ON THE MASS CULTURE OF ALGAE. III. LIGHT DIFFUSERS; HIGH VS LOW TEMPERATURE CHLORELLAS[1, 2]

JACK MYERS & JO-RUTH GRAHAM

DEPARTMENTS OF BOTANY & ZOOLOGY, UNIVERSITY OF TEXAS, AUSTIN

The present work is introduced by a previous report (4). The yield of algae under sunlight irradiance is limited by the characteristic of light-saturation. The Chlorellas and all other algae studied to date become light-saturated at values of irradiance far less than that of full sunlight. The resulting efficiency of light utilization is considerably lower than that achievable under low incident irradiance. For mass culture under sunlight illumination attempts to increase yield become attempts to minimize or circumvent the limitations due to light-saturation. The present work is concerned with two possible approaches: A, the use of a cone diffuser as a physical means of light attenuation and B, the use of an alga with a higher temperature optimum and a presumed higher irradiance for light-saturation.

METHODS

We have chosen to study yield (mg/day) in cultures of Chlorella maintained under controllable conditions in the laboratory. Most of the methods used have been described in detail (4). Continuous light from a tungsten lamp, filtered through water and copper sulfate, was presented to the open horizontal surface of a culture as an approximately collimated beam of irradiance equivalent to full sunlight. The culture vessel was a flat-bottomed glass cylinder of 67 mm ID containing 1,010 ml of algal suspension at a normal working depth of 267 mm. It was aerated with 5 % CO_2 in air and stirred just sufficiently to prevent cell sedimentation. Further details of the culture vessel and subsequent modifications are presented in figure 1.

The information sought required comparison of cultures, or series of cultures, each illuminated at approximately the same incident irradiance but differing in terms of one defined variable. Each culture was operated under a chemostat (6) system of a constant rate of dilution with fresh medium and an equal rate of withdrawal of algal suspension. Such management provided a steady-state system similar to that which would be a method of choice in any practical large-scale culture. Unfortunately, the low specific growth rates of algae make the chemostat system sluggish in reaching a steady-state and in practice each culture had to be maintained for 7 to 12 days.

DIFFUSING CONE

One attack upon the problem of light-saturation is to find some physical method of attenuating the high irradiance of sunlight without energy loss, i.e., by spreading it out over a greater area as viewed by the cells of the culture. Of the several methods which have been suggested (3, 9) the most attractive is the use of diffusing cones held base-up and projecting into a deep culture. The culture vessel was designed to compare yields in a culture with and without a diffusing cone. In initial control experiments without use of the cone it became apparent that yield was dependent upon cell concentration. The preceding paper (4) describes yield as a function of cell concentration and therefore provides the control or base line for study of effects of adding the diffusing cone.

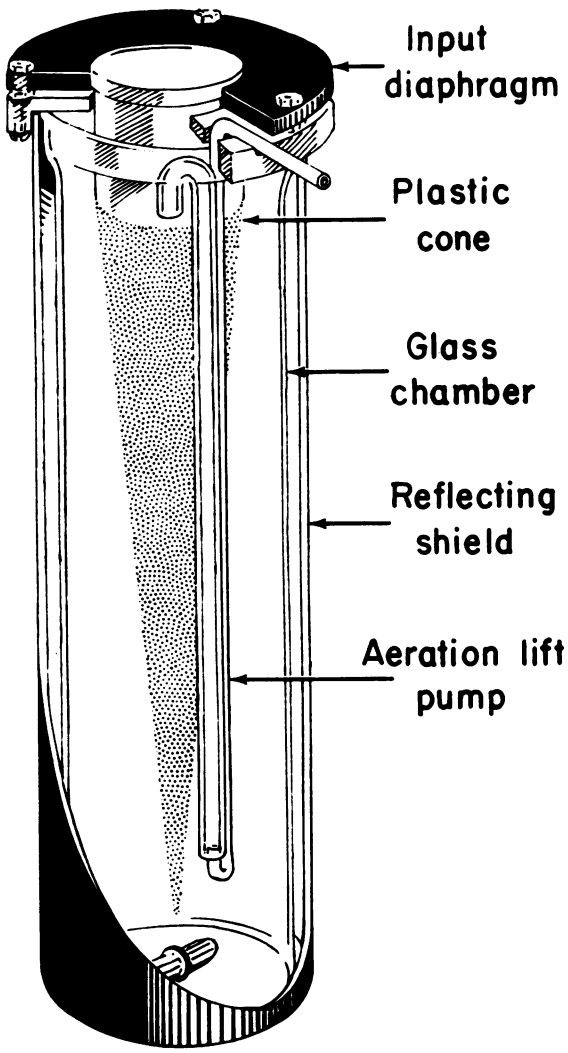
Our first cone was turned from a solid lucite rod, leaving a very finely turned thread to give a diffusing surface. The diameter of the base was 50 mm and the height of the conical portion was 244 mm. The cone was held in an opaque diaphragm of 50 mm diameter. The diffusing conical surface for light output had an area of 192 cm^2 as compared to a base input area of 19.6 cm^2. The intent was to achieve a 10 : 1 attenuation of input irradiance. We attempted to obtain a profile of the output irradiance over the surface of the cone but were able to do this only in a relative fashion. When immersed in water and illuminated through the base most of the light emerged from the lower third of the cone.

