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THE GROWTH RATE OF MICROORGANISMS AS A FUNCTION OF THE CONCENTRATION OF A SINGLE LIMITING SUBSTRATE*

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August 1970

ABSTRACT

We have developed a simplified model of a cell consisting of a series of linked reversible enzymatic reactions dependent on the concentration of a single external substrate. The general mathematical solution for this system of reactions is presented. This general solution confirms the concept of a rate limiting step, or "master reaction", in biological systems as first proposed by Blackman.[18] The maximum rate of a process consisting of a series of consecutive enzymatic reactions is determined by, and equal to, the maximum rate of the slowest forward reaction in the series.

Of practical interest in modeling the growth rate of cells are three cases developed from the general mode. The simplest special case results in the Monod equation:[1,2]

$$\mu = \frac{\mu_{max}[S]}{B + [S]}$$

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where μ is the specific growth rate, time^{-1}; μ_{max} is the maximum specific growth rate, time^{-1}; $[S]$ is the concentration of the external limiting substrate, mass/volume; and B is a constant, mass/volume. The Monod equation results when the maximum forward rate of one enzymatic reaction in the cell is much less than the maximum forward rate of any other enzymatic reaction.

More realistic is the case where the maximum forward rates of more than one enzymatic reaction are slow. When two slow enzymatic reactions are separated from each other by any number of fast reactions that overall can be described by large equilibrium constant, the Blackman form results:

$$\mu = [S]/A, \text{ when } [S] < A\,\mu_{max}$$

$$\text{and } \mu = \mu_{max}, \text{ when } [S] > A\,\mu_{max}$$

where A is a constant, mass × time/volume.

A third case is that in which two slow enzymatic steps are separated by an equilibrium constant that is not large. Unlike the Monod and Blackman forms, which contain only two arbitrary constants, this model contains three arbitrary constants:

$$[S] = \mu A + \frac{\mu B}{(\mu_{max} - \mu)}$$

where the specific growth rate, μ, is implicitly expressed. The Monod Blackman forms are special cases of this third form.

Twelve sets of experimental data from the literature were examined. The Monod equation gave poorer fit of the data than the Blackman form in nine of the twelve cases, as determined by a non-linear least-squares fitting technique.

It is concluded that workers modeling the growth of microorganisms should not accept the Monod equation without question, as many apparently do today.

I. INTRODUCTION

Current kinetic analysis of the growth of microorganisms, both batch-wise and continuous culture, is primarily based on the relationship between limiting substrate concentration and growth rate first propsed by Monod:[1,2]

$$\mu = \frac{\mu_{max}[S]}{B + [S]} \tag{1}$$

where μ = specific growth rate, time^{-1}; μ_{max} = maximum specific growth rate, time^{-1}; [S]= limiting substrate concentration, mass/volume; B = a constant, mass/volume. This Monod equation, as it is usually called, is analogous to the Michaelis-Menten equation, which expresses the relation between substrate and rate in a single enzymatic reaction.[3]

Several criticisms of the Monod equation have been raised that will not be considered here, having to do with required maintenance energy,[4] substrate inhibition,[5] and effects of cell density.[6] These particular criticisms, if valid, will also apply to the equations given in the rest of this paper. More important is the fact, recognized by Monod,[2] that the above equation describes an oversimplified model. As will be shown later, much of the experimental data in the literature, including Monod's original data, fails to fit the simple hyperbolic form of Eq. (1).

Several empirical relationships have been proposed. These have not been widely used by workers concerned with the kinetics of growth of microorganisms. The form proposed by Teissier[7] is:

$$\mu = \mu_{max}(1 - e^{-T[S]}) \tag{2}$$

where k is an arbitrary constant, volume/mass. This form is not easy to handle mathematically, but some workers find that their data is better fit by this form[8,9] than by the Monod equation. Another correlation was proposed by Moser[10] that contains, instead of two, three arbitrary constants, μ_{max}, B, and r:

$$\mu = \frac{\mu_{max}[S]^r}{B + [S]^r} \quad . \tag{3}$$

The three arbitrary constants allow this form to fit a greater variety of curves. However, Powell[11] points out that this model may be unrealistic at very low substrate concentrations, since

$$d\mu/d[S] = \infty \quad \text{at} \quad [S] = 0, \quad \text{if } r < 1,$$

while

$$d\mu/d[S] = 0 \quad \text{at} \quad [S] = 0, \quad \text{if } r > 1.$$

Simple diffusion is probably not important in the transport of large organic substrate molecules such as sugars into a cell, since permeases are necessary for the transport of these molecules. But diffusion may be important for smaller molecules where no permeases exist.

The case of simple diffusion followed by a single irreversible Michaelis-Menten enzyme has been treated by several workers.[11,12,13] The resistance to diffusion may be the cell wall, the cell membrane, cytoplasm, or a combination of these. The model is shown diagrammatically in Fig. 1 as a cell that contains a resistance to the diffusion into the cell of external substrate of concentration $[S_e]$. The cell contains a single irreversible Michaelis-Menten reaction, which produces the product, new cellular material.

The solution to this problem is given, for example, by Reiner's Equation XI. 3),[13] where the velocity of the enzymatic reaction, v, is implicitly expressed. Reiner's solution may be made more general by noting that there is not necessarily a one-to-one correspondence between substrate consumption and new cell material formation. A yield coefficient, Y, relating μ to V, may be introduced. The following form is obtained with μ implicitly expressed:

$$[S] = \frac{B\mu}{\mu_{max} - \mu} + \frac{\mu}{k_c aY} \qquad (4)$$

where k_c is the substrate mass transfer coefficient between the bulk liquid phase and the cell interior, where the substrate is consumed, length/time; Y is the yield coefficient for the substrate and microorganism in question, cell volume/mass substrate; and a is the surface area/cell volume. If, as is generally assumed, the denominator of the last term, $k_c aY$, can be considered to be constant, this equation has the same form as the three constant model proposed later in this paper. However, the three constant model proposed later results from a different situation, where diffusion is not considered to be a limiting factor.

Reiner[13] also presents the kinetics of disappearance of substrate when there is a permease followed by a Michaelis-Menten enzymatic reaction. His model considers leakage out of the cell to be in direct proportion to the internal substrate concentration. In order to convert substrate utilization, v, to specific growth rate, μ, a yield coefficient, Y, must be inserted into Reiner's Equation XI. 6). There are a total of five arbitrary constants, making this model of little use in correlating experimental data.

II. A SIMPLIFIED MODEL OF A CELL

Our simplified model of a cell consists of N consecutive reversible Michaelis-Menten enzymatic reactions. The model is oversimplified in that double substrates, coenzymes, and allosteric effects are not considered. The case of a single enzyme-substrate complex undergoing successive transformations, as described by Hearon,[14] should not be confused with this case in which the product of each enzymatic reaction is the substrate for the next. There is one external limiting substrate, S. The model is described by the following systems of N equations:

$$
\begin{aligned}
&S + E_1 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} SE_1 \underset{k'_{-1}}{\overset{k'_1}{\rightleftharpoons}} P_1 + E_1 \\
&P_1 + E_2 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} P_1E_2 \underset{k'_{-2}}{\overset{k'_2}{\rightleftharpoons}} P_2 + E_2 \\
&P_{n-1} + E_n \underset{k_{-n}}{\overset{k_n}{\rightleftharpoons}} P_{n-1}E_n \underset{k'_{-n}}{\overset{k'_n}{\rightleftharpoons}} P_n + E_n \\
&P_{N-1} + E_N \underset{k_{-N}}{\overset{k_N}{\rightleftharpoons}} P_{N-1}E_N \underset{k'_{-N}}{\overset{k'_N}{\rightleftharpoons}} P_N + E_N \quad .
\end{aligned}
\tag{5}
$$

E denotes the enzyme, P denotes the products of the enzymatic reactions, and k denotes the rate constants. P_N is the final product, considered here to be new cell material. Catalysis occurs in the substrate-enzyme complexes, such as $P_{n-1}E_n$. In this general case no reaction steps are considered

to be irreversible; irreversibility is approached only by virtue of a large negative free energy change.

The velocity of the nth reaction, v_n, is found by assuming that steady state is obtained. Steady state may be assumed even if we consider an autocatalytic process such as balanced cellular growth, if we express enzyme concentrations per unit of cellular volume. The solution[15] is given here with v_n implicitly expressed:

$$[P_{n-1}] = \frac{[P_n](V_{Bn} + v_n) + K'_n v_n}{K'_n/K_n(V_{Fn} - v_n)} , \qquad (6)$$

where $[P_n]$ and $[P_{n-1}]$ are concentrations of species P_n and P_{n-1}; mass/volume; $V_{Fn} = k'_n E^o_n$, the maximum forward rate, mass/time/volume; $V_{Bn} = k_{-n} E^o_n$, the maximum backward rate, mass/time/volume; E^o_n is the total amount of the nth enzyme, mass/volume; $K_n = (k_{-n} + k'_n)/k_n$, mass/volume; $K'_n = (k_{-n} + k'_n)/k'_{-n}$, mass/volume. Note that V_{Fn}, V_{Bn}, K_n, and K'_n are related to the equilibrium constant for the transformation of P_{n-1} to P_n, K_{eqn}, through the so-called Haldane relation,[15] $K_{eqn} = V_{Fn} K'_n / V_{Bn} K_n$.

All of the individual reactions give equations of the form of Eq. (6). We obtained the solution for the whole system of N equations by realizing that at steady state $v = v_1 = v_2 = \ldots\ldots = v_n = \ldots\ldots = v_N$ and by making successive substitutions until the concentration of S, [S], is related only to v and $[P_n]$ the concentration of final product. If P_N is a cellular constituent and cellular growth is balanced, then $[P_N]$ is constant with time (when expressed per unit of cellular volume). The solution is:

$$[S] = \sum_{n=1}^{N} \frac{K_n \prod_{i=1}^{n} (V_{B_{i-1}} + v)}{\prod_{i=1}^{n} \frac{K'_{i-1}}{K_{i-1}} (V_{Fi} - v)} + \frac{[P_N] \prod_{n=1}^{N} (V_{Bn} + v)}{\prod_{n=1}^{N} \frac{K'_n}{K_n} (V_{Fn} - v)} , \qquad (7)$$

where V_{Bo} is defined equal to zero, and K'_o/K_o is defined equal to one. If, for example, we have three enzymatic reactions, N = 3, and:

$$[S] = \frac{K_1 v}{(V_{F1} - v)} + \frac{K_2 v (V_{B1} + v)}{\frac{K'_1}{K_1} (V_{F1} - v)(V_{F2} - v)} + \frac{K_3 v (V_{B1} + v)(v_{B2} + v)}{\frac{K'_1}{K_1} \frac{K'_2}{K_2} (V_{F1} - v)(V_{F2} - v)(V_{F3} - v)}$$

$$+ \frac{[P_3] (V_{B1} + v)(V_{B2} + v)(V_{B3} + v)}{\frac{K'_1}{K_1} \frac{K'_2}{K_2} \frac{K'_3}{K_3} (V_{F1} - v)(V_{F2} - v)(V_{F3} - v)} . \qquad (8)$$

If one wishes to convert Eqs. (7) and (8) to [S] as a function of μ by using the relation $\mu = Yv$, then these new equations would be of exactly the same form with the same number of arbitrary constants.

Eqs. (7) and (8) include the case where a permease functions to bring substrate into the cell. A permease is simply a "directional" enzyme, using its active site to move substrate in one direction.

III. THE MASTER REACTION CONCEPT

In Eq. (7), the maximum rate of forming new cell material is set by, and cannot exceed, the smallest V_{Fn}, since [S] approaches infinity as v approaches the smallest V_{Fn}. Such a rate limiting step, or "master reaction", cannot occur in a sequence of chemical reactions of higher than zero-order (see Denbigh et al.[16] for a discussion of first-order chemical systems). But as Perret[17] has pointed out, a rate limiting step must occur in a sequence of enzymatic reactions, since an enzymatic reaction becomes zero-order with respect to the concentration of its substrate when the enzyme is completely saturated with its substrate. Blackman[18] is usually given credit for the master reaction concept when he stated in 1905 without proof, "when a process is conditioned as to its rapidity by a number of separate factors, the rate of the process is limited by the pace of the 'slowest' factor". The validity of this statement has been questioned by Burton[19], Monod[2], and others. Burton, arguing on the basis of first-order transformations, concluded that a rate limiting step cannot exist in a sequence of consecutive reactions at steady state.

Unfortunately, Burton's dismissal of Blackman's axiom has been accepted by many workers in the biological sciences as the final word on this matter, despite Perret's[17] criticism that it was based on irreversible uncatalyzed first-order reactions. Likewise, the statement of Monod,[2] "A master reaction could take control only if its rate were very much slower than that of all the other reactions", has also served to mislead workers in biology. Equation (7) provides a mathematical proof for Blackman's axiom, which Blackman himself did not offer.

Care must be used not to carry the master reaction concept too far. Even though the slowest reaction sets the maximum rate at which the overall process may proceed, it does not entirely determine the relationship of overall rate as a function of substrate concentration. At low substrate concentrations more than one of the reactions in the series may influence the overall rate.

An exception to the master reaction concept does exist. Consider two parallel reaction sequences producing the same product. Two enzymes, one in each of the parallel sequences, must reach their maximum rates before the process reaches its maximum rate. But if we treat these two limiting steps as a single enzyme, then the above arguments hold. Similar situations result when there are more than two parallel sequences producing the same product.

IV. A SINGLE SLOW ENZYMATIC REACTION: THE MONOD EQUATION

Equation (1), the form for specific growth as a function of substrate concentration suggested by Monod,[1,2] will arise from the general form, Eq. (7), in two different cases. The first case, which will not be examined further, occurs when all the arbitrary constants (the V and K values) are of the same order of magnitude, but fortuitously have the right values so that Eq. (7) collapses into the Monod form. The probability of this occurring is small.

The second case arises when the maximum forward velocity of one of the enzymatic reactions is much smaller than any of the other enzymatic reactions, and there is a large negative standard free energy change between this slow reaction and the ultimate product formation of new cell material. A negative standard free energy change between the slow step and the formation of product is likely, since the probability of the last enzymatic step of the series being the slow one is small, and since negative standard free energy changes are associated with spontaneous biochemical reactions. Since all reactions are assumed to be very fast and reversible, except for the one slow reaction, the substrates and products of the fast reactions can be assumed to be in equilibrium with each other. This means that all irreversibilities occur in the one slow reaction.

The Monod equation can be found from the general form, Eq. (7), or from the following, using the above assumptions:

$$S \longleftrightarrow P_1 \longleftrightarrow P_2 \longleftrightarrow \ldots\ldots \longleftrightarrow P_{n-1} \tag{9a}$$

$$P_{n-1} + E_n \underset{k_{-n}}{\overset{k_n}{\rightleftharpoons}} P_{n-1}E_n \underset{k'_{-n}}{\overset{k'_n}{\rightleftharpoons}} P_n + E_n \quad \text{(the slow step)} \tag{9b}$$

$$P_n \longleftrightarrow P_{n+1} \longleftrightarrow \ldots\ldots \longleftrightarrow P_N \quad . \tag{9c}$$

For Reaction (9a) there is an equilibrium constant relating the concentrations of S and P_{n-1}, $[P_{n-1}] = K_{eq}[S]$. For Reaction (9c) there is a similar relationship, $[P_n] = [P_N]/K'_{eq}$. However, since a large negative standard free energy change has been assumed for this step, K'_{eq} approaches infinity, and $[P_n]$ approaches zero. As $[P_n]$ approaches zero, the rate of the back reaction associated with k'_{-n} also approaches zero, and the last step in Reaction (9b) may be treated as an irreversible one; therefore:

$$v_n = \frac{V_{Fn}[P_{n-1}]}{[P_{n-1}] + K_n} \quad . \tag{10}$$

This is the irreversible Michaelis-Menten equation. Making use of the equilibrium relation for Reaction (9a) and the fact that all the new velocities are equal, the Monod equation is obtained relating overall rate to substrate concentration:

$$v = \frac{V_{Fn}[S]}{[S] + K_n/K_{eq}} \tag{11}$$

where K_n/K_{eq} is an apparent Michaelis constant, which will be smaller the larger K_{eq} becomes. K_{eq} can be expected to increase as n increases because of the usual negative standard free energy change associated with biological processes.

