

AD720401

1

SECOND ANNUAL PROGRESS REPORT

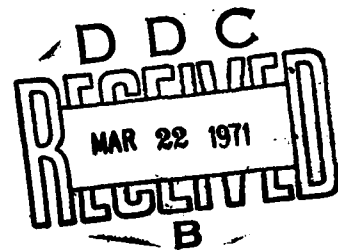
April 1, 1971

Leslie Ralph Berger
Department of Microbiology
University of Hawaii
2538 The Mall
Honolulu, Hawaii 96822

ASSISTED BY: Kumar C. Korapatl
Dan H. Pope

WORK UNIT: NR-306-820

CONTRACT: N00014-67-A-0387-0008



"EFFECTS OF HYDROSTATIC PRESSURE ON PHOTOSYNTHESIS AND
GROWTH OF UNICELLULAR MARINE ALGAE AND DIATOMS."

ABSTRACT

Light-dependent oxygen production and growth of algal cultures have been measured at 25°C at various light intensities and hydrostatic pressures.

A device which maintains a desired concentration of dissolved oxygen during growth and oxygen evolution by photosynthetic organisms is described. The system uses a modified rate measuring oxygen electrode in conjunction with an oxygen concentration monitoring system.

Period Covered by this Report: 1 April, 1970 - 31 March, 1971

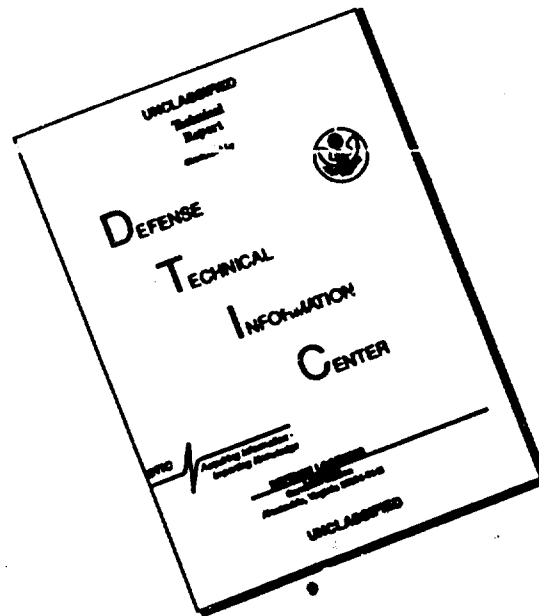
Reproduced by
**NATIONAL TECHNICAL
INFORMATION SERVICE**
Springfield, Va. 22151

DISTRIBUTION STATEMENT A

Approved for public release;
Distribution Unlimited

13

DISCLAIMER NOTICE



THIS DOCUMENT IS BEST
QUALITY AVAILABLE. THE COPY
FURNISHED TO DTIC CONTAINED
A SIGNIFICANT NUMBER OF
PAGES WHICH DO NOT
REPRODUCE LEGIBLY.

All work was done at 25°C.

I. Oxygen production by algae at increased hydrostatic pressure.

A. Apparatus.

We are using two types of polarographic oxygen electrodes. The first type, described in the First Annual Progress Report, measures the concentration of dissolved oxygen in the environment without measurably affecting that concentration. This unit depends on an oxygen gradient across a membrane placed between the environment and the electrode per se. Three types of membranes have been used: dimethylsilicone, previously described, Teflon, and polyethylene. We currently use polyethylene.

The second electrode system measures directly the rate of light-dependent oxygen evolution by algal cells. The principle of the unit is basically that described by Chandler and Vidaver (1). A relatively large platinum electrode is placed directly against a membrane filter on which is layered a known number of cells. The cells are supported by dialysis membrane and the whole assembly is immersed in growth medium. The assembled unit is placed in windowed cylinders and may be pressurized to 1000 atmospheres, maximum. Illumination of the cells is external.

B. Results.

The test organism has been a strain of unicellular blue-green alga, Anacystis nidulans for most experiments.

Figure 1 shows the effect of light intensity on the rate of oxygen production. Subsequent studies were done at 750 foot candles. At about this intensity the rate of oxygen production is proportional to the cell concentration over the range 10^7 - 10^8 cells per membrane filter.

The effects of hydrostatic pressure on these organisms is shown in Figure 2. Within 10 to 20 seconds after the light is turned on a very rapid increase in the rate of oxygen evolution is observed. The actual rate of increase is probably greater than that which can be recorded by our apparatus. The plateau in the curve represents the maximum rate of oxygen production and a constant rate of photosynthesis. Four to five seconds after extinguishing the light the rate of oxygen evolution begins to drop and rapidly reaches a base-line value. Most of the time taken to reach the base-line value is probably instrumental - an artifact of the experimental system.