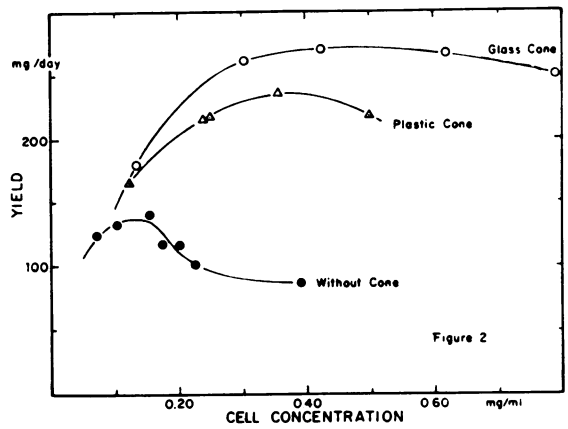
Since an algal suspension is itself a diffusing layer it appeared that an inverted cone might give similar results even with a clear surface. Accordingly, our second model was a hollow glass cone. It was held under an opaque diaphragm of 47 mm diameter in order to restrict the input beam to the ID of the top opening. The OD was 50 mm and the height of the conical portion was 227 mm. The light input area was 17.35 cm^2 and the output area 178 cm^2.

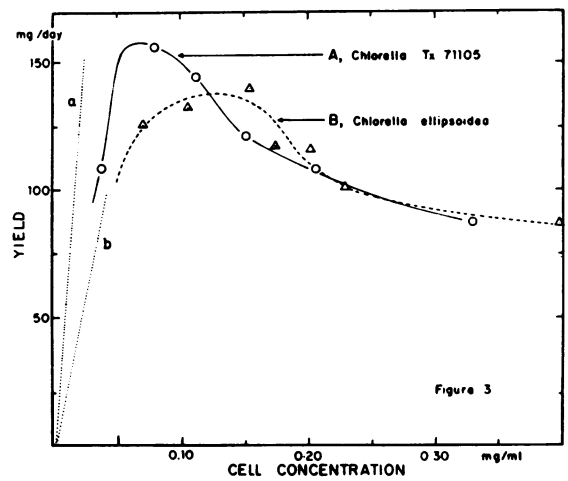
The arrangement of the cones in the culture chamber is shown in figure 1. By use of the cones the culture volume was decreased by about 150 ml from the original volume of 1,010 ml.

[1] Received December 19, 1960

[2] This work was supported by a grant from the Rockefeller Foundation.







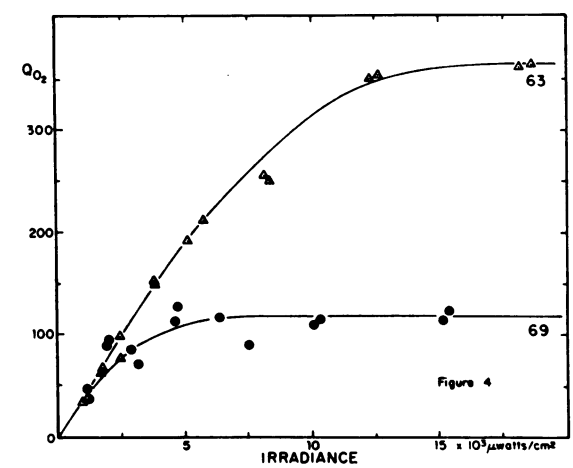


FIG. 1 (*left*). The growth chamber with plastic cone in place. The cutaway drawing shows details of the cone, the top input diaphragm, the glass chamber, and the surrounding reflecting shield. The inserted glass tubes made a lift pump delivering aerated suspension to the top of the culture with minimum splashing.