V. THE CASE OF TWO SLOW ENZYMATIC REACTIONS: THE THREE CONSTANT FORM

This case assumes that two enzymatic reactions have slow maximum forward rates. As in the preceding case it will be assumed that all other enzymatic reactions are fast and in equilibrium, and that there is a large negative standard free energy change (and hence a large equilibrium constant) between the second slow enzymatic reaction and the formation of P_N, new cell material. The system is:

$$S \longleftrightarrow P_1 \longleftrightarrow P_2 \longleftrightarrow \ldots\ldots \longleftrightarrow P_{m-1} \tag{12a}$$

$$P_{m-1} + E_m \underset{k_{m-1}}{\overset{k_m}{\rightleftharpoons}} P_{m-1}E_m \underset{k'_{-m}}{\overset{k'_m}{\rightleftharpoons}} P_m + E_m \tag{12b}$$

$$P_m \longleftrightarrow P_{m+1} \longleftrightarrow \ldots\ldots \longleftrightarrow P_{n-1} \tag{12c}$$

$$P_{n-1} + E_n \underset{k_{-n}}{\overset{k_n}{\rightleftharpoons}} P_{n-1}E_n \underset{k'_{-n}}{\overset{k'_n}{\rightleftharpoons}} P_n + E_n \tag{12d}$$

$$P_n \longleftrightarrow P_{n+1} \longleftrightarrow \ldots\ldots \longleftrightarrow P_N \quad . \tag{12e}$$

For Reaction (12a) there is an equilibrium constant relating the concentrations of S and P_{m-1}, $[P_{m-1}] = K_{eq}[S]$. A similar relation holds for Reaction (12c), $[P_{n-1}] = K'_{eq}[P_m]$. $[P_n]$ approaches zero, beacuse of the large equilibrium constant assumed for step (12e). The irreversible Michaelis-Menten equation results:

$$V_n = \frac{V_{Fn}[P_{n-1}]}{[P_{n-1}] + K_n} \quad . \tag{13}$$

For Reaction (12b), however, irreversibility is not assumed, and the back reaction must be considered:[15]

$$v_m = \frac{V_{Fm}K'_m[P_{m-1}] - V_{Bm}K_m[P_m]}{K'_m[P_{m-1}] + K_m[P_m] + K_mK'_m} \quad . \tag{14}$$

Making use of the equilibrium relations and rearranging the above two equations, they become respectively:

$$[P_m] = \frac{v_nK_n/K'_{eq}}{V_{Fn} - v_n} \tag{15}$$

$$[S] = \frac{[P_m](V_{Bm} + v_m) + K'_mv_m}{(K_{eq}K'_m/K_m)(V_{Fm} - v_m)} \quad . \tag{16}$$

Substituting Equation (15) into Equation (16), and since at steady state $v_m = v_n = v$:

$$[S] = \frac{K_nK_m}{K_{eq}K'_{eq}K'_m}\frac{v(V_{Bm} + v)}{(V_{Fm} - v)(V_{Fn} - v)} + \frac{K_m}{K_{eq}}\frac{v}{(V_{Fm} - v)} \quad . \tag{17}$$

Equation (17) has five arbitrary constants and hence is of limited value in fitting experimental data. Two more assumptions will be made in order to reduce Eq. (17) into a more usable form with only three arbitrary constants. These assumptions are that V_{Bm} and V_{Fm} are large in comparison to V_{Fn}. With these assumptions the first slow enzymatic reaction is largely responsible for setting the effective Michaelis constant, while the second slow reaction corresponds to the slowest of the enzymatic reactions in the chain. It is

unlikely that a single enzymatic reaction sets both of these quantities, as the previous case that yielded the Monod equation assumed.

The above assumptions are reasonable. The reaction that sets the effective Michaelis constant would be expected to occur early in the series of reactions, because in general the effective Michaelis constant decreases (and K_{eq} increases) the farther a step is from the beginning of the series. It is also probable that the slowest enzymatic reaction occurs after this step, since there are so many enzymatic reactions involved in cell growth.

If V_{Bm} and V_{Fm} are large in comparison with V_{Fn}, Eq. (17) reduces to:

$$[S] = \frac{K_n K_m V_{Bm}}{K_{eq} K'_{eq} K'_m V_{Fm}} \frac{v}{(V_{Fn} - v)} + \frac{K_m}{K_{eq} V_{Fm}} v \quad . \tag{18}$$

Equation (18) has three arbitrary constants and is of the same form as Eq. (4), the case where simple diffusion is important. Therefore one cannot distinguish between these two different cases on the basis of curve shape.

Even though the above three constant form will be used later in the fitting of experimental data, where it is called "the three constant form", other three constant forms are possible.

For instance, if K'_{eq}, the equilibrium constant between the two slow reactions, is very large, we obtain from Eq. (17):

$$\begin{aligned} [S] &= \frac{K_m}{K_{eq}} \frac{v}{(V_{Fm} - v)} , \quad \text{when } [S] < \frac{K_m}{K_{eq}} \frac{V_{Fn}}{(V_{Fm} - V_{Fn})} \\ [S] &= \infty , \quad \text{when } [S] > \frac{K_m}{K_{eq}} \frac{V_{Fn}}{(V_{Fm} - V_{Fn})} \quad . \end{aligned} \tag{19}$$

Equation (19) results in a Michaelis-Menten shaped curve at low substrate concentrations, but a sharp bend to the saturated rate occurs when V_{Fn} is reached. Johnson,[20] without mathematical support, has postulated that this form might be expected in a series of enzymatic reactions containing two slow enzymatic reactions widely separated from each other. Among the twelve sets of data that we have examined, this behavior was not found.

However, for photosynthesis in the alga Chlorella, behavior close to the model expressed by Eq. (19) has been found. Figure 2 shows the light response curves of photosynthesis that were obtained by Myers and Graham[21] for Chlorella grown at differing specific growth rates. All sets of data give nearly the same initial curve shapes, but the light saturated plateaus bend sharply from the initial curve. Similar data gathered in our laboratory[22] are shown in Fig. 3.

The kinetics of photosynthesis are complicated, since the two light reactions involved in photosynthesis must be considered. This case is treated elsewhere.[22]

A third possible three constant form results from Eq. (17) when V_{Bm} and V_{Fn} are small compared to V_{Fm}:

$$[S] = \frac{K_n K_m}{K_{eq} K_{eq} K_m V_{Fm}} \frac{v^2}{(V_{Fn} - v)} + \frac{K_m}{K_{eq} V_{Fm}} v \ . \qquad (20)$$

Equation (20) is exactly the same form as Eq. (18) except that v^2 replaces v in the first term on the right side.

VI. THE BLACKMAN FORM

If, in Eq. (18) K'_{eq} becomes very large because of a large negative standard free energy change between the two slow reactions, then:

$$[S] = \frac{K_m}{K_{eq} V_{Fm}} v \quad , \text{ when } [S] < \frac{K_m V_{Fn}}{K_{eq} V_{Fm}}$$

$$[S] = \infty \quad , \text{ when } [S] > \frac{K_m V_{Fn}}{K_{eq} V_{Fm}} \; . \tag{21}$$

This form was first proposed by Blackman,[18] without mathematical justification, for the rate of photosynthesis as a function of carbon dioxide concentration or light intensity. The model gives first-order behavior with respect to substrate concentration that, as substrate concentration is increased, suddenly changes to zero-order behavior.

A saturated rate must result, since no matter how large K'_{eq} becomes, the first term on the right hand side of Eq. (18) approaches infinity as v approaches V_{Fn}. A large value for K'_{eq} can be expected if the two controlling enzymatic reactions are not situated close together in the reaction series. Since hundreds, if not thousands, of enzymatic reactions are involved within the cell, this sort of behavior can be expected often.

There is a simple physical explanation for the sharp break in the Blackman form. Since the equilibrium constant K'_{eq} is very large, the product of the first slow enzymatic reaction, P_m, is present at a concentration approaching zero, being instantly swept away by the fast reactions between the two slow steps. The second slow reaction keeps pace with the first and exerts no influence on the first reaction until the substrate concentration is

raised to the point where the second slow step is working at its maximum rate. Now the concentrations of intermediates in the chain between the two slow reactions rise until the net rate of the first slow reaction is equal to the maximum rate of the second slow reaction. Thus, the sharp change from first-order to zero-order behavior occurs because the second slow reaction exerts no effect on the first slow step until the maximum rate of the second is reached. When this point is reached, the second step completely controls.

VII. ANALYSIS OF EXPERIMENTAL DATA

The three forms that will be tested against twelve sets of data are given by Eqs. (11), (21) and (18), which represent the Monod, Blackman, and three constant forms, respectively. We now introduce the yield coefficient, Y (relating substrate consumption to cell material production), to express the equations in terms of specific growth rate, μ, rather than rate of substrate consumption, v. Introducing the yield coefficient does not change the form or the number of arbitrary constants in the equations.

Equation (11), the Monod equation, becomes:

$$[S] = \frac{\mu B}{\mu_{max} - \mu} \tag{22}$$

or equivalently:

$$\mu = \frac{\mu_{max}[S]}{[S] + B} \tag{23}$$

where $\mu_{max} = YV_{Fn}$, and $B = K_n/K_{eq}$.

A property of the Monod equation is that the slope of the tangent to the curve at $\frac{1}{2}\mu_{max}$ must be $\frac{1}{4}$ of the initial slope (where [S] and μ are zero). This can be used as a rough check to see if data fit the Monod equation.

Equation (21), the Blackman form, becomes:

$$\begin{aligned} [S] &= \mu A \ , \quad \text{when } [S] < A\mu_{max} \\ [S] &= \infty \ , \quad \text{when } [S] > A\mu_{max} \end{aligned} \tag{24}$$

or equivalently:

$$\mu = [S]/A \ , \quad \text{when } [S] < A\mu_{max}$$
$$\mu = \mu_{max} \ , \quad \text{when } [S] > A\mu_{max} \tag{25}$$

where $\mu_{max} = YV_{Fn}$, and $A = K_m/YK_{eq}V_{Fm}$.

An alternate way to express the Blackman form so that a continuous function is obtained is:

$$[S] = A\mu + \frac{\varepsilon\mu}{\mu_{max} - \mu} \tag{26}$$

where ε is very much smaller than A. In the computer fitting of data ε was set equal to 10^{-20}.

Equation (18), the three constant form, becomes:

$$[S] = \mu A + \frac{B\mu}{\mu_{max} - \mu} \tag{27}$$

or equivalently:

$$\mu = \frac{B + A\mu_{max} + [S] - \sqrt{(B + A\mu_{max} + [S])^2 - 4A\mu_{max}[S]}}{2A} \tag{28}$$

where $\mu_{max} = YV_{Fn}$, $A = K_m/YK_{eq}V_{Fm}$, and $B = K_nK_mV_{Bm}/ K_{eq}K'_{eq}K'_mV_{Fm}$.

The negative square root must be taken in Eq. (28) in order to have a physically meaningful situation.

A non-linear least squares iterative procedure was used to determine the constants of best fit for the three forms. The computer program used was a Lawrence Radiation Laboratory Computer Library program, E2 BKY LSQVMT. The minimization procedure is an iterative gradient method that uses a variable metric.[23] Subroutines must be furnished by the user giving the fitting functions and their first derivatives with respect to the fitting constants. The subroutines used are given in Appendix I. The quantity to be minimized is the sum of the squares of the errors, SUMSQ:

$$\text{SUMSQ} = \sum_{i=1}^{N} \left[\mu_i - F(S_i, \mu_{max}, A, B)\right]^2 \text{weight}_i \qquad (29)$$

where F is the fitting function, corresponding to either the Monod, Blackman, or three constant forms, and N is the number of data points. The weighting factor, weight_i, was set equal to 1.0 in all cases. Equation (29) assumes that the independent variable, S_i, is well known, and that all scatter is in the dependent variable, μ_i. This is probably a reasonable assumption for the data examined.

Table I shows the results corresponding to the best fit to each of the three models for the twelve sets of data examined. The arbitrary constants μ_{max}, A, and B are given along with three criteria for goodness of fit: (1) the sum of the squares of the errors, SUMSQ, (2) the standard deviation, s. d., and (3) the modified coefficient of determination, CODET. The sum of the squares of the errors is probably the best criterion to use in comparing the appropriateness of each of the three models against a given set of data. Indeed, this is the quantity minimized in determining the arbitrary constants.

The standard deviation, s. d., was defined by:

$$s.\ d. = \sqrt{\frac{SUMSQ}{N - d.\ f.}} \tag{30}$$

where d. f. is the degrees of freedom available in the fitting fuctions; d. f. = 2 for the Monod and Blackman forms, and d. f. = 3 for the three constant form.

The modified coefficient of determination, CODET, is equal to the "explained variation" divided by the "total variation".[24] CODET is defined here as:

$$CODET = \frac{\sum_{i=1}^{N} (\mu_i - \bar{\mu})^2 - SUMSQ}{\sum_{i=1}^{N} (\mu_i - \bar{\mu})^2} \tag{31}$$

where $\bar{\mu}$ is the average of the μ_i values, $\bar{\mu} = \sum_{i=1}^{N} \mu_i/N$. The reader is referred to Appendix II for the problem of defining a coefficient of determination for non-linear equations, such as encountered here with all three equation forms.

Appendix III gives the Fortran IV computer program used in determining all three of the above statistical quantities. Appendix IV gives the twelve sets of examined data points.

The cases given in Table I will now be discussed individually. The data were examined using the same units for the variables as appear in the original articles, so that the reader may check the results against the original articles. Table II gives the units for the numbers appearing in Table I. It should be kept in mind while looking at the following data that the three constant form will always give better fit than the Monod or Blackman forms since it includes the Monod and Blackman forms as special cases.

A. Monod's Glucose Data

These data[1] are often quoted as support for the Monod equation. However, the Blackman and three constant forms, as shown in Table I and Fig. 4, fit these data better. In obtaining these data, Monod used sugar substrate solutions of known initial concentrations. A very dilute suspension of *Escherichia coli* was then allowed to grow over an interval short enough so that an insignificant amount of substrate was consumed. Knowing the time interval, Monod was able to calculate growth rates in divisions per hour (the specific growth rate, μ, = (ℓn 2) (divisions/hour)). This same technique was used by Monod in the next two sets of experimental data.

The best fit of the Monod equation gave an effective Michaelis constant, B, of 11.97 mg/l (eℓ). This is three times higher than the value suggested by Monod.[1] It is clear, however, that these data do not fit the Monod equation.

B. Monod's Mannitol Data

These data,[1] also on *E. coli*, are given in Fig. 5 and again fit the Blackman and three constant forms better than the Monod equation. However, this set of data is not ideal, since there are no data points in the region between very low and very high values of μ.

C. Monod's Lactose Data

Even though μ_{max} for *E. coli* is reached at higher concentrations of lactose than with glucose or mannitol, the Monod equation form again gives the worst fit of the data.[1] This case is shown in Fig. 6.

D. Nitrate Data of Shelef, Oswald, and Golueke

These data,[9] taken on *Chlorella pyrenoidosa* 71105 at 35° C in continuous culture, had nitrate as the only limiting substrate. The data given in Fig. 7 show that the Monod equation is better than the Blackman form, but that the three constant form fits the data best.

E. Arginine Data of Novick

These results from an E. coli mutant[25] are fit well by the Blackman form (Fig. 8).

