DETERMINING THE KINETIC PARAMETERS CHARACTERISTIC OF MICROALGAL GROWTH

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Due to the importance of biotechnology in today's world, the subject "biochemical engineering" has now been introduced into almost all syllabi for chemical engineering studies. It is therefore a worthwhile endeavor to increase the number of practical sessions pertinent to this discipline in chemical engineering laboratories.

Of particular interest in the study of the kinetics of microorganism growth is the situation where a nutrient, administered to the culture medium at a constant rate, limits growth. This may occur when restrictions are imposed by nutrient gases or by the light energy source in the case of photosynthetic cultures. In the former case, the transfer rate of the nutrient must be taken into consideration and in the latter the reduction of light intensity in the culture must be considered.

The basic aim of this practical session is to enable students to study experimentally the kinetics of growth in the unicellular algae *Chlorella pyrenoidosa* cultures under light-restricted conditions.

BASES

When unicellular algae grow under low light intensities, a linear relationship is observed between the specific growth rate, μ, and the light intensity, I. At high values of light intensity the specific rate, however, becomes constant. The most usual kinetic models for reproducing this variability are

Hyberbolic Model by Tamiya *et al.*[1]

$$\mu = \frac{\alpha \mu_m I}{\mu_m + \alpha I} \qquad (1)$$

Exponential Model

$$\mu = \mu_m \left[1 - \exp(-I / I_s)\right] \qquad (2)$$

The high extinction coefficients of microalgae in suspension determine a considerable reduction in light intensity according to the depth of the growth chamber. Moreover, if the cultures are developed

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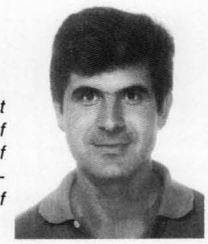

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By following the variation in the biomass concentration with time in an autotrophic culture of* Chlorella pyrenoidosa *with no limitations imposed by nutrients or CO_2, we ask that students obtain a growth curve for the algae, identify the exponential and linear growth phases, and calculate the parameters which characterize both phases.

discontinuously using artificial light, even though the incident light intensity is constant, then the intensity within the suspension varies according to position and time.

Since the specific growth rate is an average value for the whole culture, the spatial variation of the light intensity determines that the relationship between the average growth rate and the distribution of the light may be expressed as follows[2]

$$\bar{\mu} = \int\int\int_{V_c} \frac{(I)\cdot dV}{V_c} \qquad (3)$$

or

$$\bar{\mu} = \mu(I_m) \qquad (4)$$

where

$$I_m = \int\int\int_{V_c} \frac{I\cdot dV}{V_c} \qquad (5)$$

depending on the adaptation rate of the cells to the changing light intensities and the degree of mixing within the culture medium.

EXPERIMENTS

By following the variation in the biomass concentration with time in an autotrophic culture of *Chlorella pyrenoidosa* with no limitations imposed by nutrients or CO_2, we ask that students obtain a growth curve for the algae, identify the exponential and linear growth phases, and calculate the parameters which characterize both phases.

The overall interpretation of the results obtained by the students, using different incident light intensities, allows each of the two distinct kinetic models to be distinguished and their parameters to be obtained.

The integration of the kinetic model based on the two extreme situations possible for the distribution of light (Eq. 3 or 4) and its comparison with experimental results, concentration-time, would allow a quantitative difference to be established between the two situations.

MATERIALS REQUIRED

The cultures are developed with equipment as shown in Figure 1.[3] Air is pumped in by a compressor (1), through a hydrophobic cotton filter (2), a stabilizing column (3) and a flow gauge, and passes into the tank (5) where the air is humidified and mixed with CO_2 from the bottle (6).

By means of a gas distributor (8), the gaseous mixture (which is prepared in 5% CO_2 [v/v]) bubbles in the culture medium in the growth vessels. Filters by valves (13) are inserted into the three branches of the distributor.

The culture vessels (9), placed on magnetic stirrers (14), are three cylindrical containers with a capacity of one litre, covered by a jacket for the circulation of thermostatically-controlled water (10,11,12). These vessels have a glass top with two openings, one in the center for the bubbbler and the other on one side for the loading of the culture (which

