were observed at 20°C, whereas at 40°C somewhat lower contents than at 30°C occurred.

TABLE 5

Yield and efficiency of light energy conversion of *Chlorella*, strain 14–10 at a limiting and a saturating light intensity, and at different temperatures. Cultures of 30 ml (28.3 cm² [*cf.* p. 233]), inoculated with an amount of cells, equivalent to 2 mg chlorophyll. Cells previously (during about 1 month) grown at the experimental temperatures and at 0.25 cal/cm².min (*cf.* p. 231). Expts. of January 1954.

Temp., °C	No. of expts.	Inoculum		Incident intensity, cal/cm².min.			
				0.05 (limiting)		0.50 (saturating)	
		Mg of cells	Chlorophyll content, %	Yield, mg cells per 10 hrs	Efficiency, %	Yield, mg cells per 10 hrs	Efficiency, %
20	6	73 ± 11	2.7 ± 0.1	28 ± 1	18.9	60 ± 1	4.1
30	7	51 ± 5	3.9 ± 0.3	30 ± 2	20.4	110 ± 4	7.6
40	3	55 ± 6	3.6 ± 0.4	27 ± 1	18.3	107 ± 6	7.3

At a light intensity of 0.05 cal/cm².min no differences in yield and efficiency between 20°, 30° and 40°C were observed. The maximum efficiency is of the same order as that in cultures of *Chlorella*, strain A, at 25°C (*cf.* Table 4). The values are, however, somewhat lower, which may be due to appreciable respi-

ration losses at high cell densities (*cf.* p. 248). At saturating light intensities lower efficiencies were observed, and an increase of about 85 per cent in yield and efficiency of light energy conversion is found with an increase of the temperature from 20 °C till 30 °C. No differences in yield and efficiency between 30° and 40 °C are observed, in agreement with earlier results (38). Other results possibly may be expected with high temperature strains (79, 86).

Curves of yield versus the intensity of incident light obtained with *Chlorella*, strain 14–10 in three typical experiments at 20°, 30° and 40°C are shown in fig. 8A. At low light intensitiès a linear, temperature independent yield curve is found. At 20 °C light saturation occurred at a lower intensity than at 30° and 40 °C. At the latter temperatures saturation hardly is attained at 0.6 cal/cm².min. The increase in growth up to high intensities is in agreement with the results reported in Chapter III, and with the calculations given in section 2 of this Chapter. The equality of the yield curves at 30° and 40 °C is quite remarkable. In some other growth experiments, light intensity series were carried out at 20°, 25°, 30°, 35°, 40° and 45 °C. The yield curve at 25 °C is intermediate between those at 20° and 30 °C. No significant differences are found between the curves at 30°, 35° and 40 °C, while at 45 °C lower yield values are observed.

In fig. 8B the efficiency of light energy conversion is given. The more or less horizontal part of the curves represents the linear range, which is extended to higher light intensities at 30° and 40 °C. With increasing light intensities the relative differences in efficiency between 30° and 40 °C on the one hand, and 20 °C on the other hand are increased, so that, *e.g.*, at 0.5 cal/cm².min, at 20 °C only 55 per cent of the efficiency at 30° and 40 °C is found. A comparison of the results given in fig. 8B with those of Table 4 is not exactly possible, because different temperatures and different light sources were used. Moreover, absorption was different. The maximum efficiency as well as the linear range of *Chlorella*, strain 14–10 and *Chlorella*, strain A, however, seem to be of the same order. So, similar light curves could be expected (*cf.* fig. 6). In experiments, in which the cells were grown during 24 hours at 30 °C, and under various light intensities, indeed similar curves were found.

In fig. 8C the increase of the total amount of chlorophyll in the cultures is given in dependence of the incident light intensity. At low intensities the same linear range (extended to 0.05 cal/cm².min) at 20°, 30° and 40 °C is found. At 20 °C the increase of chlorophyll attains its maximum value at about 0.05 cal/cm².min, whereas at still higher light intensities less chlorophyll was found. With higher temperatures higher values are attained at higher light intensities. At 30 °C somewhat higher values than at 40 °C are found. These results are in accordance with those of the chlorophyll content given in Table 5.

With thin suspensions, TAMIYA *et al.* (84) also observed fading at high light intensities, and at low temperatures. This fading was accompanied by inhibition of growth; with dense suspensions, the same phenomenon was found (76). This is in accordance with the lower rates of photosynthesis found with cells containing less chlorophyll, reported earlier by some authors (16, 21).

In order to obtain information about the photosynthetic capacity of cells from cultures with high cell density as those described above, photosynthesis of such cells has been measured. After some growth periods at two distinctly different light intensities, chlorophyll and dry weight determinations of the cells were carried out. An amount of cells equivalent to a certain amount of chlorophyll then was suspended in a carbonate bicarbonate buffer of pH 8.7 (2% carbon

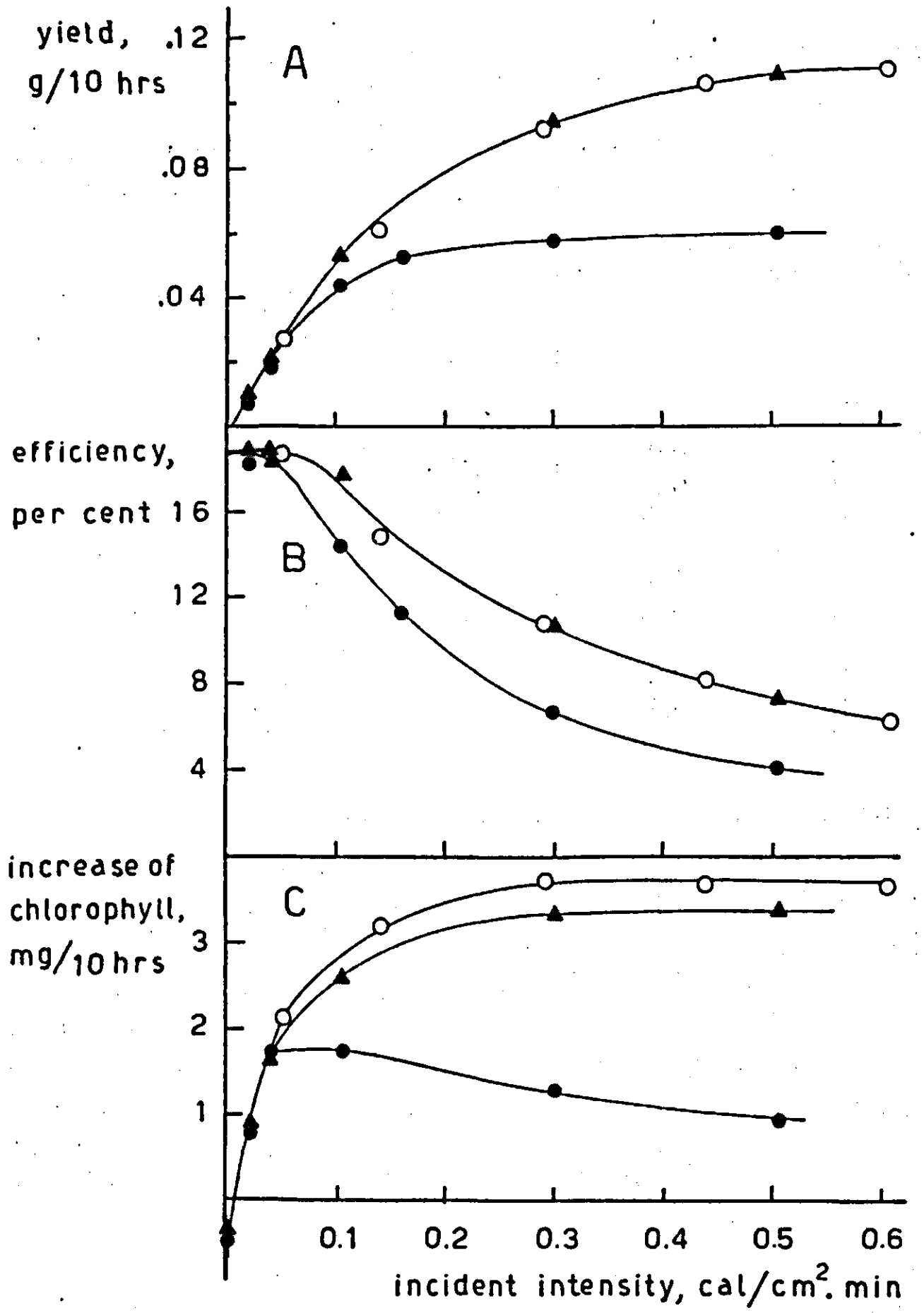

FIG. 8. Some characteristics of growing cultures of *Chlorella*, strain 14–10, at various light intensities, and at 20 °C (●——●), 30 °C (○——○) and 40 °C (▲——▲). A) Yield in g dry weight per 10 hours; B) efficiency of light energy conversion in per cent, C) increase in chlorophyll in mg per 10 hours. Cells for each experiment pre-cultivated at the experimental temperature; cultures (30 ml, 28.3 cm^2) inoculated with an amount of cells containing 2 mg of chlorophyll. Expts. of January 1954.

dioxide), in WARBURG vessels. Oxygen evolution has been measured in red light obtained from incandescent lamps (Philips Attralux) by the filter RG2 (Schott and Gen.). The quantum efficiency was determined in the light limiting range, including light absorption measurements. At these low values of light absorption (17 %) the saturation intensity can be obtained from the rates of oxygen evolution at low light intensities, and at high ones (intersection point of $f(I) = R_m$ and $f(I) = E_m I$, *cf.* fig. 4). The saturation level has been related to μg of chlorophyll.

Some preliminary results are given in Table 6. During growth at high light intensities the chlorophyll content decreased to low values, and the more so when the growth period is extended. Especially at 20 °C, the chlorophyll content reaches low values (see also Table 5). The quantum efficiencies (determined in

TABLE 6

Influence of conditions of previous growth upon chlorophyll content, and some photosynthetic characteristics of *Chlorella*, strain 14–10. Dense cultures as indicated in Table 5. Photosynthesis measured in suspensions of 17% absorption of red light (incandescent light filtered through RG2 [Schott and Gen.]), at the same temperature as that of growth. The saturation intensity is determined by intersecting $f(I) = R_m$ and $f(I) = E_m I$ (*cf.* fig. 4). Expts. of January 1954.

Conditions of growth			Chlorophyll content, %	Max. quantum efficiency, mol O_2/hν	Saturation intensity, cal/cm².min	Saturation rate, μlO_2/min, per μg chlorophyll
Temp., °C	Duration in hrs	Intensity, cal/cm².min				
20	12	0.046	3.71	0.154	0.023	0.076
	10	0.56	1.63	0.099	0.051	0.107
	17	0.56	1.35	0.063	0.091	0.121
30	12	0.046	5.71	0.123	0.032	0.084
	10	0.56	2.70	0.102	0.047	0.101
40	12	0.046	4.20	0.140	0.037	0.110
	10	0.56	3.15	0.121	0.050	0.128
	17	0.56	3.12	0.077	0.095	0.154

the light limiting range) of cells from different pre-cultures show remarkable differences. At all temperatures, cells grown at high light intensities have lower quantum efficiencies than those grown at low ones. Extension of the growth period at 20°C and 40°C causes still lower efficiencies. The energy transfer system of such cells, involving the primary photochemical reactions of photosynthesis, seems to be damaged by prolonged exposure to high light intensities. The simultaneous decrease of the chlorophyll content may be connected herewith. The saturation intensity is increased by culturing at high light intensities, involving lower chlorophyll contents. In spite of the lower quantum efficiencies of the cells grown at high light intensities, the saturation rate in fact is highest with cells exposed to high light intensities. If the saturation rate is related to mg of cells (instead of chlorophyll) the reverse is found. These values may be calculated by multiplying the saturation rate per μg chlorophyll with 10 times the chlorophyll content in per cent. The values are of the same order of magnitude as those obtained by KOK (36).

The results given in fig. 8 have shown that at high light intensities, and at 20°C, the yields were lower than those which might be expected from theoretical considerations (*cf.* section 2). Exposure to such light intensities was accompanied by a marked decrease in chlorophyll content. In view of the observations discussed above, we may now accept that the quantum efficiency has been unfavourably affected. In the experiments of fig. 8, which were started with equal amounts of cells, a decrease of the saturation rate per mg of cells may also have affected the results. A full explanation of these effects requires further investigation.