F. Tryptophane Data of Novick and Szilard

Similar to the preceding, these data[26] on E. coli mutant strain B/1, limited by tryptophane, fit the Blackman form much better than the Monod equation (Fig. 9).

G. Phosphate Data of Hinshelwood

These data,[27] taken on Bact. lactis aerogenes with phosphate limiting, were obtained by taking tangents from the batch growth curve as it went from the exponential into the stationary phase. Separate experiments to determine the yield coefficient, Y, allowed an estimation of the concentration of substrate, while the slopes of the tangents gave the growth rate. Best fit is given by the Blackman and three constant forms, which fall on top of each other (Fig. 10).

H. Schaefer Glucose Data

The slow growing Mycobacterium tuberculosis is growth limited by glucose, except at very high concentrations.[28] The effective Michaelis constant is some 500 times larger than for E. coli. This is one of the three cases where the Monod equation gives better fit than the Blackman form (Fig. 11). The data were obtained by a method similar to the one used by Monod.

I. Johnson Oxygen Data

The data presented are one of three sets obtained by Johnson.[20] All three sets have similar form, differing only in the saturated rate. The data shown in Fig. 12 were obtained using an oxygen electrode to follow the concentration of oxygen as a function of time in a closed system containing the

organism Candida utilis. Oxygen consumption rates were obtained by taking tangents from the resulting curve. In this case (Fig. 12) and the following cases the dependent variable is the consumption (or production) of oxygen, a quantity that should be proportional to the specific growth rate.

An important result found by Johnson is that continuous and batch data cannot be directly compared. The continuous culture data of Johnson suggested that the Monod equation almost perfectly fit the data, while batch experiments of the type mentioned in the previous paragraph gave results very close to the Blackman form. Johnson explained this on the basis of adaptation of the yeast's oxygen-saturated respiration rate according to the oxygen concentration under which it is grown in continuous culture. In Johnson's batch-type experiments, however, the yeast did not have time to adapt. Myers and Graham[21] also found that adaptation phenomena in algae prevent the direct comparison of batch and continuous culture data.

J. The Carbon Dioxide Data of van der Honert

These data[29] were taken on the blue-green alga Hormidium flaccidum and result in a curve close to the Blackman form. Only one set of van der Honert's data is shown in Fig. 13. The other two sets, taken at a lower temperature and a lower light intensity, also closely approximate the Blackman form.

K. The Carbon Dioxide Data of Emerson and Green

Emerson and Green's data[30] give a result similar to that found by van der Honert above, in that the Blackman form gives much better fit than the Monod equation. These data were obtained on Chlorella pyrenoidosa in M/25 phosphate buffer, pH 4.6, at 25° C in a Warburg type respirometer with light intensity not limiting. The results are shown on Fig. 14.

L. The Carbon Dioxide Data of Whittingham

These data[31] were taken on the same algal species and under the same experimental conditions as the preceding data of Emerson and Green.[30] The only experimental difference was that Whittingham used a recording infrared spectrophotometer (recirculating the gas) to measure carbon dioxide concentration. The results are similar in that the saturated rates of photosynthesis are nearly the same. However, the initial slope of this data (Fig. 15) is higher than it was for the data of Emerson and Green (Fig. 14). Also, these data fit the Monod equation almost perfectly, in contrast to the preceding data, which fit the Blackman form.

Since the algae were the same species and the experimental conditions were the same, one must wonder why these experimental results differ. The answer may be found by referring to an earlier work of Briggs and Whittingham.[32] They found that the effective Michaelis constant dropped as the carbon dioxide concentration to which the algae were acclimated during their growth was dropped. Thus, we suspect that the algae of Emerson and Green, shown in Fig. 14, were not grown under the same conditions as the algae used by Whittingham, shown in Fig. 15. Teleologically it is logical to expect that as the carbon dioxide concentration during growth is dropped, that the algae would compensate by manufacturing more of the CO_2 fixing enzyme, carboxydismutase. This would be expected to cause an increase in the initial slope of the curve.

VIII. DISCUSSION AND CONCLUSIONS

The shape of the curve, growth rate (or equivalently respiration rate or photosynthetic rate) as a function of substrate concentration, can lead to basic information on the mechanisms associated with cellular growth.

For instance, the magnitude of B relative to $A\mu_{max}$ in the three constant form may give indication of the proximity of the two slow enzymatic reactions to each other, since B approaches zero as the equilibrium constant (K'_{eq} in Eq. (18)) between these two reactions increases. Recent data obtained by Terui and Sugimoto[33] suggest that the availabliity of electrons, which ultimately come from organic substrate, sets the maximum rate of respiration in the yeasts Saccharomyces cerevisiae and Hansenula anomala. However, they maintain that the effective Michaelis constant is set by the cytochrome system close to the point of oxygen utilization, perhaps by cytochrome oxidase. Terui and Sugimoto argue against the possibility of an oxygen diffusion limitation as was proposed by Johnson.[20] The fact that Terui and Sugimoto obtain curve shapes approaching the Blackman form supports their argument that the two controlling enzymatic steps are widely separated from each other.

The Blackman form may be approached more often among individual cells than is indicated by data obtained on unsynchronized populations of cells. The cells in unsynchronized cultures are not identical. If all cells as individuals obey the Blackman form, but the parameters μ_{max} and A vary (perhaps because of different enzyme concentrations at different life cycle stages), then a summation of individual contributions is obtained. This summation is not of the Blackman form, but is rounded in the transition from first-order to zero-order behavior. This could be why much of the examined data, such as

that of Johnson shown in Fig. 12, show rounding in the transition region. The third arbitrary constant in the three constant form allows us to fit such data well, even though the reason for the rounded shape may not be the mechanistic interpretation used to derive the three constant form.

Another problem in ascribing a model to a particluar organism growing on a particular substrate is caused by adaptive phenomena: the time required for an organism to adapt to a new environment. For this reason batch culture data cannot be used to predict continuous culture performance, or vice versa. This has been demonstrated experimentally by Johnson[20] with Candida utilis and by Myers and Graham with Chlorella ellipsoidea.[21] The reason is simple: cells in continuous culture have sufficient time to adapt their enzyme levels to their environment, but cells growing in batch culture do not. Thus, Johnson's Candida, growing with oxygen as the limiting substrate in continuous culture, exhibited apparent Monod equation behavior. However, cells taken from continuous culture at any given steady state approximated Blackman behavior. Adaptation effects do not appear to have been fully appreciated in various studies concerned with the kinetics of microorganism growth.

It is logical to wonder what the effect of branching in biochemical pathways has upon the results presented here. The basic system expressed by Reaction (5) assumed a linear sequence in the formation of new cellular material from substrate. Branching and network reaction schemes have been discussed by Perret.[17] Branching should have a minimum effect on the results presented here, since the slowest reaction determines the maximum rate of growth, regardless of the branch it is in. However, if there are two or more branches leading to the same product, then a single slow step in one of these branches will not limit cell growth, since there are one or more other paths leading to the same product.

Equations (7) and (8) show how a master reaction can arise in cellular growth. In the discussion of the master reaction concept it was pointed out that the slowest enzymatic reaction in the cell determines the maximum possible growth rate of the cell. One might wonder why the cell doesn't adjust its enzyme levels so that all reactions have the same maximum rate. There are at least two reasons.

If a cell is growing in an extremely low concentration of the limiting substrate (at a low growth rate), it has reason to want to manufacture large numbers of permease sites for that substrate so that it might absorb that substrate at as high a rate as possible. But this cell would be expected to manufacture small amounts of the internal enzymes associated with growth, since it cannot grow at a fast rate anyway. Taking this cell and placing it in a environment with high concentrations of all substrates should result in the decrease of the number of permease sites and an increase in the enzymes associated with growth, since it is now able to grow at an increased rate. Such an adaptive response would be beneficial to the organism if it is going to be able to compete and survive according to the Darwinian law of survival of the fittest. Such adaptation has been found experimentally in the case of Chlorella with light as the limiting substrate.[21,22] In this case of algae limited by light, chlorophyll is analogous to the permease sites mentioned above, since chlorophyll (and to a lesser extent other pigments such as the carotenoids) is responsible for trapping the substrate, light. In Chlorella the chlorophyll content was found to be higher by a factor of three or four when the algae were grown at low intensities, compared to cells grown at high light intensities.

It has been shown that all enzymatic reactions in a metabolic sequence do not proceed at more or less the same maximum forward rates. Racker[34] points out that some of the enzymes of glycolysis exceed by hundreds of times the concentration one would think would be needed. The cell is not foolish, however, and the purpose of these seemingly high concentrations is to ensure that those enzymatic steps are both fast and reversible. Mahler and Cordes[35] discuss this point in their chapter on the metabolism of carbohydrates:

> The initial and final reactions in most metabolic sequences, be they anabolic or catabolic, are frequently rigged in such a fashion as to render them virtually irreversible thermodynamically; i.e. they possess $\Delta G^{\circ\prime}$ values (which we recall as the standard free energy change at pH 7) equal to ≤ -4 kcal/mole. Teleologically the reason for this is not hard to understand. It provides for easy flux through the pathway and minimizes the possibility of a logjam of intermediates somewhere along the line. The enzymes responsible for these essentially reversible and unique steps have often been referred to as pacemaker enzymes.

Mahler and Cordes also discuss these pacemaker enzymes in regard to induction and repression, and activation and inhibition:

> We shall see that frequently the most sensitive points for controls of this general nature are those that stand at the beginning or the end of specific metabolic sequences, i. e. the pacemaker enzyme mentioned earlier.

It was previously noted that the general solution to a series of reversible enzymatic reactions as given by Eq. (7) includes the case where a permease functions to bring substrate into the cell. A permease is simply a "directional" enzyme, using its active site and energy, probably in the form of adenosine triphosphate, to move substrate in one direction. The model presented here, Eq. (7) and subsequent simplifications, unlike the model of Reiner[13] previously discussed, does not allow for leakage of substrate from the cell through "holes" in the cell membrane by diffusion, but allows for

leakage only by virtue of the reversibility of the enzymatic permease reaction. For large organic substrate molecules, such as sugars, loss by diffusion through holes is probably unreasonable, since such holes are probably too small.

The results presented in this paper strongly suggest that the Blackman and three constant forms deserve closer attention by those concerned with the kinetics of bacterial growth. These equations may be used in the examination of both continuous and batch culture of microorganisms in the same manner that the Monod equation has been used in the past. Before any of the three models can be applied, it must be ascertained that wall growth is not a problem in the culture vessel and that adequate mixing of substrate is achieved in the case of continuous culture. Corrections for substrate inhibition, maintenance energy, and cell density may be applied to the Blackman and three constant forms as easily as they have to the Monod equation in the past.

NOMENCLATURE

a	microorganism surface area, area/cell volume
A	a constant appearing in the Blackman and three constant forms, mass time/volume
B	a constant appearing the Monod and three constant forms, mass/volume
CODET	coefficient of determination
d. f.	degrees of freedom
E	enzyme
F	fitting function used in LSQVMT computer program
k	reaction rate constant
k_c	mass transfer coefficient, length/time
K	equilibrium constant
P	product
r	arbitrary constant in the Moser equation
S	substrate
s. c.	standard conditions
s. d.	standard deviation
SUMSQ	sum of the squares of the errors
T	arbitrary constant in the Teissier equation, volume/mass
v	velocity of a single enzymatic reaction or a sequence of such reactions, mass/time/volume
V	maximum velocity, either forward or backward depending subscript, of a single enzymatic reaction, mass/time/volume
weight	weighting factor that may be used in LSQVMT
x	independent variable

y	dependent variable
Y	yield coefficient relating cellular material formation to substrate consumption, cell volume/mass substrate
ε	value of B that yields the Blackman form as a continuous function; set equal to 10^{-20} in computer program, mass/volume
μ	specific growth rate, $time^{-1}$
[ ]	concentration of the item enclosed mass/volume

Superscripts

o	total amount of all states or forms
'	differentiates between equilibrium or rate constants
-	mean value

Subscripts

B	backward
calc	calculated value
eq	equilibrium
F	forward
i	indexing subscript
max	maximum
1, 2,...m...n...N	number of the step in the sequence

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Table I. The best fit of twelve sets of data against the Monod, the Blackman, and the Three Constant Forms- (dimensions are given in Table II).

	μ_{max}	A	B	Number of data points	SUMSQ	s.d.	CODET
Monod-glucose[1]							
Monod Form	1.440	0.	11.97	27	0.363	0.1204	0.925
Blackman Form	1.229	17.63	0.		0.207	0.0909	0.958
Three Constant Form	1.256	16.46	0.701		0.1941	0.0899	0.960
Monod-mannitol[1]							
Monod Form	1.273	0.	3.70	30	0.746	0.1632	0.831
Blackman Form	1.153	6.51	0.		0.400	0.1196	0.906
Three Constant Form	1.155	6.46	0.0123		0.400	0.1218	0.906
Monod-lactose[1]							
Monod Form	1.267	0.	24.9	29	0.223	0.0909	0.948
Blackman Form	1.008	39.5	0.		0.1982	0.0857	0.954
Three Constant Form	1.072	29.5	3.69		0.1646	0.0795	0.962
Shelef *et al.*-nitrate[9]							
Monod Form	5.41	0.	2.09	24	1.297	0.243	0.979
Blackman Form	4.09	0.748	0.		1.844	0.289	0.971
Three Constant Form	4.61	0.412	0.585		0.985	0.217	0.984
Novick-arginine[25]							
Monod Form	0.653	0.	0.496	4	0.01043	0.0722	0.942
Blackman Form	0.560	1.893	0.		0.00310	0.0393	0.982
Three Constant Form	0.564	1.350	0.0305		0.0001728	0.01314	0.999

(continued)

Table I (continued)

	μ_{max}	A	B	Number of data points	SUMSQ	s.d.	CODET
Novick and Szilard-tryptophane[26]							
Monod Form	1.224	0.	2.24	5	0.00782	0.0511	0.978
Blackman Form	0.820	3.00	0.		0.001974	0.0257	0.994
Three Constant Form	0.854	2.52	0.1191		0.000311	0.01248	0.999
Hinshelwood-phosphate[27]							
Monod Form	1.088	0.	0.001195	6	0.0341	0.0924	0.950
Blackman Form	0.927	0.001758	0.		0.00394	0.0314	0.994
Three Constant Form	0.927	0.001758	0.		0.00394	0.0362	0.994
Schaefer-glucose[28]							
Monod Form	0.0369	0.	0.0275	7	1.343×10^{-5}	0.001639	0.982
Blackman Form	0.0295	1.338	0.		3.55×10^{-5}	0.00267	0.951
Three Constant Form	0.0342	0.616	0.01249		0.885×10^{-5}	0.001487	0.988
Johnson- oxygen[20]							
Monod Form	1.116	0.	0.823	15	0.0440	0.0582	0.954
Blackman Form	0.886	1.499	0.		0.0212	0.0404	0.978
Three Constant Form	0.935	1.248	0.0851		0.00493	0.0203	0.995
van der Honert-CO_2[29]							
Monod Form	124.0	0.	13.42	15	892.	8.28	0.834
Blackman Form	98.0	0.268	0.		292.	4.49	0.951
Three Constant Form	102.1	0.244	0.551		129.1	3.28	0.976

(continued)

Table I (continued)

	μ_{max}	A	B	Number of data points	SUMSQ	s.d.	CODET
Emerson and Green-CO_2[30]							
Monod Form	22.6	0.	3.53	10	49.2	2.48	0.856
Blackman Form	20.9	0.381	0.		5.36	0.818	0.984
Three Constant Form	20.9	0.381	0.		5.36	0.875	0.984
Whittingham-CO_2[31]							
Monod Form	19.87	0.	0.810	8	1.845	0.555	0.984
Blackman Form	15.64	0.0842	0.		26.6	2.11	0.767
Three Constant Form	19.84	0.00077	0.801		1.845	0.607	0.984

Table II. The dimensions for the twelve sets of data given in Table I.

