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IMPROVED YIELDS IN ALGAL MASS CULTURES

BY

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During the past few years considerable attention has been given to economic possibilities, offered by large scale cultures of green algae. Most of the earlier work (uptil 1952) in this field was surveyed in a recent publication of the Carnegie Institution of Washington [1]. In brief it could be said, that the many promising aspects of algae cultures, as compared to conventional methods of producing food, are to a certain extend balanced out by the higher cost and investments needed. In this respect, the yields obtainable per unit area are of primary importance—at least as long as the bulk material is more important than special products are.

The best yields actually observed by a number of workers ranged from 8–12 grams per m^2 exposed area per day [1] pp. 60, 113, 134,[1] 147,[1] 175, 271, 280. Optimal yields obtained earlier in our group during short periods amounted to 12,5 gr/m^2 day [1] p. 60. [2] This represented the fixation of about 4 % of incident solar radiation, in the range of 400–700 mμ. It was felt, that the main yield limiting factor was that the intensity of bright sunlight could not be used efficiently by the algae studied.

We hoped to improve outdoor yields by using strains of algae with higher photosynthetic capacity in utilizing strong light. Therefore a number of strains collected was tested under conditions of high light intensity at various temperatures. By prolonged exposure to such conditions it was tried to further adapt algal material.

During the summer of 1953 the growth yields of a number of apparently favourable strains were studied in outdoor cultures.

I. SELECTION OF ALGAL STRAINS

Method of cultivation

Glass-walled water tanks (50 × 25 × 25 cm) were arranged in such a way, that their temperatures could be kept at any desired

[1] The figures given by the authors were calculated on a basis of the cross sectional area of the cylindrical culturing devices used. A correction with $2/\pi$ cf. p. 000 probably has to be applied in these cases.

value between 15 and 50° C. By using two regulators in each bath and switching from one to the other every 12 hours, a choice of "day" and "night" temperatures was available. In three tanks the "day" temperatures were set at 40, 30 and 20° C. respectively, while the corresponding "night" temperatures were 25, 20 and 15° C. During the "day" periods the algae were illuminated with 500 Watt incandescent lamps with internal reflectors (Philips Altrilux). Each lamp yielded an intensity of ca. 0.25 cal/cm² min. of photosyntizable radiation ($\lambda < 700$ mμ), corresponding to about 40 klux over an area of ca. 200 cm². This area was sufficient to illuminate 16 test tubes, each containing 10 ml of algal culture or a smaller number of larger vessels. Per tank 4 of these lamps could be used. The cultures were grown in inorganic nutrient solution (cf. 1, pg. 59), containing 0.05 m KNO_3 and were continuously flushed with air, enriched with 5 % CO_2.

In order to have individual cells exposed to as high an intensity as available, care was taken to keep the cultures thin. The test tubes were often—the fast growing ones daily—diluted by simply discarding the contents and adding fresh sterilized medium. The frequent handling of the cultures and their continuous aeration exposed these experiments to hazards of contamination. First of all, however, our main aim was a practical one: to obtain fast growing strains under the conditions chosen. The results proved quite reproduceable: repetition of exposures starting afresh from the pure cultures kept on agar slants in a light cabinet, usually yielded identical results.

Methods Used for Studying Growth Rates

For a study of algae with respect to usefulness for massculture application, measurement of growth in terms of dry weight (preferably expressed per unit irradiated surface or still better per unit absorbed light) may be considered as most straight forward, since it yields the sumtotal of all intricate processes involved.

A semi-quantitative judgement of the growth rates in thin sterile cultures was obtained by the following procedure:

From each culture to be tested we equally inoculated a number of test tubes provided with culture medium. Two test tubes were placed in each of the tanks, and a parallel sample of the inoculum was used for estimating its dry weight.

After the samples had grown dense enough to absorb about 80 % of the incident light, their dry weight was estimated. From initial and final dry weights (Wi and Wf) and the time of exposure to the light (t) a "generation time" τ was computed according to:

$$\tau = -0{,}3\ t \log Wi/Wf \qquad (1)$$

The procedure followed was a little arbitrary since the size of the inoculum and the moment of harvesting were not exactly defined. Not too much weight therefore can be given to the absolute time values found and only data obtained within one experiment were compared.

