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MEASUREMENT OF ALGAL GROWTH UNDER CONTROLLED STEADY-STATE CONDITIONS[1]

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For reasons set forth in a following paper it has become desirable to measure growth of a unicellular alga as a function of intensity and intermittency of illumination. Conventional methods cannot be used since growth of an algal culture is accompanied by increasing mutual shading of cells. Even though the incident illumination is held constant the intensity may vary greatly between the front and back surface of the culture and the average intensity per cell decreases continually during the course of an experiment. Some special means of measuring growth must be devised.

The present paper describes an apparatus for measuring the growth rate of an alga under conditions of constant population density and constant volume in a small chamber which may be illuminated under careful control in a focused optical system. As a means of measuring growth under steady-state conditions, the method is related to the *continuous-culture apparatus* of Myers and Clark (3) and the *chemostat* of Novick and Szilard (4).

The lucite growth chamber in its final form is diagrammed in figures 1 a and 1 b. The culture cell C, 5.7 cm I. D. × 1.0 cm thick, is sandwiched between two chambers, A, for temperature control by thermostated water circulated through ports, B. Complete details of the culture cell are shown in figure 1 b. The algal suspension is stirred by a one-fourth-inch stainless steel shaft, S, driven by a small air turbine. A 4 % carbon dioxide in air gas mixture is delivered continuously into the upper portion of the cell and fresh medium is added intermittently under control of a photometric device described below. The glass overflow tube, O, serves as an automatic siphon to maintain a constant volume of suspension in the chamber. Earlier arrangements of the overflow device shown in figures 1 c and d were abandoned for reasons which will be presented later.

The complete apparatus is shown in figure 2.

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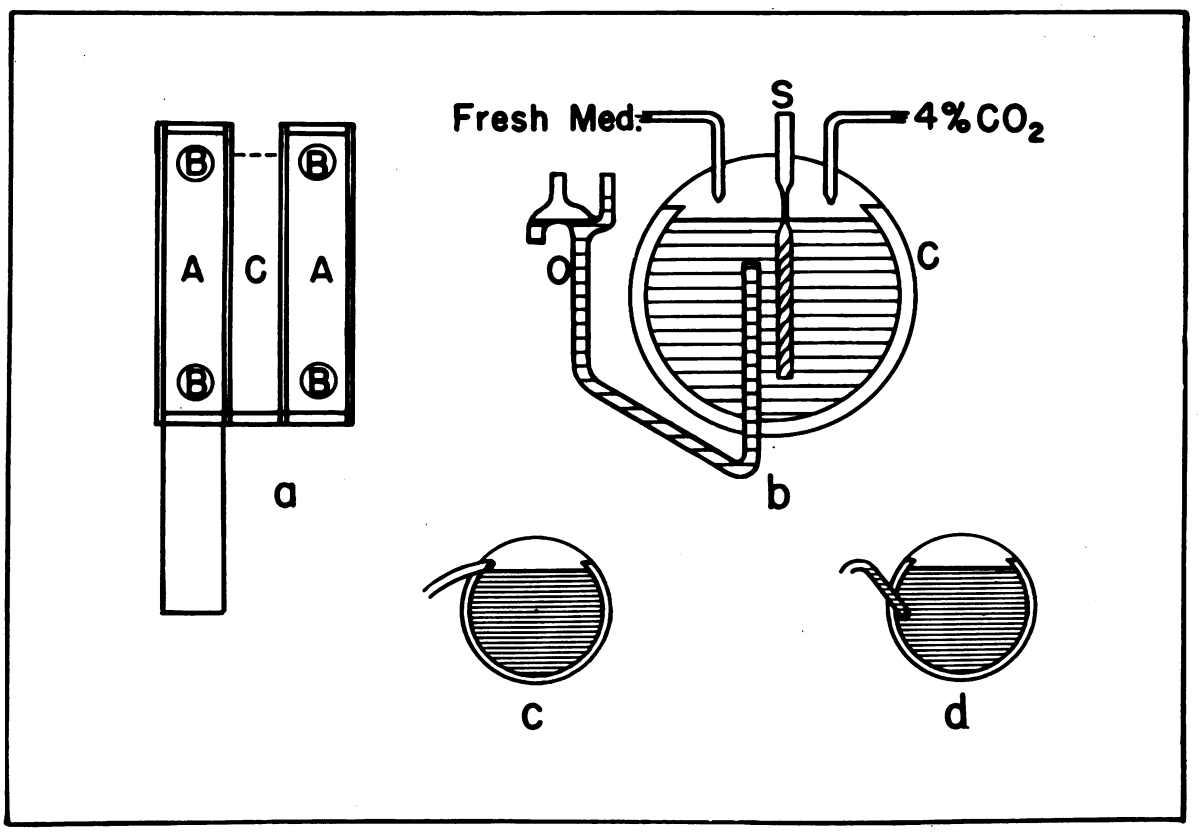

FIG. 1. Details of the growth chamber.