	μ_{max}*	A	B
Monod - lactose	divisions/hr	$\frac{mg \cdot hr}{\ell \cdot divisions}$	mg/ℓ
Monod - mannitol	divisions/hr	$\frac{mg \cdot hr}{\ell \cdot divisions}$	mg/ℓ
Monod - lactose	divisions/hr	$\frac{mg \cdot hr}{\ell \cdot divisions}$	mg/ℓ
Shelef *et al.* - nitrate	day^{-1}	mg · day/ℓ	mg/ℓ
Novick - arginine	hr^{-1}	γ hr/ℓ	γ/ℓ
Novick and Sziland - tryptophane	hr^{-1}	γ hr/ℓ	γ/ℓ
Hinshelwood - phosphate	divisions/hr	$\frac{mM \cdot hr}{\ell \cdot divisions}$	mM/ℓ
Schaefer - glucose	divisions/hr	$\frac{M \cdot hr}{\ell \cdot divisions}$	M/ℓ
Johnson - oxygen	μM/ℓ/min	min	μM/ℓ
van der Honert - CO_2	relative units	$\frac{vol\% \times 10^3}{relative\ units}$	$vol\% \times 10^3$
Emerson and Green - CO_2	μℓ/μℓ cells/hr	$\frac{\mu M \cdot \mu\ell\ cells \cdot hr}{\ell\ \mu\ell}$	μM/ℓ
Whittingham - CO_2	μℓ/μℓ cells/hr	$\frac{\mu M \cdot \mu\ell\ cells \cdot hr}{\ell\ \mu\ell}$	μM/ℓ

* the units of s.d. are the same as μ_{max}, and the units of SUMSQ are the same as μ_{max}^2.

FIGURE CAPTIONS

Fig. No.

1 The case of simple diffusion followed by a single Michaelis-Menten enzymatic reaction.

2 Photosynthetic rate as a function of irradiance at 25°C as measured in Warburg buffer consisting of 0.19 M $KHCO_3$ and 0.01 M Na_2CO_3. The flasks contained 0.12 mg dry weight per cm^2 of *Chlorella ellipsoidea*. Data are replotted from Myers and Graham.[21]

3 Photosynthetic rate as a function of irradiance at 25°C as measured in Warburg buffer consisting of 0.19 M $KHCO_3$ and 0.01 M Na_2CO_3. The flasks contained *Chlorella pyrenoidosa* with 4.2 μg total chlorophyll per $cm.^2$ Data from Dabes, Wilke, and Sauer.[22]

4 Doubling rate of *E. coli* at 37°C limited by glucose concentration. Data from Monod.[1]

5 Doubling rate of *E. coli* at 37°C limited by mannitol concentration. Data from Monod.[1]

6 Doubling rate of *E. coli* at 37°C limited by lactose concentration. Data from Monod.[1]

7 The specific growth of *Chlorella pyrenoidosa* 71105 at 35°C in continuous culture as a function of nitrate concentration as limiting substrate. The light intensity was above saturation. Data from Shelef, Oswald, and Golueke.[9]

8 The specific growth rate of an *E. coli* mutant at 37°C as a function of the concentration of arginine. Data from Novick.[25]

9 The specific growth rate of *E. coli* mutant strain B/1 at 37°C as a function of tryptophane concentration. Data from Novick and Szilard.[26]

Fig. No.

10 Doubling rate of Bact. lactis aerogenes at 40° C limited by phosphate concentration. Data from Hinshelwood.[27]

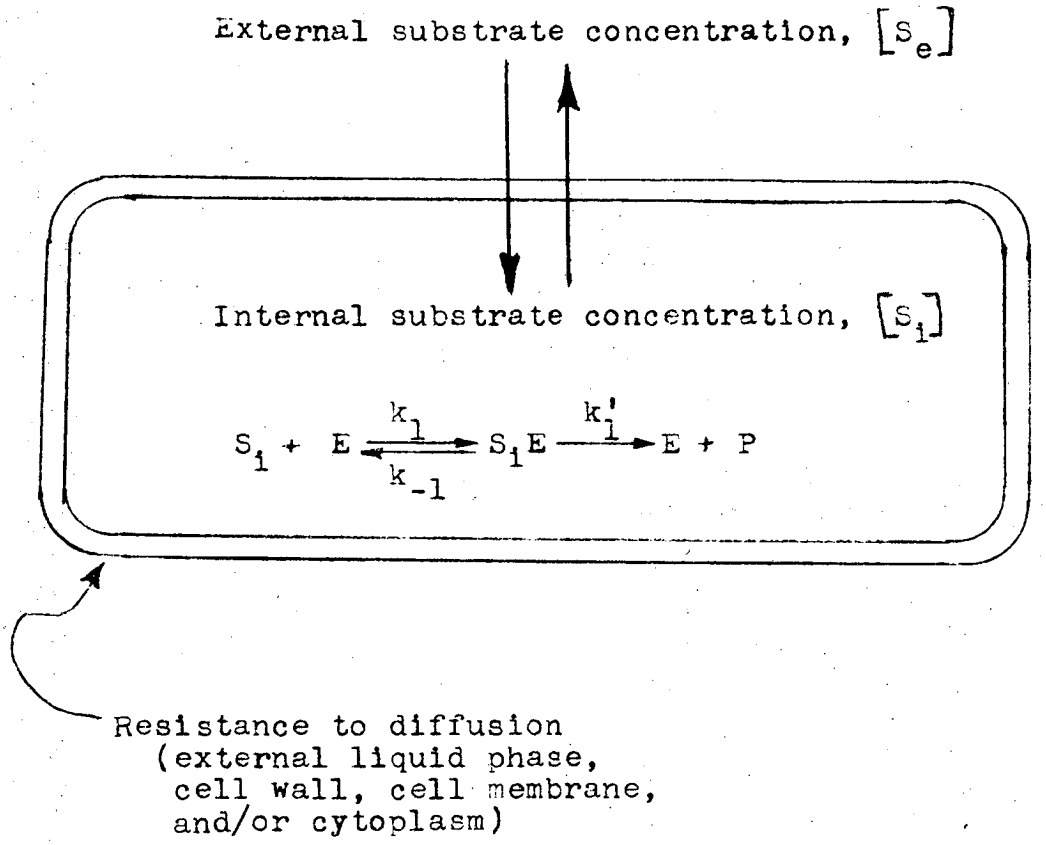
11 Doubling rate of Mycobacterium tuberculosis at 38° C limited by glucose concentration and grown on Dubos' medium. Data from Schaefer.[28]

12 The rate of oxygen uptake of Candida utilis at 30° C as a function of oxygen concentration. The data presented here are for cells grown at 0.97×10^{-6} M O_2. Data from Johnson.[20]

13 The rate of photosynthesis of Hormidium flaccidum at 20° C as a function of CO_2 concentration. Data from van der Honert.[29]

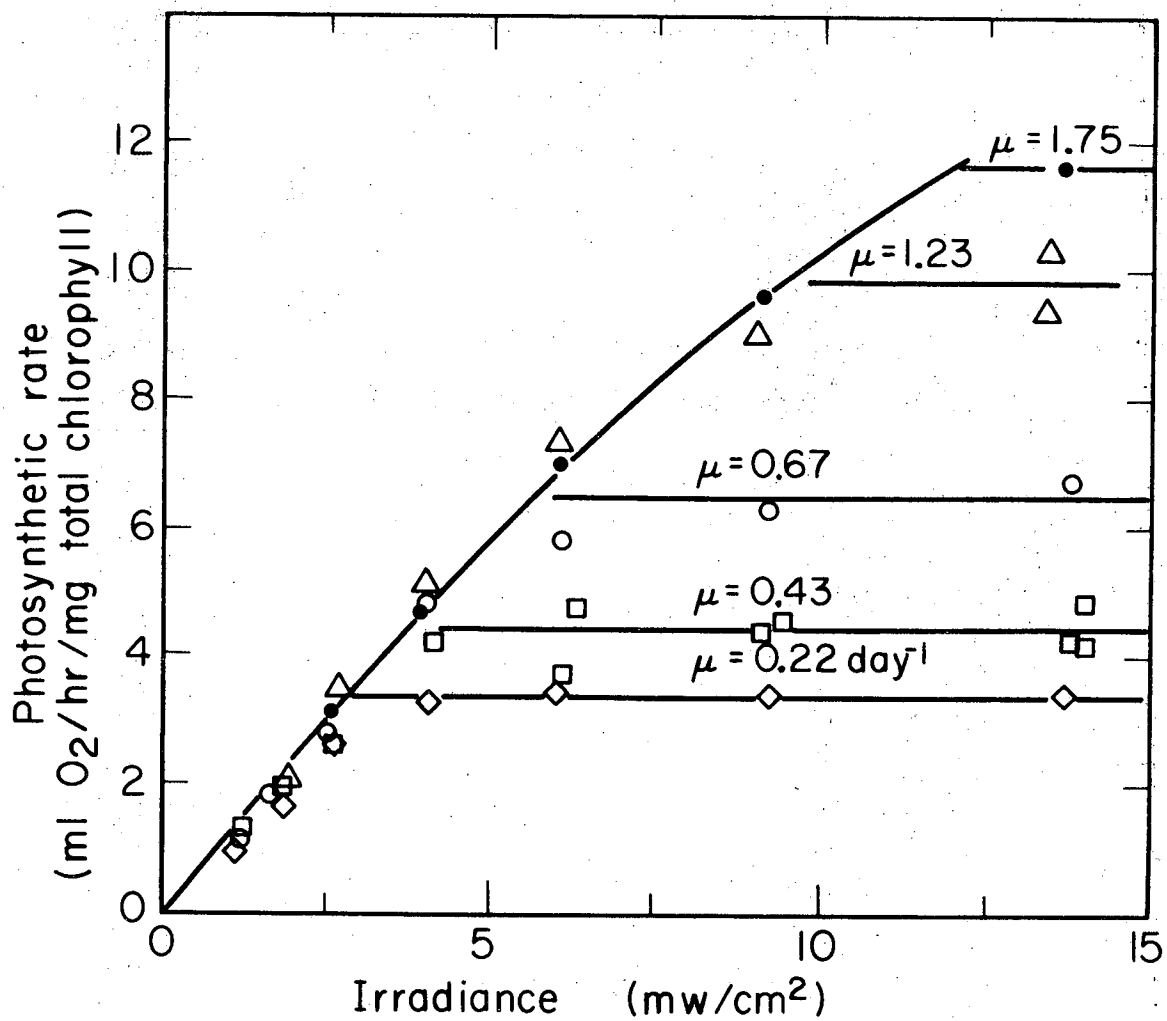
14 The rate of photosynthesis of Chlorella pyrenoidosa at 25° C in M/25 phosphate buffer at pH 4.6 as a function of CO_2 concentration. Light intensity was saturating. Data from Emerson and Green.[30]

15 The rate of photosynthesis of Chlorella pyrenoidosa at 25° C in M/30 phosphate buffer at pH 4.6 as a function of CO_2 concentration. Light intensity was saturating. Data from Whittingham.[31]



