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AN ANALYSIS OF THE PRODUCTIVITY OF A CELSS CONTINUOUS ALGAL CULTURE SYSTEM

R. Radmer, P. Behrens, E. Fernandez, K. Arnett
Martin Marietta Laboratories
1450 South Rolling Road
Baltimore, Maryland 21227-3898

One of the most attractive aspects of using algal cultures as plant components for a CELSS is the efficiency with which they can be grown. Although algae are not necessarily intrinsically more efficient than higher plants, the ease with which they can be handled and manipulated (more like chemical reagents than plants), and the culturing techniques available, result in much higher growth rates than are usually attainable with higher plants. Furthermore, preliminary experiments have demonstrated that algal growth and physiology is not detectably altered in a microgravity environment,(1) whereas the response of higher plants to zero gravity is unknown.

In an earlier communication,(2) we described a series of studies on long-term cultures of *Scenedesmus obliquus* maintained in an annular air-lift column operated as a turbidostat. The primary finding was that productivity vs dry weight rose linearly to a maximum before slowly declining.

In order to rationally design and operate such culture systems, it is necessary to understand how the macroparameters of a culture system, e.g., productivity, are related to the physiological aspects of the algal culture. In this communication we describe a "first principles" analysis of this culture system and derive a mathematical model that describes the relationship of culture productivity to the cell concentration of light-limited cultures. The predicted productivity vs cell concentration curve agrees well with the experimental data obtained to test this model, indicating that this model permits an accurate prediction of culture productivity given the growth parameters of the system.

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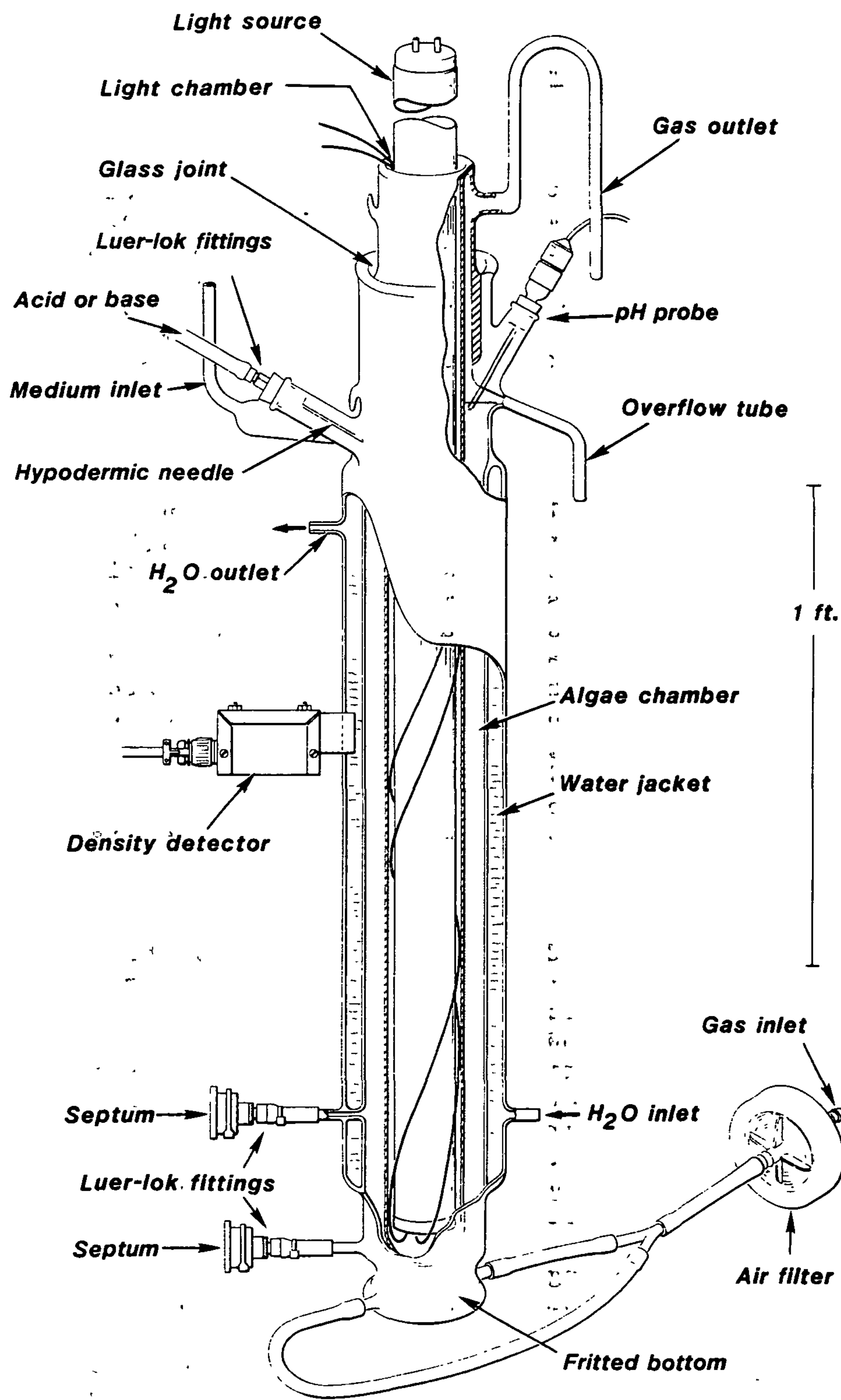

