• • • • • SOLAR ILLUMINATED PHOTOSYNTHETIC GAS EXCHANGER

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INTRODUCTION

During recent years much effort has been expended in attempting to develop principles, techniques, and instrumentation leading to the ultimate goal of utilizing photosynthetic organisms for the support of man in an extraterrestrial environment. Most of the studies accomplished to date have been concerned with single-celled algae as the photosynthetic organism. (1,2,3) All systems studied have one common feature - this is the use of artificial light as the energy source. The incorporation of an artificial source of illumination in a photosynthetic gas exchanger has two major objections. These are: (A) Systems constructed around artificial light are quite large and bulky; and, (B) Electrical energy must be provided to operate the artificial lights.

A considerable amount of work has been accomplished in studying the growth of algae under natural sunlight. (4,5,6,7) However, these studies have not been directed toward possible use of solar illuminated algal cultures in extraterrestrial environments.

A system employing natural illumination will have an advantage in that it will operate at significantly lower power level because the illuminative energy will be "free".

This study was designed to determine if solar illuminated photosynthetic gas exchange systems would be feasible and to provide sufficient data to determine if further consideration of this approach would be warranted.

EXPERIMENTAL PROCEDURE

During the summer of 1960, preliminary investigations were accomplished at the Arctic Aeromedical Laboratory. In this preliminary study, simple culture cylinders (approximately 1.5 liter capacity) of Chlorella in a suitable nutrient media were continuously exposed to the near 24-hours of Arctic summer daylight. The high light intensities did not appear to harm the Chlorella as long as the culture was agitated. Growth rates were consistently high.

Based upon these observations, two types of photosynthetic solar illuminators were designed, constructed, and tested at the Arctic Aeromedical Laboratory during the summer of 1961. One type of illuminator was a hemispherical dome (Fig. 1) and the other was a flat panel (Fig. 2). Four domes and two panels were tested.

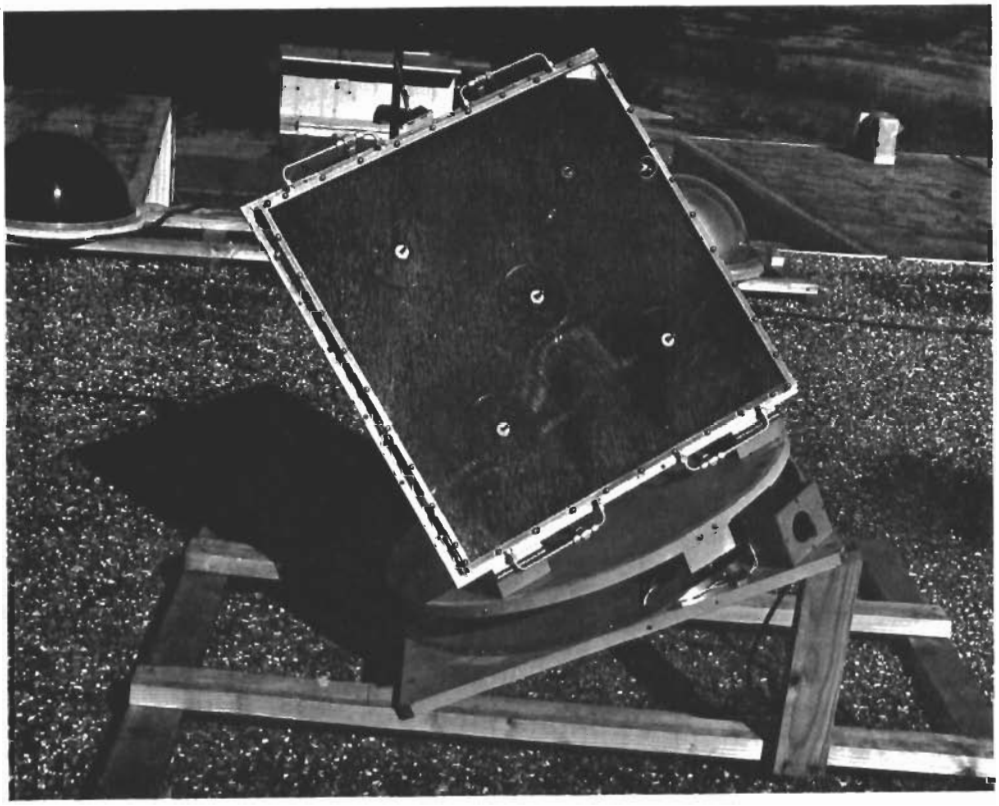
Note: This study was accomplished in part under USAF Contract AF 41(657)389

Figure 1. (Left)

Hemispherical Dome Illumina

Figure 2. (Below)

Flat Panel Illuminator



The domes were mounted in a fixed position, whereas the panels were mounted on tilted motor-driven turntables and remained perpendicular to the sun's rays throughout the day. The surface area of both types of illuminators was $0.5M^2$. Both the domes and the panels were constructed similarly. Each had an inner surface of aluminum and an outer surface of Plexiglas. The space between these surfaces was coupled by 1-inch Tygon tubing to a liquid pump and four liter Wolff bottle containing a sintered glass sparger. The panels were later modified with rotary "scrubbers" as it was observed that one type of alga studied (Chlorella) had a tendency to adhere to the surface of both the front and back plate.

The film depths varied in both the domes and the panels. Two domes had a 2 cm. film depth; one had a 1 cm. film depth; and one had a 4 cm. film depth. Both panels were initially designed with a 2 cm. film depth. One of the panels was later converted to a 1 cm. depth by the addition of a 1 cm. Plexiglas insert which was fixed to the aluminum back plate. The illuminators were mounted on the roof of the Laboratory with the related equipment installed on the upper floor of the Laboratory.

In routine operation, the units functioned as follows: The algal suspension was circulated between the Wolff bottle and the illuminator on the roof. Aluminum coil heat exchangers in the return lines were immersed in constant temperature water baths. A mixture of air and CO_2 was pumped through the sparger in the Wolff bottle. CO_2 concentration was the same for all cultures and varied between 5% and 10%. A schematic diagram of a single unit is shown in Fig. 3.

Two algal species, Chlorella pyrenoidosa, TX 71105 (8) and Synechococcus lividus (9), were studied. They were cultured in tris buffered medium E. (9) Chlorella was cultured at 40°C. and Synechococcus was cultured at 50°C.

Growth was measured by periodic sampling of the suspension and determing optical density on a Klett colorimeter at 540mμ. Samples used for optical density determinations were always diluted to produce readings below 0.25. The reported optical density is the observed value times the dilution factor.

