COMMUNICATIONS TO THE EDITOR

A Structured Model of Photosynthesis in Anacystis nidulans

In 1955, Myers and Kratz[1] presented a paper on photosynthetic characteristics of the blue-green alga *Anacystis nidulans*. In their study, they grew *A. nidulans* in continuous turbidostat-type cultures to obtain samples of cells of known and reproducible physiological characteristics. The growth rate controlling "substrate" was light, and steady-state systems were run at both 25 and 39°C. The cells were harvested and analyzed for cell volume, cell dry weight, pigment content, and light intensity curves of photosynthesis (Q_{O_2} as a function of light intensity) at both 25 and 39°C. The photosynthesis curves were similar to those shown in Figure 1. The photosynthesis curves saturated at high light intensities, producing a $Q_{O_2\max}$ for each set of cells grown under different conditions. The initial slope of the photosynthesis curves was directly related to the pigment content. However, the $Q_{O_2\max}$ values went through a maximum with the highest value being associated with cells which were grown at intermediate conditions of both growth rate and chlorophyll content. No explanation of this interesting phenomenon was found.

This appeared to provide an interesting case for attempting to do some structured modeling. The production of O_2 from photosynthesis requires both a light absorption step and a series of dark reactions.

These reactions may be written as follows:

$$\text{Chl} \underset{k_1}{\overset{I}{\rightleftarrows}} \text{Chl}^* \qquad \text{(light reaction)} \tag{1}$$

$$\text{Chl}^* \xrightarrow[k_2]{} \text{Chl} + YP \qquad \text{(dark reaction)} \tag{2}$$

where Chl and Chl* represent chlorophyll in its ground and activated state, P is a product of photosynthesis (O_2 in this case), and Y is a yield coefficient. In the first reaction, chlorophyll absorbs light and is raised to a higher-energy state. The return to the ground state may then occur by fluorescence via the k_1 reaction (normally small) or by the dark irreversible enzyme-type reaction which produces the product.

Differential equations may be written for these reactions as follows:

$$\frac{d\text{Chl}}{dt} = -A\phi\alpha I_0(1 - e^{-\epsilon \text{Chl} W}) + k_1\text{Chl}^* + k_2\text{Chl}^* \tag{3}$$

$$\frac{d\text{Chl}^*}{dt} = -\frac{d\text{Chl}}{dt} \tag{4}$$

$$\frac{dp}{dt} = Yk_2\text{Chl}^* \tag{5}$$

where for a spherical cell which is 2 μm in diam:A is the cross-sectional area of the cell (3.14×10^{-8} cm^2). $\phi = 3.33 \times 10^{18}$ photons/J for a light wavelength of 0.665×10^{-6} m. α is the photons required to activate one chlorophyll molecule ($\alpha = 1.0$). I_0 is the light intensity in W/cm^2 = J/sec cm^2. ϵ is the extinction coefficient which equals 3.05×10^{-9} cell/Chl molecule-μm. Chl is the number of chlorophyll

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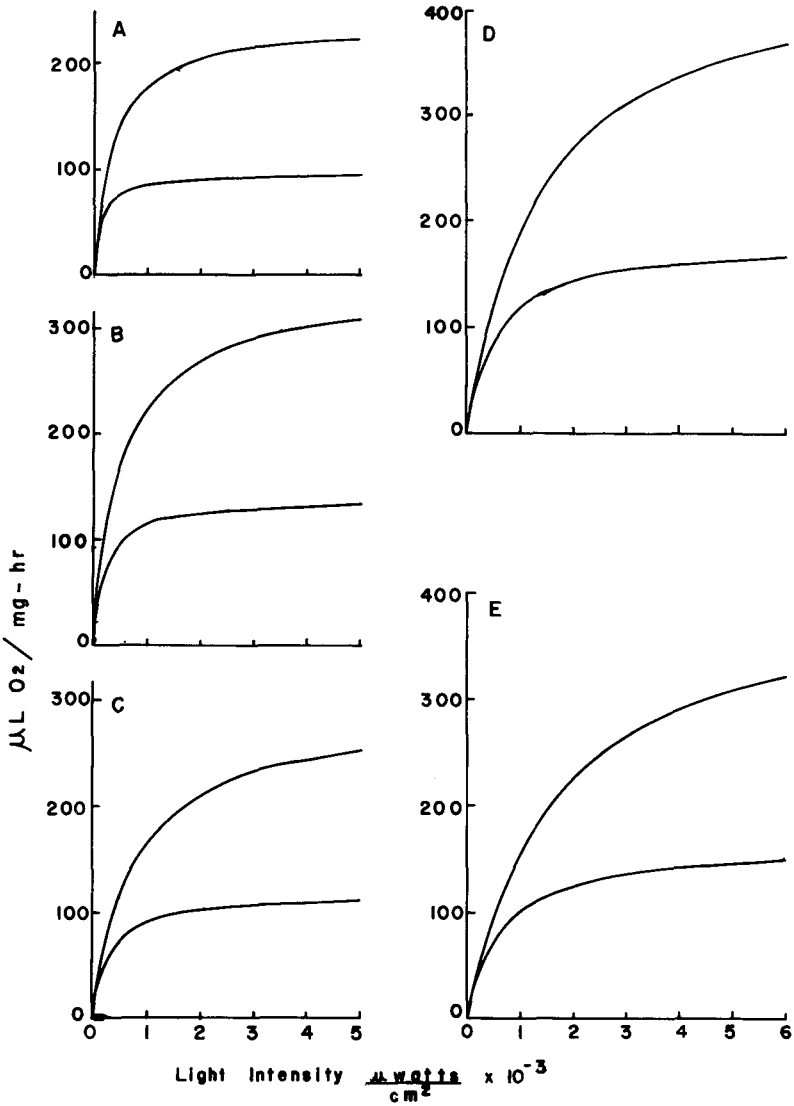