From growth experiments described in this section, the efficiency of light energy conversion has been computed. For that purpose, the energy content of the cell material which is produced under special growth conditions must be known (*cf.* p. 232). In the experiments described earlier in this section, light intensity and temperature were varied. Contradictory results concerning the effect of light intensity upon chemical composition have been reported (1,

53). This may be due to differences in strains and methods used. To elucidate absolute effects of light intensity and temperature upon chemical composition during growth, cultures have to be chemostated (*cf.* [56]). We are, however, merely interested in the energy content of the cells, grown at the different conditions used. In some experiments in sodium light (as those given in Table 4), and in white light (*cf.* Table 5 and fig. 8) the chemical composition, determining the energy content, was estimated from elementary analysis.

TABLE 7

Protein, carbohydrate, and lipid content of cells of *Chlorella*, strain A, grown during 48 hours at 25 °C, and at the indicated light intensities (sodium light). Cultures similar to those given in Table 4 (*cf.* p. 249) Expt. of 8–5–52.

	Inoculum	Light intensity, cal/cm².min			
		0.013	0.040	0.056	0.072
Proteins, %	66	64	67	67	64
Carbohydrates, %	12	13	11	11	15
Lipids, %	22	23	22	22	21

Results of one experiment with *Chlorella*, strain A, grown in sodium light are given in Table 7. The chemical composition is independent of the light intensity under the conditions used. Kok (35), however, reported a decrease of protein content with increasing intensities up to 0.03 cal/cm².min. Using a medium with less nitrogen than our usual one, we observed the same. Obviously, this is connected with nitrogen exhaustion of the medium (*cf.* Chapter VI). Provided sufficient nitrogen was supplied (50×10^{-3} m KNO_3), and not too long culture periods were taken, effects of high light intensities (up to 0.40 cal/cm².min), and different temperatures (20°–40 °C) were neither found with *Chlorella*, strain 14–10 (experiments of December 1953).

4. ADAPTATION PHENOMENA DURING GROWTH

In the preceding section, the yield and the efficiency of energy conversion have been determined under various conditions of light intensity and temperature. In these experiments the same temperatures have been maintained both in the pre-cultures, and in the experimental cultures. As has been reported recently (38), the temperature characteristics of some strains largely depend upon the temperature at which the cells were grown previously. Cells of *Scenedesmus* 3, and *Chlorella*, strain 14–10, derived from cultures which were grown at 20°, 30° and 40 °C and at 0.25 cal/cm².min during 7 months, showed different growth rates. At 20 °C, growth of cells, pre-cultivated at 20 °C was better than that of cells previously grown at 30° and 40 °C. At 30 °C, no differences in growth between cells of the same strain taken from the various pre-cultures were observed, while at 40 °C, cells from cultures previously grown at 20 °C grew slower than cells from pre-cultures at 30° and 40 °C.

Similar adaptation phenomena are given in fig. 9. Cells of *Chlorella*, strain 14–10 were pre-cultivated during 4 months at 20°, 30° and 40 °C and about 0.25 cal/cm².min in cycles of 12 hours, alternated with dark periods of 12 hours and temperatures of 15°, 20° and 25 °C respectively (*cf.* p. 231). From these cells, growth was studied at various light intensities and temperatures in experiments

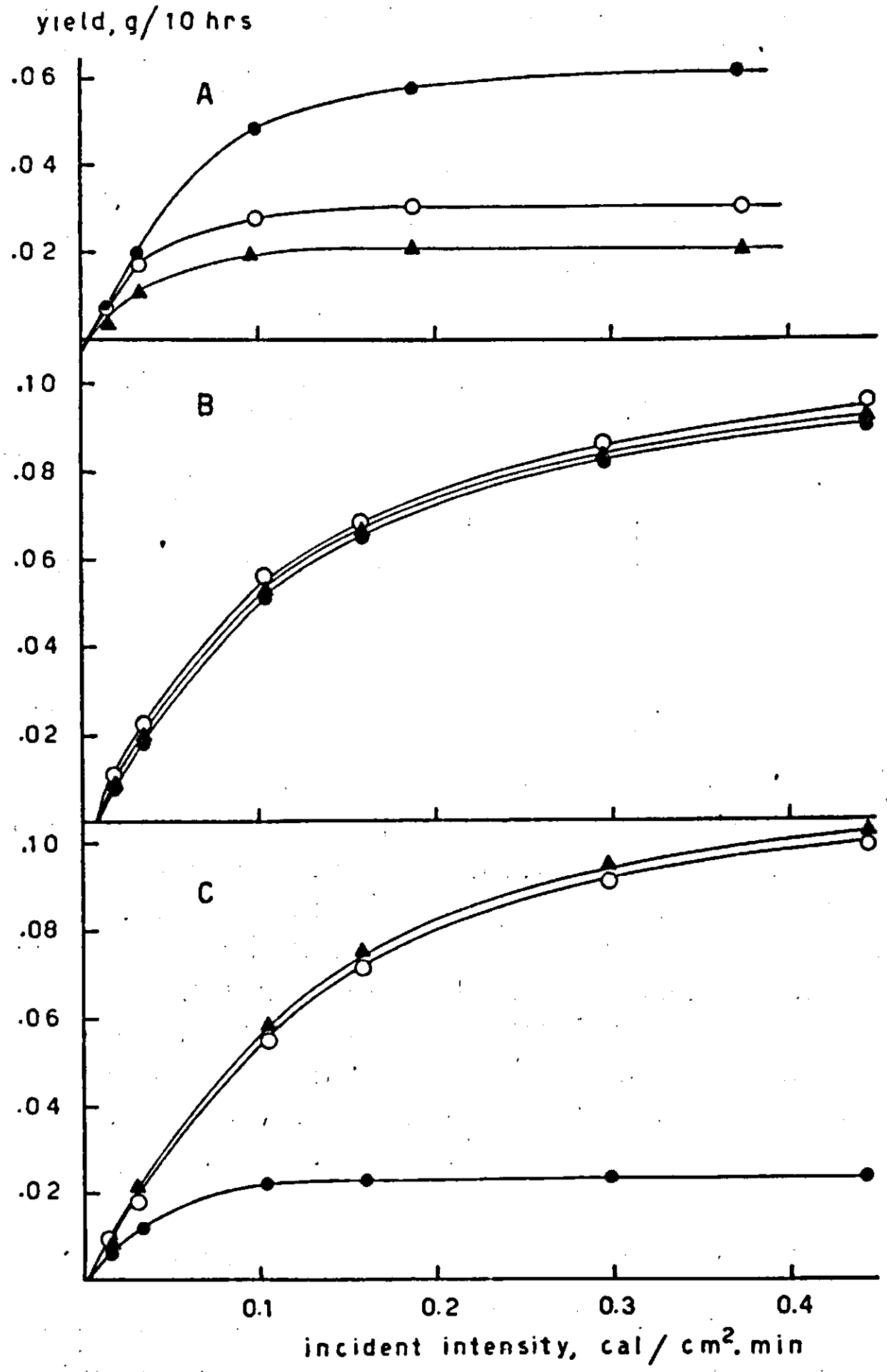

FIG. 9. Growth of *Chlorella*, strain 14–10, during 10 hours at various light intensities, and at temperatures of 20 °C (A), 30 °C (B), and 40 °C (C), as affected by 4 months' pre-cultivation at temperatures of 20 °C (●——●), 30 °C (○——○), and 40 °C ▲——▲). Experiments with cultures of 30 ml (28.3 cm^2); initial density 2 mg of chlorophyll (corresponding to 0.04–0.06 g cells) Expts. of December 1953.

of 10 hours duration, using Erlenmeyer flasks as culture vessels and Philips Attralux lamps as light sources (*cf.* p. 233). The flasks, containing 30 ml nutrient solution (*cf.* p. 233, with 50×10^{-3}m KNO_3) were inoculated with an amount of cells equivalent to 2 mg chlorophyll, yielding practically complete absorption of the incident light. Owing to differences in chlorophyll content after cultivation at different temperatures (*cf.* Table 5), this corresponds to 40–60 mg dry weight of cells.

At 20°C (fig. 9A) a smaller growth rate is found if the temperature of pre-cultivation had been higher. Primarily, the saturation level seemed to be affected (*cf.* pre-cultivation at 30°C), while at larger differences between temperature of pre-cultivation and experiment the growth at low light intensities is also de-

creased seriously. At 30 °C (fig. 9B), the growth curve versus light intensity is no affected by the temperature of pre-cultivation. At 40 °C (fig. 9C), no differences were found between cells from 30° and 40 °C, whereas cells previously grown at 20 °C showed lower rates both at low and at high light intensities. The results at 20 °C cannot be ascribed to differences in chlorophyll content, by which lower contents cause higher saturation rates per amount of chlorophyll (see Table 6). One might expect then higher growth rates of cells from 20 °C at all temperatures. In fact, however, this has not been observed. An explanation of these phenomena requires further investigation.

It has been shown earlier that much shorter periods (of about one week) are sufficient to establish temperature adaptation. Also for the reversal of these effects, short periods are sufficient as is illustrated in Table 8. These cultures were

TABLE 8

Growth rate of cultures of 30 ml of *Scenedesmus* 3, and *Chlorella*, strain 14–10, during 7 days receiving alternately 12 hrs incandescent light of 0.32 cal/cm².min at 40 °C, and 12 hrs darkness at 25 °C, as dependent upon temperature of previous growth during 4 months. Cultures kept at 40–80 mg per 30 ml. Expt. of 11–9–53.

Strain	*Scenedesmus 3*		*Chlorella*, strain 14–10	
Temp. of previous growth	20 °C	40 °C	20 °C	40 °C
Average growth, mg/10 hrs:				
during first 3 days	58 ± 11	110 ± 11	23 ± 8	114 ± 8
during subsequent 4 days .	95 ± 9	109 ± 4	112 ± 16	115 ± 8

grown in the same way as those described above. During the first days similar results as those of fig. 9 were found. After some days of exposure the growth rate of cells from cultures grown at 20 °C increased gradually to that of cells from cultures at 40 °C. This gradual increase of the growth rate is the main reason for the large statistical error of the values given in Table 8.

On the other hand previous adaptation to light intensity did not result in higher yields with *Scenedesmus* 3 and *Chlorella*, strain 14–10, not even after previous exposures of 4 months to a high intensity, as has been reported recently (38). These results were confirmed in further experiments: the curves of the yield versus incident intensity of these strains, previously exposed to high or low intensities, were similar. Manifestations of adaptation as reported in section 3 were not found in the present type of experiments. Probably they do not persist for a considerable time after transfer to new conditions.

5. DISCUSSION

The question, which of the curves of fig. 4, describing the relation between the rate of photosynthesis and the light intensity in suspensions of low cell density, is the best from a theoretical view-point, is still unsolved. A comparison of these curves with those actually found, showed that curves A and B of fig. 4 too much simplify the situation. The yield and efficiency values calculated on the basis of curve A are too high, because actually a transition range between the two parts of the curve occurs. A rectangular hyperbolic function for the rate of photosynthesis in relation to light intensity (curve B, fig. 4) implies that, even at low light intensities, the quantum efficiency decreases with increasing light intensities, and that a light-independent rate of photosynthesis is attained only at

extremely high light intensities. A comparison with experimental curves shows that this function does not apply here. In consequence, the yield values calculated on the basis of this curve (TAMIYA *et al.* [84]) are lower than those actually found. At 25°C, the results of SASA and NIHEI (76) show a similar divergence between experimental yield values, and those calculated on this basis. At 15°C the authors observed a better agreement. This may have been caused by the fact that at this temperature growth is affected unfavourably (*cf.* p. 250). The exponential curve C (fig. 4) seems to be a rather good approximation; the calculated yield values are higher than those calculated from curve B (see fig. 6). However, it fails by deviation from linearity at light intensities lower than actually found. This deviation from linearity is less pronounced than with the hyperbolic function, while a light-independent rate of photosynthesis is attained at lower light intensities. In view of the deviation from linearity of curve C at low light intensities, it seems most likely that actual yield values should be situated between those calculated from curves A and C (*cf.* fig. 4, see also fig. 6).