Photosynthesis was measured by two different methods. In the first, the system was closed with a fixed volume (22 liters) of air containing 8% to 10% CO_2. In the second method, the system was closed with a measured volume of air containing 4% to 6% CO_2 in a chain compensated gasometer. Throughout the run, additional CO_2 was added thereby increasing the gas volume but maintaining the internal pressure and the CO_2 concentration. Because of the limited and fixed quantity of CO_2 available, the first method was adequate for only short term studies of one to three hours. The second method was more suited for long term experiments. In both methods, a sample portion of the gas was continuously circulated through a Beckman model F - 3 O_2 analyzer and a Liston-Becker IR CO_2 analyzer. The output of each analyzer was recorded on Brown Strip Chart recorders either intermittently or continuously, as required by the specific test.

The studies were so designed (see Fig. 4) as to obtain certain basic information and to compare:

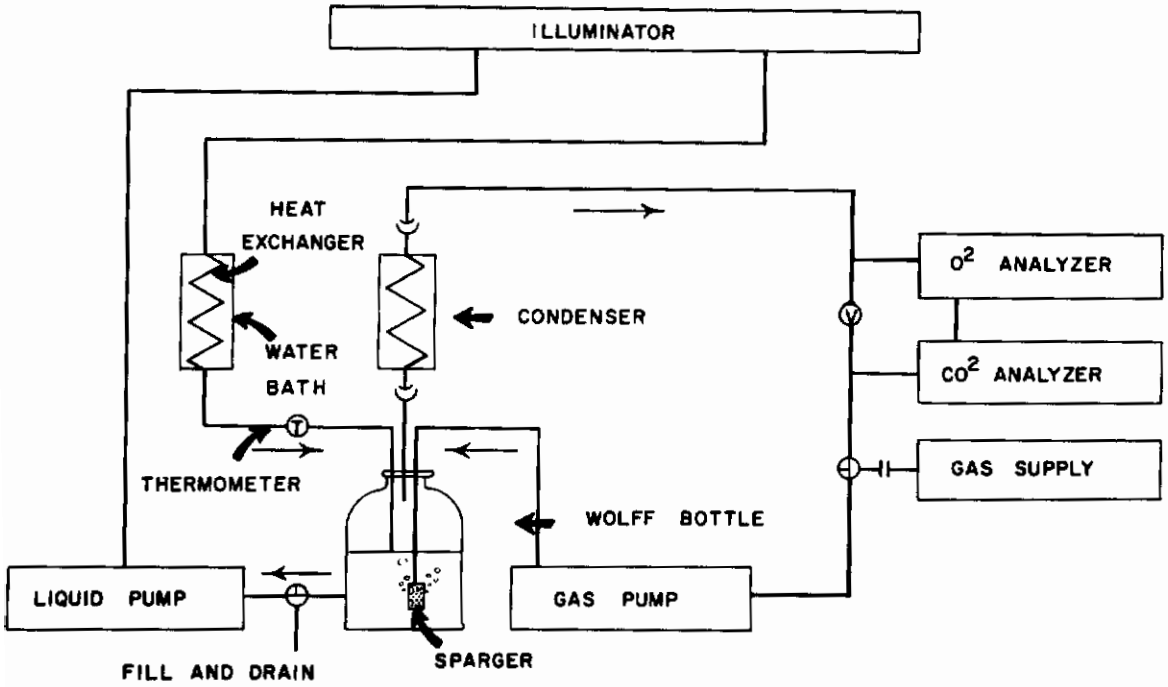


Figure 3. Schematic diagram of experimental set-up.

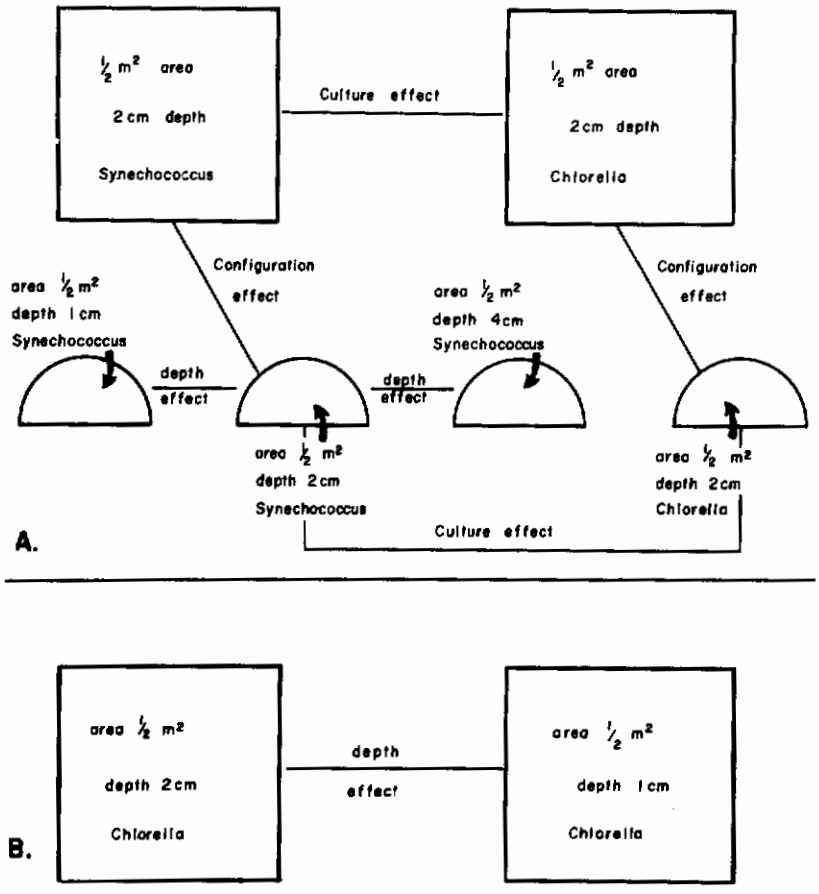


Figure 4. Experimental Design

1. Effect of depth of algal film - a comparison of 1, 2 and 4 cm. film depths was made in the hemispherical configuration.

2. Effect of configuration of the algal film - a flat panel of 0.5 square meter area was directly illuminated by solar light and compared to a hemispherical panel of equal area illuminated partially by direct and partially by indirect solar illumination.

3. Effect of algal culture selection - 2 cm. film depths of Synechococcus lividus, a blue-green thermophilic alga, and Chlorella pyrenoidosa (TX 71105), a green thermophilic alga, were compared in both hemispherical and flat panels.

4. Effect of depth of algal film in panel illuminators of 2 and 1 cm. depth.

RESULTS

A. Growth

Data obtained on 19, 20, and 21 June on the optical density of the four cultures in the hemispherical domes are plotted in Fig. 5 along with a transcription of the incident energy plot obtained from the Epply pyrheliometer. Temperature control of the Chlorella culture (Fig. 5.c.) was not adequate during the first six or eight hours of 20 June and culture temperatures reached 44° to 46°C. The growth data was, therefore, not representative of this culture under the optimum temperature condition of 39° - 40°C. (On 16 June a linear growth rate of 0.040 optical density units per hour was observed over a seven hour period for the 2 cm. Chlorella dome.)