Light from the source, L, and spherical mirror, M, passes through a 5 cm water cell, W_1, and is focused by two 6-inch condensing lenses, CL. The uniform light field in the plane, S, is focused upon the culture cell, C, through an 18.5 cm water cell, W_2, by a Tessar-type field lens, FL, of 3 inches diameter and 7 inches focal length. Light intensity is controlled by insertion of Wratten neutral filters at F or insertion of a ground glass diffusing plate at S. Intermittency can be provided by a rotating sector placed at S. All of the apparatus behind the lens, FL, is protected from stray light so that illumination on the chamber can be cut to zero by insertion of an opaque plate, P.

The photometric control is operated by two GE barrier-type photocells: P_1, which looks through the algal suspension, and P_2, which looks directly at a portion of the final light field. A galvanometer-type relay operates from the current-balancing photocell circuit. The meter G is a Rubicon #3402 spotlight galvanometer, sensitivity 0.04 μamp/mm, with an RCA #922 phototube mounted in place of the usual glass scale. The phototube feeds into an electronic relay employing a #2050 vacuum tube and designed so that increase in light on the phototube energizes the relay. In practice, photocells P_1 and P_2 are screened so that each delivers about 100 μamp and the circuit is balanced when the transmission of the cell is about 80 %. The photometric control is similar to that previously developed by Myers and Clark (3).

The system works as follows. Multiplication in the algal suspension causes increased shading of photocell P_1. The off-balance current of the photocell circuit trips the galvanometer relay, actuating the solenoid valve, SV, and allowing fresh medium to flow into the chamber from reservoir MF until photocell balance is again obtained. Stability and sensitivity of the system are good enough to hold the density of algal suspension constant to ± 1 % for a period of several days. Concurrent with each addition of fresh medium, an equivalent small and representative sample of the algal suspension flows out of the chamber via the constant level siphon into a tared flask. The weight of suspension in the chamber ($\sim$ 20 gm) is determined at the beginning, and rechecked at the end, of each experiment. Cumulative weighings of the collected effluent are recorded periodically as a function of time.

The specific growth rate, k, is calculated by the simple relation derived as follows. The growth equation for the condition of exponential growth may be written:

$$k = \frac{1}{N}\frac{dN}{dt} \qquad (1)$$

in which dN/dt is the increment in cell number per unit time when the cell number is N. When the volume, V, in the growth chamber is held constant and when the cell number per unit volume in the chamber,

N/V, and the cell number per unit volume in the effluent, $\Delta N/\Delta V$, are held constant and equal, equation (1) can be rewritten:

$$k = \frac{1}{V}\frac{\Delta V}{\Delta t} \qquad (2)$$

in which $\Delta V/\Delta t$ is the rate of effluent accumulation. During steady-state growth $\Delta V/\Delta t$ is constant.

Attainment of the condition that $\Delta N/\Delta V$ in the effluent be identical with N/V in the chamber depends upon the design of the overflow device. In one early model (figure 1 c) in which the overflow was withdrawn from the surface of the suspension, the effluent was found to contain a higher population density than the chamber, leading to low values of $\Delta V/\Delta t$ and k. In a second model (figure 2 d), withdrawal via an inclined tube allowed return of some of the cells to the chamber during periods between dilutions, leading to a less dense effluent and high values of $\Delta V/\Delta t$ and k. The final arrangement (figure 1 b) withdraws a representative sample. Some cells do settle out in the lower part of the tube before discharge so that the collected effluent is of lowered density, but this is not important so long as the sample leaving the chamber is a representative sample.

