ABSTRACT

An algal culture apparatus was designed to fit inside the 2-1/2-in. bore of a large electromagnet capable of providing fields exceeding 100,000 gauss. Photosynthesis and respiration rates of Chlorella pyrenoidosa (7-11-05) were determined by analyzing the input and effluent gases for oxygen. Two experiments were conducted: (a) the effect of 10,000 gauss on photosynthesis, and (b) the effect of 100,000 gauss on respiration. In neither case did the magnetic field have any effect.

PROBLEM STATUS

This is an interim report; work on this problem is continuing.

AUTHORIZATION

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EFFECT OF HIGH MAGNETIC FIELDS ON RESPIRATION AND PHOTOSYNTHESIS IN ALGAE

INTRODUCTION

A systematic study of factors controlling oxygen production by algae has been in progress at NRL for several years. Of primary interest to this Laboratory is the possibility that mass cultures of algae may be adapted to submarine use for the absorption of CO_2 and the production of oxygen; but much of the information gained is pertinent to the problems involved in space flight. In a previous report (1), the algal system as a regenerative oxygen source was shown to be highly reliable, and the estimated volume of the equipment was at least competitive with systems now used in nuclear submarines, i.e., electrolytic O_2 production and absorption of CO_2 by monoethanolamine. The principal objection to the use of algae is the excessive electrical power requirement of the high-intensity incandescent lamps needed in mass cultures. If the algal system is to occupy minimum volume, light sources providing very high intensities must be used and with the use of present equipment, the expenditure of approximately 32 kw per man would be necessary.

Pending the development of more efficient light sources, research on factors affecting algal growth is continuing. Magnetic fields are of interest because an algal photosynthetic gas exchanger used in space flight may be subjected to intense, naturally occurring magnetic fields or to magnetic fields generated within the spaceship as a protection against small meteorites. Many effects attributable to magnetic fields have been exhibited by plants and other biological systems. Davis' review (2) contains many references of this type, and some examples from this and other sources are shown below.

Brown (3) found that snails and worms responded to a weak magnetic field (5 gauss) and also distinguished direction in it. Stronger magnetic fields make it more difficult for the mechanism which resolves the direction of the field to perform its function. Audus (4) subjected roots of Lepidium sativum seedlings to a magnetic field, and in a large number of experiments there was a marked growth curvature along the magnetic gradient. He detected a slight shift of starch grains down the magnetic gradient also, suggesting some cause and effect relationship.

More pertinent to the study reported here is the research by Commoner et al. (5). Chloroplasts from White Burley tobacco exhibited, in the presence of light, paramagnetic resonance similar to that of a standard free radical such as diphenylpicrylhydrazyl. This indicates that the unpaired electrons present in illuminated chloroplasts have gyromagnetic properties which are indistinguishable from those of the great majority of the organic free radicals investigated thus far. The authors state that no metabolite product of photosynthesis is known to contain unpaired electrons, therefore the paramagnetic resonance effect must be attributed to either or both an excited state of chlorophyll and oxidation-reduction intermediates.

In the study reported here, oxygen production of the algal culture was measured to determine whether the magnetic field used had any effect. This measurement provides an index of physiological activity sufficiently sensitive to detect changes in a matter of minutes, whereas several hours may be required if suspension density is the criterion.

EXPERIMENTAL

Source of Algal Cells

The organism used was the Sorokin strain of Chlorella pyrenoidosa (7-11-05), known for its fast growth rate (6). Suspensions were harvested from a mass culture unit operated continuously at a given light intensity, dilution rate, and CO_2-input rate. The excellent reproducibility of this unit is described by Hannan and Patouillet (7). A freshly harvested suspension was diluted with an equal volume of culture medium to give approximately a 1-percent suspension, as determined by hematocrit, and 400 ml of this were placed in the culture apparatus. The composition of the culture medium is shown in the Appendix of this report.

Culture Apparatus

Figure 1 is a diagram of the apparatus used in this study. The culture chamber, 16 in. long, was designed to fit inside the 2-1/2-in. bore of a large electromagnet which is a facility of the Solid State Division, NRL. Stainless steel nipples extended out of the core sufficiently to permit the connection of flexible stainless steel tubing extending from a centrifugal pump which circulated the suspension. Maximum magnetic flux was imposed at the middle of the illuminated portion of the culture chamber which consisted of three concentric glass tubes held in grooved Lucite blocks. The outer diameters of the tubes were 16, 39, and 59 mm; water was passed through the inner annulus to dissipate the heat from a high intensity light source* suspended within the inner tube, and the algal suspension was circulated through the outer annulus, 7.5 mm deep. The light source was 8 in. long and the algal suspension filled the outer annulus to this height.