For most of our quantitative studies we used thick algal suspensions (i.e. in which practically all incident light of a wavelength $<$ 700 mμ was absorbed). In such suspensions the increase in dry weight is largely independent of the cellular concentration. Moreover, data obtained in this way, have general significance and bear directly upon the conditions of outdoor algal cultures (for which also thick suspensions are used). These experiments were carried out as follows:

In a thermostated bath a series of 6 identical 150 ml Erlenmeyer flasks could be exposed to white light of accurately known intensities. The vessels were illuminated with 24V, 150 Watt incandescent lamps with internal reflectors (Philips Attralux), mounted underneath the bath. These lamps each yielded uptil 0.7 cal/cm^2 min. of photosynthizable radiation over an area of about 30 cm^2. A sufficient amount of the algae to be studied was first grown in a larger vessel, exposed in one of the tanks. Each flask then was filled with 30 ml of a thick suspension of algae and continuously shaken and aerated with air containing 3 % CO_2. Samples for dry weight estimations were taken at definite time intervals.

Temperature Characteristics of Various Strains

A number of strains, obtained as pure cultures from several sides [1] and kept on agar slants in a light cabinet, were tested in the described culture tanks and compared with strains, collected in Holland and used in earlier work.

The first screening consisted of a subjective judgement of the growth in the test tubes. Several strains grew in neither of the

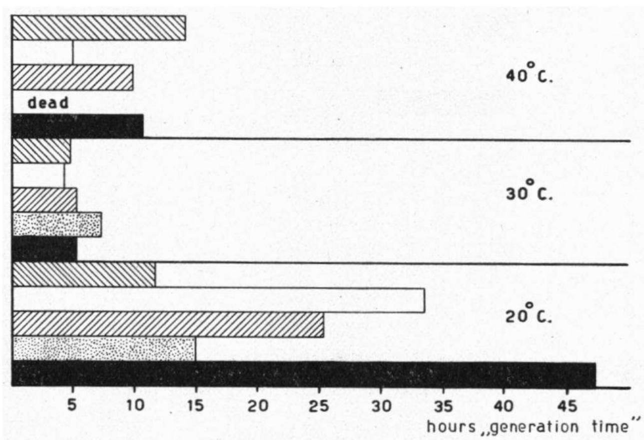

Fig. 1. "Generation-time" (see text) of a few strains of algae, grown in thin suspensions at a light intensity of 0.25 cal./cm^2 min., and temperatures as indicated, during the light periods: *Chlorella vulgaris* (strain Cornell), ▬ *Chlorella vulgaris var. viridis* (Yale) wild type ▒, *Chlorella pyrenoidosa* (Emerson's strain) ▨ *Chlorella* Tx 1105 ▭, *Scenedesmus* 3 ▧.

[1] We wish to most gratefully acknowledge Drs Appleman, Davis, Meffert, Myers, Pringsheim and Wassink, for putting these strains at our disposal.

conditions; these were generally characterized by slow growth on the agar slants in the light cabinet as well, and were not used in further work.

An illustration of the quantitative differences in growthrate as observed in the three tanks, is given in fig. 1. For this experiment the test tubes were inoculated with algae obtained directly from slants in the light cabinet. (Similar results were found in case the algae had been pre-cultivated during a few days in liquid cultures at low light intensity and medium temperature).

For most strains, the optimal growth rate is found in the 30° C. tank. High temperature strain Tx 1105 grew about as fast at 40° C. as it did at 30° C. and in other experiments the shortest generation time was found at 40° C. *Chlorella vulgaris var. viridis* appears to be a low temperature strain, it grew relatively fast at 20° C., slower than the other strains at 30° C. and survived in no case at 40° C.

A peculiarity encountered in this type of experimentation was that the temperature characteristics of algal growth proved to be largely dependent upon the temperature of pretreatment.