The method purposely uses only very small quantities of cells. The chamber contains about 5 mg dry weight and the total collected effluent of an experiment contains only 10 to 20 mg. It is important, therefore, that no loss of cells from the chamber occurs except via the effluent. In early attempts stirring and carbon dioxide were provided by bubbling a carbon dioxide-in-air mixture through the suspension. The breaking of bubbles at the surface resulted in a small but significant loss of cells by splattering on the upper dry walls of the chamber. In the final arrangement stirring is provided by a stainless steel rotor. Carbon dioxide is provided by saturating the influent medium with 4 % carbon dioxide in air; the medium contains about half of the total carbon requirement of the cells produced, and the remainder is furnished by diffusion into the suspension from the 4 % carbon dioxide in the gas phase maintained at the surface. With this arrangement no carbon dioxide limitation of growth was expected, nor observed even at very high light intensities.

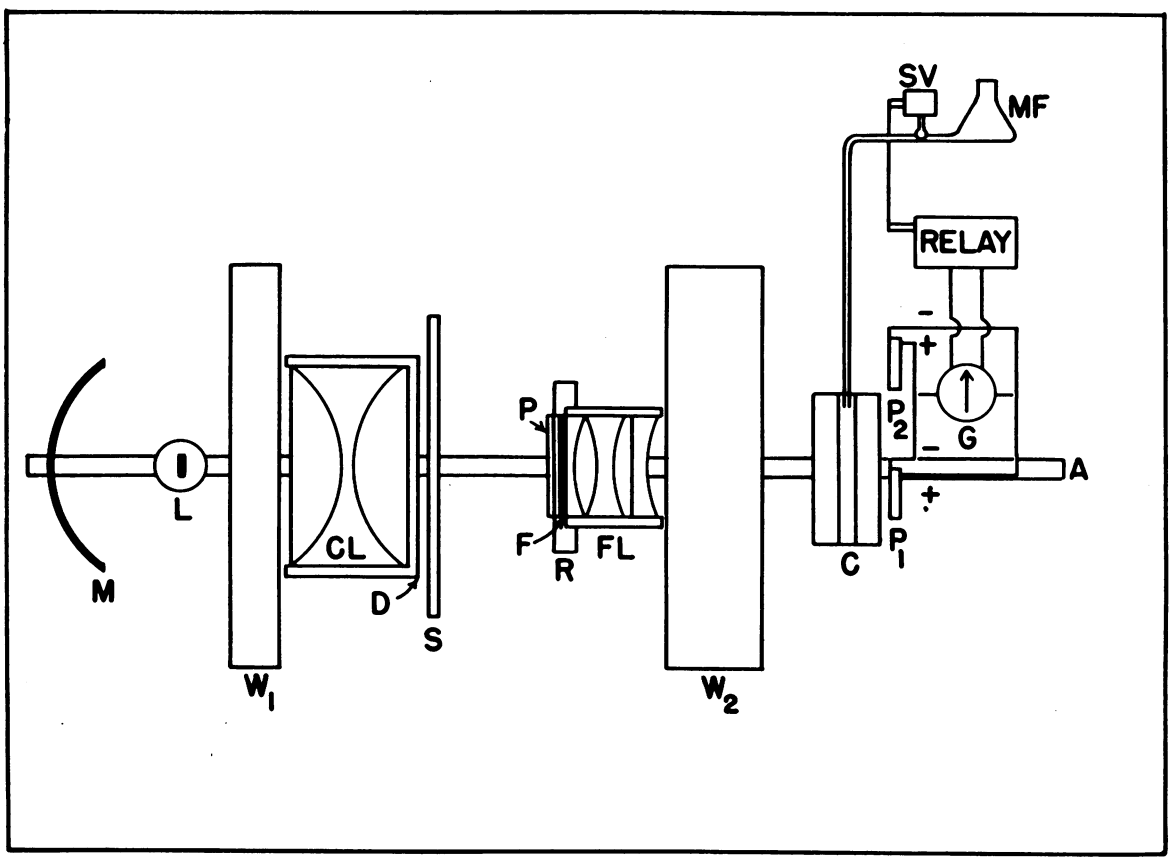

FIG. 2. Details of the complete apparatus.

In initial trials the light source was a 1000-watt, 120-volt projection lamp operated at 100 volts from a voltage stabilizer and variable transformer. Light intensity was measured by a calibrated large surface Moll thermopile or a calibrated photronic cell inserted in place of the growth chamber. Variation in intensity due to lamp decay could be held to $\pm$ 2 % of the mean value over a three-day period. Light intensity

over the surface of the growth chamber, checked by scanning the beam with a small vacuum thermocouple having a 2 mm diameter receiver, proved uniform to ± 2 %.