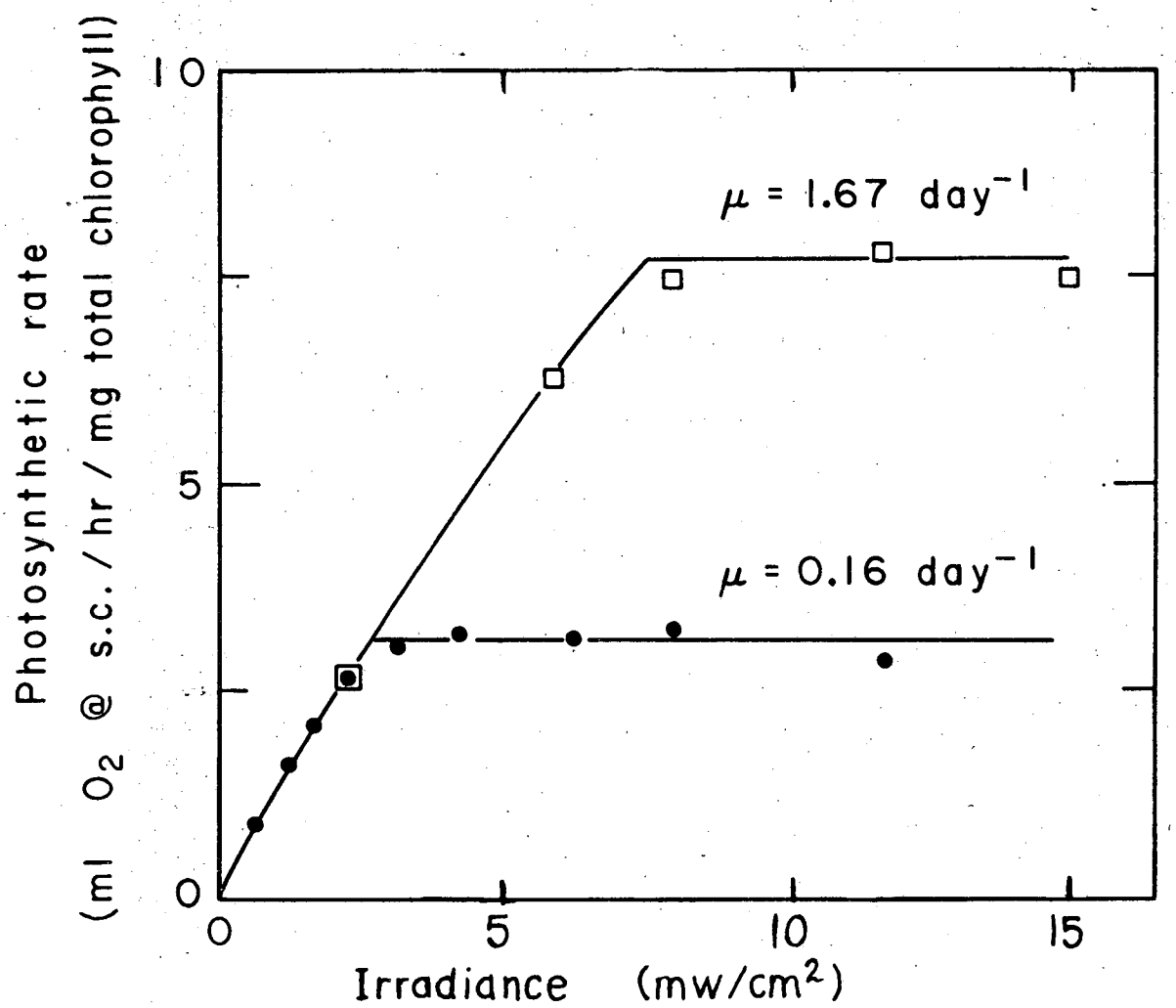
XBL 708-1912

Fig. 1



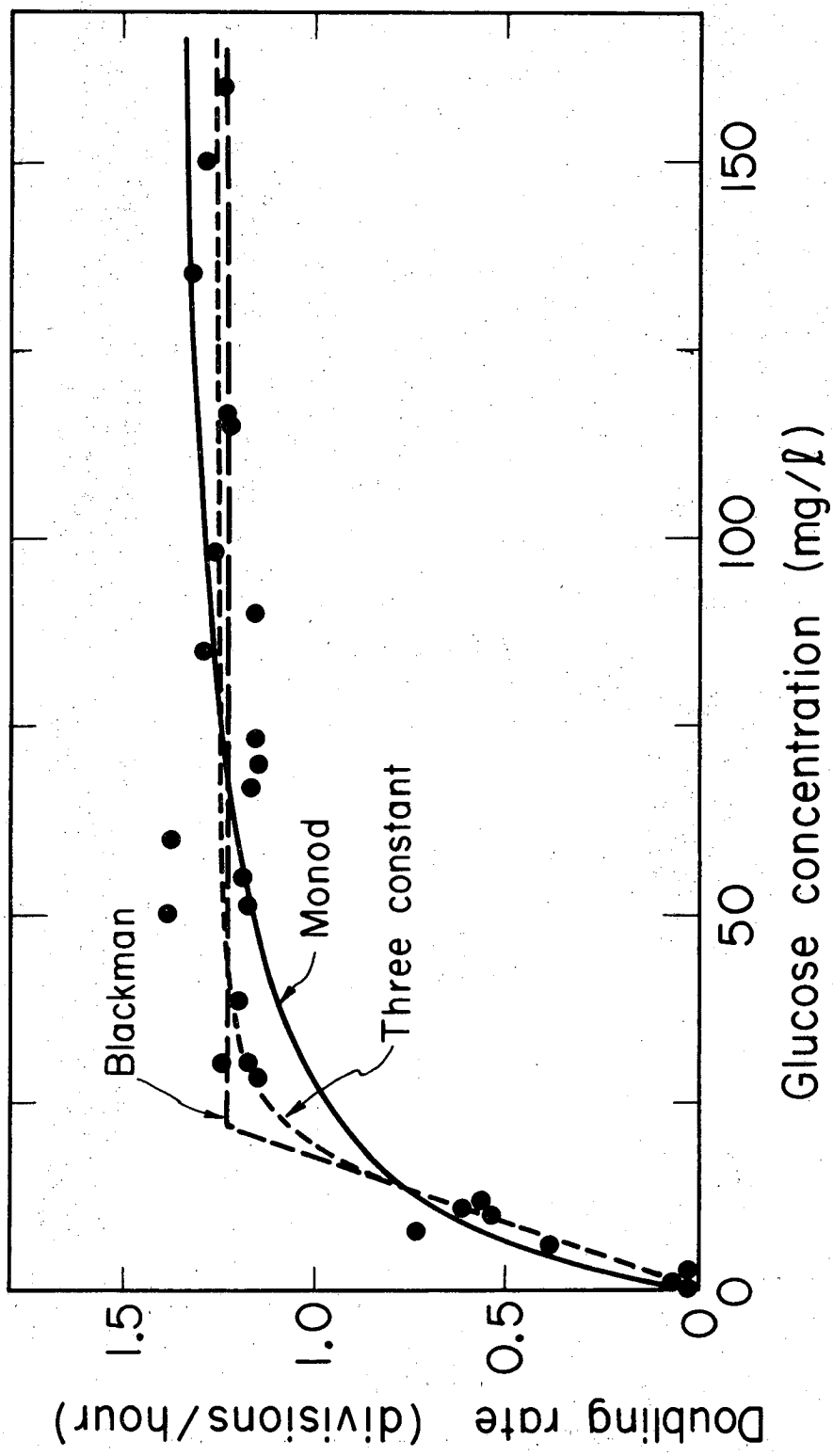
XBL698-3636

Fig. 2



XBL695-2709

Fig. 3



XBL698-3637

Fig. 4

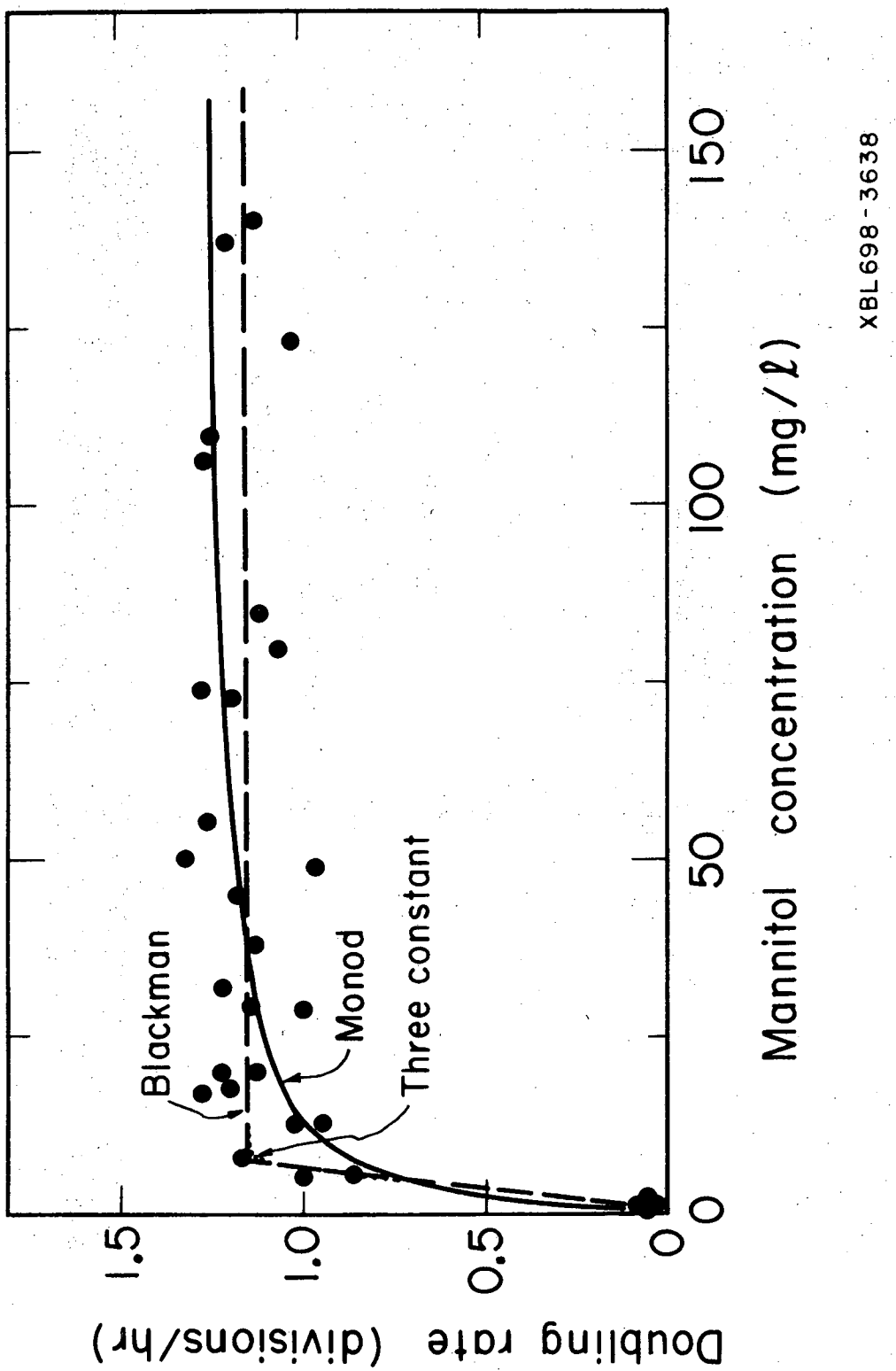
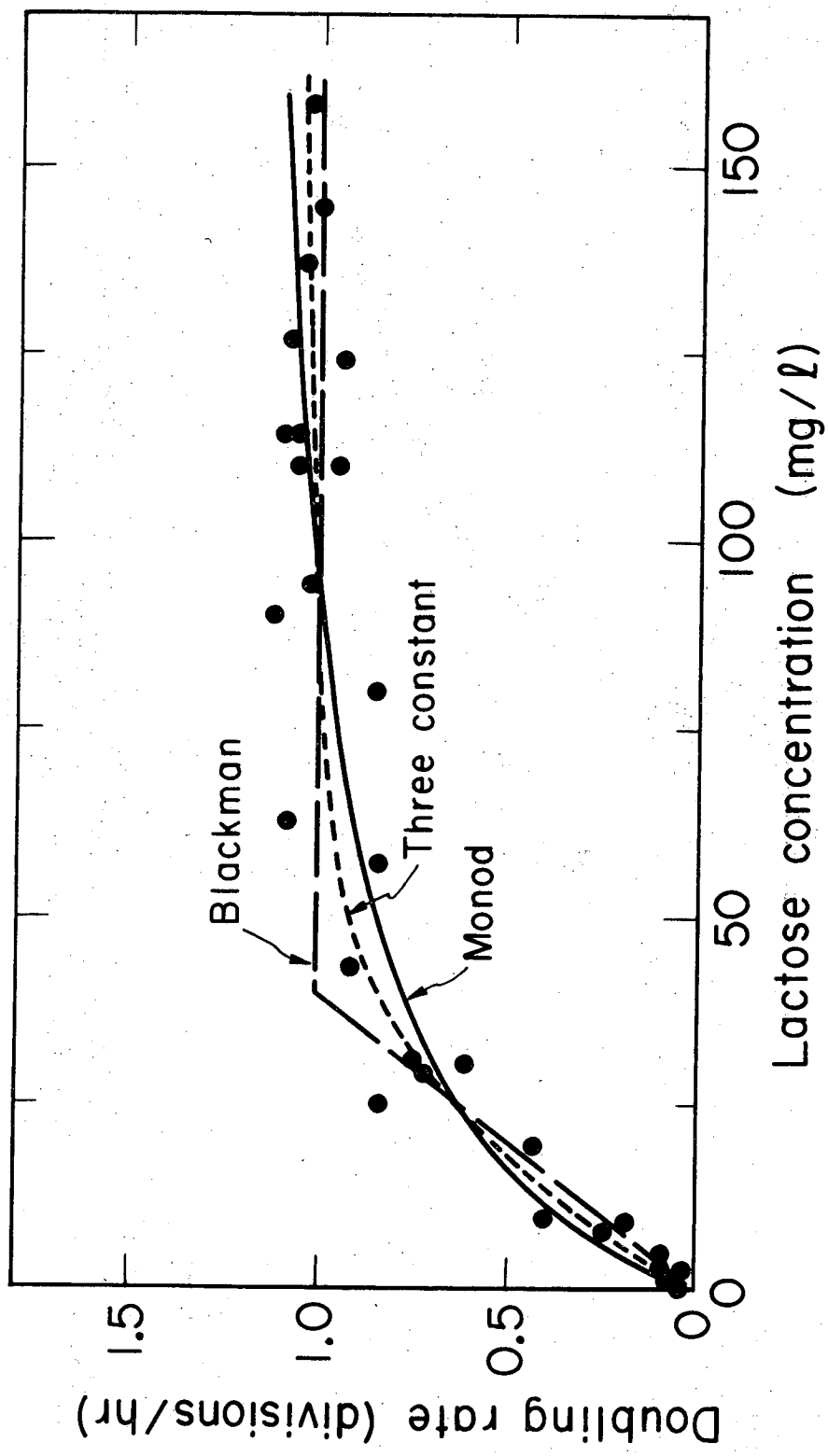
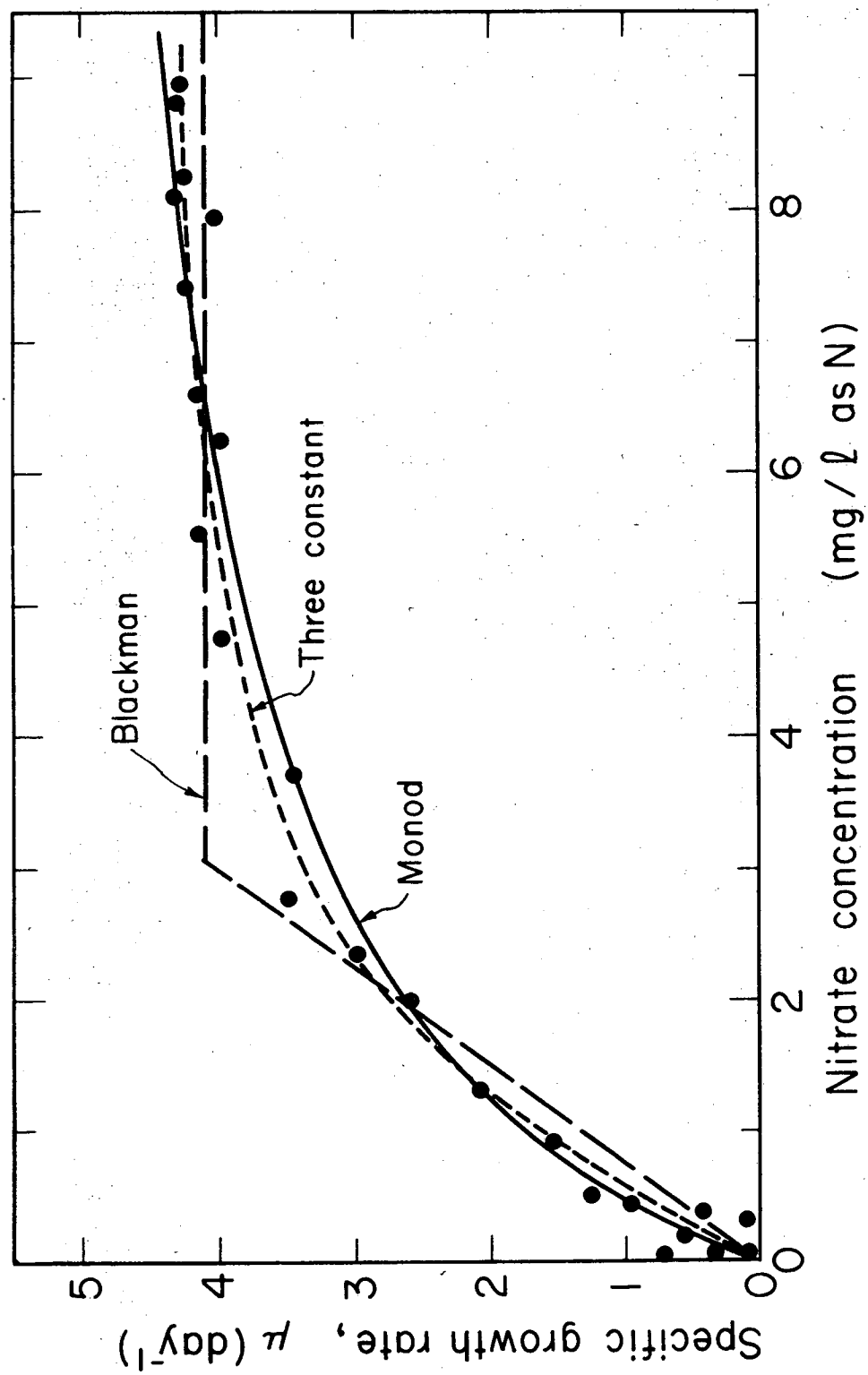

Doubling rate (divisions/hr)
1.5
1.0
0.5
0
0
50
100
150
Mannitol concentration (mg/ℓ)
Blackman
Monod
Three constant
XBL698-3638