Two brass tubes within the water annulus served a double purpose, i.e., as tie rods to hold the assembly together and as water inlet and outlet. The bottom of each tube was filled with metal and brazed closed, then the outer surface was threaded to accommodate a hex nut. The portions within the annular space were perforated, one at the bottom and one at the top, to allow the passage of cold water. A permanently attached flange at the top of each tube, above the Lucite block, provided tension on the assembly when the brass nuts at the bottom were tightened. Small O-rings at top and bottom prevented leakage of the cooling water past the tubes.

A swirling motion of the suspension within the annulus was provided by its circulation through a plastic tube bent at a 90-degree angle. The temperature of the suspension was maintained between 36° and 38°C by a thermoregulator probe which extended into the outlet of the pump. When the temperature reached 38°C, a relay activated a solenoid valve, permitting ice water to flow through the cooling jacket surrounding one of the flexible steel tubes.

Extending from the top of the unit was a brass tube, 4-1/2 mm I.D., which served as a gas inlet and outlet. The inlet gas passed through a 3-mm-O.D. polyethylene tube which extended through one arm of a glass Y-tube, then through the brass tube, and down to the bottom of the suspension where it was released. Enough clearance remained between the polyethylene tube and brass tube to allow the effluent gas to pass upward and through the other arm of the Y-tube; it was necessary however to wrap a heating tape around the Y-assembly to prevent condensation, otherwise the cyclic formation and disruption of the condensate within the narrow opening of the brass tube caused an erratic behavior of the flow meter used to measure the input gas rate.

*General Electric Quartzline lamp, 1500 watts.

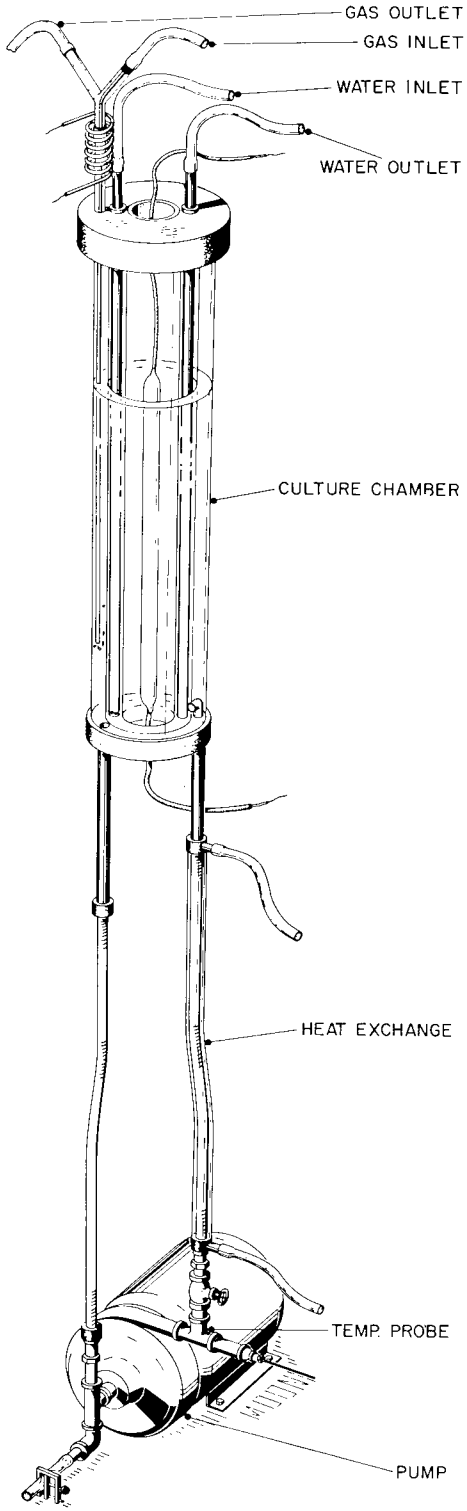

Fig. 1 - Equipment used in magnetic field study

CO_2-Air Mixture

The input gas used in this study was a mixture of 1.60 percent CO_2 in air, dispensed from a large cylinder at a rate of 500 ± 5 cc per minute. At the light intensity used, this rate was sufficient to saturate the suspension with CO_2, i.e., the algae were getting all the CO_2 they could use. In experiments such as this, it is desirable to use as low a flow rate as possible, thereby maximizing the difference between the O_2 content of the inlet and outlet gases.