Figure 1 Continuous cell culture apparatus

Materials and Methods

Scenedesmus obliquus (Gaffron strain D_3) was cultured in medium containing 60.0mM KNO_3, 2.0mM $MgSO_4 \cdot 7H_2O$, 1.1mM K_2HPO_4, 0.6mM KH_2PO_4, 68.0μM $CaCl_2 \cdot 2H_2O$, 46.0μM H_3BO_3, 18.0μM $FeSO_4 \cdot 7H_2O$, 12.0μM Na_2EDTA, 9.1μM $MnCl_2 \cdot 4H_2O$, 1.6μM $Na_2MoO_4 \cdot 2H_2O$, 0.8μM $ZnSO_4 \cdot 7H_2O$, 0.3μM $CuSO_4 \cdot 5H_2O$, and 0.2μM $Co(NO_3)_2 \cdot 6H_2O$ and adjusted to pH 6.5. Cultures were bubbled with a gas mixture of 2% CO_2 in air, illuminated with fluorescent light (40W high output, cool white, F24T12), and maintained at 32°C.

The continuous culture apparatus used in this work, shown in Fig. 1, is similar to that described earlier.(2) The apparatus is made of glass and consists of three concentric, cylindrical chambers: the innermost chamber houses the light source, the middle chamber contains the algal culture, and the outer chamber is a temperature-regulated water jacket. Cell density is maintained by monitoring the light transmission through the culture with a photocell. When the output of the photocell exceeds a preset reference value, a microprocessor activates a peristaltic pump, and a defined volume of fresh medium is added to the apparatus. With this system the cell concentration can be continuously maintained within a few percent of a desired value.

Cell density was determined using a Coulter Counter Model TA II with PCA II accessory. Chlorophyll concentration was determined by adding an aliquot of algal culture to a 1:1 mixture of Triton X-100 and 5% KOH in methanol, heating at 63°C for three minutes, and centrifuging. Optical density was determined at 645 nm. Dry weight determinations involved filtering a 10 ml aliquot of algal culture through a glass fiber filter (approximate retention 2.6 μm), rinsing thoroughly with distilled water, drying at 105°C overnight, and cooling to room temperature in a dessicator. [The filter paper was previously dried at 105°C and weighed]. The number of doublings of a culture per 24 hours was determined by measuring the increase in cell density as a function of time. The growth rate of a continuous culture was determined by measuring the volume that overflowed during a defined time period. The productivity ($mg \cdot hr^{-1}$) of a continuous culture is defined as the product of the dry weight ($mg \cdot ml^{-1}$) of the culture and the overflow rate ($ml \cdot h^{-1}$).

Photon flux was measured with a LiCor LI-190SB Quantum Sensor probe; the flux is expressed in units of $\mu E \cdot m^{-2} \cdot s^{-1}$. The extinction coefficient of the algae was calculated by measuring the photon flux in the presence and absence of algae.

Net O_2 evolution was measured polarographically using broad-band saturating, orange-red light (Schott filter OG530 and appropriate heat filters).(3) Oxygen exchange (evolution and uptake) was measured with a quadrupole mass spectrometer, using isotopically labeled O_2 (98 atom% [$^{18}O_2$], Cambridge Isotope Laboratories). The instrumentation and methods for data analysis and calibration were as described previously (4), except that the data were stored on magnetic tape and processed by a Sym-1 microcomputer. Illumination for these measurements was supplied by fluorescent bulbs having a photon flux comparable to that used to grow the culture. All experiments were performed at 32°C.

Derivation of Model Equation

The productivity Y of a photoautotrophic algal culture will be equal to its rate of photosynthesis (P) minus its rate of respiration (R), i.e., Y = P - R. Ideally, for a fixed incident light intensity the total photosynthetic yield of the culture would increase asymtotically with increasing cell concentration. However, this increase in photosynthesis with increasing cell concentration would be counterbalanced by an increase in the total respiration of the culture. Therefore, one would predict that a plot of the overall productivity of the system as a function of cell concentration will go through a maximum.