Fig. 5



XBL698-3639

Fig. 6



XBL698-3640

Fig. 7

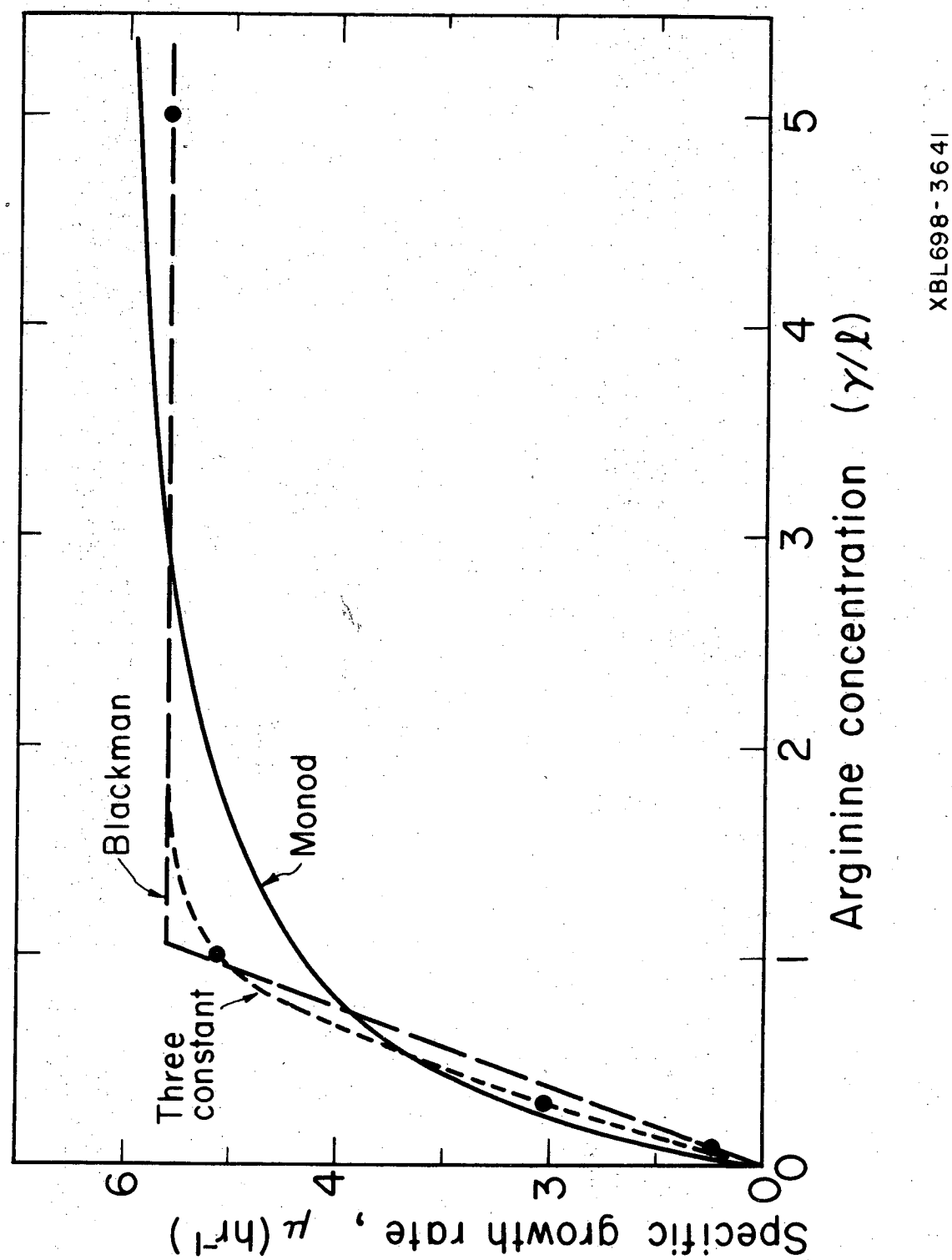
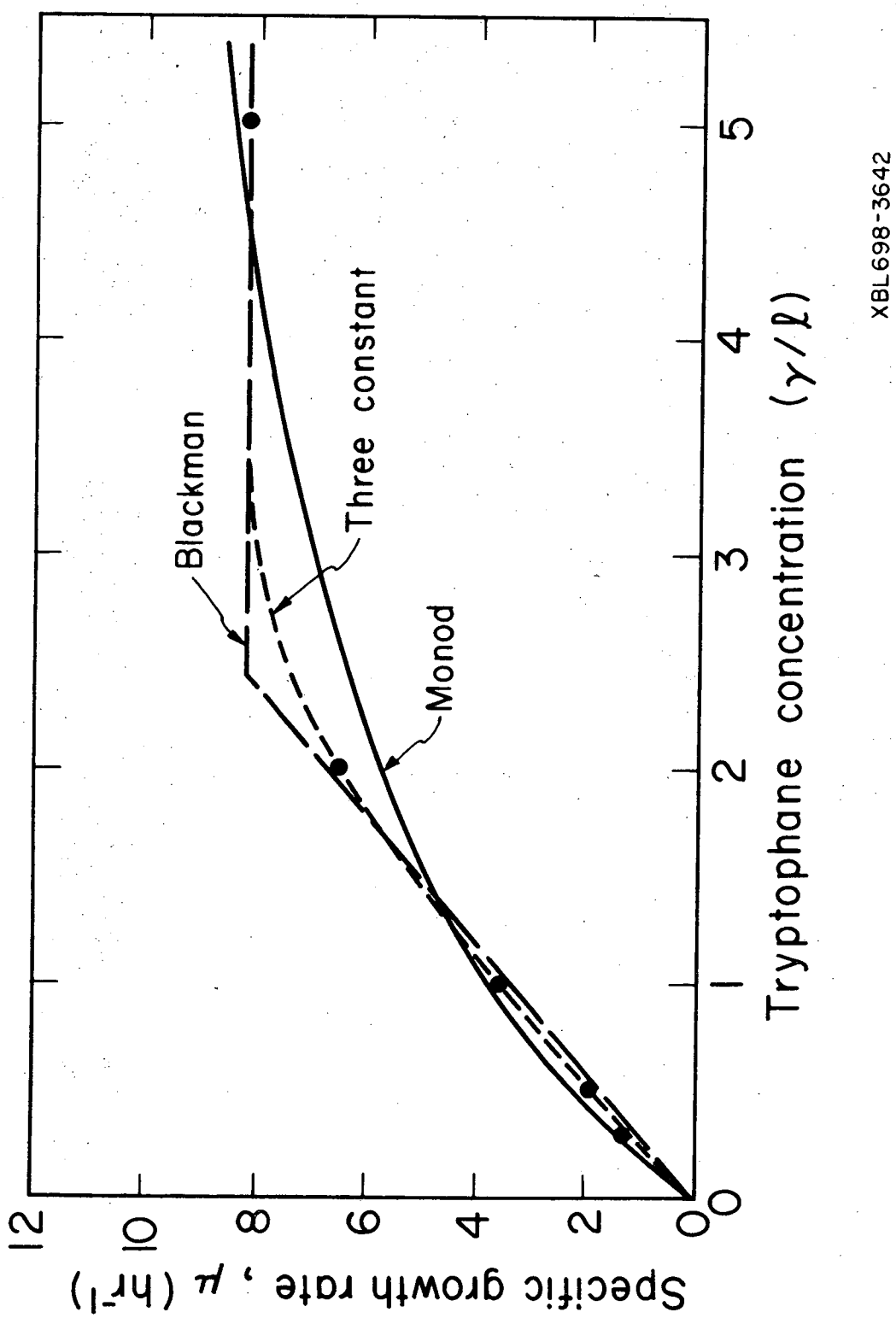
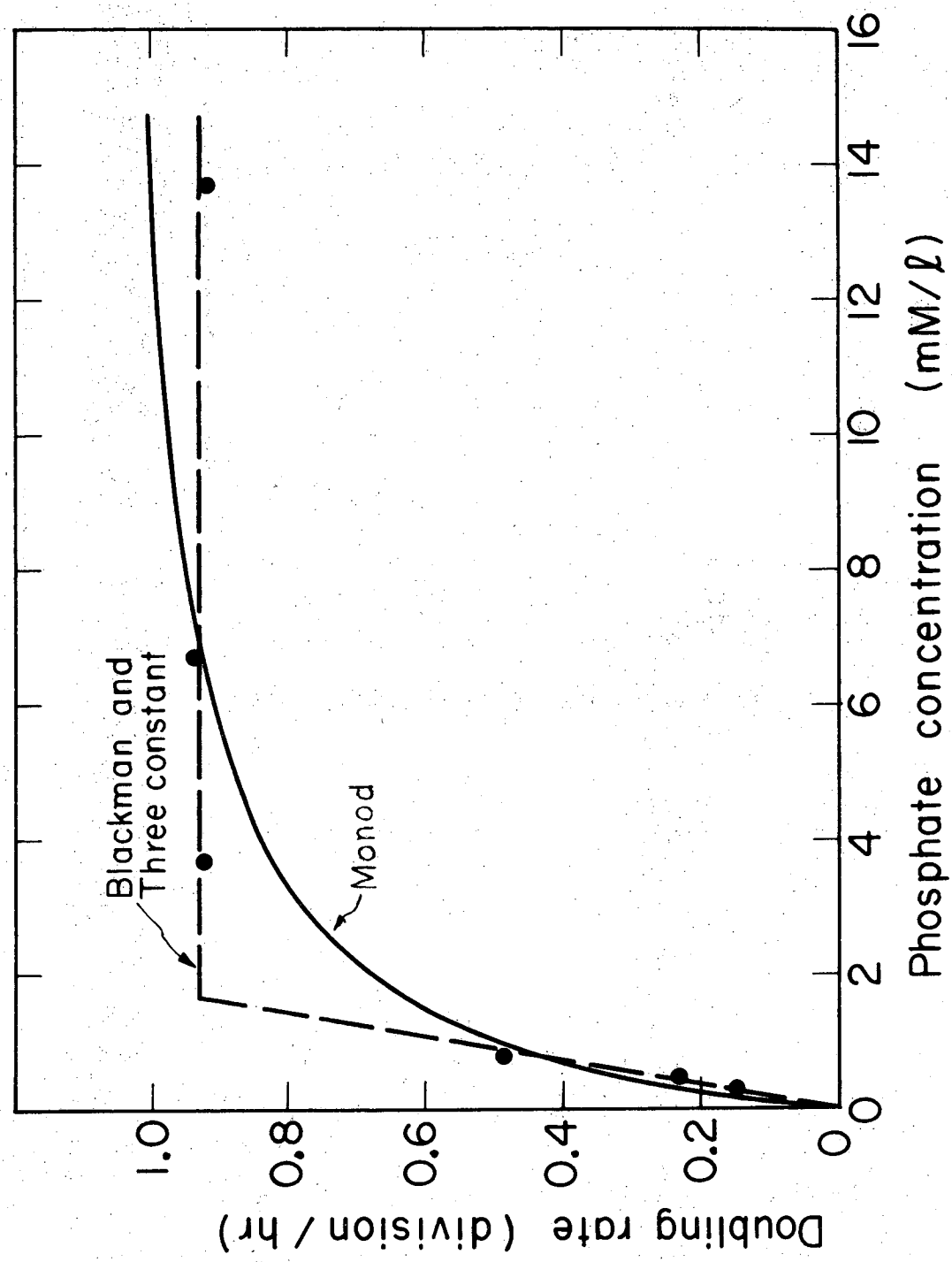

Blackman
Monod
Three constant
Arginine concentration (γ/ℓ)
Specific growth rate, μ (hr⁻¹)
0
1
2
3
4
5
0
3
4
6
XBL698-3641