The discontinuities on the bottom two curves are the result of large dilutions with fresh medium at about 1400 hours on 20 June.

Consider Figs. 5.a., 5.b., and 5.d. During the daylight hours of 20 June, these cultures were growing at linear rates indicating a light limited condition. Between 0800 and 1100 all three cultures maintained steady growth rates. In terms of optical density, the rates were 0.0651 O.D. units per hour for the 1 cm. depth culture; 0.0338 O.D. units per hour for the 2 cm. depth culture; and 0.0168 O.D. units per hour for the 4 cm. depth cultures. These optical density values are plotted against the reciprocal of the film depth in Fig. 6. If one considers only that portion of the culture in the illuminator, the volumes of each culture are directly proportional to the culture depth. It is concluded that the absolute production rate of algae was the same in all cultures. In other words, over the range of film depths and densities considered, algal production rate in these units was independent of liquid volume. An entirely similar observation was previously made by Tamiya (10) in artificially illuminated cultures of Chlorella ellipsoidea.

As expected, higher growth rates were observed in the 4 cm. depth culture at low optical density (c.f. Fig. 5d. during the morning of 19 June). Also, the high growth rate of the 1 cm. depth culture was maintained at higher optical densities (Fig. 5.a.).

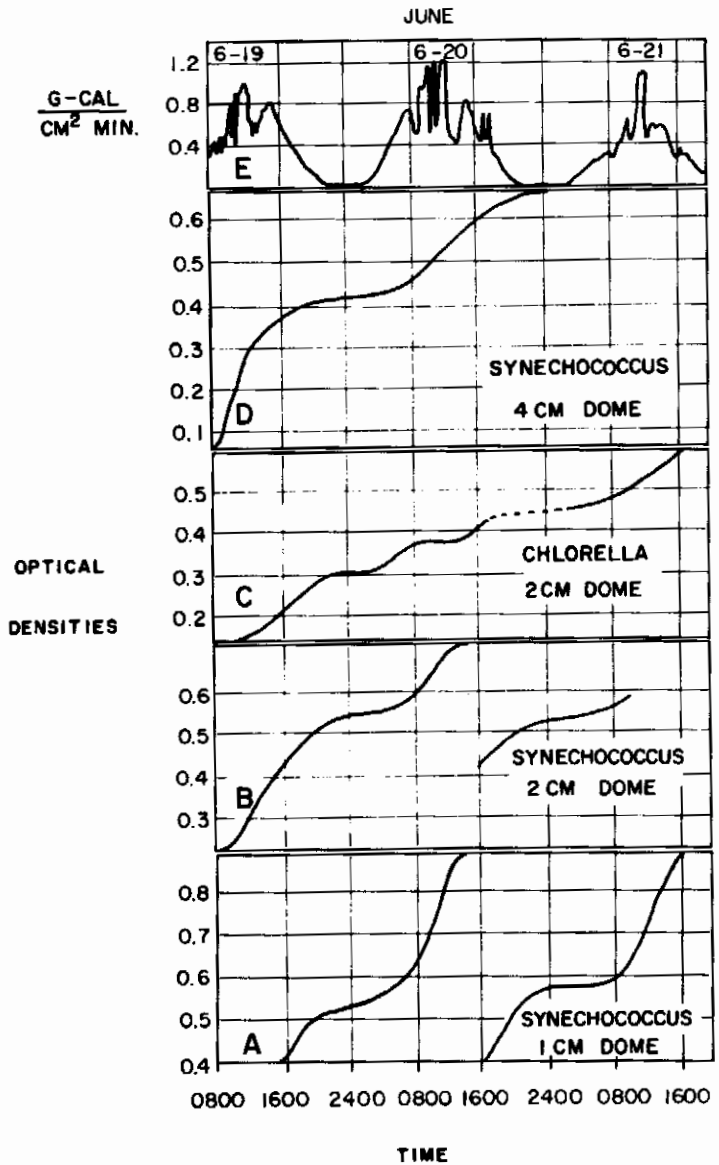


Figure 5. A plot of optical densities of Synechococcus and Chlorella cultures in the hemispherical domes vs time during parts of 19-21 June. Also included is a plot of incidental solar radiation during that period.

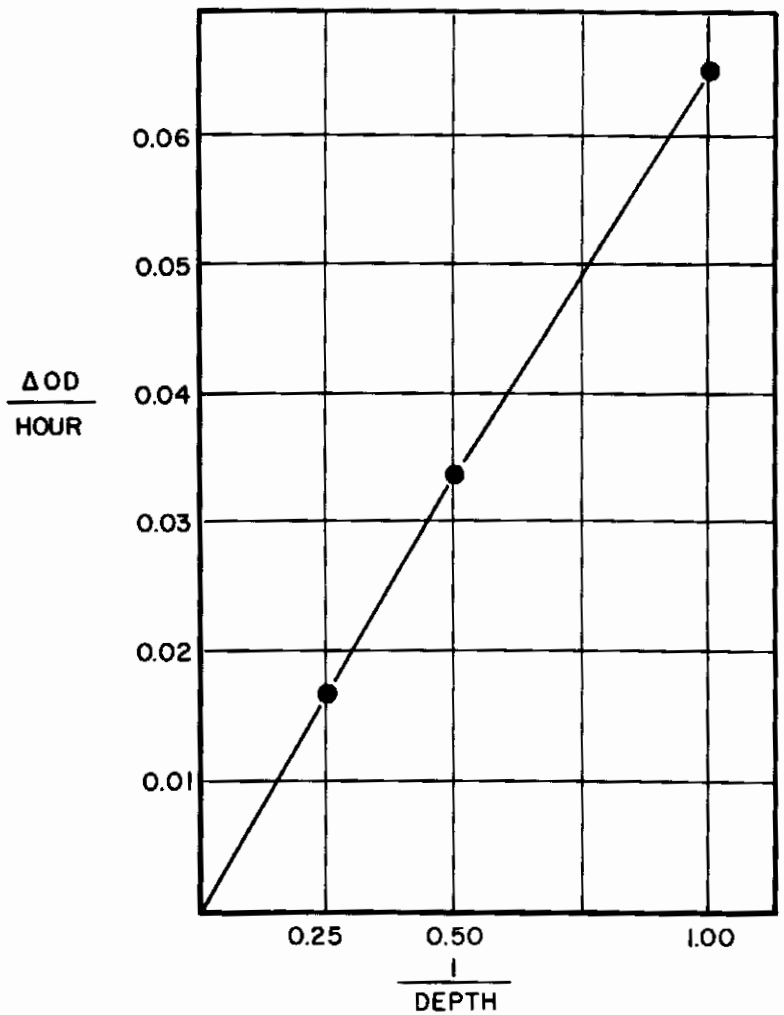


Figure 6.