Under conditions in which the photosynthetic apparatus is not light saturated, the maximum rate of photosynthesis of a culture, i.e., when every photon of light is absorbed, is given by the expression $E_m I_o AK$, where E_m is the maximum theoretical conversion of the energy of the absorbed light into chemical energy by a photosynthetic system, I_o is the light intensity available to the culture, A is the illuminated area of the culture, and K is a conversion factor for the energy equivalent of the algae. In cases in which not all of the available light is absorbed, the actual rate of photosynthesis will be the maximum rate minus a term that describes the amount of light

transmitted (i.e., not absorbed) by the culture. The expression describing the amount of light transmitted is a form of Beer's Law,(5) in which the transmitted light intensity I, is equal to $I_o e^{-\varepsilon c l}$, where ε is the extinction coefficient, c the cell concentration, and l the path length. Therefore, the rate of photosynthesis of the culture is given by:

$$P = E_m I_o AK - E_m I_o AKe^{-\varepsilon c l} \qquad (1)$$

or

$$P = E_m I_o AK\ (1-e^{-\varepsilon c l}) \quad . \qquad (2)$$

The respiration term R' is the product of the cellular respiration rate, R, the cell concentration, c, the total culture volume, V, and G, a term related to the amount of carbon per cell. Therefore, the productivity of the algal culture can be defined by:

$$Y = E_m I_o AK\ (1-e^{-\varepsilon c l}) - GRcV \qquad (3)$$

where

- Y = Yield [(g cells)·hr^{-1}]
- E_m = 0.2, the maximum attainable photosynthetic conversion on an energy basis
- A = Illuminated area (m^2)
- K = 0.156 [(g cells)·hr^{-1}·$watt^{-1}$)], the energy equivalent of the algae
- I_o = Light intensity (watts·m^{-2})
- ε = Extinction coefficient ($l \cdot cm^{-1} \cdot g^{-1}$)
- c = Cell concentration ($g \cdot l^{-1}$)
- l = Light path (cm)
- R = Respiration rate [g carbon·(g cells)$^{-1}$·hr^{-1}]
- V = Culture volume (l)
- G = Ratio of g cells to g carbon (2.04).

For the culture apparatus described in Fig. 1, the illuminated area is 0.1471 m^2, the culture volume is 1.2 l, the light intensity is 12.9 $W \cdot m^{-2}$ (59.6 $\mu E \cdot m^{-2} \cdot s^{-1}$), and the light path is 1.25 cm. The cells were 49% carbon, so that G = 2.04. The value for K (0.156 g cells·hr^{-1}·W^{-1}) was determined calorimetrically,