Light Intensity

Throughout these experiments 150 v were applied to the light source, providing an intensity of 45,000 foot-candles at the surface of the cooling jacket. The intensity was measured by a Weston light meter with suitable neutral density filters.

Magnet

A description of the magnet is contained in Ref. 8. It is sufficient to say here that magnetic fields greater than 100,000 gauss are obtainable with this equipment, and at this high field strength the power consumption of the magnet is approximately 2.5 megawatts.

Procedure

Two hundred ml of freshly harvested suspension were diluted with an equal volume of culture medium and placed in the culture apparatus. With the light source on, the CO_2-air mixture was passed through the suspension and then a portion was diverted through a drying column before entering a Beckman oxygen analyzer.* Oxygen production was computed by multiplying the flow rate by the difference between the O_2 content of the inlet and outlet gases. Consecutive measurements of the inlet and outlet gases minimized any possible error caused by "drifting" of the analyzer. After approximately an hour of operation, the culture unit had reached a steady state and constant oxygen production was obtained, at which point the magnet was turned on.

Two studies were made: (a) oxygen production (photosynthesis) was determined at a magnetic field strength of 10,000 gauss for a 40-min period, and (b) oxygen uptake (respiration) in the dark was measured at 100,000 gauss. In the latter study, several light and dark sequences were applied to determine whether any cumulative effect could be detected.

It was not possible to study the effect of magnetic fields higher than 10,000 gauss on photosynthesis. Even at this field strength the filament of the light source vibrated severely because of the 60-cycle ac passing through it, and after 40 minutes it snapped. At a field strength of 25,000 gauss, the filament snapped after about 2 min. There was no limitation, of course, on the field strengths used during the respiration study.

RESULTS

Each experiment was designed to determine the performance of the culture with and without the magnetic field, but the comparison suffered slightly because of the difficulty in maintaining the culture at 38°C when the magnet was energized. Thermal equilibrium was established between the culture unit and the wall of the magnet during the hour or

*Model F-3, Beckman Instruments Inc., Pasadena, California.

more which elapsed from the time the light source was first turned on until the magnetic field was applied. However, cooling water was passed through the magnet for approximately 10 min before it was energized, and this provided enough heat loss across the 1/16-inch air gap between the culture and the wall of the magnet to significantly affect the temperature. When current was passed through the magnet, some of this heat loss was compensated by joule heat, but the overall drop in temperature affected the performance of the cells. Therefore the oxygen production just prior to the time the magnet was energized was lower than it had been during the long "warm up" period.

Oxygen Production at 10,000 Gauss

Figure 2 contains a record of the oxygen production during the course of the experiment. In previous experiments with this culture apparatus, the oxygen production was 120 cc per hour, and the culture was producing this amount, within the error of reading the instrument* for approximately 40 min before the magnetic field was applied. The oxygen contents of the inlet and outlet gases were 20.70 and 21.10 percent respectively.