The expt. given in fig. 2 was made with algae, which originated from the same agar slant, but had been cultivated at different

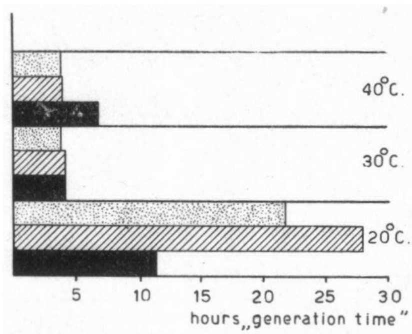

Fig. 2. *Scenedesmus* 3, cells cultivated during 7 months at: 20° C. (black), 30° C. (hatched), 40° C. (dotted). Thin suspensions, exposed to a light intensity of 0.25 cal./cm² min., and the indicated temperatures. Expt. of November 1953.

temperatures during several months. In this case the algae which had been precultivated in the two warm tanks grew poorly at 20° C., and about equally fast at 30 and 40° C. The cells, pretreated at 20° C., gave similar results as the ones directly obtained from the light cabinet, and used for exp. fig. 1.

The same adaptation phenomena are illustrated in fig. 3, which shows the results of a growth experiment in which thick suspensions were used: Three samples of cells, adapted to 20, 30 and 40° C. respectively, were first exposed to 20° C., thereafter to 30° C., and finally to 40° C. It appears, that fastest growth occurred in case the temperatures of pretreatment and exposure were identical. The difference between 30 and 40° C. was—as usually—not very marked, though. The growthrate of the cells, which were pretreated at 20° C., did not increase after the temperature was raised to 30° C. During the first day of exposure to 40° C. growth even ceased completely.

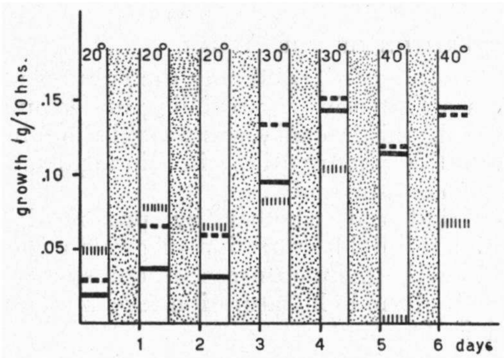

Fig. 3. *Chlorella*, 14–10, cultivated during 7 months at: 20° C. ||||||||, 30° C. ||||, and 40° C. ▬▬. Intermittently exposed to 0.35 cal/cm² min. (10 hours light, 14 hours dark. Temperature during the light periods as indicated, temperature during darkness in all cases 20° C. Initial cell density: 0.1 g/30 ml. Expt. of November 4–14, 1953.

The algae used for expts. 2 and 3 had been preexposed to the three temperatures for over seven months. It was found, however, that for establishing (respectively reversing) the described temperature adaptations much shorter pretreatments sufficed. Usually, no further significant changes could be noticed after exposures during one or a few weeks.

From this we may conclude that a sharp characterisation of algal strains in terms of their relation between growthrate and temperature is not simple. But on the other hand, the *absolute* growthrate of "high temperature" strains, even after prolonged adaptation, was generally lower at 20° C. than that of "low temperature" strains and conversely, several "low temperature" strains did not survive at all in the warmest tank.

Strain *Scenedesmus 3* appeared quite flexible in its temperature characteristics; at 20° C. it was the fastest growing strain tested and after short adaptation periods it grew about as well as any other alga at temperatures uptil 45° C.

It can be noticed in figs. 1–3, that in all cases growth at 30° C. was much faster than at 20° C. At the lower temperature the cells showed a tendency for clumping and turning pale. The latter effect has to be ascribed to photo oxidation by the strong light in the thin suspensions. It is also noteworthy, that the growth rates observed in either thick or thin suspensions were hardly different at 30° and 40° C. and more or less equal for the (five) suitable strains tested so far at these temperatures.