Fig. 8



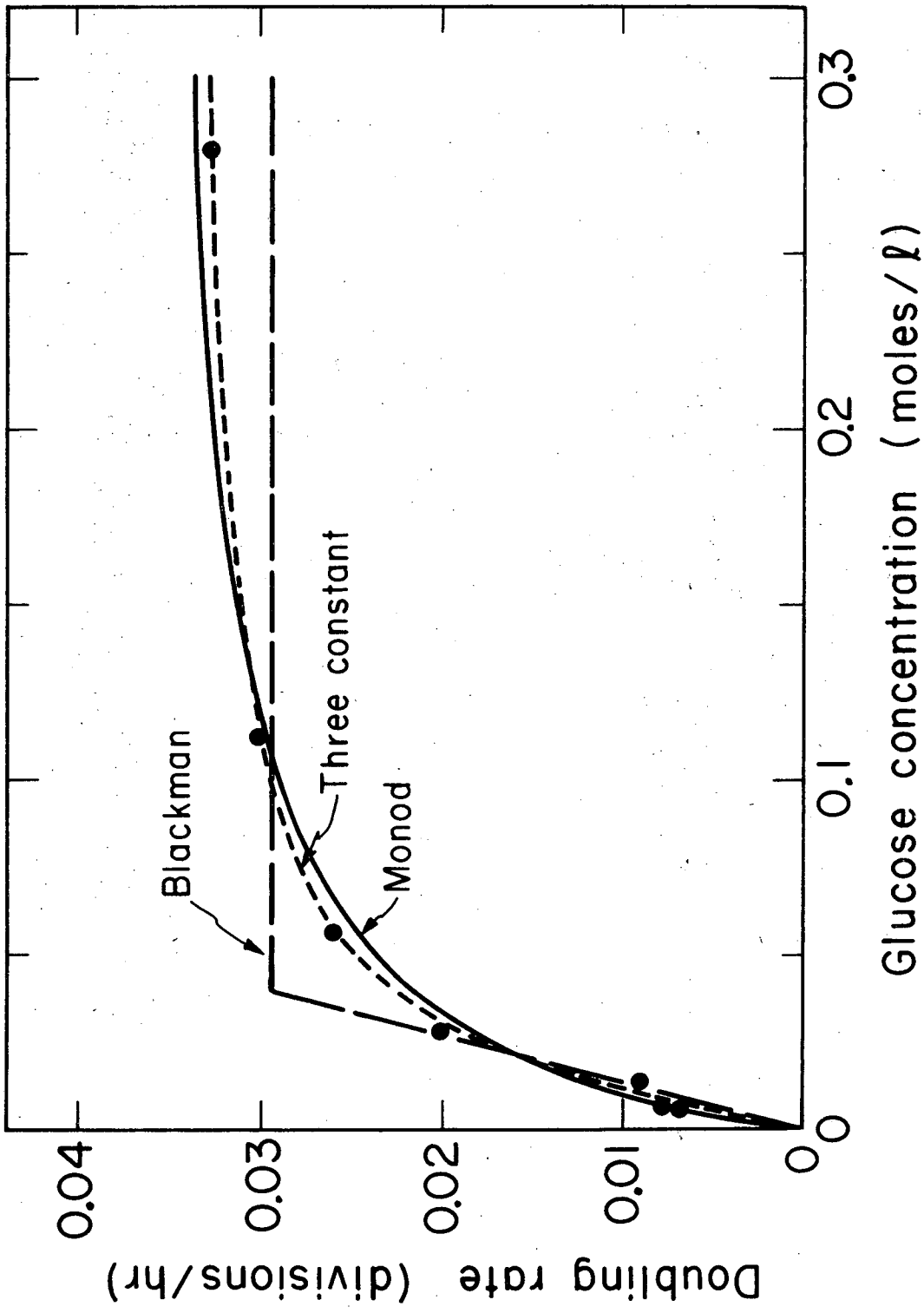
XBL698-3642

Fig. 9



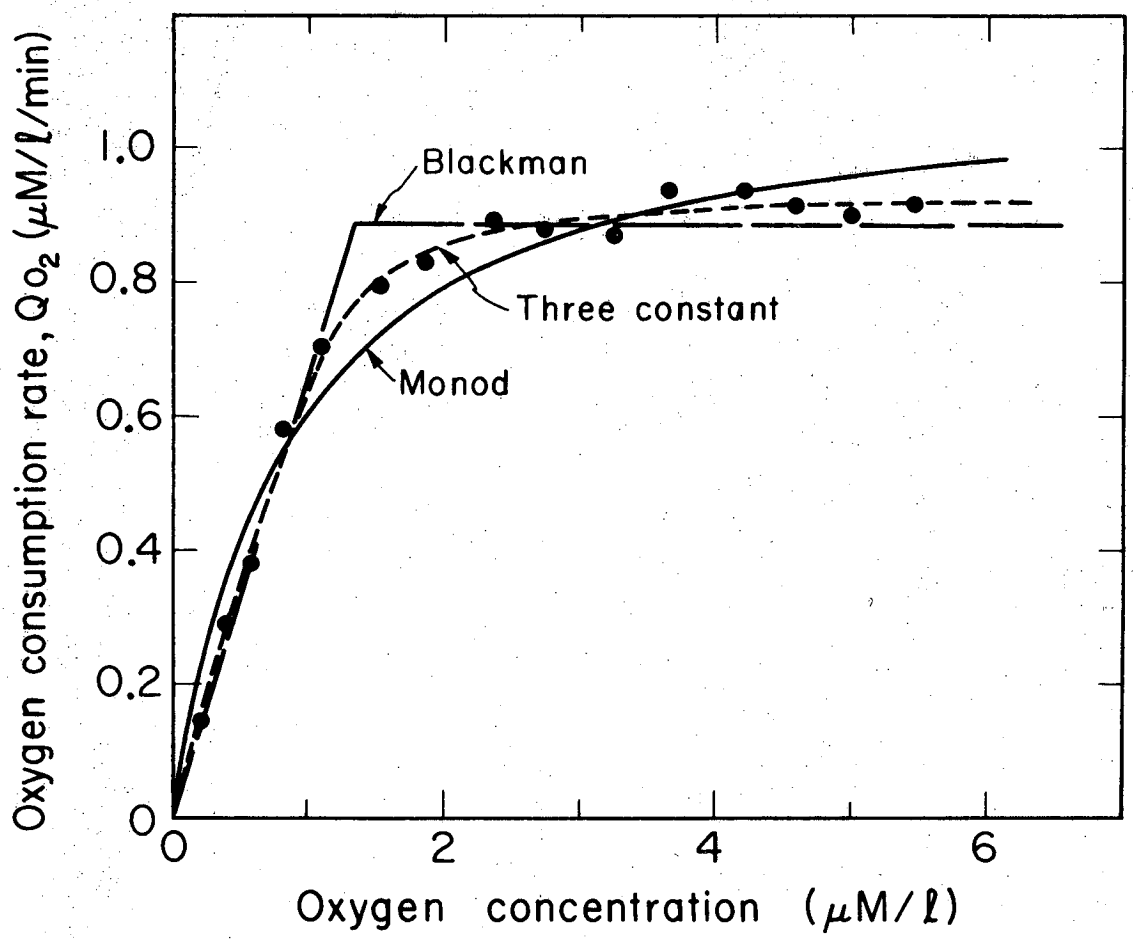
XBL698-3643

Fig. 10



XBL698-3644

Fig. 11



XBL698-3645

Fig. 12

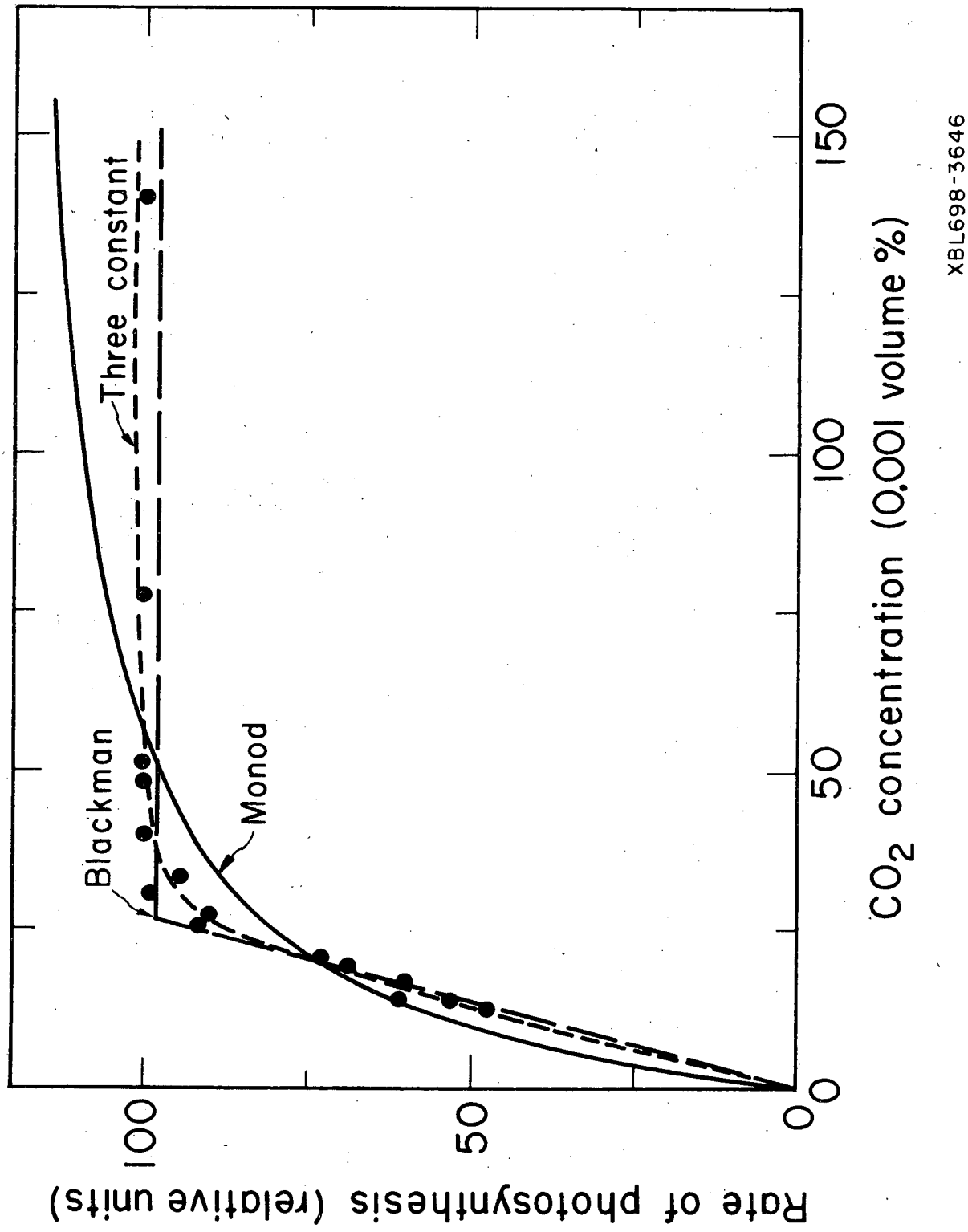

Three constant
Blackman
Monod
Rate of photosynthesis (relative units)
100
50
0
0
50
100
150
CO_2 concentration (0.001 volume %)
XBL698-3646

Fig. 13

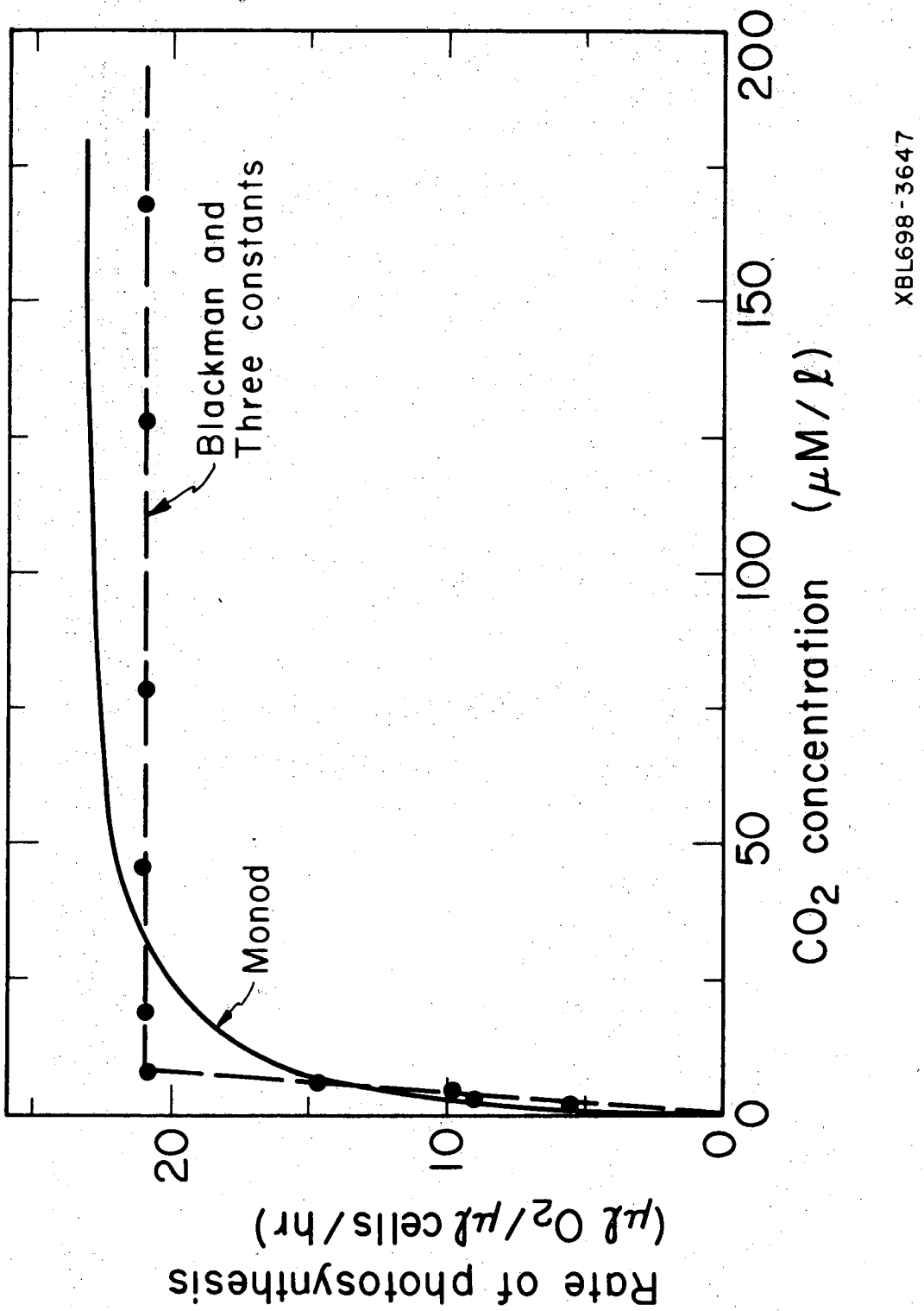
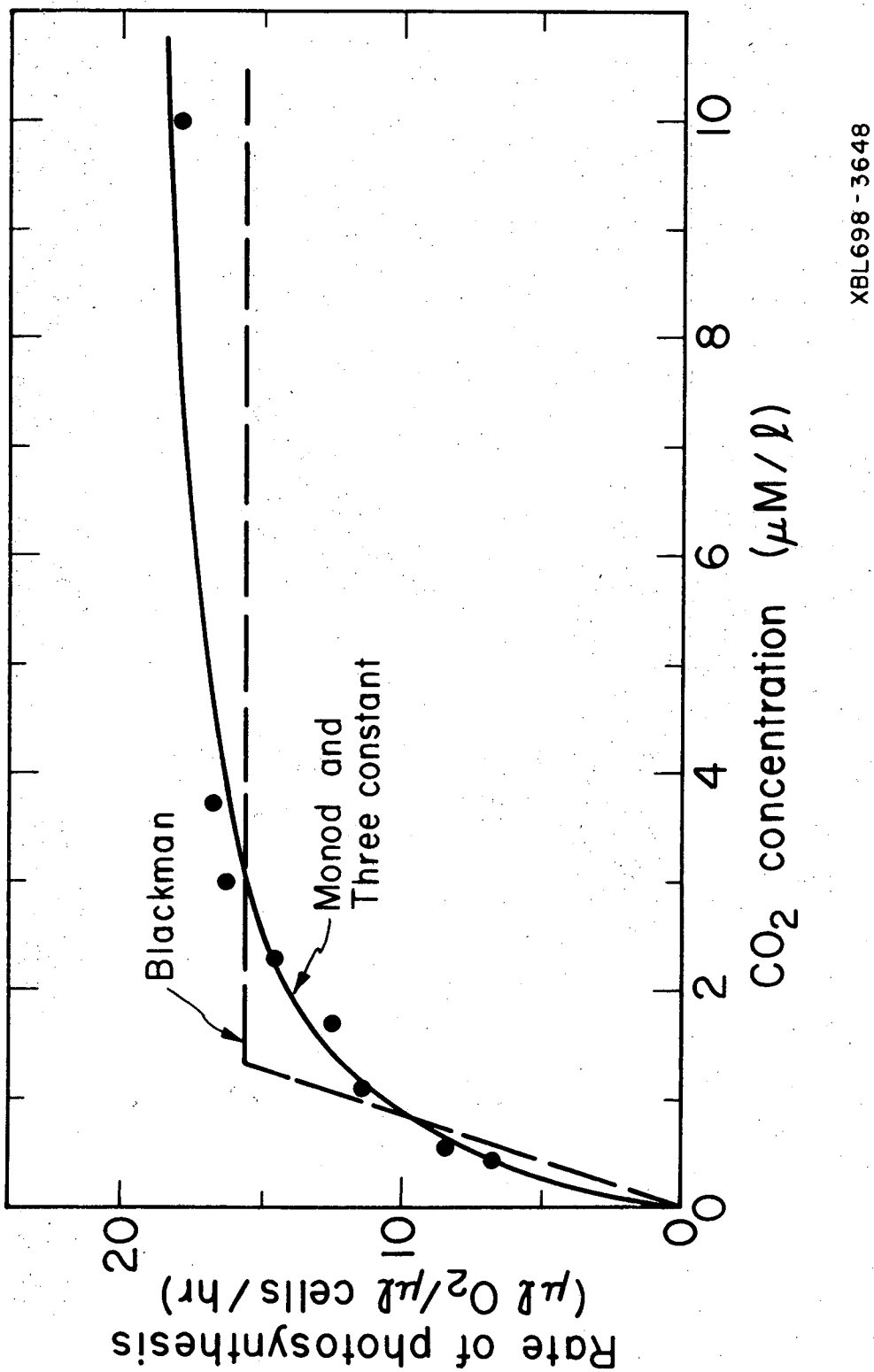

Rate of photosynthesis
(μl O2/μl cells/hr)
0
10
20
0
50
100
150
200
CO2 concentration (μM/ℓ)
Monod
Blackman and
Three constants
XBL698-3647

Fig. 14



XBL698-3648

Fig. 15

APPENDIX I

Subroutines used with LSQVMT, a Non-Linear Least Squares Iterative Procedure

The Monod Form

```
      SUBROUTINE TABLE (F,G,X,T,M1)
      DIMENSION X(2), G(2)
      IF( M1 .EQ. 1 ) RETURN
      F = X(1)*T/(X(2) + T)
      G(1) = T/(X(2) + T)
      G(2) = -X(1)*T/(X(2) + T)**2
      RETURN
      END
```

The Blackman Form

```
      SUBROUTINE TABLE (F,G,X,T,M1)
      DIMENSION X(2), G(2)
      IF(M1 .EQ. 1 ) RETURN
      ARS=SQRT(ABS((1.E-20+X(1)*X(2)+T)**2-4.*X(1)*X(2)*T))
      ARF=(1.E-20+X(1)*X(2)+T)
      F=(ARF-ARS)/(2.*X(1))
      G(1)=(X(1)*(X(2)-(X(2)*ARF-2.*X(2)*T)/ARS)-ARF+ARS)/(2.*X(1)**2)
      G(2)=0.5-(0.5*ARF-T)/ARS
      RETURN
      END
```

The Three Constant Form

```
      SUBROUTINE TABLE (F,G,X,T,M1)
      DIMENSION X(3), G(3)
      IF( M1 .EQ. 1 ) RETURN
    5 ARG = (X(2)+T)**2 - 4.*X(1)*X(3)*T
      IF (ARG) 10, 10, 20
   10 X(3) = 1.E-20 + X(1)*X(3)
      GO TO 5
   20 ARS = SQRT(ARG)
      F=((X(2)+T)-ARS)/(2.*X(1))
      G(1)=((2.*X(1)*X(3)*T)/ARS-X(2)-T+ARS)/(2.*X(1)**2)
      G(2)=(1.-(X(2)+T)/ARS)/(2.*X(1))
      G(3)=T/ARS
      RETURN
      END
```

APPENDIX II

The Calculation of the Coefficient of Determination, CODET

The "total variation" in y is defined as $\sum_{i=1}^{N} (y_i - \bar{y})^2$, where $\bar{y}$ is the mean y-value; $\bar{y} = \sum_{i=1}^{N} y_i/N$. With least squares lines or curves of the form $y = a + bx + cx^2 + dx^3 + \ldots\ldots$, it can be proved that:

$$\sum(y_i - \bar{y})^2 = \sum(y_i - ycal_i)^2 + \sum(ycalc_i - \bar{y})^2 \qquad (a)$$

where $ycalc_i$ is the y-value of the point on the least squares line or curve whose x-value is x_i The first term on the right in the above equation, $\Sigma(y_i - ycalc_i)^2$, is simply the sum of the squares of the errors, SUMSQ, and is also called the "unexplained variation". The second term on the right, $\Sigma(ycalc_i - \bar{y})^2$ is called the "explained variation", since this is the variation of the points $ycalc_i$ on the line or curve. The ratio of the explained variation to the total variation, $\Sigma(ycalc_i - \bar{y})^2/\Sigma(y_i - \bar{y})^2$, is called the "coefficient of determination", and it acts as a measure of goodness of fit, reaching one when all data fall on the curve and approaching zero when there is no correlation. For a least squares line the coefficient of determination is equal to the square of the correlation coefficient, r.