Growth rate expressed as change in optical density per hour versus the receiprocal of film depth for Synechococcus cultures in hemispherical domes.

In Fig. 7, a comparison is made between the growth rates of Synechococcus in the 2 cm. depth hemispherical dome and in the 2 cm. depth solar oriented flat panel. The maximum linear growth rate observed in the panel was 0.0597 optical density units per hour. This is not quite twice the value of 0.0338 observed in the 2 cm. depth hemisphere, indicating that not only direct solar illumination but incident skylight also contributed to growth in the domes.

Another observation made clear by Fig. 7 is the fact that cultures in the solar oriented panels continued to grow almost until the sun was below the horizon. (The shaded lines on the 0.8 O.D. line indicate periods of near complete darkness.) Furthermore, growth began much earlier after sunrise. The long lag in the solar oriented panel on the morning of 21 June can be attributed to a somewhat low culture temperature (36°C) and to a rather extensive cloud cover which was effective most of the day.

As previously stated, temperature control of the Chlorella cultures failed during the first part of the day on 20 June. The cultures were not returned to an actively growing state until late on 21 June. At the same time, the Synechococcus cultures were put on a periodic dilution regime so that maximum steady state growth rates for Chlorella and Synechococcus in the flat panels under identical conditions of illumination were not obtained. Steady state growth was observed in the Chlorella culture between 0800 and 1600 hours on 16 June and on 22 June. The observed rates were 0.0480 and 0.0435 optical density units per hour respectively. These are representative of the maximum linear growth rates that could be achieved with Chlorella in the solar oriented panels in this kind of experiment.

When comparing growth rates of algae between species, the use of optical density values is misleading because of the different absorption characteristics of the cells. As a matter of fact, the observations on optical density, to a large extent, reflect not so much the absorptivity of the suspension as the light scattering characteristics. Since Chlorella and Synechococcus are quite different in both their absorption characteristic and the size and shape of the cells (which effect light scattering characteristics), direct comparison of optical density defined growth rates is not meaningful.

A better parameter for comparing growth rates is the absolute algal production in terms of dry weight. Unfortunately, dry weight determinations, as obtained in these experiments are not as precise as are the optical density measurements. This is shown graphically in Fig. 8, using the first portion of the upper optical density growth curve taken from Fig. 7.

Corresponding dry weight determinations are included in this Figure. These data are typical of all the comparative O.D. - dry weight data and clearly indicate the relative precision of the optical density parameter as compared to dry weight.

An attempt has been made to correlate all the optical density and dry weight data accumulated in order to arrive at some reasonable conversion factor. All the points have been plotted on correlation charts in Fig. 9. The points are severely scattered for both Chlorella and for Synechococcus. Correlation lines have been drawn visually.

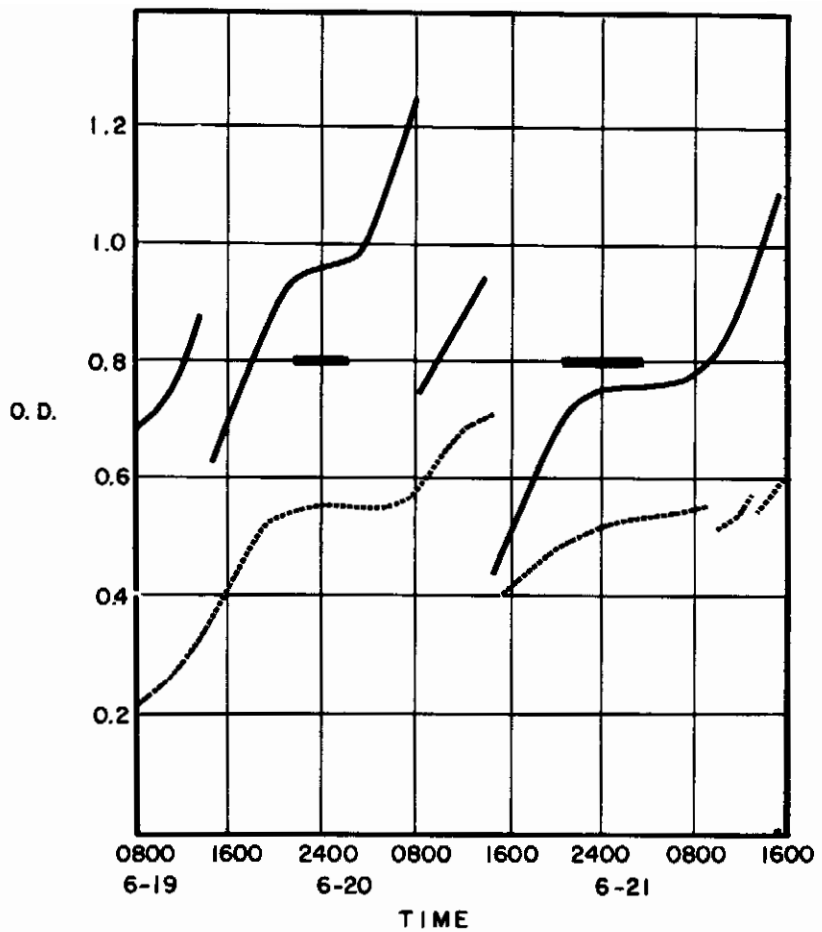


Figure 7. Optical density of 2 cm. depth cultures of Synechococcus in the solar oriented panel (___) and the hemispherical dome (....) versus time. Dark periods are indicated by bars on the 0.8 O.D. line.