For the calculation of the yield per unit area and time, the values of the maximum efficiency of light energy conversion (E_m), and the saturation intensity (I_s) must be derived from photosynthesis experiments at certain temperatures. These values were found to depend upon the conditions of previous growth of the cells, such as duration of the growth period, and intensity of illumination (*cf.* Table 6). So, the saturation intensity of photosynthesis and the maximum efficiency, and, therefore, the yield per unit time are likely to change during the growth period. Effects of the light intensity during previous growth upon the rate of photosynthesis have also been found by MYERS (53). Effects of intercalation of dark periods may be expected. The differences in photosynthetic capacity of 'light' and 'dark' cells, found by TAMIYA *et al.* (85) and NIHEI *et al.* (60) may be due to similar phenomena, suggesting photo-inhibition. The saturation intensity strongly depends upon temperature; however, also upon the temperature of previous growth (*cf.* section 4).

In view of these considerations, it is evident that an exact calculation of the yield and efficiency values of growing cultures simply by way of photosynthesis measurements is still impossible. The light intensity curves of the yield found at 30° and 40°C (*cf.* fig. 8A) approximately agree with those calculated (*cf.* fig. 6), showing an increase of the yield up to high light intensities. So, relatively high values for the conversion of light energy were found at high incident light intensities (*cf.* fig. 8B). The calculations of TILSTRA (87) pointed to similar results. The different shape of the curve at 20°C (*cf.* fig. 8A) may have been due to photo-inhibition; the lower chlorophyll content of the cells observed is in agreement with this supposition. The degree of extension of the horizontal parts of the efficiency curves as shown in fig. 8B, and compared with the theoretical ones (*cf.* fig. 7), indicates that the actual curve will be situated between the theoretical curves A and C (*cf.* fig. 7, see also fig. 4).

CHAPTER VI

LIGHT ENERGY CONVERSION AND CELLULAR COMPOSITION AS AFFECTED BY NITROGEN SUPPLY

1. COMPARISON OF AMMONIUM AND NITRATE AS NITROGEN SOURCE

Considerable attention has been given to the comparison of various nitrogen sources in green plants. Because they are less complicated in structure, we will mainly restrict ourselves to the results obtained with algae. Of the various nitrogen sources which have been investigated, inorganic ones seem of primary importance with regard to efficient mass-culturing of algae. Nitrate or ammonium ions are likely to affect the pH of the culture solution during growth, uptake of nitrate being accompanied by an increase in pH, and uptake of ammonium by a decrease. This was used as a criterion in studies, leading to the conclusion that absorption of ammonium is preferent in various *Chlamydomonas* species (43). The same was observed for *Chlorella* and *Scenedesmus acuminatus* by way of nitrogen determination (*e.g.*, [44], [68], [89]). With the tracer technique similar results were obtained with *Chlorella vulgaris* (77). Mixed populations of phytoplankton gave the same results (30, 65). Detrimental effects of ammonium, however, were also found (*Nitzschia* [74], *Scenedesmus quadricauda* [63]). High concentrations of ammonium may inhibit growth (*Spirodela polyrhiza* [8], some algal species [3]). Inhibition of photosynthesis of *Chlorella* at high concentrations of ammonium sulphate was found by GREENFIELD (27).

Before being assimilated into amino acids and proteins, nitrate nitrogen has to be reduced to the ammonium level. Although the pathways of the reduction are only partly known so far, it is evident that assimilation of nitrate consumes more energy than an equivalent amount of ammonium, the difference being the energy required for reduction of nitrate to ammonium. This will result in higher yields with ammonium containing media. Indeed this was observed by GEOGHEGAN (26), who adjusted pH during growth. PRATT and FONG (68), however, found better growth with nitrate. In their experiments, low pH may have reduced the growth in the media containing ammonium, as was observed in ours. TAMIYA *et al.* [84], and DAVIS and DEDRICK (*cf.* DAVIS *et al.* [13], p. 119) compared the growth in media with nitrate or urea (which do not cause fast shifts in pH), and observed higher growth rates with urea. Actual differences in efficiency can only be determined if the absorption of the light is also measured. Otherwise, a higher growth rate may result in higher light absorption, which causes higher yields.

In our experiments on the conversion of light energy with nitrate or ammonium, *Chlorella*, strain A was used. By growth in WARBURG vessels and illumination with sodium lamps (*cf.* p. 233), the production of oxygen and the absorption of light were determined during growth.

With ammonium, a fast decrease of pH with time was observed, so that sometimes the cells were killed. In the following experiment nitrate and ammonium were compared. The culture medium with nitrate was composed of 0.01 m KNO_3, 0.002 m $MgSO_4.7H_2O$ and 0.01 m KH_2PO_4; the composition with ammonium was 0.0036 m $NH_4H_2PO_4$, 0.0032 m $(NH_4)_2HPO_4$, 0.002 m $MgSO_4.7H_2O$, 0.0032 m KH_2PO_4 and 0.0168 m KCl. Both were supplied with iron and micro-nutrients (*cf.* p. 233). The quantum efficiency during growth decreased

TABLE 9

Quantum efficiency and pH of cultures of *Chlorella*, strain A, after various growth periods in ammonium or nitrate media. Sodium light, 0.036 cal/cm².min; temperature, 25 °C. Culture media see p. 257. Initial pH with ammonium: 7.1, with nitrate 4.5. Cell density: 1.5 μl/cm² irradiated area. Expt. of 30–9–52.

Culture medium with	20 hours		43 hours		
	Quantum efficiency	pH	Quantum efficiency	pH	Overall efficiency
ammonium . . .	0.127	6.3	0.073	3.1	19.9%
nitrate	0.120	6.3	0.115	7.0	17.4%

with decreasing pH, as is shown in Table 9. During the first period, practically the same quantum efficiency was found with ammonium and nitrate. After 43 hours, a lower quantum efficiency was observed in the ammonium containing medium, while pH had decreased to 3.1. Obviously, the overall efficiency with ammonium has been affected by the lower quantum efficiency during the latter period. This conclusion is supported by the observation that in similar experiments, in which low pH values were avoided by readjusting pH to the original level once a day, overall efficiencies of 21.8 per cent with ammonium and 18.8 per cent with nitrate were found.

ALGEUS (3) used a culture medium, buffered with 0.0067 m phosphate. We used 0.03 m phosphate and obtained a reasonable buffering capacity without inhibition of growth. In the ammonium containing medium a pH of 6.9, decreasing to 5.6 during growth was obtained, if a 0.03 m phosphate mixture consisting of 39 per cent KH_2PO_4 and 61 per cent $Na_2HPO_4.2H_2O$ was added. In the nitrate medium a similar mixture consisting of 87 per cent KH_2PO_4 and 13 per cent $Na_2HPO_4.2H_2O$ yielded a pH of 5.8, which increased to 6.9 during growth. In both media the same concentration of different elements (except those of Cl) was applied. The composition (in mol/liter) was as follows:

	NH^+_4-medium	NO^-_3-medium
KNO_3	zero	0.0161
$NH_4H_2PO_4$	0.0039	zero
$(NH_4)_2HPO_4$	0.0061	zero
KH_2PO_4	0.0078	0.0261
$Na_2HPO_4.2H_2O$	0.0122	0.0039
$MgSO_4.7H_2O$	0.0020	0.0020
NaCl	zero	0.0166
KCl	0.0344	zero

With these media some growth experiments were performed at two different, relatively low intensities of sodium light. The results of one of these is given in Table 10. At both intensities, and with continuous and discontinuous illumination, similar relative differences in the efficiency of light energy conversion between ammonium and nitrate are found. With ammonium containing media 24 to 32 per cent higher efficiencies were obtained. With ammonium the assimilation quotient (calculated from balance equations, *cf.*, *e.g.*, below) ranged from 0.91 to 0.99, while that obtained with nitrate was lower, *viz.*, 0.77 to 0.79. The protein content was hardly affected by the nitrogen source, while no effects

TABLE 10

Efficiency of light energy conversion with reference to dry matter production, and chemical composition of *Chlorella*, strain A, grown in buffered media with NH_4^+ or NO_3^- (see text). Two light intensities of sodium light (the highest one somewhat above saturation, *cf.* Table 4); continuous and discontinuous illumination (7 hrs light, 8 hrs dark); temperature, 25 °C. Initial cell density: 1.5 μl/cm^2 irradiated area, light energy absorbed per culture: 3.8 kcal. Expt. of 16–10–52.

Illumination, cal/cm^2.min	Efficiency, per cent	CO_2/O_2 ratio	Proteins, per cent	Carbo-hydrates, per cent	Lipids, per cent
41 hrs cont. at 0.033					
NH_4^--medium	19.0	0.99	60	22	18
NO_3^--medium	14.6	0.79	52	31	17
22 hrs cont. at 0.064					
NH_4^+-medium	17.1	0.91	61	22	17
NO_3^--medium	12.9	0.78	54	26	20
22 hrs discont. at 0.064					
NH_4^+-medium	17.4	0.96	58	24	18
NO_3^--medium	14.0	0.77	54	29	17

of discontinuous illumination upon efficiency and protein content were found. The further chemical composition neither was significantly affected.

If chemical composition and dry weight are known, balance equations for the overall metabolism can be written. For the cultures grown at 0.033 cal/cm^2. min (*cf.* Table 10) these equations are:

$$0.197C_{6.17}H_{9.80}O_{2.55}N + 5.26CO_2 + 2.30H_2O + 0.76NH_4^+ \rightarrow 0.960C_{6.73}H_{10.66}O_{2.86}N + 5.29O_2 + 0.76H^+.$$

$$0.197C_{6.17}H_{9.80}O_{2.55}N + 4.20CO_2 + 3.72H_2O + 0.55NO_3^- \rightarrow 0.751C_{7.21}H_{11.74}O_{3.47}N + 5.31O_2 + 0.55OH^-.$$

Starting from the same type of cells, a nearly, equal chemical composition was attained with ammonium and with nitrate (*cf.* also Table 10). Since energy is required for reduction of nitrate, more algal material has been produced with ammonium for the same amount of light energy. Although more carbon dioxide has been fixed, the same amount of oxygen is evolved, so that equal quantum efficiencies (mol O_2 per hν) are observed (*cf.* also Table 9), the CO_2/O_2 ratios being different. In nitrate containing media the CO_2/O_2 ratio is low (*cf.* Table 10 and [11], [35], [57]); so that it makes differences to calculate the efficiency in terms of dry weight, or as quantum efficiency (mol O_2 per hν). Evidently, the efficiency calculated on a dry weight basis is preferable in our case.

The results of Table 10 have shown that an enhancement of the efficiency of light energy conversion of algal cultures by about 30 per cent is possible if ammonium instead of nitrate is used. This percentage has been predicted by KOK (35) from theoretical balance equations, while from such equations given by CRAMER and MYERS (11) the same value can be derived. Higher plants placed in darkness, and growing with glucose and ammonium or nitrate showed relative differences in energy conversion of the same order of magnitude (90). Similar phenomena were found with heterotrophic micro-organisms (9).

2. EFFECTS OF NITROGEN DEFICIENCY

The influence of the mineral composition of the medium upon growth of algae has been investigated for *Chlorella*, (54, 67), *Scenedesmus* (39, 63) and *Ankistrodesmus* (73). A wide variation in composition (67) and concentration (54) may obtain without effects upon growth. SPOEHR and MILNER (81) reported a value of 0.001 m nitrogen with *Chlorella pyrenoidosa* to result in good growth, and cells with a high nitrogen content. The length of the growth period, however, must be taken into account. If cultures are growing with a limited amount of nitrogen, deficiency may occur during the culture period which may results in a shift of the chemical composition. (1, 24, 29, 31, 50, 81). In this section, effects of nitrogen deficiency upon chemical composition are reported, and attention have been given to the efficiency of light energy conversion, which is connected with nitrogen deficiency. Recently, some results have been briefly communicated (62).