The rate of oxygen evolution is essentially unaffected by pressure up to 200 atmospheres. A slightly reduced rate occurs at 400 atm. and only approximately 50% of the ambient rate is evident at 600 atmospheres. Figure 3 and Table 1 summarize these data including experiments made at hydrostatic pressures up to 1000 atm. Marked decreases in the rate of oxygen evolution is observed at higher pressures; no oxygen was evolved at 1000 atmospheres.

The above data are maximum rates of oxygen evolution. Figure 4 shows the relative rate of oxygen evolution by the algae over a 60-minute period. The data are plotted as percent of the values at ambient pressure. At 100 and 200 atm. a new steady-state rate is reached by 60 minutes; above these pressures a new steady-state rate was not reached within the period of the experiment. Presumably the rates fall to zero at pressures above 400 atmospheres (see data on growth below).

We are doing experiments to obtain rapid growth of diatoms in closed culture. To this end application of the method of Berger and Tam (2) is being used. With cultures grown in open systems (but bacteria-free) we have demonstrated that our procedures can measure the rate of oxygen evolution in the light by these organisms.

II. Growth of *Anacystis nidulans* at increased hydrostatic pressure.

Growth of the blue-green alga at various pressures was determined by placing suspensions of cells in stoppered tubes which were then incubated inside the illuminated pressure vessels. Growth was determined by increase in the density of culture. It was found that depressurization followed by removal of the tubes to determine growth and subsequent replacement and repressurization within 5 or 10 minutes had no effect on the growth rate. Growth was maintained exponentially up to 40 hours in the cylinders. The effects of hydrostatic pressure on the growth of *Anacystis nidulans* is shown in Figure 5.

III. Growth of algae at increased hydrostatic pressure at constant oxygen concentrations.

Except in open systems, cultures of organisms accumulate oxygen during photosynthetic growth. The concentration of oxygen in the environment affects the rate of growth of the organism and that of photosynthesis itself. Thus, in closed cultures measurement of these parameters is uncontrolled in ordinary laboratory experiments. Open systems are not readily feasible at increased hydrostatic pressures.

We have devised a system which we are currently testing which permits control and maintenance of a desired oxygen concentration in closed pressurized cultures of photosynthesizing algae.

In principle the device utilizes two polarographic electrode systems: a concentration-monitoring electrode whose output is displayed on a strip-chart potentiometric recorder. When oxygen exceeds a preset concentration a switch in the recorder activates the second electrode system whose capacity is large enough to reduce the oxygen to the preset concentration. At this point the second electrode is automatically switched off. An event marker records the period when the electrode is operating. From this information both the rate of oxygen production and the total oxygen produced may be computed. To prevent the large electrode system from becoming poisoned from prolonged exposure to cellular materials, a membrane of dimethylsilicone and electrode gel is used. Oxygen permeability through the membrane is not a limit to the rate of oxygen reduction by the system.

We have encountered a problem of toxicity of our O-rings to Anacystis. Apparently algae are generally sensitive to bi-products from rubbers. Coating the Buna-N O-rings with inert materials, or using silicone O-rings appears to be the simplest remedy to this problem. (This suggests a pollution problem of rubber in off-shore waters!)

CONCLUSIONS

1. It is technically feasible to measure the growth rate and rate of oxygen evolution by photosynthetic organisms at increased hydrostatic pressure.
2. It is possible to simulate the dilute marine environment in the laboratory, to introduce light into it, and to control the level of oxygen at any preset level (assuming that the rate of oxygen evolution exceeds that of respiration).
3. It is possible to measure both the rate of change and the absolute quantity of dissolved oxygen in vessels pressurized to 1000 atm. (hydrostatic).
4. The Blue-green alga, Anacystis nidulans grows at pressures exceeding 200 atm. without significant reduction in rate; up to 400 atm. with decreased rate of growth at 25°C, the temperature at which all work was done.

FUTURE DIRECTIONS OF THIS STUDY

No further development of techniques are envisaged during the remainder of the Contract period. We plan to obtain data on the photosynthetic activities of a variety of algae under controlled conditions of hydrostatic pressure, temperature and oxygen concentration. The rate of carbon dioxide incorporation by these organisms will be determined using ^{14}C -labelled bicarbonate.

REFERENCES

1. M. T. Chandler and W. Vidaver Stationary platinum electrode for measurement of O_2 exchange by biological systems under hydrostatic pressure. Rev. Sci. Instr. In press.
2. L. R. Berger and L. Q. Tam 1970. A method to grow obligately aerobic bacteria at increased hydrostatic pressure. Limnol. and Oceanogr. 15, 483-485.