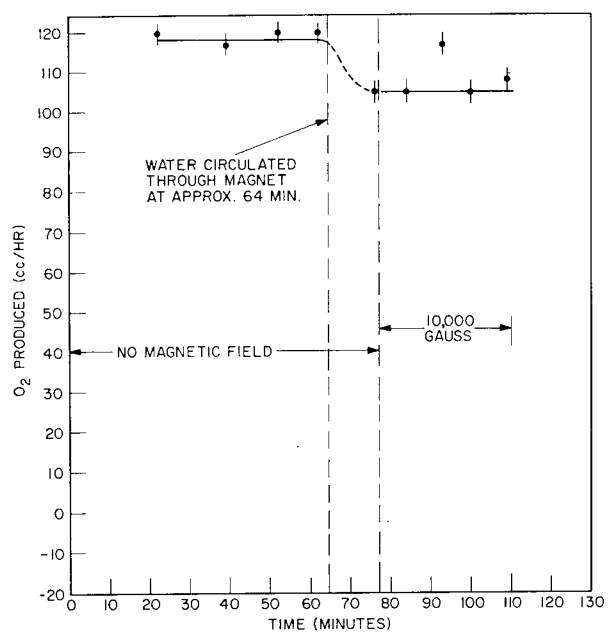

Fig. 2 - Effect of 10,000 gauss on photosynthesis

Just prior to the time the magnet was energized, the oxygen production dropped to 105 cc per hour because of the cooling effect of water passing through the magnet. At a field strength of only 10,000 gauss the cooling effect would be appreciable, because the water flow rate through the magnet is sufficient to keep it cool even when field strengths ten times greater are used. As shown in the graph, the O_2 production while the magnet was on coincided in three determinations out of four with the last reading before the magnetic field was applied. It is concluded, therefore, that the magnetic field had no effect on O_2 production.

*At the flow rate used the discrepancy would account for 3 cc per hour.

Respiration at 100,000 Gauss

Figure 3 is a representation of the results obtained when successive determinations of the respiration rate, with and without a high magnetic field, were made. Some of the reasons for the chronology of conditions imposed are outlined in the Discussion of this report, but the results may be summarized as follows: (a) the respiration rate was the same during two exposures to 100,000 gauss as it was before any field had been applied, and (b) oxygen production subsequent to respiration during exposure to the magnetic field was the same as before when allowance is made for the cooling effect explained in the preceding paragraph.

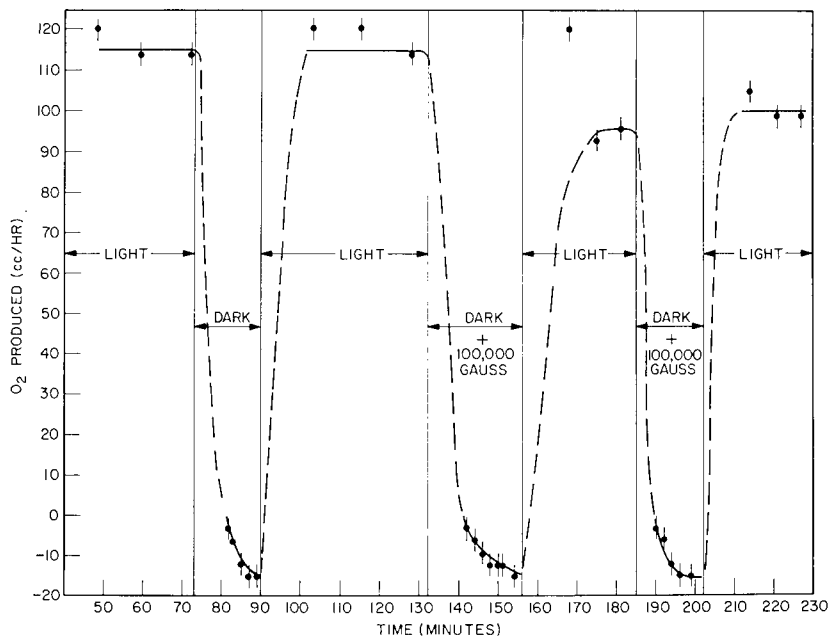

Fig. 3 - Effect of 100,000 gauss on respiration

It is concluded that respiration is unchanged by exposure to 100,000 gauss and it is felt that oxygen production subsequent to the magnetic field treatment is not affected. Perhaps some subtle change might be delineated with considerably more experimentation.

DISCUSSION

Interpretation of Results

The work by Commoner et al. (5) on the magnetic resonance of illuminated chloroplasts indicates strongly that free radicals are formed; it is assumed that the chloroplasts of the algae used in this study would form free radicals when illuminated. However, the magnetic fields used did not affect the performance of the cells, either in their respiration or photosynthesis, although they probably contained free radicals. A serious distortion of the highly oriented structure of the chloroplasts should alter their functioning; however, oxygen production was not affected, and it is concluded that chloroplast tissue is disturbed more by thermal motion than by a magnetic field acting on a number of free radicals within it.