Effects of nitrogen exhaustion upon quantum efficiency of *Chlorella* cultures

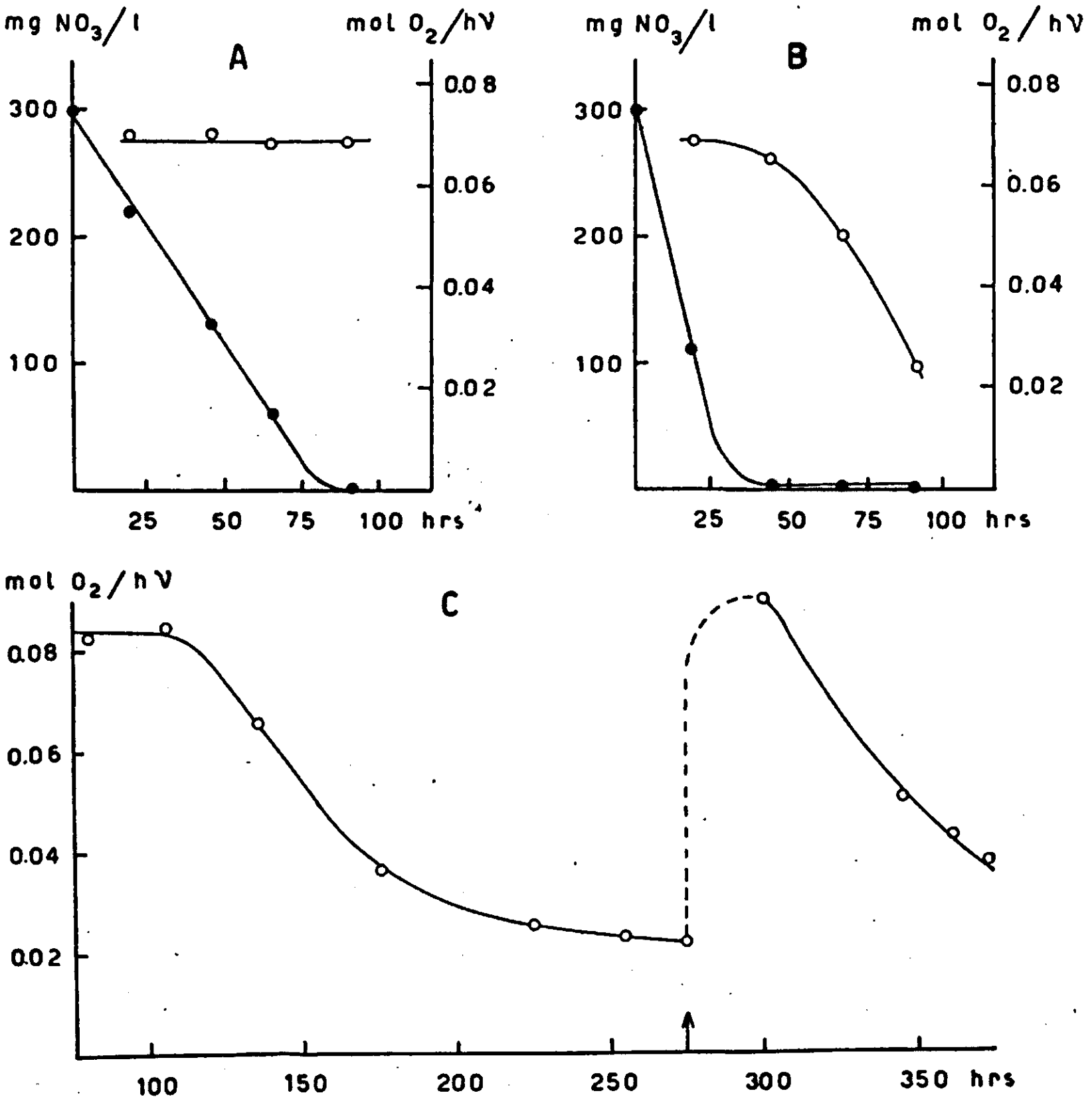

FIG. 10. Quantum efficiency (o——o) and nitrogen content of the medium (●——●) during growth of *Chlorella*, strain A, in sodium light at 25 °C. A) Light intensity 0.022 cal/cm². min, fractional light absorption 0.13 to 0.43; B) light intensity 0.017 cal/cm². min, fractional light absorption 0.55 to 0.68; C) intensity (.018 cal/cm².min, nitrate exhausted after 100 hrs of growth. At ↑ 300 mg NO_3^- per liter added, exhausted after 25 hrs. Expts. of 21–1–52, 14–1–52 and 18–2–52.

are given in fig. 10, in which typical experiments with cultures in large WARBURG vessels in sodium light are presented. The nitrate contained in the culture, shown in fig. 10A was not exhausted before the end of the culture period, and a constant efficiency is found. With faster growth (fig. 10B) nitrate is exhausted earlier, and a gradual decrease of the quantum efficiency is observed after exhaustion of nitrate. Addition of nitrate restores the quantum efficiency, as is shown in fig. 10C. It has been found in some experiments (*cf.* Table 12), that exhaustion of nitrate causes higher CO_2/O_2 ratios. The decrease of the quantum efficiency for O_2-evolution given in fig. 10, can only partly be explained by this, because at most a decrease of 40 per cent (CO_2/O_2 from 0.7 till 1.0) then might be expected, whereas larger ones are actually found.

The overall efficiency of light energy conversion is also determined by the period during which nitrogen has been exhausted. Some experiments, therefore, were carried out in media depleted of nitrogen. At low light intensities, in

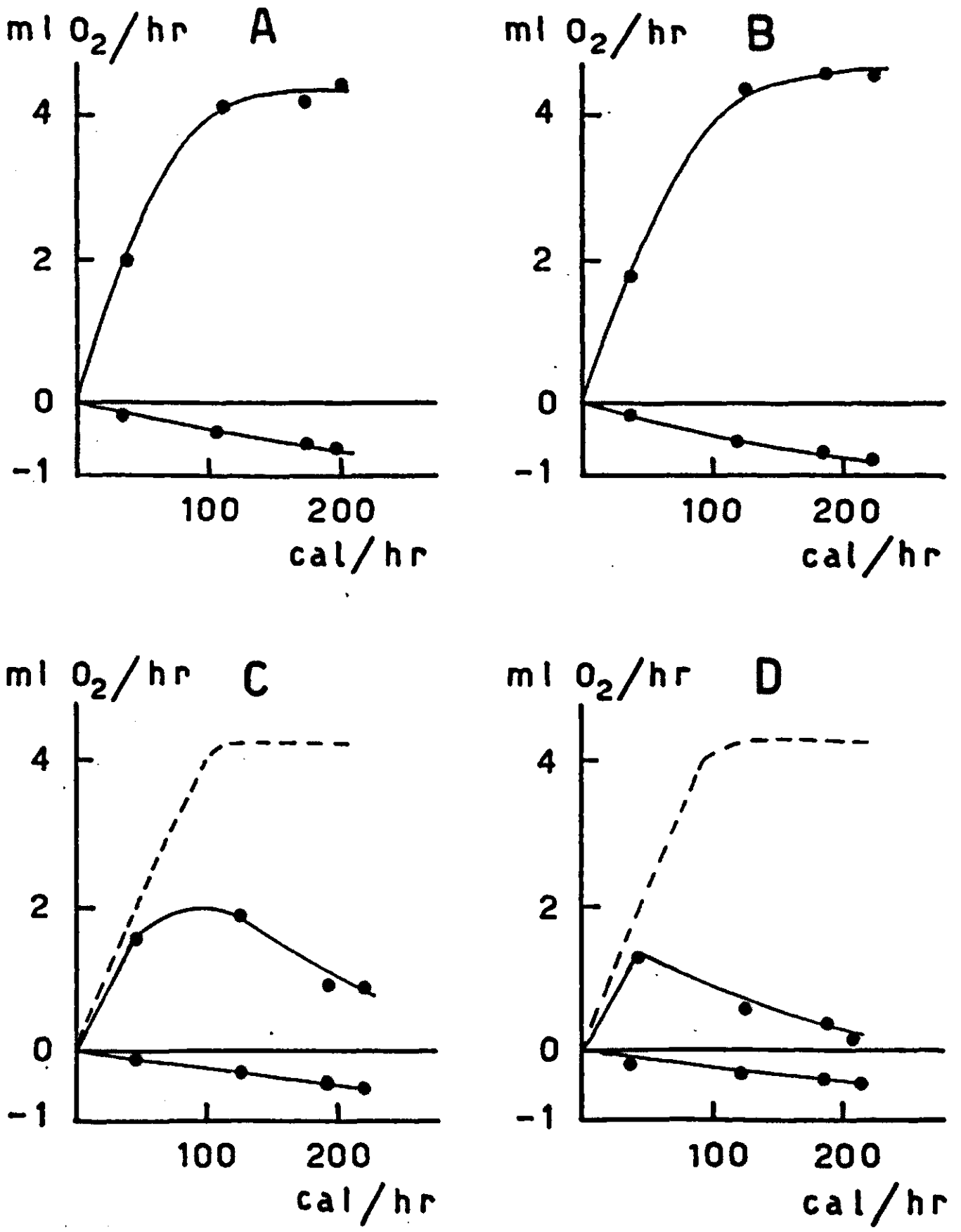

FIG. 11. Oxygen production of cultures without a nitrogen source in different light intensities, at 25 °C, as dependent upon the period of growth. A) After 2–5 hrs, B) after 17–20 hrs, C) after 31–34 hrs, and D) after 55–58 hrs. Abscissa: amount of absorbed light. Inoculum: 4.9 μl/cm² irradiated area, $\alpha = 0.76$. Expt. of 13-5-52.

sodium light, WARBURG vessels were used; at high light intensities in white light, the cell suspensions were in Erlenmeyer fasks (*cf.* p. 233).

In fig. 11, the oxygen production of nitrogen depleted cultures at some intensities of sodium light is presented. *Chlorella*, strain A was suspended in a culture medium of the following composition: 0.01 m KH_2PO_4, 0.002 m $MgSO_4.7H_2O$, iron, A_4 and B_7 solution, *cf.* p. 233. At the start of the experiment (fig. 11A), and after 17 hours of growth (fig. 11B), a curve showing light saturation of oxygen production against the amount of absorbed light energy is obtained. The respiration rate is proportional to the absorbed light energy (*cf.* KOK [35]). The oxygen production at higher light intensities was decreased after 31 hours (fig. 11C), while a further decrease was observed after 55 hours of growth (fig. 11D). The oxygen production at the lowest light intensity was not yet seriously affected. So the efficiency of light energy conversion with reference to oxygen production is affected seriously after prolonged exposure in a nitrogen-free medium, especially at high light intensities.

TABLE 11

The efficiency of light energy conversion of *Chlorella*, strain A, after 58 hours of growth in a nitrogen-free medium (see text on p. 262) at various light intensities and 25 °C, compared with that obtained in a medium with nitrate (computed from Table 4).
Expt. of 13–5–52.

Light intensity, cal/cm².min . . .	0.012	0.037	0.057	0.065
Efficiency %, without nitrogen .	21.8	10.5	7.0	6.0
Efficiency %, with nitrogen . . .	22.8	22.2	18.8	17.6

The efficiency of light energy conversion with reference to dry matter production is also affected, as is shown in Table 11. Whereas at a low light intensity the same efficiency as with nitrate is found, lower ones are observed at higher light intensities (see also [102]). As follows from fig. 11, the overall results (given in Table 11) depend upon the length of the growth period. At higher light intensities, the nitrogen content of the medium will decrease sooner by the faster growth of the cells. Just after exposure in a nitrogen-free medium (*cf.* fig. 11A), however, light saturation is also at a relatively low intensity as compared with that in nitrate containing media (*cf.* efficiency values in Table 11).

The chemical composition at the end of the growth period is given in Table 12. The initially high nitrogen content of the cells decreased to low values during the experiment. The carbohydrate content increased considerably during growth, and attained about equal values after exposure to different light intensities. No significant differences in fat contents were observed before and after the experi-

TABLE 12

Chemical composition of *Chlorella*, strain A, computed according to SPOEHR and MILNER (81), before and after 58 hours of growth in a nitrogen free medium (see text on p. 262) at various light intensities and 25 °C. Expt. of 13–5–52.

Intensity. cal/cm².min . . .	Before expt.	0.012	0.037	0.057	0.065
Nitrogen, %	10.0	3.9	2.9	2.8	2.4
Carbohydrates, %	14	54	58	58	58
Lipids, %	23	22	24	25	27

ment. Obviously, the first result of nitrogen deficiency is an increase in carbohydrate content (*cf.* also fig. 14), accompanied by a less efficient conversion of light energy. From equations for the overall metabolism, a high CO_2/O_2 ratio (0.85 to 0.91) was computed (*cf.* 11, 57).