FIG. 2 (*top right*). Effects of the plastic and glass cones. The lower curve, showing performance without the cone, is taken from the preceding paper (4) with data plotted only for the last seven experiments. *Chlorella ellipsoidea* at 25° C.

FIG. 3 (*center right*). Comparison of yields without a cone for Chlorella Tx71105 at 35° C (A) and *Chlorella ellipsoidea* at 25° C (B). The dotted curves (a) and (b) represent the respective limits imposed by maximum specific growth rates taken as 6.4 days^{-1} for Chlorella Tx71105 (a) and 3.0 days^{-1} for *Chlorella ellipsoidea* (b).

FIG. 4 (*bottom right*). Rate of photosynthesis, Q_{O_2} in μl/mg-hr, vs. irradiance as measured in Warburg buffer 0.190 M $KHCO_3$ plus 0.010 M Na_2CO_3 for cells from cultures 63 and 69 of Chlorella Tx71105 at 35° C.

TABLE I

SUMMARY DATA

EXPT.	IRRADIANCE kcal/day	CELL CONC mg/l	YIELD mg/day	EFFICIENCY* %	SPECIFIC GROWTH RATE** day^{-1}	RATE RESPIRATION μl O_2/mg-hr	CHLOROPHYLL CONTENT %
Chlorella ellipsoidea at 25° C; *plastic cone; input area* 19.64 cm²; *volume* 850 ml							
51	14.5	0.122	166	6.2	1.6	11.6	2.7
52	14.2	0.239	217	8.3	1.1	7.7	4.4
55	13.8	0.251	218	8.6	1.0	5.8	3.7
54	14.0	0.357	236	9.1	0.78	3.9	4.2
53	13.8	0.494	219	8.6	0.52	...	4.8
Chlorella ellipsoidea at 25° C; *glass cone; input area* 17.35 cm²; *volume* 856 ml							
57	12.9	0.137	180	7.5	1.5	9.4	2.7
56	13.3	0.305	262	10.6	1.0	4.5	4.1
59	13.2	0.424	270	11.1	0.74	3.6	4.3
58	13.3	0.620	266	10.8	0.50	2.7	4.9
60	12.8	0.784	251	10.6	0.37	1.8	4.6
Chlorella Tx71105 *at* 35° C; *without cone; input area* 19.64 cm²; *volume* 1010 ml							
65	13.7	0.038	108	4.3	2.9	...	...
70	13.6	0.080	156	6.2	2.0	18.2	...
66	14.0	0.112	144	5.6	1.3	16.7	...
63	13.8	0.152	121	4.7	0.79	12.1	...
67	14.4	0.207	108	4.1	0.53	8.8	...
69	14.2	0.330	87	3.3	0.27	7.4	...

* Yield × 0.0054/irradiance; heat of combustion of cells produced taken as 5.4 cal/mg.
** Yield/vol. × cell concentration.

Summarized data obtained with the cones are presented in table I [cf. (4), table II] and the yields compared to those previously obtained without the cone are shown in figure 2. Two advantageous effects of the cones are clearly evident. First, maximum yield or efficiency in utilization of input light is increased about twofold. Second, the optimum cell concentration is displaced toward higher values at which harvesting becomes more economical in terms of volume of suspension to be processed.

USE OF HIGHER TEMPERATURE ALGA. All of the experiments reported above were done with *Chlorella ellipsoidea* Gerneck at 25° C, which is close to the optimum temperature. A second series of experiments was designed to make a comparative evaluation of an alga of higher temperature tolerance. For this purpose we chose Chlorella Tx71105 (8) maintained at a temperature of 35° C. Culture management in all other respects was similar to that used in the preceding report (4) and without introduction of a cone.

The results summarized in table I and figure 3 provide comparison of performance of the two strains at temperatures close to their respective optima. Chlorella Tx71105 at 35° C gave a maximum yield about 15 % higher and achieved at lower cell concentration. Auxiliary measurements of characteristics of the cells and the cultures of Chlorella Tx71105 were attempted but were less satisfactory than those previously reported for *Chlorella ellipsoidea*. Our lead sulfide cell was more difficult to use at the higher temperature and the characteristic of light penetration into the culture could not be determined satisfactorily. Chlorophyll analyses were attempted but proved unsatisfactory for this strain because of incompleteness of extraction by boiling methanol.