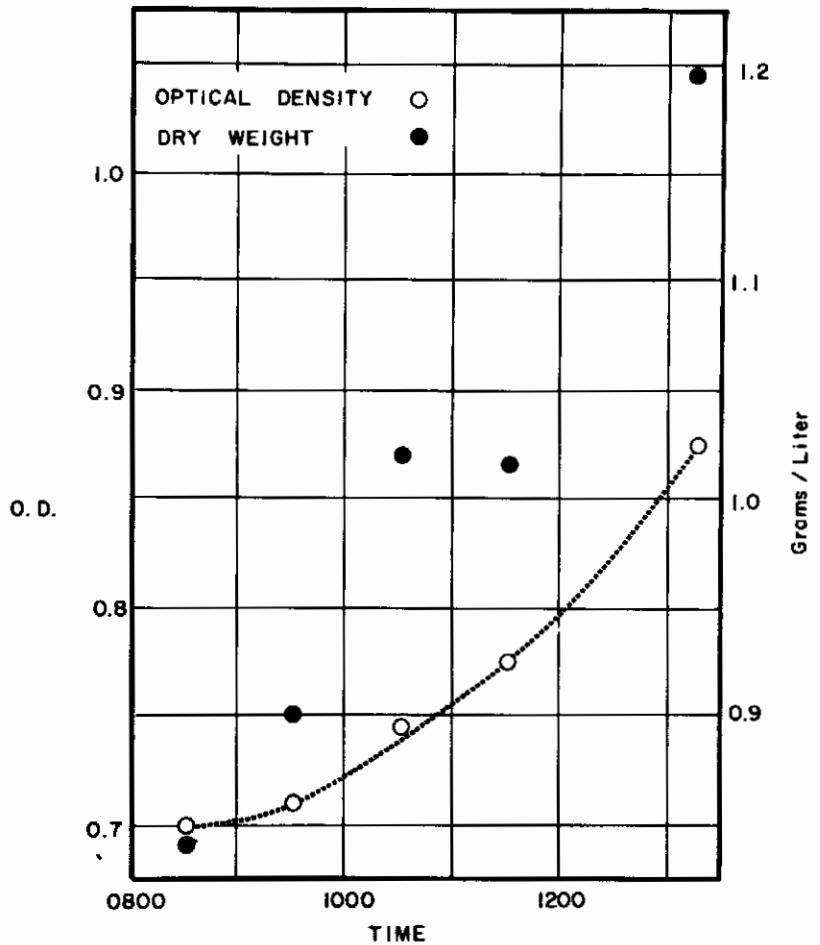


Figure 8. Comparison of optical density points (O - left ordinate) to simultaneous dry weight (O - right ordinate) versus time on a portion of a Synechococcus growth curve.

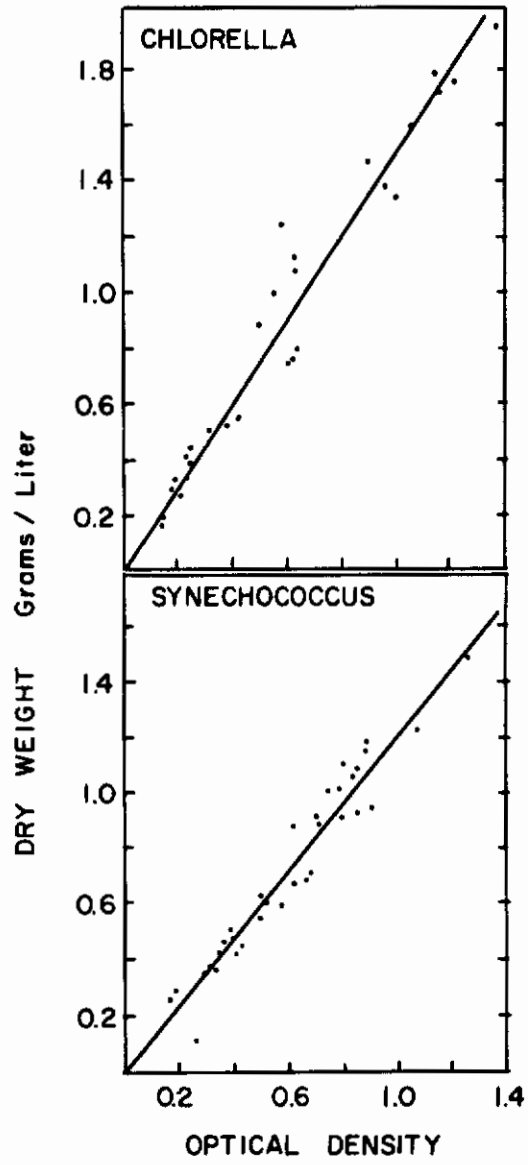


Figure 9. Correlation between optical density and dry weight for Chlorella and Synechococcus cultivated under solar illumination.

By using the correlation charts, it is possible to interpret the optical density growth rates as dry weight growth rates. The approximate comparative values are shown in Table I, using the conversion factors obtained from Fig. 8. These are: For Synechococcus, 1 O.D. unit equals 1.16 grams per liter, and for Chlorella, 1 O.D. unit equals 1.53 grams per liter.

The accuracy of these conversion factors is questionable. The qualitative nature of the observation is clear, however. Per unit dry weight, Synechococcus absorbs more light than does Chlorella when both are grown at sunlight intensities. One of the consequences of this fact is that the Chlorella cultures grew relatively better in the dome than did the Synechococcus culture. The ratio of Chlorella to Synechococcus growth rates in terms of dry weight in the domes (2 cm.) was 1.52. In the flat panels the ratio was 1.06. That is, algal production under near continuous high intensity was about equal for both species; but for lower intensity was about one and one-half times as high for Chlorella as for Synechococcus.

B. Photosynthesis

The primary purpose of these experiments was to determine the maximum oxygen production rates under the various conditions established by the experimental design. It was determined early that the photosynthetic rates observed in the solar oriented panels were significantly higher than those in the domes. Since the growth curves indicated that all cultures were light limited, this was not a surprising observation.

During the limited time available it was felt that the major effort should be concentrated on obtaining an estimate of the maximum oxygen production rate by the solar oriented panels.

The results from two experiments for each panel are tabulated in Table II, along with the estimated assimilatory quotients. Oxygen production was also measured several times in the 2 cm. Chlorella dome and in the 1 cm. Synechococcus dome. Typical data are also contained in Table II (an unidentified restriction in the gas sparger in the line of the 2 cm. Synechococcus dome caused a rather large pressure drop which made it impossible to conduct this type of experiment in that system.) The values shown in Table II are uncorrected for temperature and pressure. The generally low assimilatory quotients may result from errors in estimating CO_2 consumption due to the large buffering capacity of the medium. It is also possible that they are the consequence of an unusually high oxygen evolution rate associated with the high intensity light.

In this connection, it is of interest to examine the ratio between algal and oxygen production rates. The rate of algal production per liter from Table I may be multiplied by the liquid volume (14.16 liters for 2 cm. depth systems and 9.16 liters for the 1 cm. system) to obtain the rate per unit per day. This value is doubled to obtain the production rate per square meter per day. Oxygen production rates have been corrected to STP values and converted from liters to grams. The results are tabulated in Table III.