It was also tried to grow alga at still higher temperatures (40 a 47° C. "day", 25° C. "night" temperature). Eventually after a short adaptation, most strains which showed good growth in the 40° C. tank, thrived well uptil 45–47° C., but not better than at 40° C. Further experiments and a more detailed discussion of these matters will be given in [2].

Light Characteristics of the Algae

The light sources used for irradiating the cultures in our tanks did not yield as high an intensity as prevailing under outside conditions in full sunlight. Still, this intensity was several times higher than that required for saturating photosynthesis in thin algal suspensions—even at the highest temperature used.

We tried to find out whether by prolonged cultivation at 0.25 cal/cm^2 min. the light saturation of growth could be adapted in a similar way as the temperature characteristics of the algae. No significant changes of the light saturation level were found, however, as is illustrated in fig. 4.

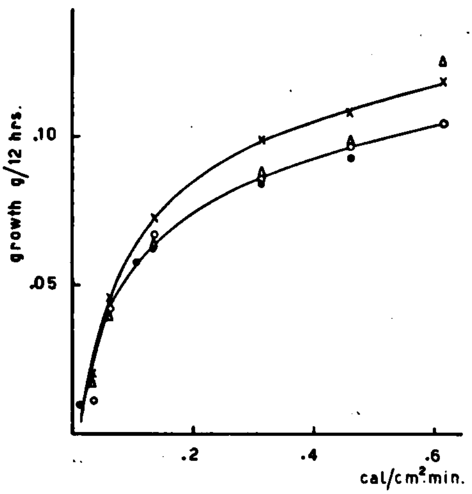

Fig. 4. Effect of prolonged exposures to 0.25 cal/cm^2 min. at 30° C. on the light saturation level of growth. Open circles: *Scenedesmus* 3, after one day exposure, closed circles: the same strain after 115 days exposure. Crosses: *Chlorella* 14–10 after one day exposure, triangles: the same strain after 115 days exposure. Cultures used for the short exposures were inoculated from agar slants and grown in weak light for a few days. Initial cell density: 0.08 g/30 ml. Temp.: 30° C. Expts. of August 7, 8, 11 and 13, 1953.

The light intensity available for our growth rate measurements in thick suspensions (0.6 cal/cm^2 min.) approached that of natural sunlight, so that it was of special interest to determine the yield of energy conversion attainable with various strains at different temperatures.

The amount of totally absorbed visible radiation ($\lambda < 700$ mμ) falling upon the vessels was determined by thermopile measurements with and without an RG 8 filter (Schott & Gen.). The increase in dry weight of the algae was expressed in calories by taking one gram of dry algal material equivalent to 5.8 kcals. [3] The percent efficiency then was calculated as:

$$\frac{\text{cals. harvested material}}{\text{cals. absorbed radiation}} \times 100$$

At temperatures over 30° C. the better strains showed quite high efficiency values. At the highest intensity used (0.6 cal cm²/sec.) 8–10 % of the absorbed light energy was converted into cell material. At 20° C. generally much lower values (4–5 %) were observed.

Outdoor Experiments

In order to compare simultaneously a number of different strains of algae, small culture flasks were used. For most exposures we used so called "Kolle" dishes (cf. fig. 5). Their flat shape facilitated an estimation of the irradiated surface (140 cm²) which was calculated as described in the legend of fig. 5.

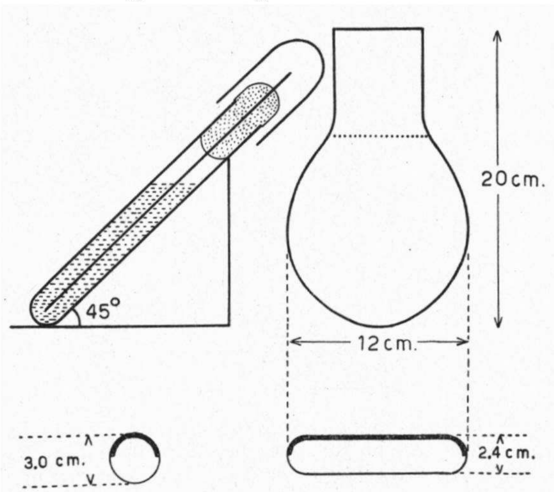

Fig. 5. To the right: a top view and a cross section of a Kolle dish. Top left: the position, in which the dishes were placed upon a blackened wooden support. Bottom left: cross section through a cylindrical vessel used. Its irradiated surface was computed by multiplying half of the circumference (heavy line) by the height of the fluid column. In the same way, for computing the exposed area of the Kolle dishes, half the circumference of the round sides was added to the area of the flat middle part.