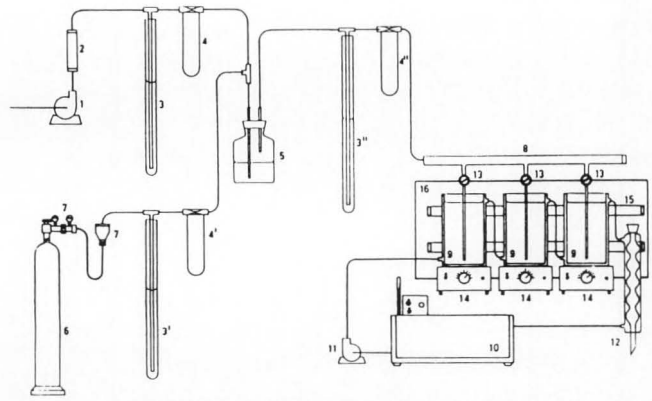

FIGURE 1. *Experimental installation.*

TABLE 1

Microorganism: *Chlorella pyrenoidosa*[1]
Culture medium: A pH: 6.5 Temperature: 30°C
Air-CO_2 : 0.5 v/v/min (5% CO_2 v/v)
I_o: 740 lux

t,h	C, g·L^{-1}	t,h	C, g·L^{-1}
0.00	0.0078	79.25	0.2246
7.75	0.0122	94.25	0.3103
22.25	0.0253	103.25	0.3593
31.25	0.0359	118.75	0.4606
48.25	0.0833	127.75	0.5031
55.25	0.1123	151.75	0.6272
70.25	0.1804	166.25	0.7432

[1] Data obtained[3] with *Chlorella pyrenoidosa*, Chick 8H Emerson, from the collection held at the School of Botany in Cambridge which was provided by the Maime Ferrán Institute of the Science Research Council. The culture medium used is medium A, as proposed by Rodríguez-López.[4]

must be previously sterilized by filtration with 0.2 μm nitrate cellulose filters), for the measurement and control of pH, and for the collection of samples. The equipment is sterlized in an autoclave before carrying out the experiment.

Two Westinghouse PLANT-GRO fluorescent light tubes (Mod. F. 40 w) (15), placed on a mobile panel (16), provide the light. The light intensity, measured with a luxometer, may be varied by altering the distance between the lamps and the growth vessels, or by introducing black metal meshes with different apertures.

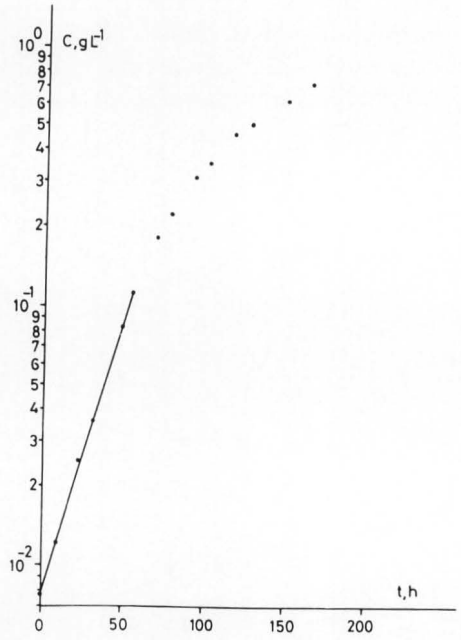

FIGURE 2. Variation of biomass concentration vs. time (semilogarithmic coordinates).

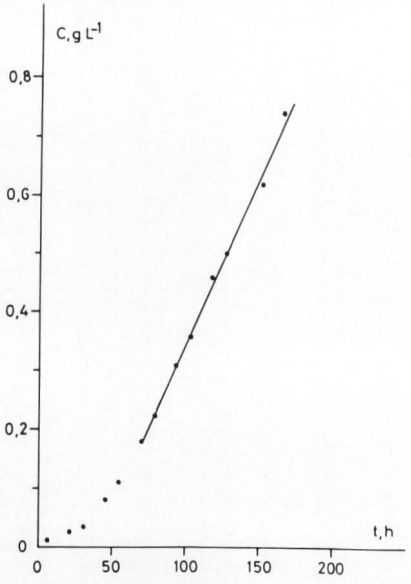

FIGURE 3.. Variation of biomass concentration vs. time (linear coordinates).

PROCEDURE AND EXPERIMENTAL CONDITIONS

1. **Preculture of the cells in a mineral medium solidified with 2% agar for four days before performing the practical session, under continuous lighting and at room temperature.**
2. **Sterilize the material.**
3. **Place the culture vessels in the equipment and adjust the light intensity desired within the range 200-2000 lux.**
4. **Prepare the culture medium, adjust the pH to 6.5, sterilize and add 500 cc to each of the culture vessels.**
5. **Adjust the temperature to 30°C.**
6. **Resuspend the preculture cells. Measure the concentration and calculate the volume to be inoculated so that each experiment is performed with an initial concentration of 0.008 gL^{-1} expressed in dry weight. Inoculate.**
7. **Adjust the composition and flow rate of the gaseous mixture added in the proportion of 0.5 v/v/min.**
8. **Collect samples throughout the experiments.**

ANALYTICAL TECHNIQUE

The cellular concentration (g dry biomass)·L^{-1} is determined indirectly after suitable dilution through the absorbance of the cell suspension measured at 600 nm. To convert absorbance to concentration, the calibration line valid up to $A_{600} < 0.5$ is used,

$$C\left(gL^{-1}\right) = 0.49\ A_{600} \qquad r^2 = 0.999 \qquad (6)$$

which has been obtained by measuring the absorbances of the suspensions of determined concentration after centrifugation, washing, and dessication at 105°C until a constant weight is achieved.