Because of the possible errors associated with their derivation, the accuracy of the ratios shown in Table III are subject to question. Their qualitative value is of considerable interest, however. The ratio of 2.0 to

TABLE I

TABULATION OF MAXIMUM GROWTH RATES OF SOLAR ILLUMINATED CHLORELLA AND SYNECHOCOCCUS CULTURES UNDER SPECIFIED CONDITIONS (see text)

Cultures & Conditions	O.D. Units Per Day	Grams/Liter Per Day
1 cm. dome Synechococcus	1.56	1.81
2 cm. dome Synechococcus	0.810	0.94
4 cm. dome Synechococcus	0.404	0.47
2 cm. panel Synechococcus	1.43	1.66
2 cm. panel Chlorella	1.15	1.76
2 cm. dome Chlorella	0.960	1.47

TABLE II

OXYGEN PRODUCTION RATES OBSERVED IN SOLAR ILLUMINATED CULTURES OF CHLORELLA AND SYNECHOCOCCUS

Date	Conditions	Oxygen Production liters per day per sq. meter of illuminated surface	Approximate AQ
6-19	Chlorella 2 cm. panel	70.6	0.64
6-20	Synechococcus 2 cm. panel	71.2	0.73
6-22	Chlorella 2 cm. panel	84.0	0.62
6-22	Synechococcus 2 cm. panel	81.6	0.58
6-16	Chlorella 2 cm. dome	68.6	--
6-22	Synechococcus 1 cm. dome	54.0	--

TABLE III

RELATIONSHIP BETWEEN OXYGEN PRODUCTION AND ALGAL PRODUCTION

Condition	Maximum 0_2 production grams/m^2 - day	Maximum algal production grams/m^2 - day	grams 0_2 / grams algae
Chlorella panel	107	50	2.14
Synechococcus panel	105	47	2.23
Chlorella 2 cm. dome	88	41.6	2.12
Synechococcus 1 cm. dome	68	33.2	2.05

2.2 grams of oxygen per gram of algae produced is somewhat higher than the values of 1.5 to 2.0 generally found in the literature (11, 12).

The low assimilatory quotients and high O_2 to algae ratios taken together imply that the algae produced under these conditions should be highly reduced. It is clear that a better understanding of these observations will depend on an elementary analysis of the cells. It would be of interest to make a special study of the fat content of the cells since it is implicit that the relative amount of lipids should be unusually high.

Inasmuch as the photosynthetic data presented above were obtained in rather short term experiments (1 - 3 hours), with limited amounts and continuously decreasing concentration of CO_2, and with a single panel depth (2 cm.), further studies without these limitations were accomplished.

Following modification of the panels to increase turbulence, comparative studies were made of 2 and 1 cm. film depth and with the system closed with a gasometer. Chlorella was used exclusively in these experiments. No attempt was made to hold the algal suspension to a steady state density as it was desired to determine the most effective algal density.

Sequential experiments were accomplished with each experiment starting within the approximate range of O.D. where the preceding experiment was terminated. Figure 10 is a plot of the data (oxygen production versus optical density) from four of these experiments, each extending over a period of six hours.

The optimum ranges of cell concentration for growth and photosynthesis of Chlorella in these panels cannot be accurately stated from the available data. The best estimate appears to be about 0.85 grams (dry weight) per liter in the 2 cm. panels and about 1.6 grams per liter in the 1 cm. panel.

In order to more clearly indicate the relative rates, the data from these experiments have been plotted as oxygen production and growth (optical density) versus time in Figure 11, and are summarized in Table IV.

The maximum oxygen production rate was observed in the six-hour experiment AII. The Chlorella culture in the 2 cm. panel produced 2.26 liters of oxygen per hour, equivalent to approximately 109 liters per square meter per day. Based on the requirement of 600 liters per day for one man, the illuminated area per man thus would be 5.6 square meters. At a film depth of 2 cm. this represents a liquid volume in the illuminator of 112 liters.

In the 1 cm. panel the maximum oxygen production rate was 1.94 liters per hour (experiment BI). Based on this figure, the one man illuminator surface area would be 6.5 square meters. However, the required illuminator volume would be only 65 liters. The weight savings associated with the development of thin film illuminators is thus apparent.

C. Discolorization

It was anticipated that the solar oriented cultures would have a tendency to bleach due to solarization. As far as could be determined, neither Chlorella

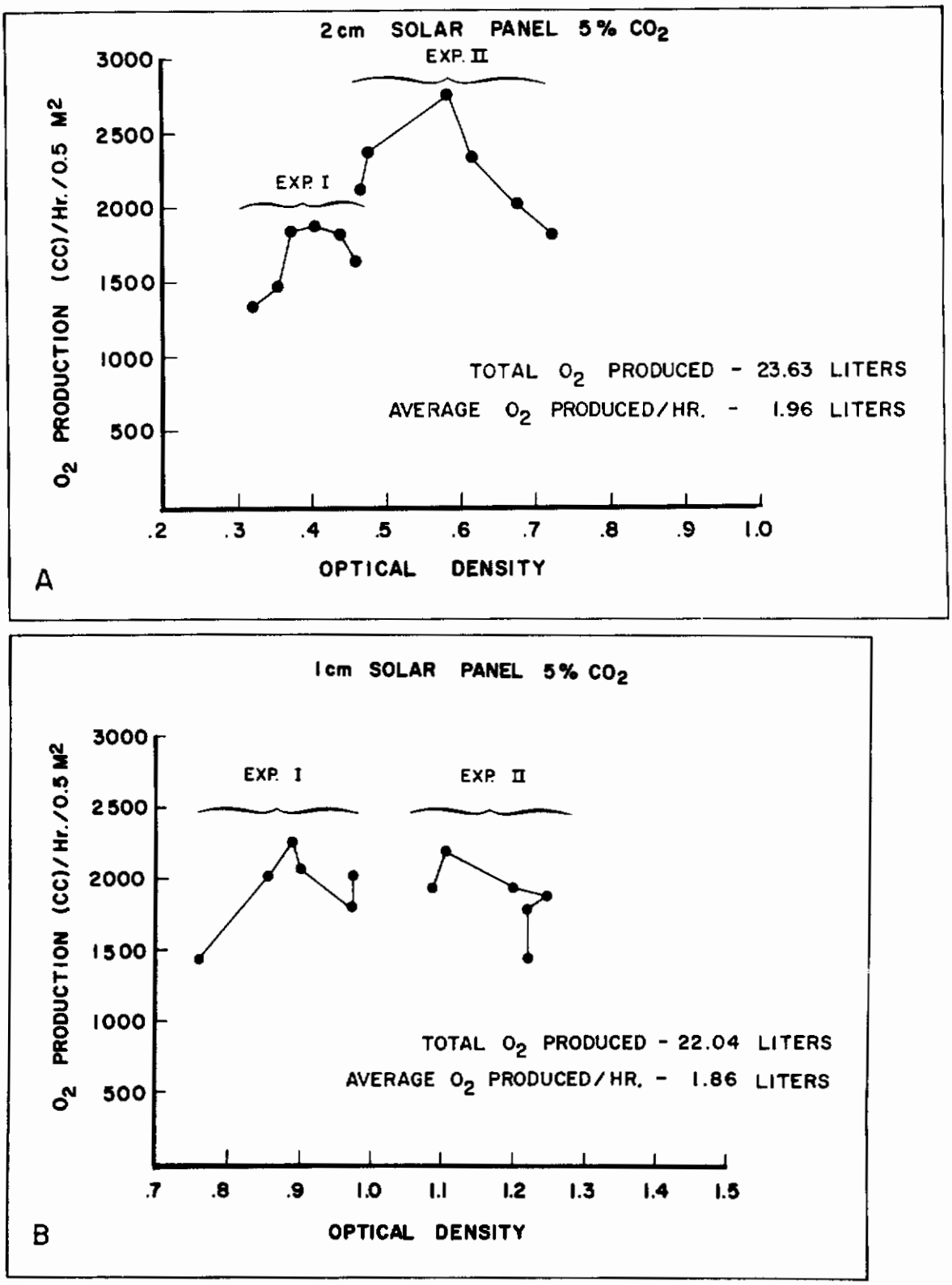


Figure 10. O_2 production by Chlorella as a function of optical density.
A. 2 cm. depth panel. B. 1 cm. depth panel.