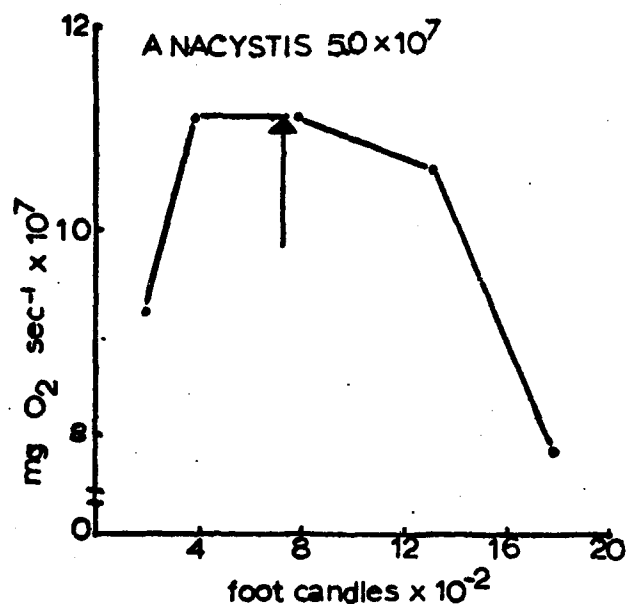


Figure 1. Effect of light intensity on the rate of oxygen evolution by *Anacystis nidulans*. 5.0×10^7 cells were used. $25^\circ C$. Ambient pressure.

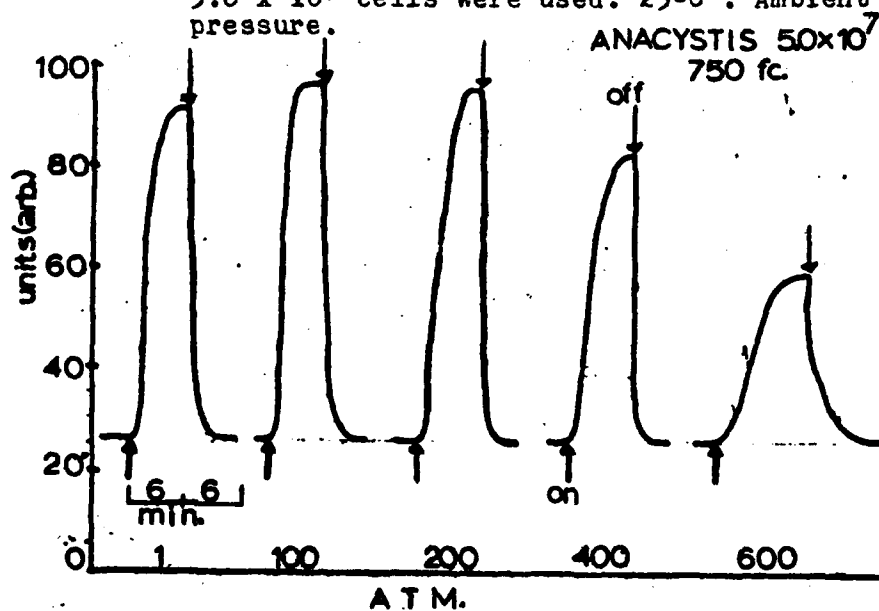


Figure 2. Relative rates of oxygen evolution by *A. nidulans* at $25^\circ C$, 750 ft. candles at various hydrostatic pressures. 5.0×10^7 cells were used per determination.

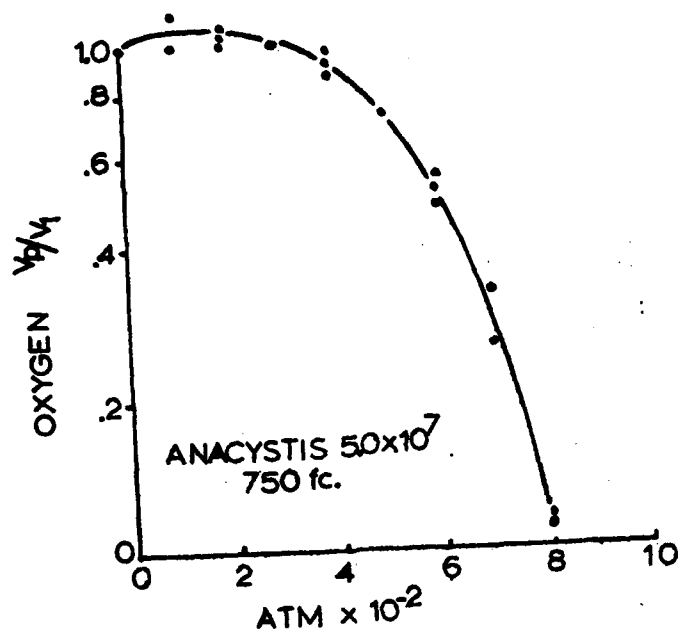


Figure 3. Relative rates of oxygen evolution by *A. nidulans* vs hydrostatic pressure. See Fig 2. for details. The ordinate is the ratio of observe rate to rate at ambient pressure.