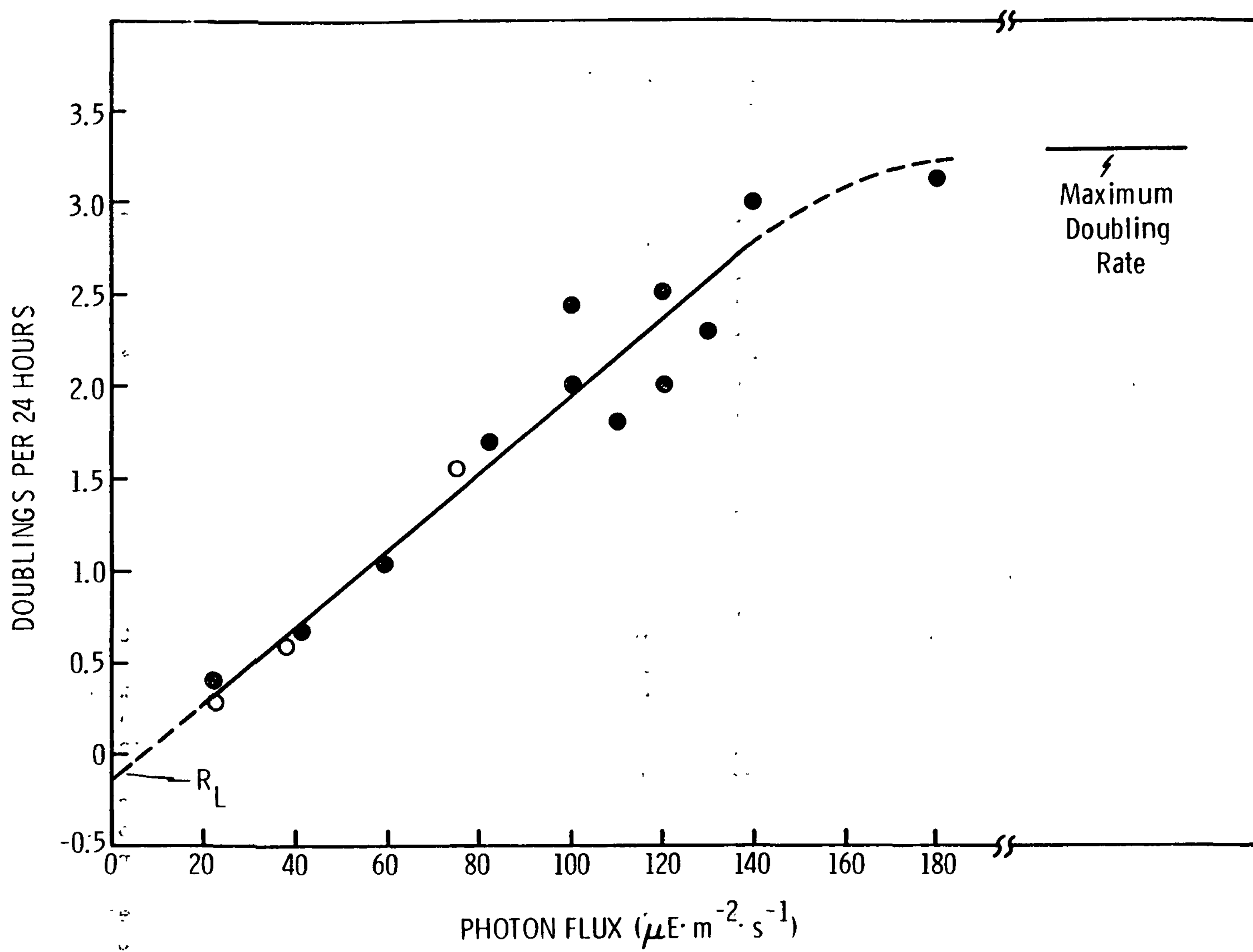

Figure 2 Growth rate (expressed as doublings·day^{-1}) of Scenedesmus as a function of photon flux in Roux culture bottles (closed circles) and continuous culture (open circles). R_L equals respiration rate extrapolated from the light curve.

and agrees with published values.(6) The maximum attainable photosynthetic conversion E_m was taken to be 20%.(7)

The above analysis assumes that 1) photosynthesis is proportional to the amount of absorbed light, i.e., light is not saturating, a necessary condition for efficient light utilization, and 2) the rate of respiration R is a constant or known function of I and c. In addition, the absorption coefficient ε is not a constant with respect to cell concentration, and thus the relationship between ε and c must be ascertained. These items are considered further in the following sections.

Results and Discussion

Light Limitation

Figure 2 shows the relationship between photosynthetic growth of Scenedesmus and photon flux using culture (Roux) bottles (closed circles) and our continuous culture system (open circles). The doublings per 24 hours increase monotonically with photon flux up to approximately 140 $\mu E \cdot m^{-2} \cdot sec^{-1}$ at which point the growth rate approaches the maximum. Note that there is very good agreement between the growth rate obtained in the continuous culture and Roux bottle growth systems. At the highest photon flux of the continuous culture system (75 $\mu E \cdot m^{-2} \cdot s^{-1}$), the growth rate is still on the linear portion of the curve. This finding indicates that the algae in the continuous culture system are not light saturated and that photosynthesis is proportional to the amount of absorbed quanta. Therefore, a necessary condition of the derived model is satisfied.

Extinction Coefficient

Figure 3 shows the relationship between the integrated (400-700 nm) average absorption coefficient (ε) and cell concentration in the culture system. Note that this relationship is neither constant nor linear, reflecting the fact that at high cell densities the predominant green light is only poorly absorbed. This phenomenon has been quantitatively rationalized in terms of Beer's Law, which is strictly valid only for monochromatic light.(5)