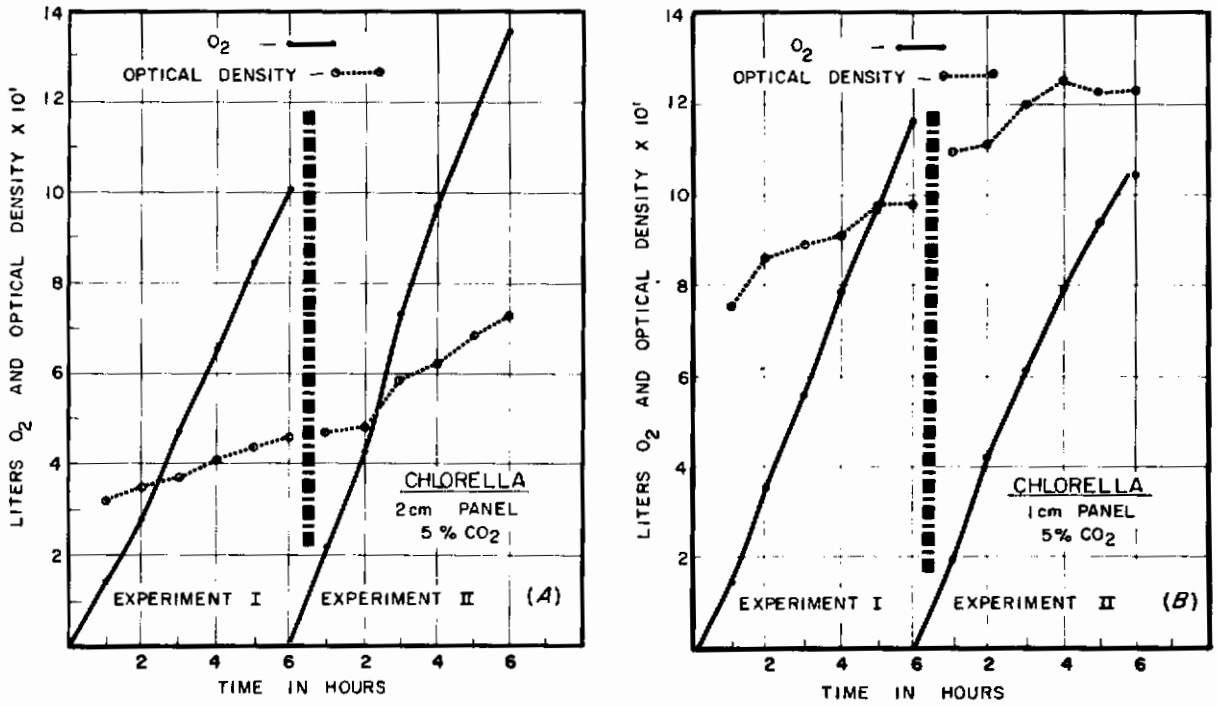


Figure 11. O_2 production and growth (optical density) by Chlorella as a function of time. A. 2 cm. panel. B. 1 cm. panel.

TABLE IV

SUMMARY OF SOLAR PANEL DATA

Experiment No.	AI	AII	BI	BII
Film Depth	2 cm		1 cm	
Panel Volume	10 liters		5 liters	
Light Intensity (range - fc)	6,500 - 10,000		5,000 - 10,000	
Light Intensity (mean - fc)	8,000		8,200	
CO_2 Concentration (range %)	3 - 6		4.5 - 7.2	
CO_2 Concentration (mean %)	4.7		5	
Duration (hours)	6	6	6	6
Δ O.D./hr (mean)	0.028	0.053	0.044	0.028
O.D. (range)	.32 - .45	.46 - .73	.75 - .94	1.0 - 1.25
O_2 Production/hr (mean - liters)	1.67	2.26	1.94	2.02

nor Synechococcus suffered solarization at sunlight intensities, even at relatively low densities.

A phenomenon was observed with the Synechococcus cultures which was apparently a function of high light intensity. We prefer to describe the phenomenon as "discolorization" rather than solarization since it appears to be different at least with respect to the pigments involved. Discolorization is not well understood. The phenomenon has been encountered in laboratory cultures grown under high light intensity when the temperature is below the optimum value or when the supply of carbon dioxide is low. The cultures under these conditions lose the rich blue-green color and appear olive-green in color. The species can apparently survive and grow even in the discolored state but growth and photosynthesis may proceed at a lower rate.

Discolorization occurred in the Synechococcus cultures early in the experiments reported here when culture temperatures ranged between 45° and 50°C. It was corrected apparently by maintaining culture temperatures between 52° and 54°C. whenever the cultures were exposed to direct sunlight.

The discolorization apparently results from the loss (or lack of synthesis) of the blue pigment, phycocyanin. The evidence for this is contained in Fig. 12. The absorption spectrum for blue-green cells grown in a culture chamber illuminated by fluorescent light is compared to that of cells grown in the solar illuminated panel before discorlorization was corrected. These spectra were made on a Beckman model DK recording spectrophotometer. The effect of light scattering was minimized by using opalescent difusers in the light path of both cell suspension and water blank cuvettes (13). This Figure also contains a plot of the relative absorption spectrum of extracted phycocyanin. (All curves drawn to arbitrary density values.) The coincidence between the difference in the algal absorption spectra and the spectrum of phycocyanin leaves little doubt that the discolored cells are lacking in this characteristic blue pigment. Some loss of chlorophyll is apparent in the spectral differences at 460 mμ and 660 mμ but the major difference in the spectra appears related to the lack of phycocyanin. It will be important to determine the factors controlling discolorization and whether discolored cells are characterized by lower photosynthetic and growth rates than are cells with a "normal" complement of phycocyanin.

DISCUSSION

In this study we have primarily considered growth and photosynthesis in two different configurations of solar illuminated systems. These were the hemispherical dome and the flat panel. Under the conditions of the experiment, it was observed that the solar oriented panels were more effective than the domes. The average surface area normal to the sun's rays was less than half that of the panels so these results are not surprising.