Besides this, we used cylindrical tubes: with a radius (r) of 1,5 cm and a height of the fluid column (h) of 12 cm; it appeared, that the irradiated area of these tubes was best computed as $2\pi rh$ (cf. fig. 5 and Geoghegan [1] pag. 185). Calculated on this basis, the dishes and the tubes gave results fitting within ~ 10 %. A further check on these estimations was obtained by growing parallel samples of algae in large flat vessels, for which commercially available "thermopane" windows (40 × 40 × 1.2 cm) were used. The vessels were exposed at an angle of 45°, facing the south and the suspensions were intensely flushed with air, enriched with carbondioxide. This arrangement made complicated stirring devices superfluous. The gas inlet tubes passed through cotton plugs, over which a glass cap served as a protection against rain.

The light intensity falling upon the cultures was recorded continuously. For this, a thermopile, enclosed in a glas hemisphere and mounted with the same tilt of 45° C. as the culture flasks, was used. The pile was connected to a galvanometer, which actuated a pen

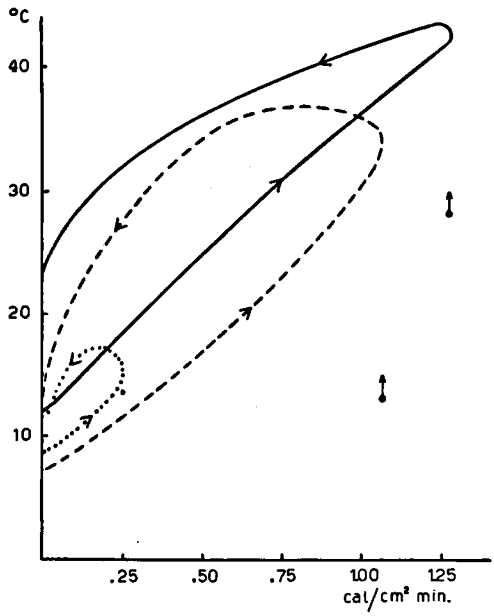

Fig. 6. Daily course of the temperature of the cultures in dependence of the light intensity. Solid line: July 25, broken line: Sept. 26, both cloudless days. Dotted line: Oct. 3, totally covered sky. The temperature of the surrounding air (dots) was measured at 1 p.m.

recorder via a photoelectric amplifier. From the area under the curves (cf. fig. 7) the sumtotal of light falling into the cultures during a given length of time could be calculated. The temperature was recorded with the aid of a simple thermograph, the feeler of which dipped into a culture tube filled with indian ink and exposed in the same way as the algal cultures. In addition, the air temperature was read twice daily.

It appeared that the sunlight falling into the vessels completely absorbed by the water and the algae, could raise the temperature considerably above the temperature of the surrounding air. On sunny days, differences over 20° C. were often observed in the middle of the day, whereas on cloudy days, an effect was hardly present. This is illustrated in fig. 6, showing the correlation between light and temperature during the course of a few days, in which no abrupt changes in intensity occurred. In fig. 7 light- and temperature records are shown for two days, with varying cloudiness. In all curves the temperature appears to follow the light intensity with a rather small time lag. In a laboratory experiment, the temperature of a black