Fig. 1. Photosynthesis curves at 39°C (higher rate) and 25°C as predicted by the model. See Table I for the conditions and parameter values associated with each curve. These curves should be compared to those presented by Myers and Kratz.[1]

molecules/cell (calculated from Myers data). Chl* is the photoactivated Chl molecules/cell. W is the average thickness of the cell (1.33 μm). Y is the number of molecules of O_2 per Chl* molecule (0.25). k_1 = 0.01443 molecules Chl/molecule Chl*·sec (assumed to be small). k_2 = molecules Chl/molecules Chl*·sec (calculated from Myers data). P is the number of molecules of O_2.

By use of a pseudo-steady-state hypothesis, eq. (4) may be solved for Chl* as follows:

$$\text{Chl}^* = \frac{A\phi\alpha I_0}{k_1 + k_2}\left(1 - \frac{e^{\epsilon \text{Chl}^* W}}{e^{\epsilon \text{Chl}_T W}}\right) \tag{6}$$

where $\text{Chl}_T = \text{Chl}^* + \text{Chl}$. This represents a trial-and-error solution. If we assume that most of the light passes through the cell, then we can assume that

$$e^{\epsilon \text{Chl} W} = 1 + \epsilon \text{Chl} W$$

and we get

$$\frac{dp}{dt} = \frac{Yk_2 \text{Chl}_T I_0 A\phi\alpha\epsilon W}{k_1 + k_2 + I_0 A\phi\alpha\epsilon W} \tag{7}$$

If $k_1 \ll k_2$ and $I_0 A\phi\alpha\epsilon W \ll k_1 + k_2$, then eq. (7) reduces to

$$\frac{dp}{dt} = Y(A\phi\alpha\epsilon W) I_0 \text{Chl}_T \tag{8}$$

This states that at low light intensities, the production of O_2 is a linear function of both the pigment content (Chl_T) and the light intensity (I_0). This agrees with the results of Myers and Kratz.

If $I_0 A\phi\alpha\epsilon W \gg k_1 + k_2$, then:

$$\frac{dp}{dt} = Yk_2 \text{Chl}_T \tag{9}$$

This states that the maximum O_2 production rate is a function of both the total chlorophyll content of the cell and the rate of the dark reaction. The dark reaction is expected to be enzymatic. Therefore k_2 actually represents a combination of an enzyme concentration and a rate constant:

$$k_2 = k'_2 \text{ Enz} \tag{10}$$

Since we cannot distinguish between the rate constant and the enzyme content of the cell, it is best to lump these together as k_2. However, one must recognize that k_2 is both a function of temperature and the physiological condition of the cell.