However, the equations considered here (Monod, Blackman, and three constant) are not of the above form, and Eq. (a) does not hold. The more general relation, which also holds for non-least squares curves, is:

$$\begin{aligned}\sum(y_i - \bar{y})^2 &= \sum((y_i - ycalc_i) - (yclac_i - \bar{y}))^2 \\ &= \sum(y_i - ycalc_i)^2 + 2\sum(y_i - ycalc_i)(ycalc_i - \bar{y}) + \sum(ycalc_i - \bar{y})^2\end{aligned} \qquad (b)$$

where the middle term on the right is not zero, which it was in Eq. (a). For the three forms examined here, Eq. (b) is true, but Eq. (a) is not, so a modified coefficient of determination, CODET, was defined as:

$$\text{CODET} = \frac{\text{total variation} - \text{unexplained variation}}{\text{total variation}} \qquad \text{(c)}$$

$$\text{CODET} = \frac{\sum(y_i - \bar{y})^2 - \sum(y_i - \text{ycalc}_i)^2}{\sum(y_i - \bar{y})^2} \qquad \text{(d)}$$

where the unexplained variation is the sum of the squares of the errors, SUMSQ.

APPENDIX III

Computer Program in Fortran IV for determining the Statistical Quantities SUMSQ, s. d., and CODET

```
      PROGRAM CODE (INPUT,OUTPUT)
C THE THREE CONSTANT FORM-GROWTH RATE AS A FUNCTION OF SUBSTRATE CONC
      DIMENSION YDATA(50), X(50), YCALC(50), FORM(12),
     *DELDEN(50), DELDESQ(50), DELY(50), DELYSQ(50), CHAR(12)
      REAL MUMAX
      READ 3, (FORM(I), I = 1,12)
    3 FORMAT (12A6)
    6 READ 7, (CHAR(I), I = 1,12)
    7 FORMAT (12A6)
      PRINT 8, (CHAR(I), I = 1,12)
    8 FORMAT (1H1, 12A6)
      READ 9, N
    9 FORMAT (I4)
      IF (N .EQ. 0) GO TO 60
      PRINT 10, N
   10 FORMAT (1H0, 27HTHE NUMBER OF DATA POINTS =, I4//)
      READ 11, (YDATA(I), X(I), I = 1,N)
   11 FORMAT (2F10.0)
      PRINT 12
   12 FORMAT (19X, 5HYDATA, 11X, 1HX//)
      PRINT 14, (YDATA(I), X(I), I = 1,N)
   14 FORMAT (14X, E13.5, 2X, E13.5)
      PRINT 16, (FORM(I), I = 1,12)
   16 FORMAT (1H0, 12A6)
C THE CALCULATION OF THE AVERAGE OF YDATA, YBAR
      YSUM = 0.
      DO 20 I = 1,N
      YSUM = YSUM + YDATA(I)
   20 CONTINUE
      YBAR = YSUM/N
      PRINT 22, YBAR
   22 FORMAT (1H0, 6HYBAR =, E13.5)
      READ 23, MUMAX, A, B
   23 FORMAT (3F10.0)
      PRINT 24, MUMAX, A, B
   24 FORMAT (1H0, 7HMUMAX =, E13.5, 5X, 3HA =, E13.5, 5X, 3HB =, E13.5)
C CHECK OF SUM OF SQUARES OF ERRORS, SUMSQ
      SUMSQ = 0.
      DO 30 I = 1,N
      YCALC(I) = ((B+A*MUMAX+X(I))-SQRT((B+A*MUMAX+X(I))**2-4.*A*MUMAX*X
     *(I)))/(2.*A)
      DELY(I) = YDATA(I) - YCALC(I)
      DELYSQ(I) = (DELY(I))**2
      SUMSQ = SUMSQ + DELYSQ(I)
   30 CONTINUE
      PRINT 32, SUMSQ
   32 FORMAT (1H0, 34HSUM OF SQUARES OF ERRORS, SUMSQ, =, E13.5)
C CALCULATION OF TOTAL VARIATION, DENOM
      DENOM = 0.
      DO 40 I = 1,N
      DELDEN(I) = YDATA(I) - YBAR
      DELDESQ(I) = (DELDEN(I))**2
```

```
      DENOM = DENOM + DELDESQ(I)
   40 CONTINUE
      PRINT 41, DENOM
   41 FORMAT (1H0, 29HTHE TOTAL VARIATION, DENOM, =, E13.5)

C THE CALCULATION OF THE COEFFICIENT OF DETERMINATION, CODET, AND THE
C CORRELATION COEFFICIENT, R
      CODET = (DENOM - SUMSQ)/DENOM
      R = SQRT(CODET)
      PRINT 42, CODET
   42 FORMAT (1H0, 42HTHE COEFFICIENT OF DETERMINATION, CODET, =, E13.5)
      PRINT 44, R
   44 FORMAT (1H0, 32HTHE CORRELATION COEFFICIENT, R, =, E13.5/)

C THE CALCULATION OF THE STANDARD DEVIATION, STDEV
      STDEV = SQRT(SUMSQ/(N - 3))
      PRINT 46, STDEV
   46 FORMAT (1H0, 32HTHE STANDARD DEVIATION, STDEV, =, E13.5)
      GO TO 6
   60 CONTINUE
      END
```

APPENDIX IV

The Twelve Sets of Examined Data

BEST FIT OF MONOD GLUCOSE DATA YDATA=DIV/HR X=MG/L

THE NUMBER OF DATA POINTS = 27

YDATA	X
3.80000E-01	7.00000E+00
1.17000E+00	3.00000E+01
1.38000E+00	6.00000E+01
1.16000E+00	9.00000E+01
1.22000E+00	1.15000E+02
1.32000E+00	1.35000E+02
1.28000E+00	1.50000E+02
1.24000E+00	1.60000E+02
5.00000E-02	1.00000E+00
6.10000E-01	1.10000E+01
1.20000E+00	3.80000E+01
1.16000E+00	7.30000E+01
1.27000E+00	9.80000E+01
1.23000E+00	1.16000E+02
3.00000E-02	2.00000E+00
5.60000E-01	1.20000E+01
1.17000E+00	5.10000E+01
1.17000E+00	6.70000E+01
1.29000E+00	8.50000E+01
4.00000E-02	1.00000E+00
5.30000E-01	1.00000E+01
1.22000E+00	3.00000E+01
1.18000E+00	5.50000E+01
1.15000E+00	7.00000E+01
7.40000E-01	8.00000E+00
1.15000E+00	2.80000E+01
1.39000E+00	5.00000E+01

BEST FIT OF MONOD MANNITOL DATA YDATA=DIV/HR X=MG/L

THE NUMBER OF DATA POINTS = 3

YDATA	X
6.00000E-02	2.00000E+00
1.22000E+00	3.20000E+01
1.07000E+00	8.00000E+01
1.24000E+00	1.10000E+02
1.12000E+00	1.40000E+02
4.00000E-02	1.00000E+00
9.50000E-01	1.30000E+01
1.25000E+00	5.50000E+01
1.12000E+00	8.50000E+01
1.27000E+00	1.06000E+02
1.03000E+00	1.23000E+02
1.20000E+00	1.37000E+02
1.20000E+00	1.80000E+01
9.60000E-01	4.90000E+01
1.27000E+00	7.40000E+01
5.00000E-02	1.00000E+00
1.22000E+00	2.00000E+01
1.32000E+00	5.00000E+01
1.19000E+00	7.30000E+01
8.60000E-01	6.00000E+00
1.28000E+00	1.70000E+01
1.15000E+00	2.90000E+01
1.14000E+00	3.80000E+01
1.18000E+00	4.50000E+01
1.16000E+00	8.00000E+00
1.13000E+00	2.00000E+01
1.00000E+00	2.90000E+01
7.00000E-02	1.00000E+00
1.00000E+00	5.00000E+00
1.01000E+00	1.30000E+01

BEST FIT OF MONOD LACTOSE DATA YDATA=DIV/HR X=MG/L

THE NUMBER OF DATA POINTS = 29

YDATA	X
7.00000E-02	2.00000E+00
1.90000E-01	9.00000E+00
7.20000E-01	2.90000E+01
9.20000E-01	4.30000E+01
1.13000E+00	9.00000E+01
1.06000E+00	1.14000E+02
1.05000E+00	1.37000E+02
7.00000E-02	2.00000E+00
4.30000E-01	1.90000E+01
8.30000E-01	2.50000E+01
1.03000E+00	9.40000E+01
1.09000E+00	1.14000E+02
9.40000E-01	1.24000E+02
1.02000E+00	1.58000E+02
4.00000E-02	1.00000E+00
2.50000E-01	8.00000E+00
7.40000E-01	3.00000E+01
1.09000E+00	6.20000E+01
1.00000E+00	8.90000E+01
9.60000E-01	1.10000E+02
1.08000E+00	1.27000E+02
1.00000E+00	1.44000E+02
4.00000E-02	3.00000E+00
9.00000E-02	4.00000E+00
4.00000E-01	1.00000E+01
6.10000E-01	3.00000E+01
8.40000E-01	5.70000E+01
8.60000E-01	8.00000E+01
1.06000E+00	1.10000E+02

BEST FIT OF SHELEF NITRATE DATA YDATA=1/DAY X=MG/L

THE NUMBER OF DATA POINTS = 24

YDATA	X
4.27000E+00	8.96000E+00
4.29000E+00	8.78000E+00
4.23000E+00	8.24000E+00
4.29000E+00	8.08000E+00
4.03000E+00	7.95000E+00
4.22000E+00	7.42000E+00
4.15000E+00	6.60000E+00
3.98000E+00	6.25000E+00
4.15000E+00	5.52000E+00
3.97000E+00	4.72000E+00
3.46000E+00	3.72000E+00
3.49000E+00	2.78000E+00
2.98000E+00	2.35000E+00
2.59000E+00	1.98000E+00
2.07000E+00	1.31000E+00
1.55000E+00	9.07000E-01
1.24000E+00	5.24000E-01
9.33000E-01	4.93000E-01
3.98000E-01	4.01000E-01
8.80000E-02	3.17000E-01
5.75000E-01	1.94000E-01
3.05000E-01	1.01000E-01
7.03000E-01	9.30000E-02
6.60000E-02	9.20000E-02

BEST FIT OF NOVICK ARGININE DATA YDATA=1/HR X=GAMMA/L

THE NUMBER OF DATA POINTS = 4

YDATA	X
5.60000E-01	5.00000E+00
5.12500E-01	1.00000E+00
2.07000E-01	2.91000E-01
4.85000E-02	8.60000E-02

BEST FIT OF NOVICK THYPTOPHANE DATA YDATA=1/HR X=GAMMA/L

THE NUMBER OF DATA POINTS = 5

YDATA	X
1.28000E-01	3.00000E-01
1.86000E-01	5.00000E-01
3.55000E-01	1.00000E+00
6.48000E-01	2.00000E+00
8.20000E-01	5.00000E+00

BEST FIT OF HINSHELWOOD PHOSPHATE DATA YDATA=1/HR X=MOLE/L

THE NUMBER OF DATA POINTS = 6

YDATA	X
9.17000E-01	1.37000E-02
9.38000E-01	6.70000E-03
9.26000E-01	3.58000E-03
4.82000E-01	7.85000E-04
2.28000E-01	4.73000E-04
1.44000E-01	3.02000E-04

BEST FIT OF SCHAEFER GLUCOSE DATA YDATA=DIV/HR X=MOLE/L

THE NUMBER OF DATA POINTS = 7

YDATA	X
6.80000E-03	6.30000E-03
7.85000E-03	6.90000E-03
9.03000E-03	1.32000E-02
2.01000E-02	2.79000E-02
2.60000E-02	5.64000E-02
2.99000E-02	1.11700E-01
3.27000E-02	2.79000E-01

BEST FIT OF JOHNSON O(2) DATA YDATA=MICROMOLES/L/MIN X=MICROMOLE/L

THE NUMBER OF DATA POINTS = 15

YDATA	X
9.13000E-01	5.45000E+00
9.01000E-01	5.10000E+00
9.13000E-01	4.59000E+00
9.37000E-01	4.19000E+00
9.39000E-01	3.64000E+00
8.68000E-01	3.25000E+00
8.76000E-01	2.74000E+00
8.92000E-01	2.37000E+00
8.30000E-01	1.85000E+00
7.95000E-01	1.52500E+00
7.04000E-01	1.10200E+00
5.80000E-01	8.28000E-01
3.79000E-01	5.66000E-01
2.90000E-01	4.04000E-01
1.44000E-01	2.02000E-01

BEST FIT OF VAN DER HONERT CO(2) DATA YDATA=REL.P.S. X=MILLIVOL. P/C

THE NUMBER OF DATA POINTS = 15

YDATA	X
4.79000E+01	1.20000E+01
5.31000E+01	1.32000E+01
6.09000E+01	1.36000E+01
6.02000E+01	1.62000E+01
6.89000E+01	1.92000E+01
7.34000E+01	2.05000E+01
9.13000E+01	2.56000E+01
9.01000E+01	2.70000E+01
9.90000E+01	3.07000E+01
9.42000E+01	3.28000E+01
1.00100E+02	3.95000E+01
1.00100E+02	4.85000E+01
1.00100E+02	5.06000E+01
1.00100E+02	7.83000E+01
1.00000E+02	1.40300E+02

BEST FIT OF EMERSON CO(2) DATA YDATA=MICROL/MICROL/HR X=MICROMOLE/L

THE NUMBER OF DATA POINTS = 10

YDATA	X
5.48000E+00	1.85000E+00
9.05000E+00	2.93000E+00
9.79000E+00	4.31000E+00
1.47300E+01	5.85000E+00
2.08500E+01	7.70000E+00
2.09300E+01	1.88000E+01
2.09300E+01	4.53000E+01
2.09300E+01	7.82000E+01
2.09300E+01	1.28000E+02
2.10000E+01	1.68000E+02

BEST FIT, WHITTINGHAM CO(2) DATA YDATA=MICROL/MICROL/HR X=MICROMOLE/L

THE NUMBER OF DATA POINTS = 8

YDATA	X
1.80000E+01	1.00000E+01
1.68000E+01	3.70000E+00
1.63000E+01	3.00000E+00
1.46000E+01	2.30000E+00
1.25000E+01	1.70000E+00
1.15000E+01	1.10000E+00
8.50000E+00	5.70000E-01
6.80000E+00	4.30000E-01

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