This correlation has previously been obtained with the microorganism and the culture medium specified in Table 1, and is valid for the experimental conditions to be used in the practical session.

RESULTS AND DISCUSSION

Interpretation of Individual Results

As an example, the biomass concentration at different culture times is shown in Table 1 in an experiment performed under the conditions indicated. The representation of the experimental data in semilogarithmic and linear coordinates (Figures 2 and 3) demonstrates graphically the exponential growth phase (the straight line in Figure 2) and the linear growth phase (the straight line in Figure 3).

By integrating the biomass balance during the exponential growth phase, in which μ is constant and with the initial condition that at t = 0, C = C_0 (as no appreciable lag phase is observed, then the following is obtained:

$$\ell n(C / C_0) = \mu_{max} \cdot t \qquad (7)$$

From the slope of this line, the maximum specific growth rate in h^{-1} may be calculated.

The integration of the biomass balance during the linear growth phase, in which

$$\mu \cdot C = \text{constant} = P \qquad (8)$$

leads to

$$C = P \cdot t + b \qquad (9)$$

where b represents an integration constant. This equation provides P in $gL^{-1}h^{-1}$.

Overall Interpretation of the Results

The maximum specific growth rates during the exponential phase and the growth rates during the linear phase in experiments performed at seven different incident light intensities are recorded in Table 2. The graph representing these parameters in linear coordinates vs. the incident light intensity shows the experimental variation obtained (Figures 4 and 5).

The specific rate during the exponential growth phase seems to vary linearly with I_0 up to approximately 1000 lux, and a saturation effect is observed thereafter.

The adjustment of pairs of values μ_{max}-I_0 to the kinetic models is carried out through the least squares method to the linearized form of the hyperbolic model by Tamiya,

$$\frac{1}{\mu_{max}} = \frac{1}{\mu_m} + \frac{1}{\alpha I_0} \qquad (10)$$

and through non-linear regression to the exponential model (Eq. 2).

TABLE 2[1]

Microorganism: *Chlorella pyrenoidosa*
Culture medium: A pH: 6.5 Temperature: 30°C
Air-CO_2: 0.5 v/v/min (5% CO_2 v/v)

I_0, lux	μ_{max}, h^{-1}	P, $g \cdot L^{-1} \cdot h^{-1}$
252	0.0205	0.00241
494	0.0427	0.00348
740	0.0478	0.00577
1128	0.0727	0.0101
1194	0.0777	0.0118
1688	0.0837	0.0139
2410	0.0891	0.0184

[1] Data from [3].

The model which best reproduces the experimental variation may be selected by using the minimum from the accumulative sum of the squares of the residues as a criteria. For data collected in Table 2, the exponential model provides the optimum adjustment when the following values are used for the parameters

$$\mu_m = 0.099\ h^{-1} \quad \text{and} \quad I_s = 926\ \text{lux}$$

The linear variation P-I_0 confirms the light limitation in the linear growth phase. The adjustment of these values allows the prediction of growth rate during this phase for any value of incident light