The photosynthesis curves were reported by Myers in terms of Q_{O_2} = μl O_2/hr mg dry wt. Since the above model would give O_2 production rates in molecules O_2/cell sec, a conversion factor (C_f) is required for each of the five cases that Myers studied. These can be calculated as follows for 2 μm diam cells:

$$\frac{\text{molecules } O_2}{\text{cell-sec}} = \frac{\text{molecules } O_2}{\text{cell-sec}} \times \frac{3600 \text{ sec}}{\text{hr}} \times \frac{1 \mu\text{l } O_2}{2.69 \times 10^6 \text{ molecules } O_2}$$

$$\times \frac{1 \text{ cell}/4.19 \times 10^{-12} \text{ cm}^3}{\text{mg dry wt/cm}^3} = \frac{1 \ \mu\text{l } O_2}{C_f \text{ mg-hr}}$$

where the mg dry wt/cm^3 is obtained for each case from Myers data. The total number of chlorophyll molecules per cell can be calculated from:

$$\frac{\text{Chl}_T \text{ molecules}}{\text{cell}} = \frac{\text{mg Chl}}{\text{mg cell dry wt}} \times \frac{\text{mg dry wt}}{\text{cm}^3} \times \frac{4.19 \times 10^{-11} \text{ cm}^3}{\text{cell}}$$

$$\times 6.75 \times 10^{17} \frac{\text{molecules Chl}}{\text{mg Chl}}$$

This assumes that the molecular weight of chlorophyll is 892 g/mol.

We can write an equation for maximum O_2 production from eq. (9) if we take into account the correction factor C_f and a slight modifier on the total chlorophyll concentration. Trial-and-error solutions of eq. (6) indicate that at light intensities of 10,000 μW/cm^2, only 93% of the total chlorophyll would be in the activated state. Light intensities of this magnitude tend to be damaging to the photosynthetic apparatus. Therefore, 93% of the total chlorophyll in the activated state was set as an upper limit. The maximum O_2 production equation can then be written

$$\left(\frac{dp}{dt}\right)_{\max} = C_f Y k_2 (0.93) \mathrm{Chl}_T \tag{11}$$

Equation (11) can be used to solve for k_2 for each of the five cases presented by Myers and Kratz. The results are shown in Table I. Since some of the photosynthesis curves which were conducted by Myers and Kratz did not reach their saturation point, a temperature dependence of k_2 was calculated from only those cases which did reach saturation. This gave a ratio of k_2 (39°C)/k_2 (25°C) equal to 2.46 which represents an activation energy of 11,900 cal/mol, which is reasonable for enzyme reactions. Using the values that are presented in Table I, eq. (6), and the production equation:

$$\frac{dp}{dt} = C_f Y k_2 \mathrm{Chl}^* \tag{12}$$

the photosynthesis curves can be determined for the five cases at the two temperatures of 39 and 25°C. The results are shown in Figure 1 and are remarkably similar to the results presented by Myers.

For the three cultures which were grown at 39°C, the values of Q_{O_2}, Chl_T, and k_2 may be further plotted as a function of the specific growth rate of the cells and the light intensity to which the cells were exposed during their continuous growth (Fig. 2). As can be seen in Figure 2, k_2 appears to increase linearly with growth rate, and the chlorophyll content appears to decrease linearly with light intensity over the range for which the data were taken.

The fact that the maximum Q_{O_2} rate occurs at intermediate growth conditions then becomes more understandable. At low light intensities (low growth rate), the cells have a high chlorophyll content so as to trap as much light as possible. However, since the total amount of light energy trapped by the cell is small, they only need a relatively small dark reaction enzyme capacity. At a high light intensity (higher growth rate), they need less chlorophyll to supply their energy needs, but a much greater enzyme capacity. When such cells are removed from their normal steady-state environment and suddenly exposed to high levels of light, the dark reaction capacity limits the O_2 production of the slower growing cells and the chlorophyll content limits the faster growing cells. At intermediate conditions, there appears to be a better balance between both chlorophyll and dark reaction capacities which leads to a higher maximum O_2 production rate.

Cells which were grown at 25°C (cases D and E) had much higher dark reaction capacities (k_2's) and lower chlorophyll contents. The higher dark reaction capacity would be expected because at 25°C the dark reactions would be operating with lower rate constants so more enzyme would be required to maintain a given specified growth rate. The lower chlorophyll content may be due to a lower rate of synthesis or a lower requirement for light owing to the lower maximum specific growth rate at 25°C. The highest maximum O_2 production rate occurred in case D

TABLE I
Data and Calculations for the Model

Parameter	Myers case				
	A	B	C	D	E
Growth rate[a] (day^{-1})	0.33	1.3	2.5	0.55	0.85
Growth temperature (°C)	39	39	39	25	25
Dry wt/cell volume (mg/mm^3)	0.297	0.271	0.266	0.278	0.260
C_f [(μl O_2/mg-hr)/(molecules O_2/cell-sec)]	1.0764×10^{-4}	1.1797×10^{-4}	1.2019×10^{-4}	1.1500×10^{-4}	1.2296×10^{-4}
(Dry wt Chl/Dry wt cell) (%)	2.85	2.47	1.43	1.27	0.94
Chl_f (molecules/cell)	2.394×10^7	1.893×10^7	1.076×10^7	0.9985×10^7	0.6912×10^7
$(dP/dt)_{max}$ (μl O_2/mg-hr)	220 (39°C)	315 (39°C)	270 (39°C)	170 (25°C)	155 (25°C)
k_2 (39°C)[b] (sec^{-1})	0.3672	0.6067	0.8980	1.5665	1.9296
k_2 (25°C) (sec^{-1})	0.1493	0.2466	0.3650	0.6368	0.7844