The effects of higher light intensities were studied with white light, in cultures of 30 ml in Erlenmeyer flasks. Curves of yield versus light intensity of nitrogen deficient cultures of *Chlorella*, strain 14–10 were determined in the experiment, presented in fig. 12A. During preceding growth at 0.25 cal/cm².min and 30°C, the nitrate supplied appeared to be exhausted. The cells with a high carbohydrate content (*cf.* Table 13) were studied in the pre-cultivation medium, and the growth was determined over periods of 24 hours. The first day, a light curve with a lower saturation level as that of fig. 8A was found. During subsequent days

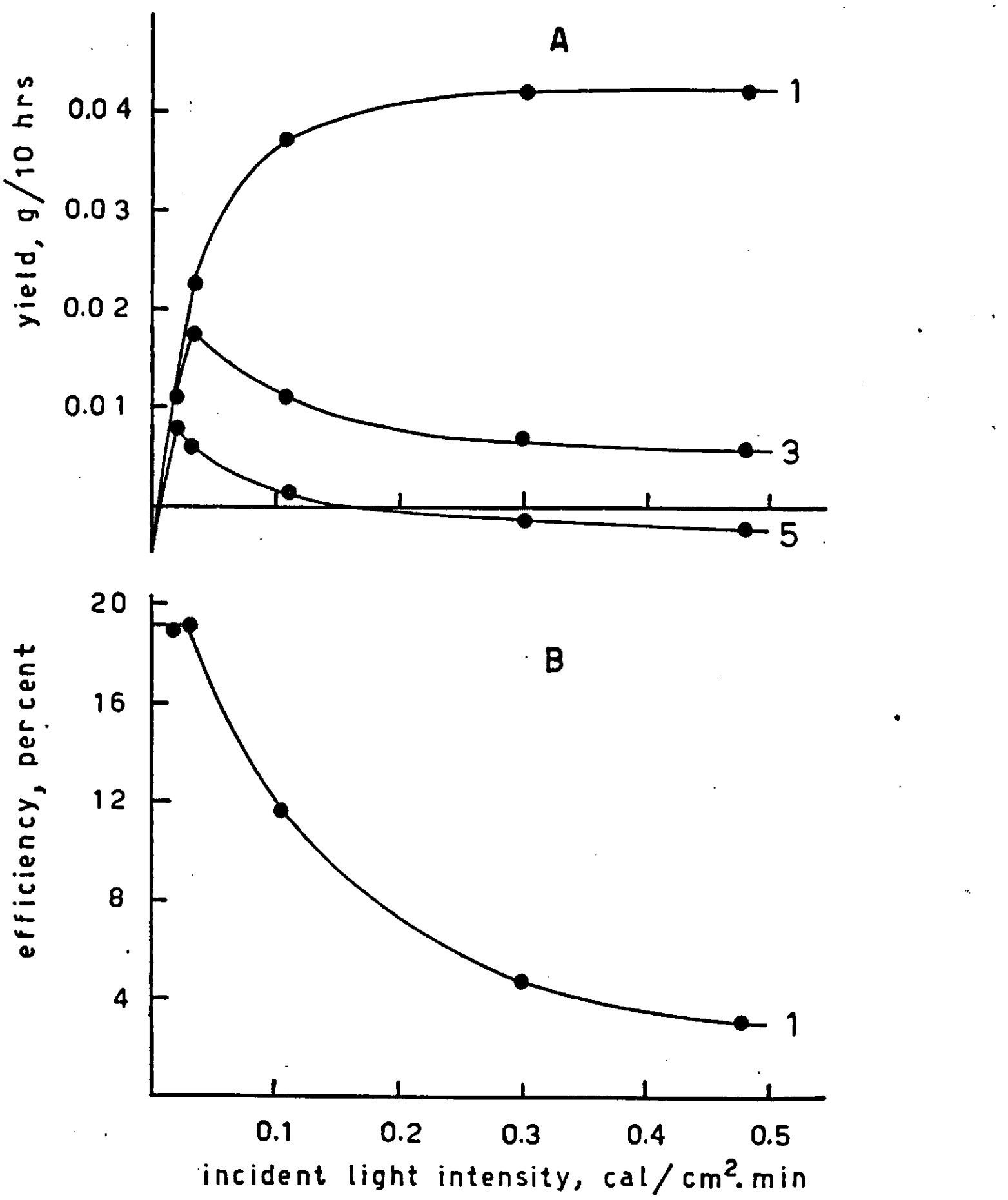

FIG. 12. Effects of light intensity upon growth and efficiency of light energy conversion in cultures of *Chlorella*, strain 14–10, the nitrogen source of which is exhausted. Cultures under continuous illumination at 30 °C, during 5 days in the medium used for pre-cultivation (initial conditions: 150 mg NO_3/liter, 3 days at 0.25 cal/cm². min and 30 °C). Inoculum: 2 mg of chlorophyll. A) Growth on the first, third and fifth day; B) efficiency on the first day. Expt. of 20–2–54.

lower growth rates at high light intensities, and even decreases of dry weight were observed. During the first day the same efficiency as with nitrate was computed at low light intensities; at higher intensities, however, lower efficiency values were found already then (fig. 12B). The efficiency during the subsequent days could not be computed, because of the decrease of the absorption occurring during the second day. It is evident from the yield curves that the efficiency decreases further with increasing length of the growth period. So, also with *Chlorella*, strain 14–10, nitrogen deficiency causes a lower saturation level, at a lower light saturation intensity.

TABLE 13

Chemical composition of *Chlorella*, strain 14–10 after 5 days of darkness, and at three light intensities, at 30 °C. See also the legend of fig. 12. Expt. of 20–2–54.

Intensity, cal/cm².min	Before expt.	Dark	0.108	0.300	0.480
Chlorophyll, %	3.8	2.2	0.8	0.3	0.0
Nitrogen, %	4.7	6.2	1.8	1.8	2.3
Carbohydrate, %	52	43	67	67	60
Lipids, %	19	18	22	22	26

The chemical composition after 5 days of growth is given in Table 13. This Table shows much resemblance to Table 12: lower nitrogen contents and higher carbohydrate contents are observed after illumination. In dark the reverse is true: the nitrogen content increases, whereas the carbohydrate content decreases. This is probably connected with respiration losses. At the high light intensities neither any significant increase in lipid content is observed. After the first day, the chlorophyll content decreased fastly, so that after 5 days of exposure at the highest light intensity no chlorophyll was found (*cf.*, *e.g.*, also [2], [73], [81]).

So far, nitrogen deficiency only caused high carbohydrate contents. In order to obtain high lipid contents the growth period of *Chlorella*, strain A was extended in some experiments. The cultures were maintained in Erlenmeyer flasks, using fluorescent tubes as a light source (*cf.* p. 231).

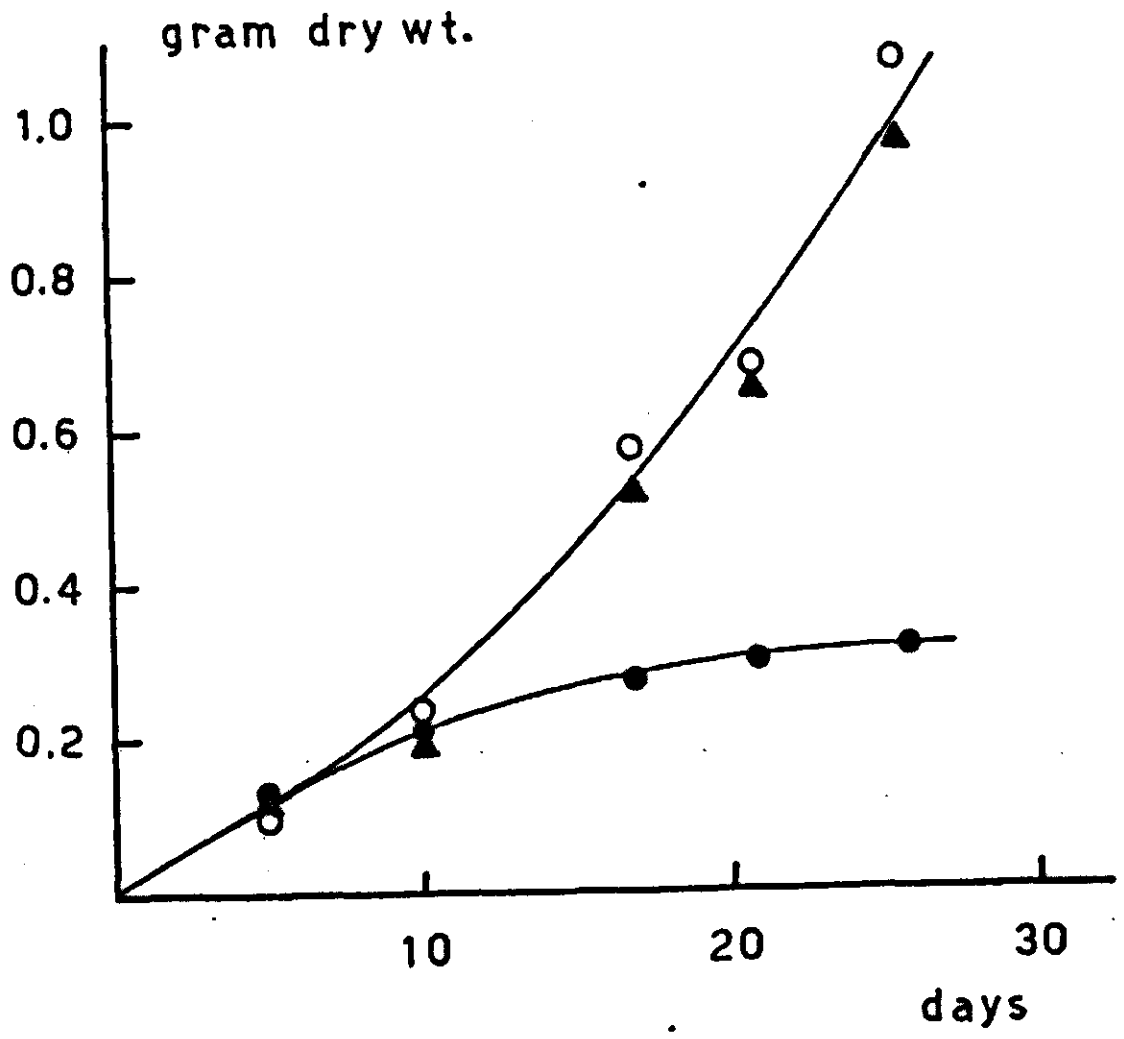

FIG. 13.
Growth of *Chlorella*, strain A, in cultures of 150 ml at a light intensity of 0.03 cal/cm².min ('daylight' fluorescent tubes, *cf.* p. 231) and at 25 °C. Throughout the whole period in the same medium: ●——●; every two days addition of KNO_3 and KH_2PO_4: o——o; every 2 days renewal of the medium: ▲——▲. Expt. of 11–2–52.

The results of a typical experiment are shown in fig. 13. A number of cultures of *Chlorella*, strain A (culture medium *cf.* p. 231, with 5.0×10^{-3}mKNO_3) were thinly inoculated, and continually exposed to 0.03 cal/cm^2. min and 25°C. Part of the cultures was maintained in the same medium for 26 days; in other ones KNO_3 and KH_2PO_4 was added every two days, while in still other cultures the medium was renewed every two days. The growth of the cultures was compared by determining cell volume at regular intervals. For cultures of the same treatment the results fitted within 5 per cent. At definite time intervals some cultures were harvested in order to determine dry weight and chemical composition. After 10 days of growth, the nitrogen deficient cultures showed a gradually decreasing growth rate, although dry weight still continued to increase (*cf.* also [81]). The addition of nitrate and phosphate resulted in a steady increase in dry weight (*cf.* also [58]), which was also observed with renewal of the medium. This suggests the absence of considerable amounts of growth inhibiting substances in these dense cultures (see also [73]). The light absorption was not determined, but after 10 days of growth about complete absorption was obvious in all cultures. Thereafter, in the nitrogen deficient cultures the absorption decreased. The shape of the growth curve indicates that the light energy is used less efficient in these cultures.

TABLE 14

Cellular composition of *Chlorella*, strain A, after various culture periods in the same medium (**1**), with addition of nitrate and phosphate (**2**), and with renewal of the medium (**3**). Cultures in Erlenmeyer flasks at a light intensity of 0.03 cal/cm^2.min and 25 °C. Expt. of 11–2–52.