It should be mentioned that there is a time lag of perhaps 6 min or more before a measurable change in the oxygen content of the effluent gas can be detected after some experimental variable is introduced. The effluent must pass through about 6 ft of tubing before a portion of it is diverted through a drying tube and then into the oxygen analyzer; and after the gas in the analyzer has reached the same composition as that coming from the culture unit a significant time is required for the oxygen analyzer to register accurately. Despite a constant flow of input gas, this time lag between the change in culture conditions and equilibration of the effluent with the oxygen analyzer is variable because the fraction of the effluent diverted to the analyzer is not always constant. This is mentioned to prevent any misinterpretations of the data on the respiration studies when varying times to reach maximum oxygen uptake were required after the light source was turned off.

Notes on Respiration Studies

Past experience has shown that the oxygen uptake by cells in the dark is constant for only a short time, not longer than approximately 30 min. Therefore, it was not feasible to determine, in one period when the cells were in darkness, their respiration with and without the magnetic field. After the first respiration determination the culture was allowed to photosynthesize for more than 40 min to replenish the food reserves drawn on by the cells during their time in darkness. This cycle was repeated a second time to provide a better comparison between the respiration found initially and that with a magnetic field.

Occasionally after a period of darkness the cells will exhibit a burst of oxygen production which lasts for just a short time. This may have accounted for the one determination of 120 cc per hour which was recorded after the second period of darkness. While 120 cc was to be expected under normal conditions, it would be a high reading compared to those when the temperature of the suspension was lower than normal. During the time the light source was turned off, no cooling water was passed through the annulus adjacent to the light source; otherwise the temperature of the suspension would have dropped to 15°C. During the respiration studies the temperature of the suspension was approximately 35°C.

Duplication of Effect of Cooling

In a separate experiment after the culture unit was removed from the magnet, oxygen production was determined at the normal temperature setting of 38°C, and then the thermoregulator was adjusted to provide several lower temperatures for the suspension. At 28°C the oxygen production had dropped to 96 cc per hour, compared to the normal of 120, but at 33°C there seemed to be no change from the normal output. This experiment was not quite a direct counterpart of the one conducted in the magnet because in this case the cells already in the dark were cooled the most; i.e., the cooling water was applied to the stainless steel tubing, whereas when the culture unit was in the core of the magnet the cooling was provided around the lighted portion. However, there is good correlation of a lowered oxygen production with a decrease in temperature, 120 cc at 38°C compared to 95 cc at 28°C. The temperature of the water leaving the magnet was 21°C. The demands made on the magnet facility are so great that the time allotted for any project is necessarily limited. If more time had been available for experimentation, the problem of maintaining the culture at 38°C could have been solved despite fluctuations in the temperature of the magnet. Because the results of the experiments conducted were negative, the study was not pursued further.

Time of Exposure to Magnetic Fields

While the magnet was energized, only 50 percent of the suspension was in the magnetic field at any time because of the large volume required for the centrifugal pump and the flexible tubing leading to it. However, the oxygen production figures represent the output of those cells within the magnetic field.

FUTURE WORK

In view of the negative results obtained, no future work on magnetic field effects is planned.

ACKNOWLEDGMENTS

It is a pleasure to acknowledge the service of Mr. E. B. Cheville and other members of the Plastics Shop for making the culture chamber. Also thanks are due Mr. J. R. Clement for providing the use of the large magnet, and to Mr. J. F. Donnelly for its operation.

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APPENDIX
COMPOSITION OF THE CULTURE MEDIUM

Major Constituents	Amount (grams/liter)
$MgSO_4 \cdot 7H_2O$	5.0
KH_2PO_4	2.5
NaCl	2.0
Urea	0.8

Micronutrients	Conc. Trace Element (ppm)	M.E.Q. Element (liter)
Fe - EDTA	1 Fe	5.36×10^{-2}
$CaCl_2$	8 Ca	0.8
H_3BO_3	1 B	0.6
$MnCl_2 \cdot 4H_2O$	1 Mn	3.64×10^{-2}
$ZnSO_4$	0.18 Zn	5.5×10^{-2}
$CuSO_4 \cdot 5H_2O$	0.04 Cu	1.25×10^{-2}
$(NH_4)_6 Mo_7 O_{24} \cdot 5H_2O$	0.019 Mo	1.19×10^{-3}
$NaVO_3$	0.84 V	8.24×10^{-2}