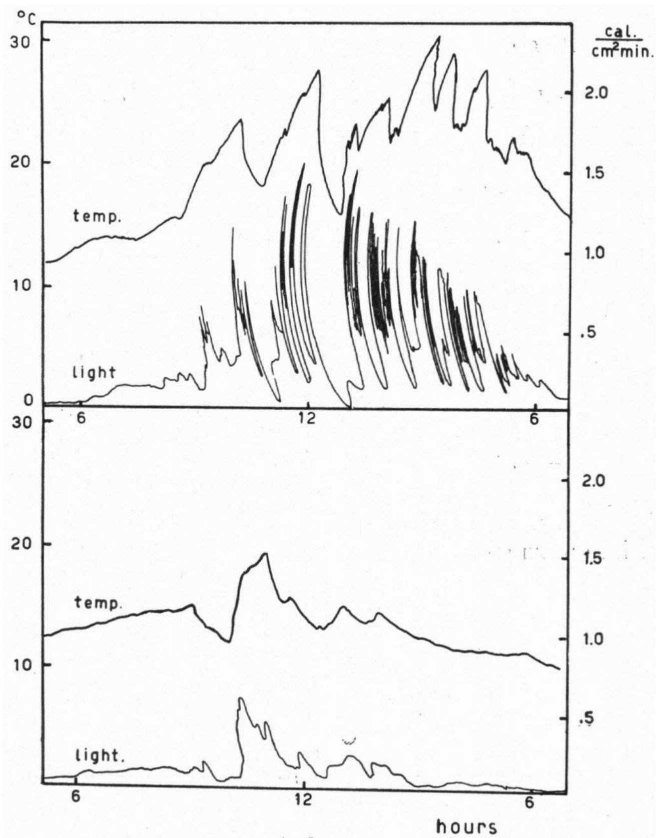

Fig. 7. Recordings of light and temperature on two days, both characterized by varying cloudiness. Top: July 30, bottom: July 31. Total radiation during these days: 199 and 66 cal/cm^2 min. respectively. In the upper figure short term temperature variations as large as 15° C. can be noticed.

liquid in a Kolle dish, if exposed to strong light, increased with a half time of about 25 minutes. Probably this parallelism between light and temperature has favourably influenced algal growth in our experiments.

If growth of the cultures was sufficiently fast, samples for dry weight estimation were taken daily. If growth was slower, this was done every other day, or at still longer intervals. At the moment a sample was taken, exactly half of the culture was discarded and replaced by fresh, sterilized medium. By this procedure, the density of the cultures was always kept sufficiently high to ensure complete light absorption, so that independency of the growth rate of the cellular concentration was obtained.

From the increase in dry weight (expressed in grams per m^2 irradiated area), the efficiency of light utilisation could be calculated

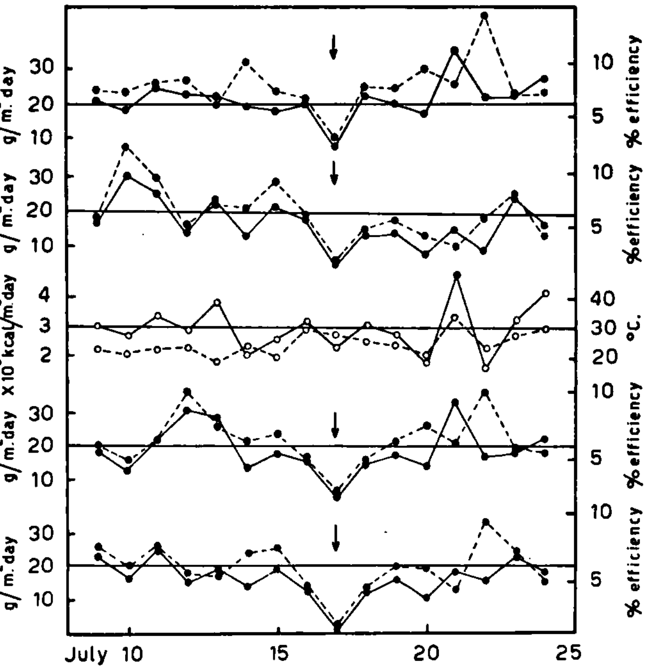

Fig. 8. Daily records from July 9–24, 1953, see text. Middle row (open circles): *total* radiation (solid lines) and average temperature (broken lines). In the other curves solid lines indicate daily yields, broken lines indicate efficiencies. From top to bottom: *Scenedesmus* 3, *Chlorella vulgaris var. viridis*, mixed culture Mt, mixed culture M 5–6–A. At ↓ the CO_2 supply failed.

for each period between two harvests. This calculation was made as described on page 539. We assumed that half of the amount of light measured by the thermopile was of a wavelength between 400 and 700 μ and therefore usable for algal growth, which is a rather conservative estimation.