The apparatus can be applied to growth rate studies of any unicellular alga which suspends uniformly in liquid culture. To date it has been applied only to the Emerson strain of *Chlorella pyrenoidosa* at 25°C. Additional details of operation and typical experimental results may be cited for this alga.

The culture medium was a modified Knops solution now used routinely in this laboratory. It contained 0.010 M $MgSO_4$, 0.012 M KNO_3, 0.009 M KH_2PO_4, 0.5 gm per l of the chelating agent, ethylene diamine tetraacetic acid, and trace elements in parts per million as follows: 30 Ca, 20 B, 10 Fe, 20 Zn, 4 Mn, 4 Mo, 1 Co. The pH was adjusted to 6.8. The solution was used full strength in a continuous culture apparatus (3) which provided a uniform and pure culture inoculum for each experiment. In the growth-measuring apparatus the medium was used at a 1 : 3 dilution to minimize formation of slight precipitates. It has been demonstrated that growth rate is independent of total concentration of Knops solution within this dilution range (2). No attempt was made to maintain pure culture technique in the growth-measuring apparatus. Under the conditions employed, contamination by other microorganisms was not significant.

The data of five successive experiments run at different light intensities are presented in figure 3 and table I. The linear character of the curves of figure 3 provides an over-all measure of the stability and sensitivity of the system in maintaining steady-state conditions. Precision of the slope of each curve was estimated by the Student t distribution using a 95 % confidence limit; in order to use the Student t distribution, a normal distribution of errors in slope from the true slope was assumed. In practice, the values of k determined graphically are regarded as accurate to ± 0.05 day^{-1}. Treatment of growth rate as a function of light intensity is reserved for the following paper.

TABLE I

REPRESENTATIVE DATA ON GROWTH OF *Chlorella pyrenoidosa* AT 25°C UNDER DIFFERENT LIGHT INTENSITIES

EXPERIMENT *	V	ΔV/Δt	k **
no.	*ml*	*ml/day*	*day^{-1}*
54 a	22.1	42.3	1.92 ± 0.05
55 b	21.7	32.0	1.48 ± 0.09
57 c	22.0	23.5	1.07 ± 0.05
58 d	22.0	15.0	0.68 ± 0.07
56 e	21.9	8.16	0.37 ± 0.01

* Letters designate corresponding curves of figure 3.
** The dimension of specific growth rate of day^{-1} is that required by equation (1) and is equivalent to a dimension of $\log_e$ units/day for the integrated form of equation (1). The ± variation indicated is that calculated for a 95 % confidence limit of the regression coefficient for ΔV/Δt.

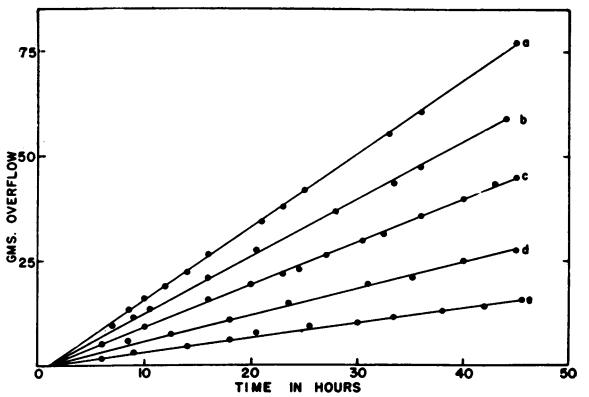

FIG. 3. Plots of the data of five experiments. Accumulated overflow versus time. Measured weight in grams is taken as equivalent to volume in ml. The slopes of the curves give the corresponding ΔV/Δt values cited in table I. Data for curves d and e were extended beyond the time period shown.

DISCUSSION

The apparatus described is one of several which provide a constant density of population and allow study of growth under steady-state conditions. The *chemostat* of Novick and Szilard (4), applied in principle also by Monod (1), may be described as a continuous-dilution, constant-volume device. Rate of growth (which always must be less than the maximum rate) is established by a constant dilution or washing-out rate, $\frac{1}{V} \times \frac{\Delta V}{\Delta t}$. The population density then adjusts itself to the maximum value permitted by some limiting component of the influent medium. The *chemostat* is particularly adapted to the study of growth and mutations of microorganisms under deficiency conditions.