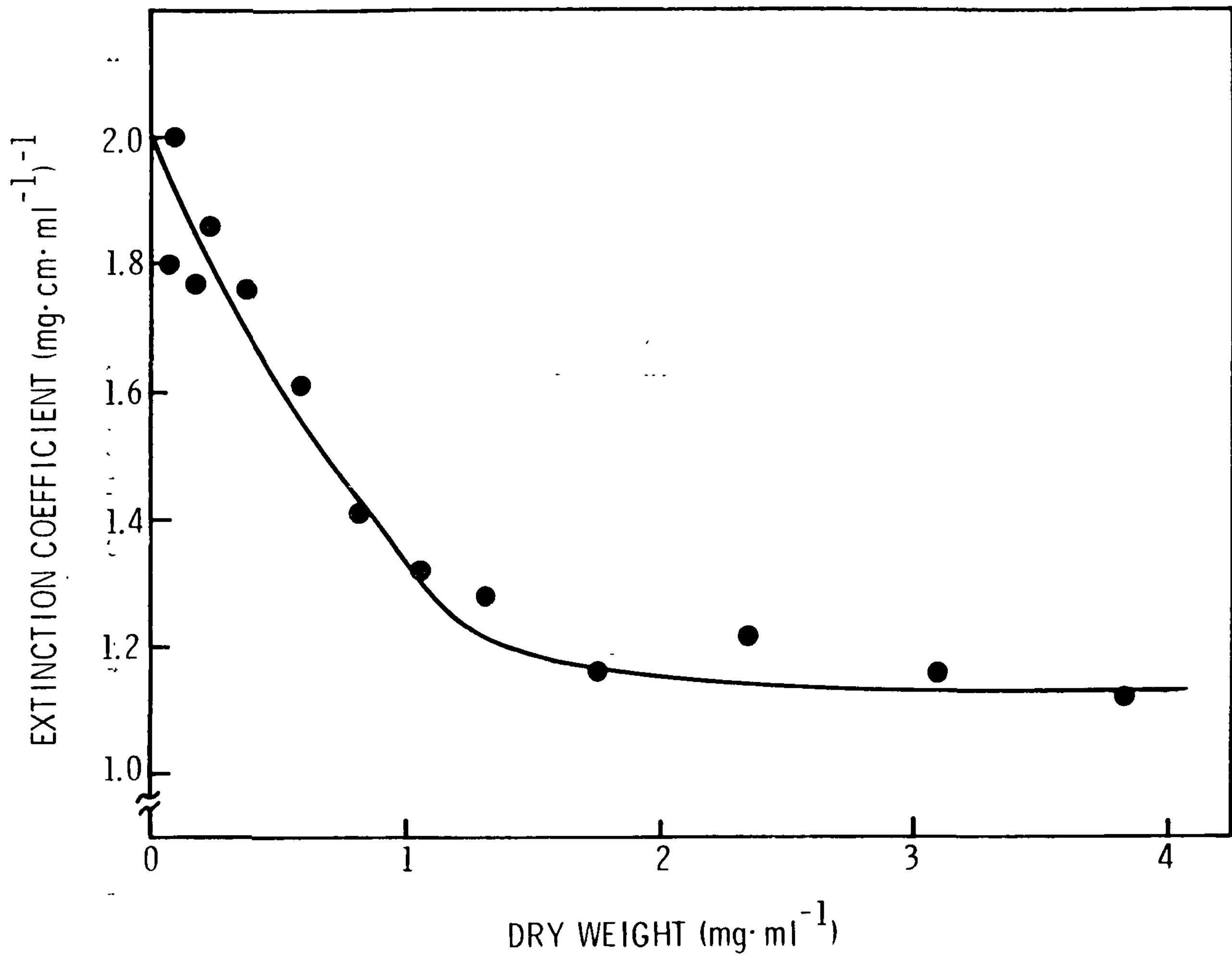

Figure 3 Integrated (400-700 nm) absorption coefficient of *Scenedesmus* as a function of cell density (expressed as g dry wt·ml^{-1}).

Cellular Respiration

Because it is difficult to accurately measure respiration in a photosynthesizing system, we used three different approaches. In the first, we extrapolated the data from Figure 2 to zero light flux to measure the respiration rate, R. In this case, the growth rate at zero light is -0.12 doubling per 24 hours, which corresponds to a respiration rate of 4.1 μmoles CO_2 evolved• mg chl^{-1}•hr^{-1} or 0.0024 g carbon•g $cells^{-1}$•hr^{-1}.

The second approach involves measuring oxygen consumption of a darkened culture using an oxygen polarograph. As shown in Fig. 4, thoroughly dark-adapted cells had a low respiration rate; ca. 2.8 μmoles O_2 consumed•mg chl^{-1}•hr^{-1} (0.0016 g carbon evolved• g $cells^{-1}$ • hr^{-1}). When illuminated, the respiration rate was masked by a high rate of photosynthetic oxygen evolution. Immediately after the cessation of illumination, there was a high rate of respiration (13.9 μmoles O_2 consumed•mg chl^{-1}•hr^{-1} or 0.0081 g carbon evolved•g $cells^{-1}$•hr^{-1}) which, after ten minutes in the dark, declined to about the same rate as before illumination. High respiration rates immediately after illumination have been previously observed.(8) Although these observations are not new, these values provide limits for the respiration rate needed for the mathematical model.

Figure 5 shows the results of an experiment in which a specially constructed mass spectrometer inlet system was used to measure the cellular respiration rate <u>during</u> illumination. The mass spectrometer can measure the concentration of two different oxygen isotopes ($^{16}O_2$ and $^{18}O_2$) simultaneously in solution. Therefore, this third approach provides a means to distinguish between respiratory oxygen consumption and photosynthetic oxygen evolution. The experiment in Fig. 5 was performed under the same conditions used for continuous culture apparatus; thus, the oxygen exchange (consumption and evolution) should closely mimic that occurring in the culture system. Upon illumination, there was an initial burst of oxygen uptake that subsequently leveled off and reached a steady state that corresponds to 8.5 μmoles O_2 consumed•mg chl^{-1}•hr^{-1} (0.0049 carbon evolved•g $cells^{-1}$•hr^{-1}). Concurrently, there was a sustained, light-dependent rate of oxygen evolution (60 μmoles O_2 evolved•mg chl^{-1}•hr^{-1}), a value consistent with the calculated growth rate based on the productivity of the culture.