Irradiance curves for the harvested cells were measured in a light beam of the same spectral character used for the cultures. The single light beam available required use of the Warburg carbonate-bicarbonate buffers and repeated measurements on a single vessel at randomly ordered values of irradiance. In spite of lack of precision, which reflects known limitations in the procedure, the irradiance curves followed the same trend and demonstrated the same phenomenon previously observed with *Chlorella ellipsoidea*. Two curves presented in figure 4 were chosen from the set because of their greater reliability; each curve contains data from duplicate experiments on different days. The curve for culture 69, describing cells grown at high cell concentration and low average irradiance per cell, shows a low irradiance for light-saturation and a low maximum rate of photosynthesis.

DISCUSSION

The experimental conditions were purposely chosen to study the special or limiting case of an algal culture under continuous sunlight irradiance. Evalu-

ation in terms of practical application requires additional consideration of limitations in the experimental conditions used.

DIFFUSING CONE: The results demonstrate a twofold increase in yield and efficiency of input light utilization by a diffusing cone of arbitrarily chosen geometry. As applied to the practical case of a large culture under diurnal solar illumination there are opposing factors which would enhance or reduce the observed gain in yield. Obviously the gain will be reduced under diurnal sunlight since the most serious effects of light-saturation occur only during a portion of the day. On the other hand a large culture, having its surface covered with close-packed cones, would have little loss of light from the sides of the culture and could take more complete advantage of the cone principle. We have noted previously (4) the consequences of the unmeasured light losses which occurred from the sides of our culture vessel (edge effects). These are considered significant even though minimized by a surrounding reflecting shield. We failed in attempts to obtain a quantitative measure of these losses and can only record that they appeared considerably larger with the cone than without it.

The term "diffusing cone" is intended in a generic sense to describe a static optical device for distributing the incident surface irradiance over a larger area within a deep culture. The choice of optimum geometry has not been examined. For a large culture the use of pyramids rather than cones would allow closer packing and complete use of the surface. And it is not clear that either pyramids or cones should have straight sides for optimum effects. For attainment of maximum yield the intent is only that the incident light be so distributed that no cell of a culture is ever light-saturated. Visual inspection of our experimental cones showed that the cone surface did not radiate uniformly. Hence the desired 10:1 attenuation was not achieved and some areas of light-saturation did occur.

In short, the observed twofold gain in yield by use of the cone was obtained in spite of increased edge losses and in spite of lack of attainment of uniform light distribution. It would appear that practical test of the diffusion cone principle is merited for mass cultures under diurnal sunlight illumination. For the proposed use of algal cultures as gas exchangers in space vessels the diffusing cone principle should be an even greater advantage.

HIGH-TEMPERATURE STRAIN: The small gain in optimum yield observed with Chlorella Tx71105 at 35° C as compared with *Chlorella ellipsoidea* at 25° C is considerably less than might have been expected. Furthermore, even this small advantage was obtained only at low values of cell concentration. These findings suggest re-examination of the premises made for use of high temperature algal strains in mass culture.

In early attempts at mass culture the algae chosen were *Chlorella pyrenoidosa*, *Chlorella ellipsoidea*, and species of Scenedesmus, all with temperature optima at about 25° C (low-temperature strains). The first isolation by Sorokin (8) of a Chlorella with a temperature optimum as high as 39° C provided a new and important alga for mass culture. Subsequently a variety of strains of Chlorella, Scenedesmus, and other genera with temperature optima in the range of 35 to 40° (high-temperature strains) have been isolated and used in various laboratories (9). The higher temperature optima presented an important practical advantage in minimizing the requirement of cooling of cultures under sunlight illumination. Of this advantage no question can be raised. It was envisioned also that the higher rates of metabolism and growth of the high-temperature strains would allow greater yields in mass culture. The two possible bases for such an expectation should be re-examined.

As compared to the low-temperature strains, the high-temperature strains have maximum specific growth rates about threefold higher. Specific growth rate, k, is defined as $dN/N\,dt$ where N is a measure of cell quantity and t is time. For purposes of cell production in mass culture the criterion of performance is production rate or yield which for a given culture is dN/dt or kN. In general yield will be referred to some measure of culture size such as illuminated area giving dimensions such as g/m^2-day. However, for the present work the culture geometry and illumination were held constant and comparisons of yield can be made in terms of cell mass produced per day.