Culture period in days	5	10	17	26	
Conditions of medium	**1, 2, 3**	**1, 2, 3**	**1**	**1**	**2, 3**
Proteins, %	70	35	15	15	35
Carbohydrates, %	12	44	40	32	43
Lipids, %	18	21	45	53	22

The composition of the cellular material after various growth periods is of interest. In Table 14 some of these data are given. During the first 10 days all cultures show a decrease in protein and an increase in carbohydrate content. Thereafter, the cellular composition of cells in cultures with addition of nitrate and phosphate (Table 14, [2]), or with refreshment of the medium (Table 14, [3]) remained constant. In connection with the growth curves given in fig. 13, it is evident that the amount of proteins in these cultures increased continually with the length of the growth period. In cultures in which no adjustment or renewal of the medium took place (Table 14, [1]), the protein content of the cells was further decreased after 17 hours of growth, while the lipid content of the cells then had increased considerably. After 26 days of growth, the same low protein content was found, while a further increase of the lipid content, now accompanied also by a decrease of the carbohydrate content, was observed. The lack of nitrogen will be mainly responsible for these effects. In separate experiments it was observed, that large differences of protein and carbohydrate contents of the cells could be induced by growth in media which only differ in nitrogen content (*cf.* also Table 12).

From the results of fig. 13 and Table 14 we can calculate the total production of proteins, carbohydrates and lipids in the cultures grown under different conditions. After 10 days of growth practically the same amount of proteins,

carbohydrates and fats are produced under the three conditions applied. Thereafter, differences arise; the cultures with extra supply of nitrate produce more proteins and carbohydrates, while practically the same amount of lipids is formed after 17 days of growth. After 26 days the following amounts have been produced:

	mg proteins	mg carbohydrates	mg lipids
in nitrogen deficient cultures	47	99	164
in cultures with nitrogen	350	430	220

In nitrogen deficient cultures the production of proteins and carbohydrates is considerably lower than in cultures with extra supply of nitrogen. Although the lipid content is high in nitrogen deficient cultures (*cf.* Table 14), even less total lipids are accumulated. From this we may conclude, that lipids in algal culture are produced in the most efficient way under normal culture conditions with a sufficient nitrogen supply.

By still more prolonged exposures in the same medium under similar conditions as those mentioned above, we have tried to raise the lipid content further.

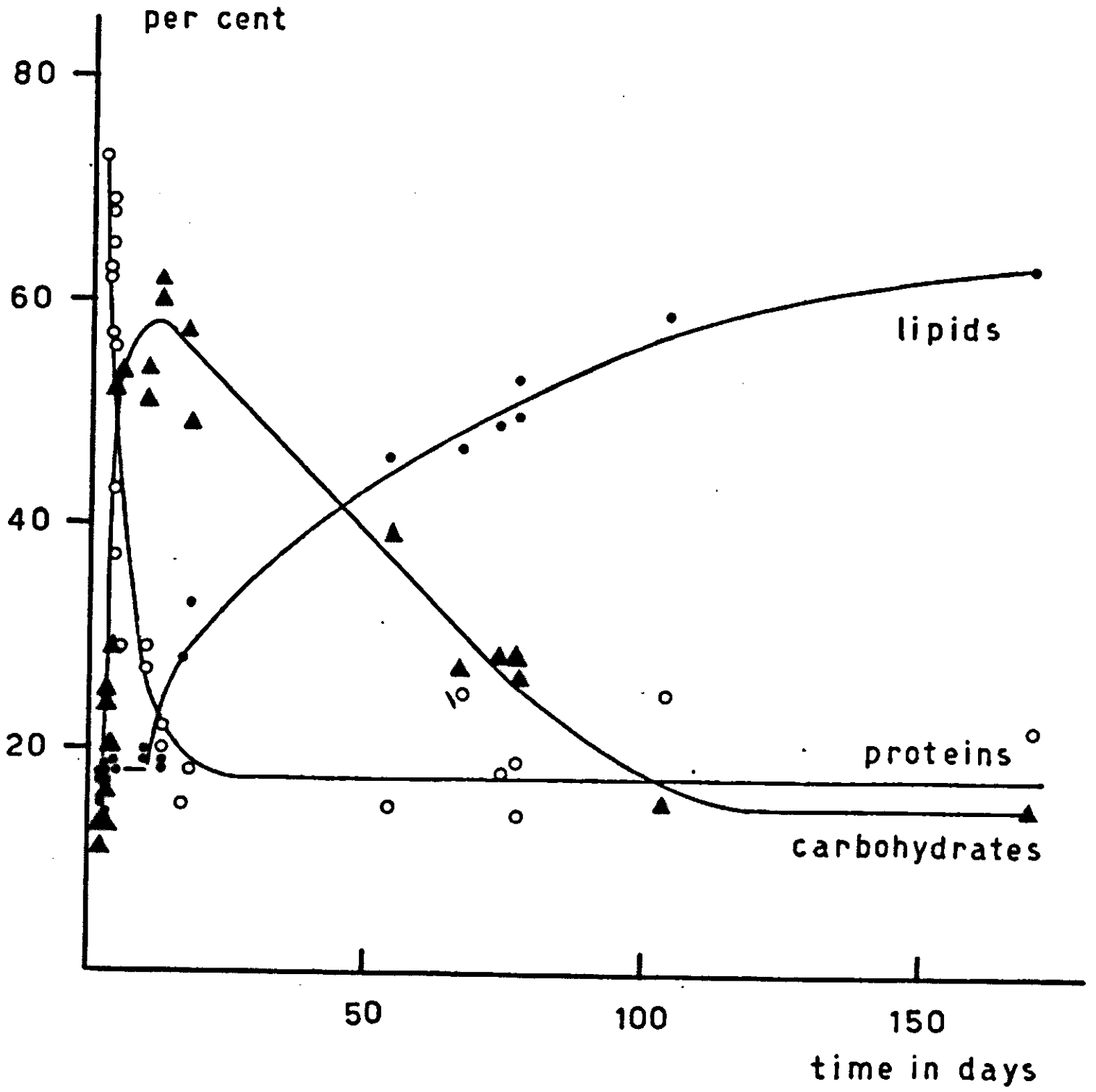

FIG. 14. Effect of duration of the growth period upon the protein content (○——○), carbohydrate content (▲——▲), and lipid content (●——●) of *Chlorella*, strain A. Cultures at a light intensity of 0.03 cal/cm². min ('daylight' fluorescent tubes, *cf.* p. 231) at 25 °C, and supplied with 150 mg NO_3^- per liter at the beginning of the growth period. Expts. of 1952–1953.

Fig. 14 gives data of the chemical composition of cells in relation to the length of the growth period. As also revealed above, the chemical composition first shifted from a high protein to a high carbohydrate content, whereas the lipid content remained fairly constant. After about 10 days a low, constant level of protein content was attained, indicating that cell division had ceased (*cf.* also [1]). Then the lipid content showed a fast increase during the next weeks (*cf.* also Table 14). If cultures grew older, this increase slowed down, so that a lipid content of 63 per cent was attained only after 170 days. With increasing lipid content, the carbohydrate content decreased to low values. Because of the constant dry weight of the cultures in these long periods (from about 60 days onward), the absolute amount of carbohydrates decreased too.

The production of cells with high carbohydrate contents was accompanied by a lower efficiency of light energy conversion (*cf.* Tables 11 and 12, fig. 12 and Table 13). The results of fig. 13 and Table 14 suggest, that also cells with high lipid contents are produced with a lower efficiency. In order to have exact values, the light absorption must be determined. Some experiments, therefore, were performed with WARBURG vessels in sodium light (*cf.* p. 233). Cells of different cellular composition (with high protein, high carbohydrate, or high lipid content) were obtained during different growth periods under fluorescent light at 25°C. These cells were suspended into media with either a definite amount of nitrate or without a nitrogen source, and exposed to a light intensity of 0.06 cal/cm².min. at 25°C. After certain periods the cellular composition was estimated, while the efficiency of light energy conversion was determined by including absorption measurements during these periods.

TABLE 15

Relation between cellular composition and efficiency of light energy conversion of cultures of *Chlorella*, strain A, exposed to 0.06 cal/cm².min (sodium lamps) and 25 °C. Cells obtained from various cultures, grown under fluorescent tubes (0.03 cal/cm².min) and 25 °C. Expts. 1, 2 and 3: cultures of 3 days; expt. 4: culture of 8 days; expt. 5: culture of 18 days. Expts. of November 1952.

Expt.	Conditions of expt.	Cellular composition in per cent			Efficiency in per cent
			Before expt.	After expt.	
1	48 hrs duration, 0.02 m nitrogen.	proteins	66	67	19.1
		carbohydrates	12	11	
		lipids	22	22	
2	71 hrs duration, 0.01 m nitrogen.	proteins	72	19	14.7
		carbohydrates	11	65	
		lipids	17	16	
3	95 hrs duration, without nitrogen	proteins	56	14	7.7
		carbohydrates	29	53	
		lipids	15	33	
4	95 hrs duration, without nitrogen	proteins	22	12	3.2
		carbohydrates	60	56	
		lipids	18	32	
5	95 hrs duration, without nitrogen	proteins	18	14	0.0
		carbohydrates	49	51	
		lipids	33	35	

In Table 15 the results of these experiments are given. In expt. 1 the culture had been supplied with sufficient nitrogen. The initially high protein content remained high during the experiment, and no shift in chemical composition was found. This culture had a high efficiency of light energy conversion (*cf.* also Tables 4 and 7). If less nitrate had been supplied (expt. 2), the composition shifted from a high protein to a high carbohydrate content during the experiment. Then the efficiency of light energy conversion was already lower than that in expt. 1. In expt. 3 no nitrogen had been supplied, while the culture period was extended. Now both the carbohydrate and the lipid content are increased, which is accompanied by an efficiency value, considerably lower than that found for the production of cells with a high carbohydrate content only (expt. 2). This points towards an even lower efficiency, if cells with high lipid contents are produced from cells with high carbohydrate contents. Therefore, cells with a high carbohydrate content (obtained from a culture of 8 days) were used in expt. 4. Now only the lipid content increased, and the efficiency of light energy conversion in this experiment indeed decreased to a very low value. In expt. 5 the cells had a high lipid content before the experiment. During the experiment no shift in composition occurred. In view of the results of fig. 14 no effect upon composition might be expected during this short period. In accordance herewith, no fixation of light energy could be demonstrated during this experiment.

Thus, the efficiency of light energy conversion decreased, when the chemical composition shifted from a high protein content to a high carbohydrate content. A further decrease was found, when the lipid content raised, so that even no light energy may be fixed by cells which already have high lipid contents.

3. DISCUSSION

The efficiency of light energy conversion with reference to dry matter production in media containing ammonium salts was 30 per cent higher than that observed with nitrate, at least if precautions were taken against the otherwise large fluctuations of pH. The quantum efficiency of oxygen production, however, was the same in both cases, which is due to differences in CO_2/O_2 ratios.

After exhaustion of the nitrogen source, the quantum efficiency gradually decreased. A decrease in growth rate of ageing cultures has been variously observed (*e.g.*, [1], [77]), while a lower rate of photosynthesis of cells from old cultures has been found (*e.g.*, [33], [98], [102]). In nitrogen-free media the same was observed (*cf.* figs. 11 and 12). In growing cultures the efficiency of light energy conversion was determined at various light intensities (*cf.* Table 11). Light saturation was attained at a low light intensity. Obviously, these results are affected by different growth rates, which cause differences in the decrease of the nitrogen content of the cells during growth. The results obtained within short periods (figs. 11A and 12), however, indicate that light saturation is attained at a lower intensity than with sufficient nitrogen supply.

In these experiments of relatively short duration, the carbohydrate content increased, while the fat content remained low. With *Chlorella pyrenoidosa* lower carbohydrate contents were found under conditions of nitrogen deficiency, while the lipid content increased fastly (1, 81). An increase of the carbohydrate content after the end of the period of exponential growth has been reported for *Chlorella vulgaris* (64). These species seem to be different in their capacity of lipid production. Differences in lipid accumulation by these and other

species have been shown (24). Possibly, *Chlorella*, strain A, and *Chlorella*, strain 14–10, are much like *Chlorella vulgaris* in this respect. With ageing cultures, the lipid content increased after high carbohydrate contents (~ 60 per cent), and low protein contents (~ 20 per cent) had been attained (*cf.* fig. 14). Then, carbohydrates were converted into lipids, and lipid contents up to 63 per cent have been reached after long growth periods. Even higher lipid contents were found with *Chl. pyrenoidosa* after shorter periods of growth (1, 81). [1])

The results presented in fig. 13 and Table 14 suggest a low efficiency of production of cells with a high lipid content for *Chlorella*, strain A. From AACH's results the same can be computed for *Chl. pyrenoidosa*. The results given in Table 15 indicate that the efficiency of light energy conversion for the production of cells with high lipid contents is even considerably lower than that for the production of cells containing much carbohydrates. Moreover, nitrogen deficiency is accompanied by a breakdown of chlorophyll (*e.g.*, [1], [2], [73], [81], and Table 13), resulting in incomplete light absorption. The reduced efficiency may be connected with this damage of chlorophyll (*cf.* also p. 252). Because even more lipid material (although of a different nature, *cf.* [50]) can be obtained from cultures normally supplied with nitrogen (*cf.* p. 266), it seems most efficient to grow algae with high protein contents. With *Chlorella pyrenoidosa*, however, a brief period of nitrogen starvation may be more favourable, as has been found with yeast (45).