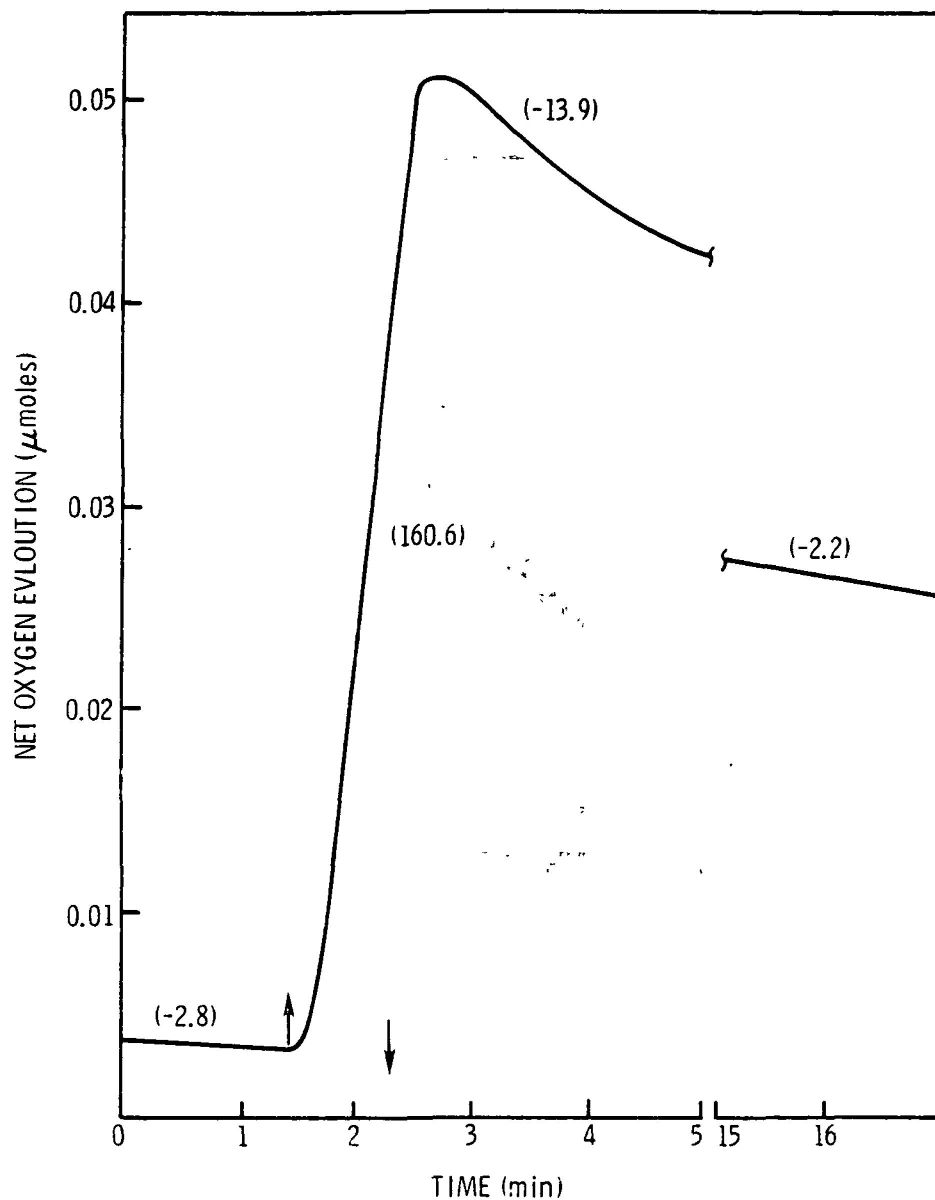

Figure 4 O_2 trace obtained when dark-adapted (>10 min) _Scenedesmus_ was illuminated (↑) and subsequently darkened (↓). Values in parentheses are computed O_2 exchange rates (in units of μmoles $O_2 \cdot$ mg $chl^{-1} \cdot hr^{-1}$).