[a] Maximum specific growth rate was 3.3 $days^{-1}$ at 39°C, 0.87 $days^{-1}$ at 25°C.
[b] k_2 (39°C)/k_2 (25°C) = 2.46.

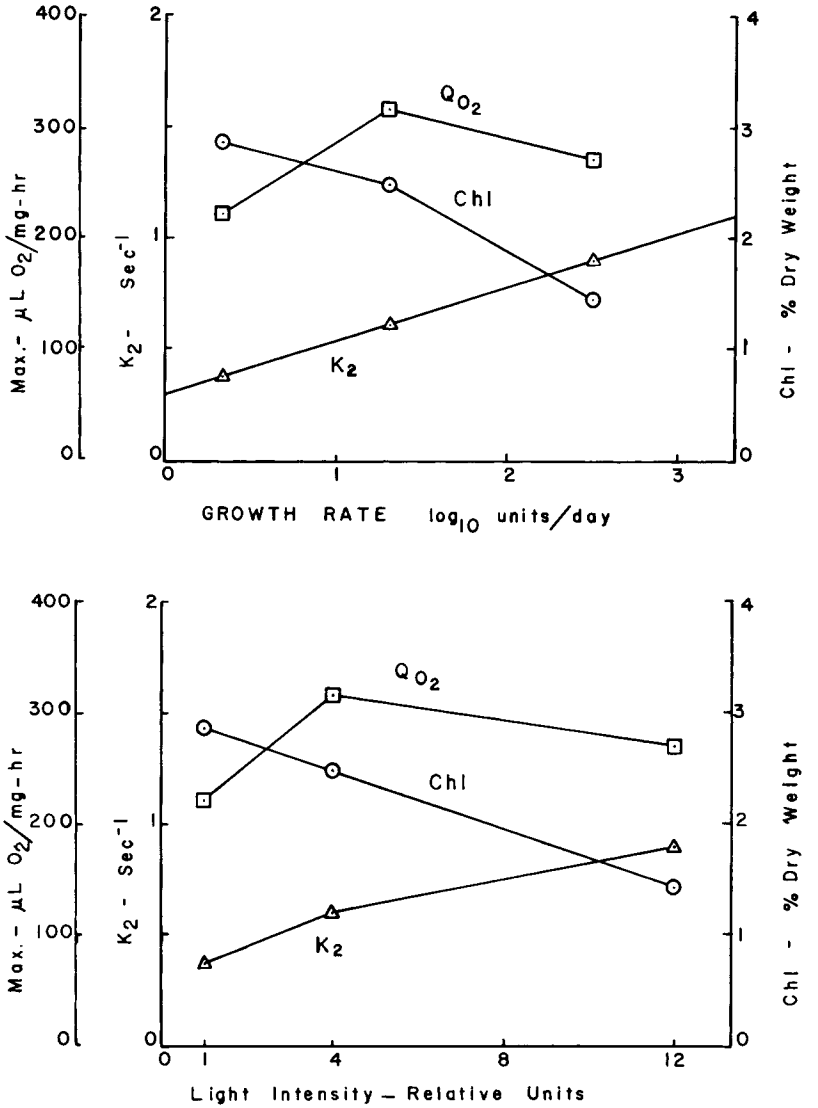

Fig. 2. Q_{O_2max}, cell chlorophyll content (Chl), and maximum dark reaction rate (k_2) plotted as a function of the growth rate of the alga and the light intensity used during growth. Data are for cells grown at 39°C.

where cells grown at an intermediate growth rate at 25°C were switched to high light intensity and 39°C.

To a certain extent, this model represents a curve fit to the data of Myers and Kratz. However, only k_2 was calculated from their data to obtain the fit. All other constants were calculated in a direct fashion prior to fitting their data. The fact that

the values of k_2 follow a logical pattern gives some credence to the validity of the model. Certainly the model is in a rather immature state, but more sophisticated data would be required to develop it further.

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References

1. J. Myers and W. A. Kratz, *J. Gen. Physiol.*, **39,** 11 (1955).

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