From experience with other microorganisms it might be reasoned that the higher maximum specific growth rates of the high-temperature strains should result in higher yield. However, the specific growth rate of an alga is governed also by the effective irradiance per cell and its maximum value is attained only at light-saturation. All the cells of a culture cannot be maintained at light-saturation without considerable transmission and loss of light. Furthermore those cells which are maintained at light-saturation cannot be working at maximum efficiency. All experimental work has confirmed the early observation of Ketchum et al (1) that "the theory of the optimum catch" applies to algal cultures, that maximum yield is attained under conditions at which the cells are growing at less than their maximum specific growth rate. Maximum yield is to be expected at a cell concentration such that most of the incident light is absorbed. It follows that yield is governed by efficiency of light utilization rather than by specific growth rate.

A second reason to anticipate advantage of the high-temperature strains under sunlight illumination lies in an expected higher point of light-saturation. For example, suppose that the point of light-saturation for the high-temperature strains lies at about 1,200 ft-c as compared to about 400 ft-c for the low-temperature strains. Then under the high illuminance of sunlight the losses attributable to light-

saturation will be lower for the high-temperature strains. The basis of this argument has been presented in the preceding paper (4).

Unfortunately the expectation of a consistently high point of light-saturation for Chlorella Tx71105 is denied by the experimental findings. The illuminance curves obtained by Sorokin (7) make it clear that cells grown at 39° C and 60 ft-c reach saturation at an illuminance about one-fourth as great as for cells grown at 400 ft-c. The data of figure 4, though incomplete and far less precise, confirm Sorokin's observations. The same effects are observable, even under an incident irradiance of full sunlight, when cell concentrations are so high as to give a low average irradiance per cell. It is now abundantly clear that a fixed irradiance curve of photosynthesis is not an intrinsic character of an algal species (2, 4, 5, 8).

The data of figure 3 show for Chlorella Tx71105 at 35° C a maximum yield only about 15 % higher than for *Chlorella ellipsoidea* at 25° C. This is a significant advantage but not one which would by itself dictate the choice of alga for mass culture. No greater advantage in yield under steady-state conditions has yet been shown for any high-temperature strain under any chosen conditions of illumination.

Summary

Yields (mg/day) of two strains of Chlorella were studied in steady-state cultures under a continuous visible irradiance equivalent to full sunlight. For *Chlorella ellipsoidea* at 25° C the maximum production rate was increased about twofold by use of plastic or glass diffusing cones designed to supply the incident energy over a greater surface at reduced irradiance. The diffusing cone principle appears to be a practicable means of circumventing the limitations of light-saturation under sunlight illumination. In cultures without the cones Chlorella Tx71105 at 35° C showed a maximum production rate only about 15 % higher than that obtained with *Chlorella ellipsoidea* at 25° C. The bases for earlier expectation of much higher production rates obtainable with high-temperature strains of algae have been re-examined.

Literature Cited

1. Ketchum, B. H., Lois Lillick, & A. C. Redfield. 1949. The growth & optimum yields of unicellular algae in mass culture. J. Cell. Comp. Physiol. 33: 267–280.
2. Myers, J. 1946. Influence of light intensity on the photosynthetic characteristics of Chlorella. J. Gen. Physiol. 29: 429–440.
3. Myers, J. 1958. Algal growth: Processes & products. Transactions of the Conference on the Use of Solar Energy, E. F. Carpenter, ed. Vol. iv. Univ. of Arizona Press, Tucson. Pp. 1–11.
4. Myers, J. & Jo-Ruth Graham. 1959. On the mass culture of algae. II. Yield as a function of cell concentration under continuous sunlight irradiance. Plant Physiol. 34: 345–352.
5. Myers, J. & W. A. Kratz. 1955. Relations between pigment content & photosynthetic characteristics in a blue-green alga. J. Gen. Physiol. 39: 11–22.
6. Novick, A. & L. Szilard. 1950. Description of the chemostat. Science 112: 715–716.
7. Sorokin, C. 1958. The effect of past history of cells of Chlorella on their photosynthetic capacity. Physiol. Plantarum 11: 275–283.
8. Sorokin, C. & J. Myers. 1953. A high-temperature strain of Chlorella. Science 117: 330–331.
9. Tamiya, H. 1957. Mass culture of algae. Ann. Rev. Plant Physiol. 8: 309–334.