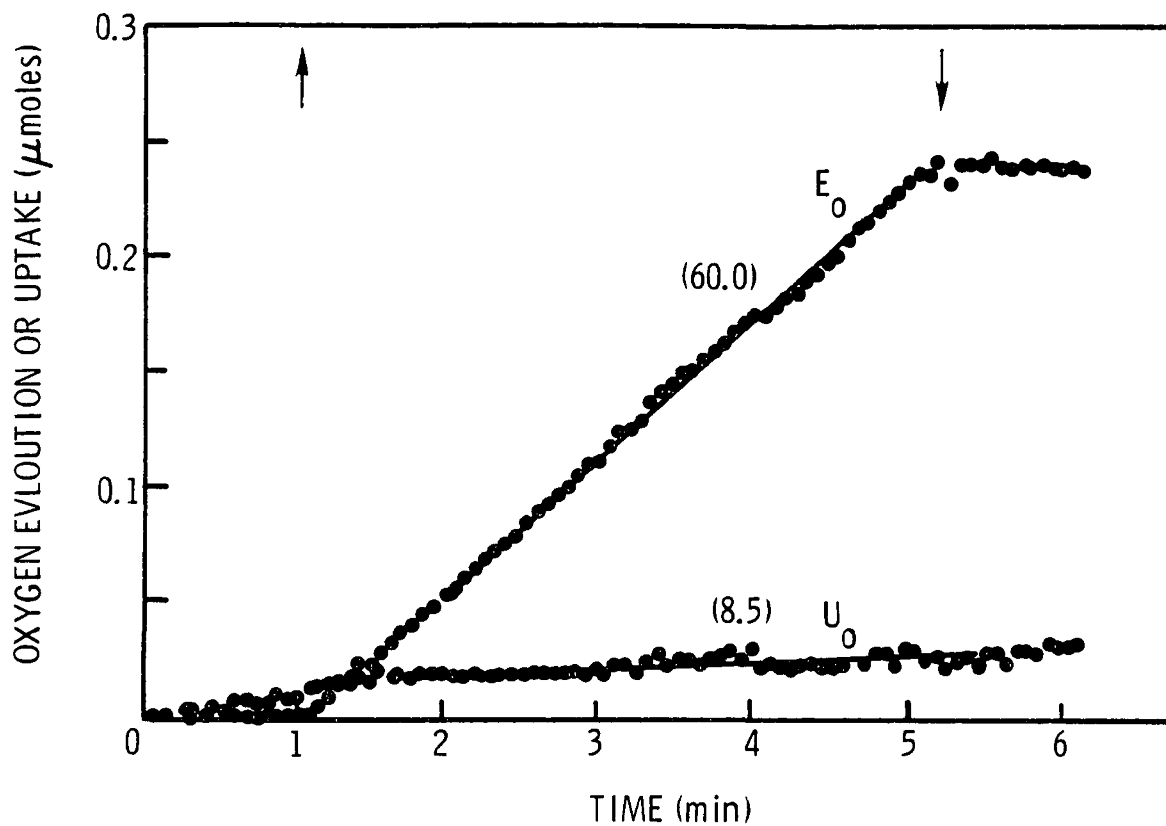

Figure 5 Computed time courses of O_2 evolution (E_o) and O_2 uptake (U_o) obtained using a mass spectrometer system to monitor the gas exchange of Scenedesmus. ↑ and ↓ indicate light-on and light-off, respectively.