CHAPTER VII

DISCUSSION AND SUMMARY

1. GENERAL DISCUSSION

From the results obtained with cultures grown under artificial light and at controlled temperatures (Chapter V), we will now try to explain the growth characteristics of large and small scale cultures (Chapters III, IV) under natural conditions. Although an exact calculation of yield and efficiency from photosynthesis is impossible (*cf.* p. 256), the experimental curves of Chapter V have shown that the results of such calculations in general may account for the values actually observed. So, relatively high efficiency values (*cf.* fig. 8) were found at high incident light intensities and 30 °C (*e.g.*, at 0.5 cal/cm².min: 7 %, which is about 37 % of the maximum efficiency at low light intensities). While no higher values were observed at 40 °C, lower values were found at 20 °C (*e.g.*, 4 % at 0.5 cal/cm².min).

Small scale cultures growing under natural conditions (Chapter IV) show somewhat lower efficiency values. They are, however, only partially comparable with those discussed in Chapter V. The complications arise from the variability of the outdoor conditions. Adaptation of growth to a certain temperature may occur (*cf.* V, 4), while, furthermore, outdoor light periods are alternated with dark periods. This may well affect overall growth (26, 47, 83); the low tempera-

[1]) In a recent paper of COLLYER and FOGG (J. Exp. Bot. 6, 256–275 [1955]), it has been suggested that water deficiency (and thus drying in vacuo at room temperature) may promote fat accumulation. In our work, such effects have not been observed in cells with high nitrogen contents (*cf.* note on p. 232); no data are available, however, about nitrogen deficient cells.

tures during these dark periods *e.g.*, may be favourable (*cf.* [13], p. 119, 129, 138). An important difference with cultures grown under controlled conditions is the large variation of light intensity and temperature during the light periods, and the close connection between both variations. This may be the main reason for the fairly constant efficiency values found during July and August (~ 8 per cent), and the fairly linear relationship between daily yield and light energy absorbed (*cf.* [38]). From the results of Chapter V, mentioned above (*cf.* also fig. 8), however, one would expect that raising the temperature above 30°C will not result in higher growth rates. These results may have been affected by adaptation to the experimental temperature, for preliminary experiments showed that the saturation level of photosynthesis of cells, pre-cultivated at 20°C, may increase with temperature up to 40°C. Because in outdoor experiments the temperature, being low at the beginning of the light periods, changed relatively quickly, such effects may occur in these cultures. A full analysis of these phenomena still has to be made, applying variable light intensities and temperatures in laboratory experiments.

The yield and efficiency values of small scale outdoor cultures are much higher than those of large outdoor cultures, as reported in Chapter III. Although another strain (*Chlorella*, strain A) was used, it seems unlikely that this is solely responsible for the higher values obtained (*cf.* also p. 242). The more indirect connection between light intensity and temperature in the large and deep cultures as compared with that in small ones, may have been less favourably. The main reason, however, for the lower yield and efficiency values in the large outdoor cultures as compared with those of small ones must be the fact that the maximum efficiency of light energy conversion[1]) in these large cultures is relatively low (*cf.* Table 3). The cause of this is not clear. Possibly, the 'semi sterile' conditions may have exerted unfavourable effects, while also the supply of the large cultures with undiluted carbon dioxide may have been harmful. As to the effect of incident light intensity upon the yield and efficiency values of these cultures, it may be stated that the results (*cf.* fig. 2) in general agree with those of the experiments of Chapter V (fig. 8): an increase of the yield accompanied by a decrease of the efficiency being found at high light intensities.

A remarkable, and unexpected phenomenon occurring with outdoor cultures, is the decline of the efficiency with decreasing light intensities at the end of the season (*cf.* figs. 1 and 3, see also Table 2). A decrease of the yield per unit area and time with decreasing temperatures may be expected at relatively low light intensities (*cf.* fig. 8), and lower temperatures were found to cause fading of cells (*cf.* p. 250). Such strong effects upon the efficiency of light energy conversion, however, are not quite clear. Possibly, algal strains adapted to high temperatures grow very slowly at low ones (*cf.* fig. 9). The results of TAMIYA (86) pointed into the same direction. No decline of the efficiency has been observed by MEFFERT and STRATMANN (48), who used suspensions of lower cell density during the autumn. It seems possible that by this procedure unfavourable effects of too high cell densities, especially occurring at lower light intensities, were avoided.

A comparison of the maximum outdoor yields observed by various authors ([13], p. 108, 113, 143; [42]) shows that most values are near 10 g/m².day (see also Chapter III), although the type of cultures and the climatic conditions were quite different. The results of TAMIYA (86), and KOK and VAN OORSCHOT ([38],

[1]) *i.e.* the efficiency under conditions of light limitation.

see also Chapter IV) point to considerably higher yields, values of 14–26 g/m².day being observed under outdoor conditions. The reasons for this discrepancy are not well known. Those between our results given in Chapter III (10–13 g/m².day), and in Chapter IV (20–26 g/m².day) are discussed above. Such high values have also been observed with large cultures (open trenches with about 4 m² area) by TAMIYA (86).

The yield values may be predicted approximately from photosynthesis (*cf.* p. 243). Using a saturation intensity (I_s) of 0.05 cal/cm².min (*cf.* fig. 8 and Table 6), a maximum efficiency of light energy conversion (E_m) of 20 per cent (*cf.* Tables 4 and 5), and 95 per cent light absorption, an efficiency of 7.5–8.5 per cent then may be calculated for an incident light intensity of 0.3 cal/cm².min. This would correspond to a yield of $\sim$ 2.5 g/m².hr (*cf.* p. 241). Hence, a yield up to 30 g/m².day seems feasible with simple culture devices. The yield of algal cultures would be improved, if the saturation rate of photosynthesis could be raised. Possibly, strains with higher saturation rates, and with higher temperature optima (79) can be isolated or developed. With more complicate culture systems, aiming to irradiate cells with short light flashes (*e.g.*, [36], [66]) or to 'dilute' solar energy within cultures (20, 87), still higher yields, corresponding to the maximum efficiency of light energy conversion, would be possible.

The results discussed so far have been obtained with cultures to which nitrate was supplied. From the results given in the first section of Chapter VI, it follows that the efficiency of light energy conversion of these cultures could have been raised by 30 per cent of its original value by using a more reduced nitrogen source (ammonium salt). On the other side, results given in the same Chapter (section 2) have shown, that sufficient nitrogen must be supplied in order to attain the yield values discussed above. Nitrogen deficiency, causing low protein contents, and high carbohydrate contents or, (after prolonged exposures) high lipid contents, is accompanied by lower yield and efficiency values. Especially, the production of cells with high lipid contents, is accompanied by a very low efficiency of light energy conversion. Because a larger total amount of carbohydrates and lipid material is produced in the same time by cultures containing sufficient amounts of nitrogen, the most efficient production of proteins, carbohydrates, and lipids will be attained with normal cultures.

From results with algal cultures as described in Chapter III, and from those with higher plants, it has been concluded earlier (100), that the efficiency of light energy conversion in higher plants and algae is nearly the same. In view of the new data on algal culture (Chapters IV and V), it seems that this conclusion was connected with the type of experiments carried out with algal cultures. Until more extensive and exact data about higher plants are available, the question whether algae can convert more solar energy than higher plants is still open. In plants with much leaves (*e.g.*, beets), mutual shading of leaves (and possibly of cells within leaves) will exert similar effects upon the yield as those found with mutual shading of cells in dense algal cultures. Especially in dense field crops such conditions will obtain, leading to increase of yield up to incident light intensities far above those for light saturation of the photosynthetic apparatus.

2. SUMMARY

The conversion of light energy in algal culture has been quantitatively investigated under various conditions of growth. For that purpose the absorbed

light energy during growth, and the energy which is fixed in organic material have been determined and computed. The efficiency of light energy conversion is expressed as the percentage of the absorbed light energy which is fixed. The energy which is stored in the cultures has been computed from dry weight determinations, and elementary chemical analysis of the cells. For computation of the absorbed light energy, incident light intensity, irradiated area, and length of the growth period have been measured. Besides this, the average of the fractional absorption, which may change during growth, had to be determined. In many experiments practically complete light absorption has been achieved by the use of cultures with high cell density or in thick culture layers.

In order to obtain as high as possible a fixation of solar energy, such dense cultures have been used under natural conditions. With large cultures grown during the periods from April to November (1951 and 1952), efficiency values ranging from 1 to 5 per cent of the incident light energy ($\lambda < 0.7\ \mu$) were observed. The corresponding yield values which are based upon ashfree dry weight, ranged from 1 to 13 g/m^2.day. Increasing the light intensity up to those of full sunlight caused lower efficiency values, while the yield values still increased (Chapter III). The temperature in small cultures under natural conditions was much more closely connected with the incident light intensity than in large cultures, so that very high temperatures occurred at high light intensities. With these cultures, high efficiency values of 8 per cent, corresponding to yields of 20 g/m^2.day and more, were found during July and August (1953), using various strains in cultures of high cell density (Chapter IV). As was found with large cultures (Chapter III), the efficiency of light energy conversion decreased when the incident light intensity and the temperature decreased during the autumn (Chapter IV). With large cultures grown under controlled and constant conditions in artificial light similar effects were observed. The maximum efficiency of light energy conversion of these large cultures determined under artificial light of low intensity, appeared to be significantly lower than that of small ones (Chapter III, 3).

From several types of mathematical description of the relation between the light intensity and the rate of photosynthesis in suspensions of low cell density, yield values were calculated for cultures with high cell densities. The calculations showed that with increasing incident light intensities up to high values, the yield increases. So, relatively high efficiency values at high incident light intensities could be expected (Chapter V, 2). The effects of light intensity and temperature upon the yield and efficiency values were experimentally investigated under controlled conditions (Chapter V, 3). The results actually obtained with cultures at various light intensities showed that in general the calculations were correct. Relatively high efficiency values were found at high incident light intensities, *e.g.*, 7 per cent at 0.5 cal/cm^2.min and 30°C. Lower values (*e.g.*, 4 per cent at 0.5 cal/cm^2.min) have been observed at 20°C, while yields at 40°C were equal to those at 30°C. An exact calculation of the yield for practical use appeared to be hampered by the fact that the rate of photosynthesis depended upon the light intensity and temperature at which the cells were grown (Chapter V, 3, 4).

The supply of nitrogen to the culture medium appeared to be of importance for the conversion of light energy (Chapter VI). Supply of ammonium salts instead of nitrates increased the efficiency of light energy conversion by 30 per cent of its original value. Nitrogen deficiency, on the other hand, causes a decrease of the efficiency of light energy conversion, while the chemical com-

position shifted from a high protein content towards a high carbohydrate content. High lipid contents were only attained after prolonged growth periods. While the production of cells with high carbohydrate contents runs with an already lowered efficiency, that with high lipid contents is accompanied by very low efficiency values. Even for an efficient lipid production with algae, non-deficient cultures are best suited.

Finally, results obtained under outdoor conditions were discussed in relation to those of cultures grown under controlled conditions of light intensity and temperature, and compared with yield values observed by other investigators (Chapter VII).

3. SAMENVATTING

De omzetting van lichtenergie in culturen van eencellige algen werd bestudeerd onder verschillende omstandigheden tijdens de groei. Zowel de hoeveelheid lichtenergie, die door 'n cultuur wordt geabsorbeerd, als de energie die in het organisch materiaal wordt gebonden, moesten daarvoor bepaald worden. Het rendement, waarmee lichtenergie wordt vastgelegd, werd dan weergegeven als het percentage van de geabsorbeerde lichtenergie, dat in celmateriaal is vastgelegd. De vastgelegde energie werd berekend uit het drooggewicht van de cellen en de energie-inhoud per gewichtseenheid. Bij benadering kon deze energie-inhoud worden berekend uit gegevens van een elementair analyse van de cellen. Ter berekening van de geabsorbeerde energie werd de opvallende lichtintensiteit gemeten en het bestraalde oppervlak en de duur van de groeiperiode bepaald. Bovendien werd de absorptie van het licht, die tijdens de groei kan veranderen, bepaald met behulp van een witte bol. In verschillende proeven werd gestreefd naar 'n benadering van volledige absorptie door de culturen in geconcentreerde suspensies of in dikke lagen te kweken.