TABLE 1.

ANACYSTIS 1.0×10^8 ml ⁻¹		
ATM.	rate oxygen evolution volts min ⁻¹	
1	0.24	0.18
50	—	0.19
100	—	0.19
300	0.30	0.18
600	0.23	0.14
1000	0.00	—
media 1	0.00	0.00
media 1000	—	0.03

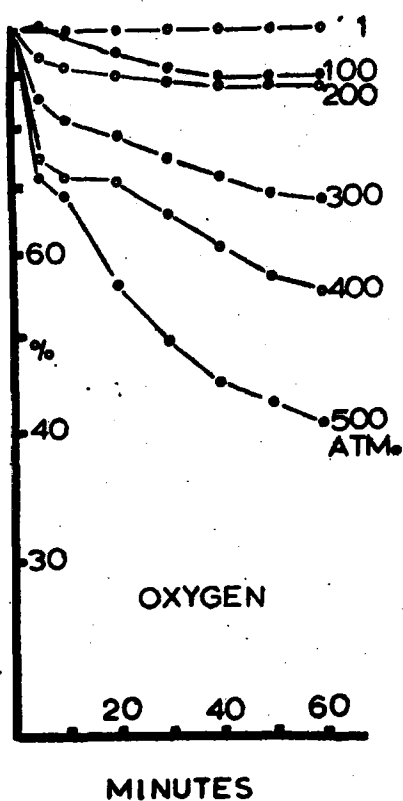


Figure 4.

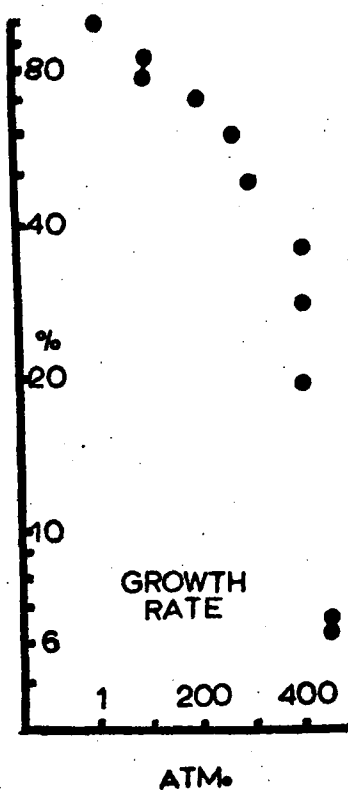


Figure 5.

Figure 4. Relative rate of oxygen evolution by *A. nidulans* over a 60 minute period. Conditions similar to those in Figure 3.

Figure 5. Relative growth rate of *A. nidulans* in pressurized cultures. Rate was measured during exponential increase of the culture. See text for details.

UNCLASSIFIED

Security Classification

DOCUMENT CONTROL DATA - R & D		
(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)		
1. ORIGINATING ACTIVITY (Corporate author) Board of Regents of the University of Hawaii Honolulu, Hawaii 96822		2a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED
		2b. GROUP
3. REPORT TITLE EFFECTS OF HYDROSTATIC PRESSURE ON PHOTOSYNTHESIS AND GROWTH OF UNICELLULAR MARINE ALGAE AND DIATOMS		
4. DESCRIPTIVE NOTES (Type of report and inclusive dates) Second Annual Progress report April 1, 1970 thru Mar 31, 1971.		
5. AUTHOR(S) (First name, middle initial, last name) Leslie R. BERGER		
6. REPORT DATE 1 APRIL 1971	7a. TOTAL NO. OF PAGES 11	7b. NO. OF REFS 2
8a. CONTRACT OR GRANT NO. N00014-67-A-0387-0008	9a. ORIGINATOR'S REPORT NUMBER(S) -	
b. PROJECT NO. NR 306-820		
c. CODE443	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report) -	
10. DISTRIBUTION STATEMENT DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED		
11. SUPPLEMENTARY NOTES -	12. SPONSORING MILITARY ACTIVITY OFFICE OF NAVAL RESEARCH 50 FELL STREET SAN FRANCISCO, CA 94102	
13. ABSTRACT Light-dependent oxygen production and growth of algal cultures have been measured at 25°C at various light intensities and hydrostatic pressures. A device which maintains a desired concentration of dissolved oxygen during growth and oxygen evolution by photosynthetic organisms is described. The system uses a modified rate-measuring oxygen electrode system in conjunction with an oxygen concentration monitoring unit.		

UNCLASSIFIED

Security Classification

UNCLASSIFIED

Security Classification

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
ALGAE						
PHOTOSYNTHESIS						
OXYGEN, DISSOLVED						
HYDROSTATIC PRESSURE						
OXYGEN, EVOLUTION						
OXYGEN, ELECTRODE						

UNCLASSIFIED

Security Classification