The *continuous-culture apparatus* of Myers and Clark (3) may be described as a controlled-dilution, variable-volume device. Dilution is controlled by a photometric device to just such a rate as will keep the density constant and balance the rate of growth. Volume is allowed to increase and effluent samples are withdrawn periodically to some fixed volume. The specific growth rate, k, can be evaluated from the integrated form of the growth equation as $\frac{1}{t - t_o} \ln \frac{V}{V_o}$, where V and V_o designate the volumes corresponding to times t and t_o. The method can be used to measure rate of growth and to provide uniform experimental material.

The apparatus herein described is a controlled-dilution, constant-volume device. Dilution is controlled by a photometric method to maintain constant density. Maintenance of constant volume by means of an overflow allows direct use of the differential form of the growth equation.

A continuous-dilution, variable-volume device utilizing the principle of Novick and Szilard is also possi-

ble in theory but would require a logarithmically increasing dilution rate.

Summary

An apparatus is described for precise measurement of growth rate of an alga as a function of intensity and intermittency of illumination. Dilution of a culture is controlled by a photometric device to just such a rate as will maintain constant density of population and balance the growth rate. Maintenance of constant volume by an automatic siphon allows direct application of the differential form of the growth equation in evaluating specific growth rate. The relationships of the new apparatus to other steady-state growth devices are discussed.

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GROWTH RATE OF CHLORELLA IN FLASHING LIGHT[1]

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Studies on the mass culture of algae under sunlight conditions have demonstrated that the most important factor limiting the yield per unit area of exposed surface lies in the characteristic of light saturation (12). Cells at the front surface of a culture use the very high light intensity of sunlight with low efficiency; at the same time cells at the back surface of a dense culture may receive no light at all. In theoretical studies of effects of intermittent light on photosynthesis it has been shown that light of high intensity may be used with high efficiency if presented in short flashes separated by long dark periods. One anticipates that some similar effect will hold for the total growth process as well as for photosynthesis alone. If, by turbulence of culture suspension, individual algal cells are moved back and forth between the high intensity of the front surface and the darkness of the back surface, an improvement in over-all efficiency of light utilization by the culture might be effected.

Reported herein is an investigation of the characteristics of growth of a representative alga, *Chlorella pyrenoidosa*, in intermittent light. In order to obtain interpretable results, rate of growth has been studied in thin layers of culture suspension of such low population density that mutual shading effects of the cells are minimized. Intermittent illumination of measurable characteristics has been provided by a mechanical optical system. As a secondary objective it has been necessary to determine also the characteristics of the light intensity curve for growth in continuous illumination.

Materials and Methods

The growth rate at each light intensity or intermittency regimen was determined by a separate experiment. Cells harvested from a continuous-culture apparatus were centrifuged out and resuspended in a ¼-strength EDTA Knops solution (13) to a population density which gave a 50 % transmission in an Evelyn colorimeter with 600 mμ glter. In the growth chamber this suspension had a transmission of 81 % as measured with a fully-illuminated photocell placed behind the suspension. Constancy of the photometric control was checked during and at the end of each experiment by readings of transmission of the suspension in the Evelyn colorimeter; all such readings fell within the range of 48–52 %. In each experiment the specific growth rate, k, was evaluated in $\log_e$ units per day as described in the preceding paper. The temperature used throughout was 25°C.

Intensity Measurement: Measurements of light intensity at the position of the growth chamber were made with a large surface Moll thermopile with a compensated Aryton shunt and Rubicon Type T galvanometer. It was calibrated without a shielding window against an NBS standard lamp. For subsequent measurements the thermopile was used with a thin glass shielding window. Estimate of the intensity of visible radiation was obtained by use of a Jena RG8 filter which transmits the near infrared and has a sharp cut-off at 7000 Å (fig 1). Readings with the thermopile were taken alternately with the system open, with the RG8 filter, and with the system closed by a shutter. (For positions of the shutter and filter in the optical system see figure 2 of the preceding paper.) Total deflection with the system open, minus deflection with the RG8 filter was taken as the measure of visible radiation. The procedure is similar to that used by Kok (8). A series of check readings made with a calibrated Weston 603 Illumination meter showed good linearity with the thermopile and RG8

[1] Received June 29, 1953.