Met dergelijke culturen zijn onder natuurlijke omstandigheden verschillende experimenten gedaan om na te gaan, welk gedeelte van de energie van het zonlicht in organisch materiaal kan worden vastgelegd. Grote culturen leverden gedurende de periode van april tot october (in 1951 en 1952) rendementen, die varieerden van 1 tot 5 procent van het ingestraalde licht. Daarbij is van de lichtenergie alleen het gedeelte met golflengten kleiner dan 0.7 μ, die bruikbaar zijn in de photosynthese, in aanmerking genomen. De met deze rendementen overeenkomende opbrengsten varieerden van 1–13 g/m^2.dag. Bij een toename van de lichtintensiteit tot die van vol zonlicht neemt de opbrengst nog steeds toe, terwijl het rendement afneemt. In kleine buitenculturen met een grote celdichtheid volgde de temperatuur de variaties in lichtintensiteit tamelijk snel, zodat hoge temperaturen konden optreden bij hoge lichtintensiteiten. Rendementen van 8 procent van de ingestraalde lichtenergie, overeenkomend met opbrengsten van 20 g/m^2.dag en meer, werden in deze culturen gevonden gedurende juli en augustus (1953). Evenals bij grote culturen werd gevonden dat gedurende de herfst het rendement, waarmee lichtenergie wordt vastgelegd, afneemt. Onder gecontroleerde omstandigheden in kunstlicht werden met grote culturen dezelfde verschijnselen waargenomen. Bij deze culturen bleek het maximale rendement, dat in het licht-limiterende gebied constant is, lager te zijn dan dat van kleine culturen onder gecontroleerde omstandigheden.

Uitgaande van drie verschillende functies voor de betrekking tussen de lichtintensiteit en de photosynthesesnelheid in suspensies van lage dichtheid, werden

opbrengsten berekend voor culturen met hoge celdichtheid. Dan zal ook boven de verzadigingsintensiteit van de photosynthese de opbrengst nog toenemen met de lichtintensiteit. Daaruit volgt, dat nog betrekkelijk hoge waarden voor het rendement zijn te verwachten bij hoge lichtintensiteiten. De invloed van de lichtintensiteit en de temperatuur op de opbrengst van algenculturen is experimenteel onderzocht onder gecontroleerde condities in het laboratorium. De werkelijke opbrengsten bij verschillende lichtintensiteiten komen bij benadering overeen met de berekende. Zodoende werden bij hoge lichtintensiteiten nog betrekkelijk hoge rendementen gevonden, b.v. 7 procent bij 0.5 cal/cm².min en 30°C. Bij 20°C zijn de waarden lager, b.v. 4 procent bij 0.5 cal/cm².min, terwijl bij 40°C de rendementen dezelfde zijn als bij 30°C. Bij hoge intensiteit, vooral als deze gepaard ging met lage temperatuur, trad photo-oxydatie op. Een exacte berekening van de opbrengst onder buitencondities bleek bemoeilijkt te worden door het feit, dat de photosynthesesnelheid afhankelijk is van de lichtintensiteit en de temperatuur, waarbij de cellen hebben gegroeid.

De stikstofvoorziening is een belangrijke factor bij de vastlegging van lichtenergie. Vervanging van nitraat als stikstofbron door 'n ammoniumzout doet het rendement toenemen met 30 procent van de waarde met nitraat. Stikstofgebrek veroorzaakt een afname van het rendement, waarmee lichtenergie wordt vastgelegd. Bovendien verandert de chemische samenstelling: in plaats van cellen met een hoog eiwitgehalte, worden cellen met een hoog koolhydraatgehalte verkregen. Pas na zeer lange groeiperioden werden ook hoge vetgehalten gevonden. Dergelijke cellen worden met een nog lager rendement geproduceerd. Voor productie van vet door algen, is het gunstiger normale culturen, waarvan de cellen een laag vetgehalte hebben, te gebruiken.

De resultaten welke zijn verkregen onder natuurlijke condities, werden vergeleken met die van culturen onder gecontroleerde omstandigheden van lichtintensiteit en temperatuur, terwijl ook een vergelijking is gemaakt met opbrengsten, die door andere onderzoekers zijn gevonden.

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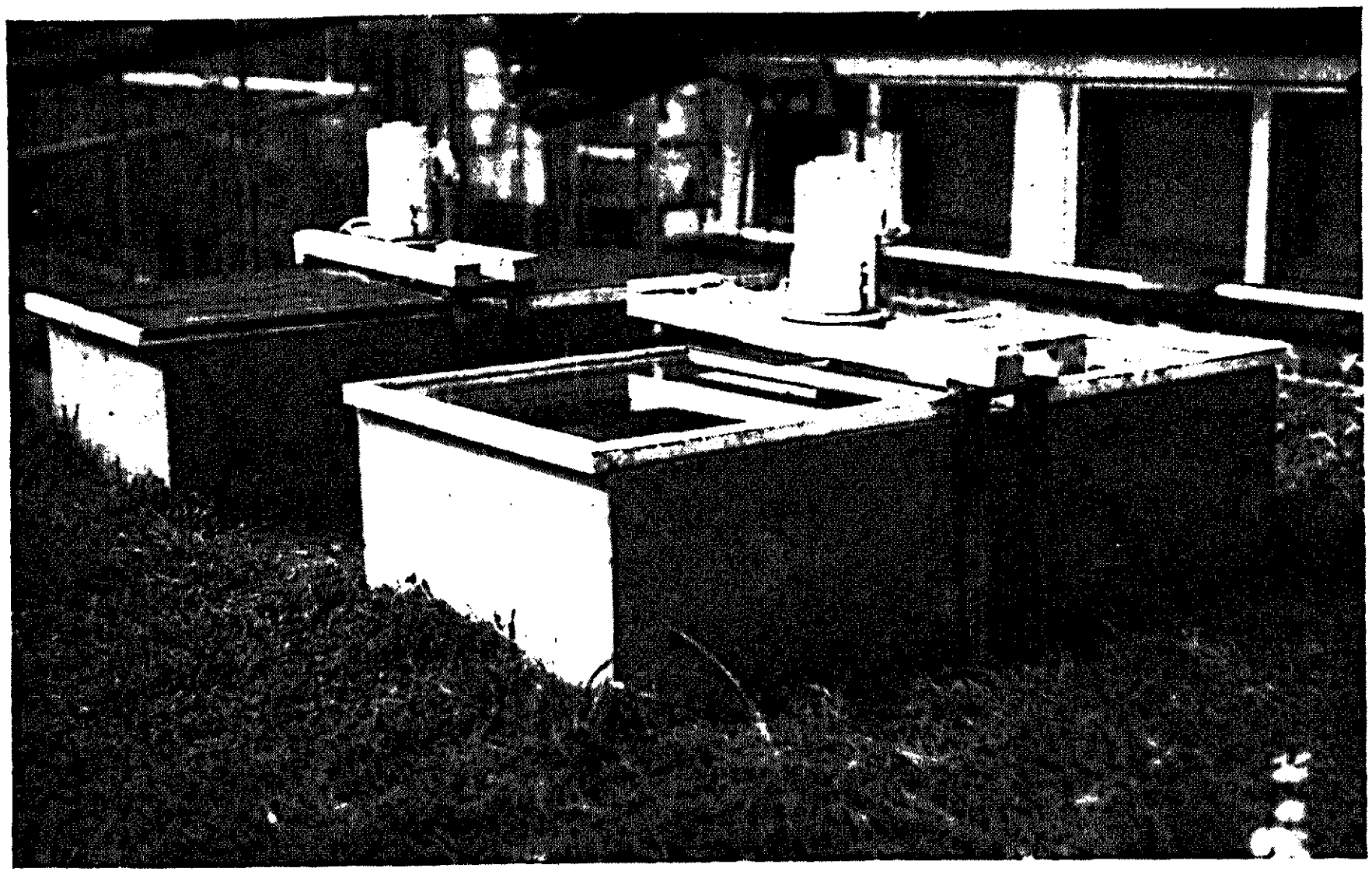

PLATE 1. Outdoor 300 l tanks (irradiated area, 1 m^2) for mass cultivation of *Chlorella* in sunlight. Tanks with glass cover and stirring motor. Taken from (97). (See Chapter III).

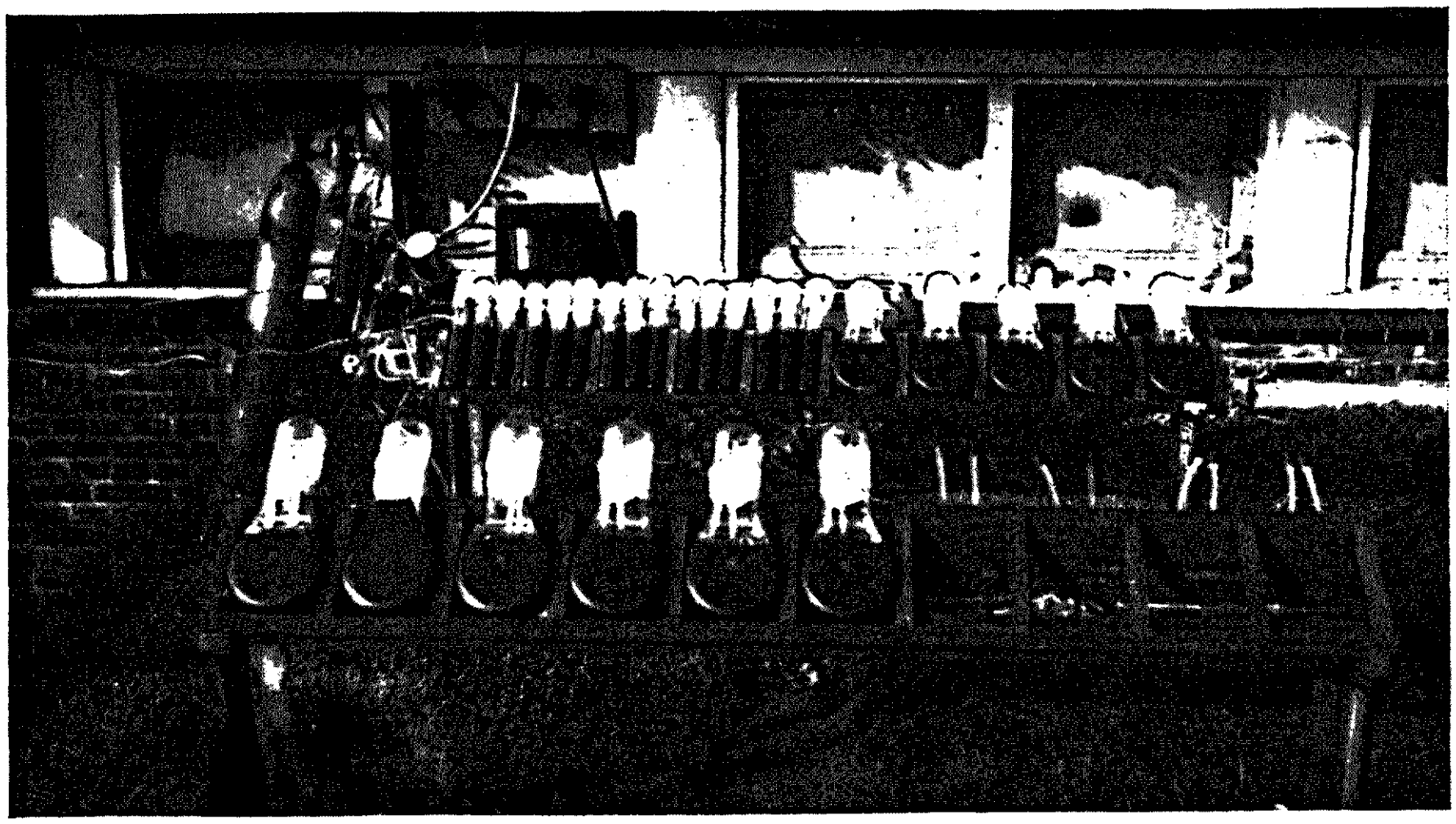

PLATE 2. Small scale cultures of algae. Tubes and Kolle dishes under an angle of 45° exposed to the south. Light intensity and temperature measurement devices at the left. (See Chapter IV).