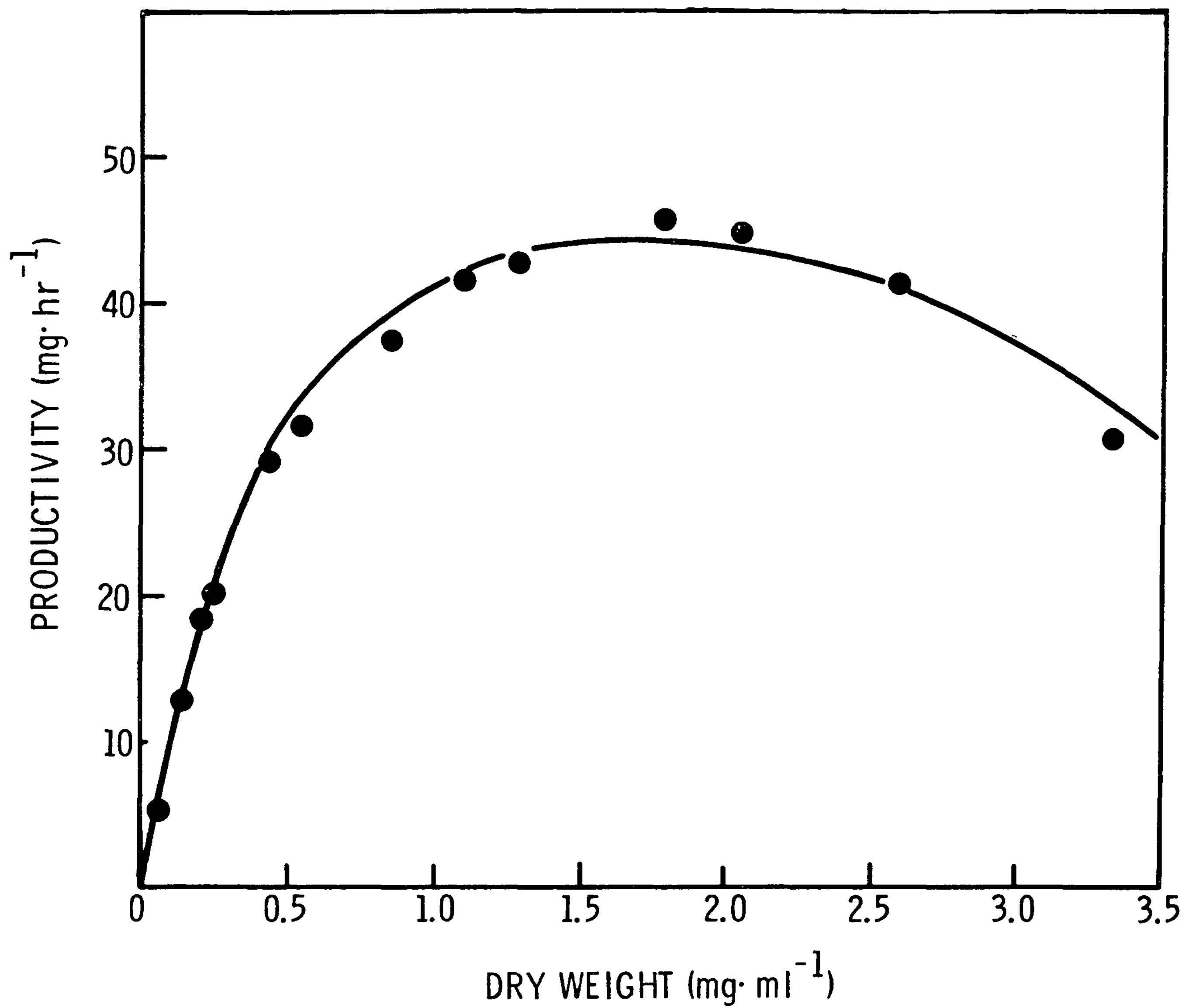

Figure 6 Relationship between culture productivity and cell density (measured as dry weight) in the continuous culture apparatus shown in Fig. 1. The closed circles show the experimental data obtained. The solid line shows the relationship predicted using the model described in the text.

Each of the three independent analytical methods used to estimate the cellular respiration rate has particular aspects that limit its accuracy for determining the respiration rate during illumination. However, together they can provide an *approximation, which, in this case, is* 5.0 μmoles $O_2 \cdot$mg chl$^{-1} \cdot$ hr^{-1} (0.0029 g carbon evolved$\cdot$g cells$^{-1} \cdot$hr^{-1}). This value will be used in the model equation.

Comparison of Observed Productivity to Theoretical Predictions

Figure 6 illustrates the observed and predicted relationships between culture productivity and dry weight. The data points were obtained in a series of experiments in which Scenedesmus was maintained in the continuous culture system. The solid line is the relationship predicted using the values assigned and derived in the previous sections. The excellent agreement between the observed and predicted values suggests that the model accurately describes the culture system in terms of physiological parameters.

At cell concentrations greater than about 1.5 mg$\cdot$ml^{-1}, the relationship between extinction coefficient and cell concentration is approximately constant (Fig. 3). Thus, between these limits we can differentiate Eq. 3 with respect to c. Under these conditions, maximum productivity will occur at a cell concentration of:

$$c_{mp} = \frac{1}{\varepsilon l} \ln \frac{GRV}{\varepsilon l E_m I_o AK}$$

where c_{mp} is the cell concentration at maximum productivity. If we use the parameter values given above, we compute that c_{mp} = 1.73 mg$\cdot$ml^{-1}, which agrees well with the data of Fig. 6.

We should emphasize that this model is completely determined and has no free adjustable parameters. Its main limitation appears to lie in the experimental determination of the respiration rate, R. Although the model accurately describes the growth of photoautotrophic algae in this system, it is limited to cases in which all growth factors except light are present in excess.

We are presently working to modify this model to account for conditions where CO_2 concentration is limiting for cell growth.

References

1. Phillips, J.N., 1979, "Experiments with photosynthetic organisms in Discoverer vehicles," In: NASA Technical Memorandum 58217, BIOSPEX: Biological Space Experiments, M. Anderson, ed., p. 166.

2. Radmer, R., P. Behrens, E. Fernandez, O. Ollinger, and C. Howell, 1985, "Algal culture studies related to a closed ecological life support system," In: The Physiologist, in press.

3. Marsho, T.V. and B. Kok, 1974, "Photosynthetic regulation by cations in spinach chloroplasts," Biochim. Biophys. Acta 333: 353-365.

4. Radmer, R. and O. Ollinger, "Measurement of the oxygen cycle: the mass spectrometric analysis of gases dissolved in a liquid phase," In: Photosynthesis and Nitrogen Fixation, Part C, Methods in Enzymology (San Pietro, A., ed.), Academic Press, pp. 547-560.

5. Shelef, G., W.J. Oswald, and C.G. Golueke, 1968, "Kinetics of algal systems in waste treatment: light intensity and nitrogen concentration as growth-limiting factors," SERL Rep. 68-4. Sanitation Engineering Research Laboratory, University of California, Berkeley.

6. Myers, J., 1957, "Algal cultures, In: Encyclopedia of Chemical Technology, R. Kirk and D. Othmer, eds., Inter-science, New York, pp. 33-51.

7. Radmer, R. and B. Kok, "Light conversion efficiency in photo-synthesis," In: Encyclopedia of Plant Physiology, Vol. 5, A. Trebst and M. Avron, eds., Springer-Verlag, Berlin, pp. 125-135.

8. Decker, J.P., 1959, "Comparative responses of carbon dioxide outburst and uptake in tobacco," Plant Physiol. 34:100-102.