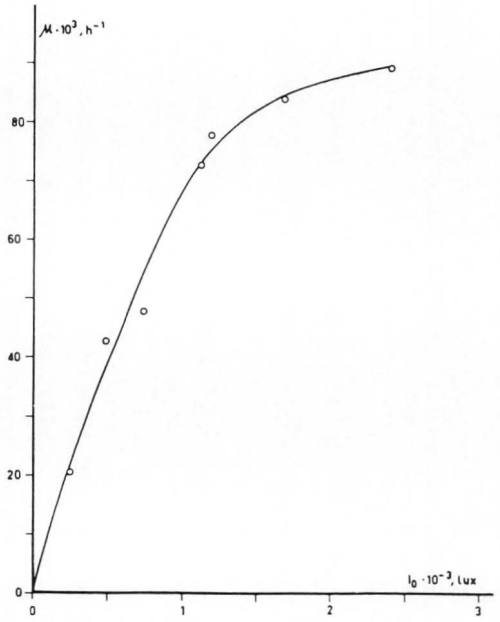

FIGURE 4 *Variation of specific growth rate vs. light intensity.*

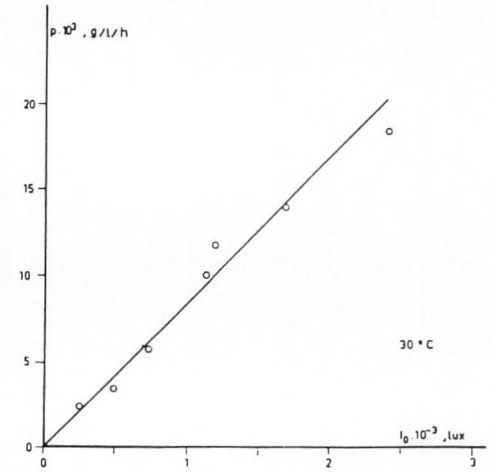

FIGURE 5. *Variation of growth rate during linear phase vs. light intensity*

intensity. In this case the relationship is

$$P = 8.45\ 10^{-6}\ I_o \qquad (11)$$

For integration of the most appropriate kinetic model, in respect of the extreme situations for light distribution, the point-by-point variation of the light intensity in the culture vessel must be known *a priori.* The difficulty in establishing this variation in a cylindrical vessel illuminated from the front leads to the need for an analogy with a parallelepipedic vessel[5] in which the point-by-point (I[x]) and average (I_m) light intensities may be calculated by means of

$$I(x) = I_o\ \exp(-k_a C x) \qquad (12)$$

$$I_m = \frac{I_o}{k_a LC}\left[1 - \exp(-k_a LC)\right] \qquad (13)$$

where k_a is the extinction coefficient with a value of 2.42 $Lg^{-1}cm^{-1}$, and L is the equivalent length of the culture chamber in centimeters.

Inclusion of Eq. (12) into Eq. (2) provides the solution to the integral of Eq. (3), and results in

$$\bar{\mu} = \frac{I_o \mu_m}{I_s k_a LC}\left[1 - \exp(-k_a LC)\right] - \frac{I_o^2 \mu_m}{I_s^2\, 4\, k_a LC}\left[1 - \exp(-2\, k_a LC)\right] + \frac{I_o^3 \mu_m}{I_s^3\, 18\, k_a LC}\left[1 - \exp(-3\, k_a LC)\right] + \ldots \qquad (14)$$

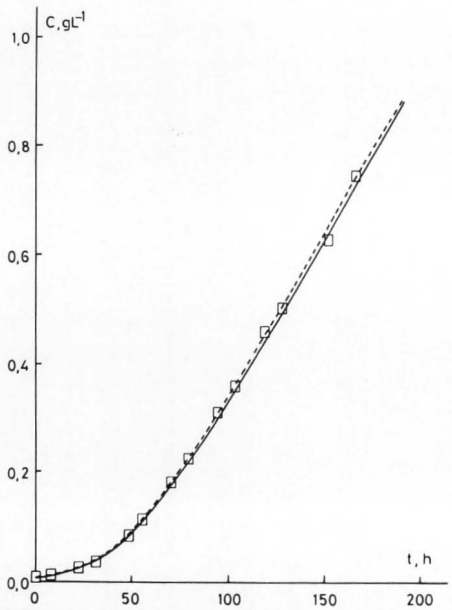

FIGURE 6. *Variation of biomass concentration vs. time (= experimental values; - - - Eq. (3), L = 4.5 cm; — Eq. (4), L = 5 cm).*

which provides the average specific growth rate as a function of the concentration when cell growth adapts to the point-by-point light intensity which the microalgae receive.

Furthermore, inclusion of Eq. (13) into Eq. (2) leads to

$$\bar{\mu} = \mu_m \left\{1 - \exp\left(-I_o\left[1 - \exp(-k_a LC)\right]\right)\right\} \Big/ \left(k_a LC\, I_s\right) \qquad (15)$$

which allows the calculation of the average specific growth rate when the cells adapt to an average value of light intensity.

In both situations, the numerical integration of the biomass balance

$$\frac{1}{C}\frac{dC}{dt} = \bar{\mu} \qquad (16)$$

through Eqs. (14) or (15) will allow the prediction of the variation in biomass concentration in time. This calculation has been carried out for both situations, modifying the value of the equivalent length until a satisfactory reproduction of the experimental results is obtained. Figure 6 shows this adjustment with a value of L = 4.5 cm, for the situation described by Eq. (3) and for the situation corresponding to Eq. (4) with a value of L = 5 cm.

The almost negligible difference between both values for L clearly shows the difficulty involved in distinguishing between the two situations. In a previous study,[6] however, using a different procedure, the value of L was found to range from 5.1 to 7.1 cm, which would seem to indicate that cellular growth adapts to average light intensities. This adaptation was proved in another study by altering the stirring rates.[5]

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