In fig. 8 the daily amounts of total incident light, the average temperature and the growth of 4 algal cultures are plotted as observed during a few weeks.

Horizontal lines are arbitrarily drawn at a yield value of 20 grams/m^2 day. On the average the daily yields of *Scenedesmus* 3 (top row) surpassed this value. The fluctuations of these yields appear to be roughly correlated with the variation in the amounts of incident light. The efficiency is found to be rather constant (~ 8 %), except for a few high values (upto 15 %), observed on days characterized by low radiation values. *Chlorella vulgaris var. viridis* (second row), a strain, which in the laboratory grew best at lower temperature (cf. fig. 1), indeed showed good yields during the first week characterized by low average temperatures. Yields decreased when the average temperature increased. Culture Mt (fourth row) contained

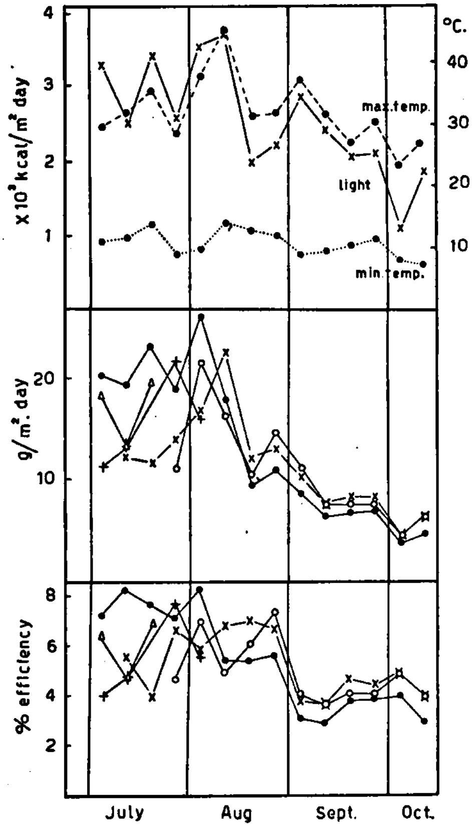

Fig. 9. Weekly averages from July to October 1954. Top section: solid line: incident light, broken line: maximum temperature, dotted line: minimum temperature. Middle section: yields observed for a few strains. Bottom section: efficiency of light utilisation as calculated from the above data. Cultures were initially inoculated with: ● *Scenedesmus* 3, ○: *Chlorella Tx 1105*, ×: *Chlorella vulg. var. vir.*, +: mixed culture 5–6–B, △: mixed culture 5–6–C.

a number of strains preselected at different temperatures in the thermostats, its growth curve resembles that of *Scenedesmus* 3 and follows the light curve still closer. Culture M 5-6 A (bottom row) contained a number of strains, preselected at 20° C. and showed lower yields on the average.

The growth of quite a number of strains was compared in exposures, which generally did not last longer than a few weeks, since cross contamination of the cultures could not be excluded definitely.

Algae, characterized by good growth and high yields, were e.g. *Scenedesmus* 3, *Chlorella* Tx 1105 and 14–10 (Myers strains [4]), *Chlorella* 7, *Chlorella* 19 and a few strains we newly isolated from tropical material.[1] Again the differences between "good" strains were only slight. The extreme variability of Dutch weather did not allow too detailed studies under outside conditions.