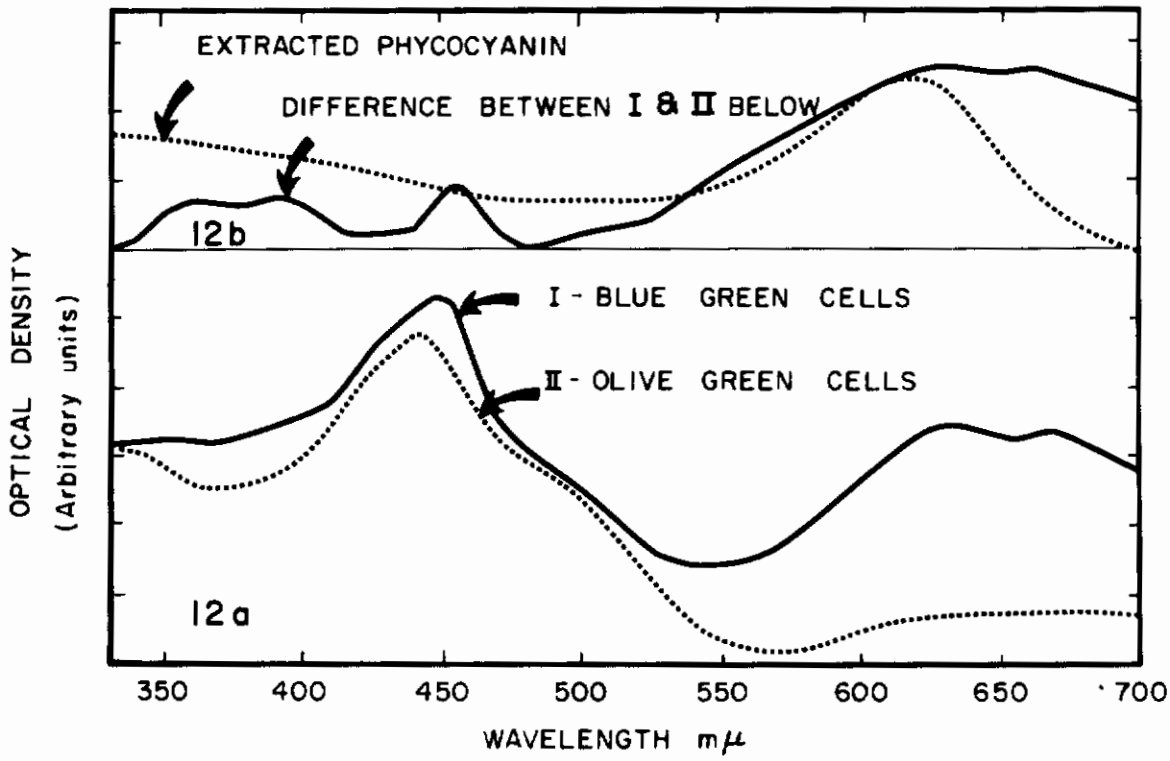


Figure 12. A. Absorption spectra of "normal" (I) Synechococcus cells compared to that of "discolored" (II) cells.
B. Difference between AI and AII compared to absorption spectrum of extracted Phycocyanin.

When our studies on this subject were initiated, it was considered that the arctic regions would be the ideal geographic location of the earth in which to accomplish such studies. Our reasoning was based solely upon the seasonal availability of near 24 hours of high light intensity. Lengthy periods of unclouded skies were a rarity during the 1961 summer season and, as a consequence, long term experiments with relatively steady state light levels were impossible. The cultures, however, were continuously exposed to sunlight of varying intensities.

During the period of these studies, no apparent changes in the Chlorella were observed. The Synechococcus did, however, change from its normal blue-green color to a more olive-green shade. The cause of this and whether or not it is detrimental to the activities of the algae must await further experimentation.

Apparently the continuous exposure to surface levels of infrared radiation was not harmful to either species. Since the polyacrylic plastic (Plexiglas) is nearly opaque to ultraviolet radiation, nothing was learned about the effect of this component. The higher levels of both long and short wave length radiation in a true extraterrestrial environment might be highly detrimental.

As stated previously, the oxygen production rates were measured in cultures which were light limited. The flow of the suspension in the panels and domes was in the laminar flow regime. The pumping velocity even in the modified panels was too low to induce turbulent flow. The rates observed thus probably represent the maximum production rates for solar illuminated cultures in static or non-turbulent films.

In this study, we have considered film thicknesses of 1 cm. minimum. As shown in Table IV, and Fig. 11, oxygen production as a function of illuminated surface area was similar in both 2 cm. and 1 cm. panels. The calculated weight of the algal suspension required to provide the oxygen for a man was reduced by approximately one-half in the 1 cm. system.

The linearity apparent in Fig. 6 further implies that film depths of less than 1 cm. can be accommodated without loss of efficiency. This is important because it bears directly on the liquid volume and, therefore, the weight of photosynthetic gas exchangers. Furthermore, the volumetric flow rate required to achieve turbulent flow is proportional to the cross sectional area so that thinner films result in lower pumping rates.

It is considered highly possible that under proper steady state conditions, i.e., culture density, light intensity, gas exchange, and turbulence, that film thickness of less than 1 cm. but high density will produce as much oxygen as a thicker film. If this be true, then the weight of the algal suspension can be reduced considerably from the calculated levels, thereby reducing the overall weight of a functional system.

Our original estimate of the value of the domes was modified early in the program. They were, however, valuable in the determination of the effect of film depth on growth rates; and they served to emphasize what

might be called the superior light utilization capability of the optically less dense Chlorella.

The ability of both species to grow well and produce oxygen at high rates in direct solar light in thin films at reasonably low densities is the important observation of these studies. The Chlorella had a tendency to "stick" on surfaces of the system. The Synechococcus did not show these tendencies. This emphasizes the requirement for development of "clean" algae strains.

It was not the intent of this study to develop a solar illuminated gas exchanger system but to determine whether or not such systems were within the realm of feasibility. If one is justified in extrapolating data obtained on the earth's surface to extraterrestrial environments, it is considered that the data presented herein conclusively proves the feasibility of utilizing solar illumination as the energy source for operation of a particular type of photosynthetic gas exchangers. The practicality of such a system must be determined by further studies. Such studies must consider overall design and weight of the hardware; long term operations of the system under the simulated extraterrestrial conditions of (a) radiations to include both ionizing and non-ionizing; (b) steady state densities and light level; (c) temperature control; (d) zero gravity; (e) indirect illumination to reduce or negate the possibility of penetration of the system by meteorites; (f) nutrient requirements of the algae; (g) degree of turbulence; (h) algal strain; and (i) film depths.

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