Fig. 9 shows weekly averages of the same type of data as given in fig. 8. Instead of the mean, the daily maximum and minimum

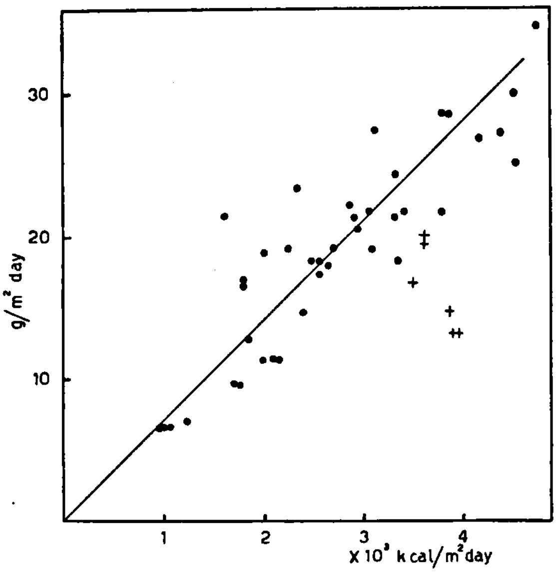

Fig. 10. Daily yields of *Scenedesmus* 3 during July and August 1953, plotted against the corresponding amounts of total radiation. Crosses represent harvests during about a week, during the first two days of which the maximum temperature of the culture surpassed 50° C. Growth rate was slowly resumed after this. The straight line indicates an efficiency of 8 %.

temperatures are given in this figure. The maximum temperature appears even more closely correlated with the light intensity, as was the mean temperature (cf. fig. 8). The minimum temperature was rather constant and low during the whole period and the often quite large differences with the maximum temperature are noteworthy.

For 3 cultures the yields and efficiencies were observed during the whole growing period. The yields of two mixed cultures, exposed during a few weeks, are plotted in addition. As also fig. 8 already showed, such cultures did not prove to be superior to monocultures and they were not used further.

[1] Additional strains with favourable light and temperature characteristics could be isolated from crude material kindly collected by Dr F. K. Vaas in Indonesia.

The curves of fig. 9 indicate high yields (up to 25 g/m^2 day) and efficiencies in the order of 6–8 % during July and the first part of August. The highest yields were observed in the first week of August, characterized by high radiation and high temperature. At the end of the second week of August, however, the maximum temperature in most cultures surpassed 50° C., which led to a decrease of the yields and bleaching of the cells. Growth was resumed only slowly. Unfortunately the solar radiation remained on a low level after this recovery period and yields as high as before were not recorded again during the latter part of the experimentation period. During September and October, a much lower and about constant efficiency of about 4 % was found. This general trend to decline during the later part of the growing season is not only shown by the yields but by the growth efficiency as well and is quite remarkable. It was found in earlier work in much the same way and will be further discussed by one of us [2]. Still, the observed yields and efficiencies at all times were considerably higher than the ones we observed earlier [1] p. 55. In fig. 10 the daily yields observed during July and August for the culture of *Scenedesmus* 3 are plotted against the total radiation. The straight line represents an efficiency value of 8 %, and serves as an index only.

The more or less linear relationship between daily yield and quantity of light uptil high values of both is striking. It may be due, at least partly, to the correlation of light intensity and temperature in the vessels. We hope that further experiments now under way will contribute to a more complete understanding of the complicated relationships between the effects of light intensity, temperature and suspension density under the conditions of these experiments.

ACKNOWLEDGEMENT

We want to express our appreciation to Miss C. Janse and Mr. A. J. Luitingh for diligent technical assistance throughout this work.

SUMMARY

Various strains of algae were tested under conditions of high light intensities at different temperatures. Differences in growth rate were studied and the better strains selected. By prolonged exposures it was tried to further adapt promising strains to such conditions. No increase of the light saturation level of growth could be noticed after seven months exposure to intensities of 0.25 cal/cm^2 min. On the other hand, for several strains an exposure during some weeks appeared to suffice for adapting the cells to a given temperature.

During the summer of 1953 growth yields of various promising strains were studied in small outdoor culture devices. Average yields of 20 g/m^2 day and efficiencies of light energy conversion of 8 % were observed during July and August. Later in the season both yields and efficiencies declined. These figures imply that during the

whole growing period the observed yields were considerably (about twofold) higher as those found in our earlier algal culture experiments.

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