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Studies on the Growth of *Spirulina platensis*

(II) Growth Kinetics of an Autotrophic Culture

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Abstract

Employing a pure culture of *Spirulina platensis*, the following growth characteristics were observed. (1) The specific growth rate (μ) is independent of pH between pH 8.5 and pH 10.5; (2) the optimum temperature lies between 35°C and 37°C; (3) with a thin cell suspension in a nutritionally sufficient medium, in which exponential growth proceeds, the relation between μ and the intensity of incident light (I_0) is expressed by

$$\mu=\mu_m I_0/(I_0+K_L), \quad \cdots\cdots (1)$$

where K_L is the saturation constant (klux) for light intensity; at 35°C, $\mu_m=2.2$ day^{-1} and $K_L=10$ klux. Based on the above Monod-type equation, the kinetic equation for the linear growth phase which follows the logarithmic growth phase was derived. For a culture having a homogeneous density of cell population and an uniform fluid thickness (h_1, measured in the direction of incident light), we obtain

$$dX/dt=(\mu_m/ah_1)\ln(I_0K_L^{-1}+1),$$

where a is the extinction coefficient of the culture fluid per unit cell concentration. Though this expression seems superficially to be in conformity with the observed constancy of the growth rate when the I_0-value is fixed, the K_L-value calculated from the linear phase equation decreased as I_0 was increased. The cause of such a discrepancy is discussed.

Introduction

As reported previously, we have succeeded in obtaining an axenic culture of *Spirulina platensis*.[1] Despite the fact that the organism as a source of nutritional protein has attracted the attention of many research workers,[2,3] little information has so far appeared on the growth kinetics of this alga due presumably to the difficulty in isolating the algal cells free from bacteria.

On the other hand, the growth kinetics of Chlorella have already been intensively studied since the pioneering research of Tamiya and his colleagues.[4,5] Rabe and Benoit[6] have proposed expressing the growth rate of microalgae as a function of "mean light intensity". Ragonese and Williams have reported that the growth rate is proportional to the number of photons absorbed per unit time.[7] Shelef *et al.*[8] have presented a few kinetic models for the reproduction of the microalga, especially in relation to continuous culture. Myers[9] studied in detail the nature of algal

growth, but gave up his intention of making a kinetic model due to the complicated nature of the relation between light and growth. The present paper describes some of the growth characteristics and kinetics of a photoautotrophic culture of *S. platensis*.

Materials and Methods

1. Strain A pure culture of *Spirulina platensis* (reported previously[1]) was employed.

2. Medium and culture conditions *S. platensis* was grown on a mineral medium.[1] For maintaining the pH within the optimal range, pure carbon dioxide or 1 N hydrochloric acid solution was added. Cultures were grown mainly in 60 ml and 700 ml Roux bottles containing, respectively, 40 ml and 500 ml of medium. The light intensity of the incident light was measured with a Toshiba photocell illuminometer. Growth was monitored by measuring the optical density at 560 mμ; a linear correlation was observed between dry cell weight and optical density. Incubation temperature was 35±1°C unless otherwise specified. In some instances, culture experiments for accumulating biomass were carried out in a 10 l jar fermenter (diameter, 20 cm).

Theory

An illuminated culture system is shown in Fig. 1. The algal culture in a vertical Roux bottle, in which the density of the cell population is uniform, has a uniform fluid depth (h_1) measured in the direction parallel to that of incident light. In the logarithmic growth phase, the light intensity at the depth of h_1 is regarded to be almost equal to I_0 as the cell density is so thin. According to the results of experiments with varying values of I_0 (*cf.* Fig. 8), the relation of the specific growth rate to I_0 can be expressed by

$$\mu=\mu_m I_0/(I_0+K_L) \quad \cdots\cdots (1)$$

where K_L is the saturation constant for the light intensity, and μ_m is the maximum growth rate.

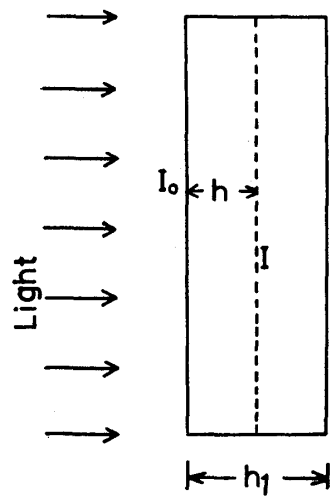

Fig. 1. Schematic diagram of illuminated culture.

As the population density becomes greater, the growth rate gradually approaches a constant value; this linear growth is maintained for a long time unless the rate is limited by the deficiency of an essential nutrient or by a metabolic change in the physicochemical environment (especially, pH). In the linear growth phase, the light intensity at a depth of h is expressed according to Beer's law by

$$I = I_0 \exp\cdot(-aXh), \quad \cdots\cdots (2)$$

where a is the extinction coefficient per unit cell concentration.

Substituting the value of I shown in Eq. (2) for I_0 in Eq. (1) to express μ as a function of h, we obtain the average (weighted mean value of) μ, denoted $\bar{\mu}$, of the culture as follows:

$$\bar{\mu} = \frac{\mu_m}{h_1}\int_{h=0}^{h=h_1} \frac{I_0 \exp\cdot(-aXh)}{I_0 \exp\cdot(-aXh) + K_L} dh, \quad \cdots\cdots (3)$$

$$\bar{\mu} = \frac{\mu_m}{aXh_1} \ln\left(\frac{I_0 + K_L}{I_0 \exp\cdot(-aXh_1) + K_L}\right). \quad \cdots\cdots (4)$$

In this phase of growth, the value of $I_0 \exp\cdot(-aXh_1)$ is negligibly small as compared with the K_L-value. Therefore, the simplified equation,

$$G_L \equiv \left(\frac{dX}{dt}\right)_{\text{linear}} = \frac{\mu_m}{ah_1} \ln\left(\frac{I_0}{K_L} + 1\right), \quad \cdots\cdots (5)$$

is obtained for practical application. Eq. (5) states that, with a fixed value of I_0, the growth rate is constant, and seemed, at first sight, to be in conformity with the experimental time course of growth. From Eq. (5), it follows that

$$K_L = \frac{I_0}{\exp\cdot\left(\frac{ah_1}{\mu_m}\cdot G_L\right) - 1}, \quad \cdots\cdots (6)$$

Eq. (5) is basically the same as Tamiya's equation,[5] but the process of derivation is different and is much simpler. The test for the fitness of Eq. (6) with the practical results is considered to raise some problems inherent in this type of culture.

Results and Discussion

1. Growth characteristics of *S. platensis* in submerged cultures Some examples of time courses are shown in Fig. 2. In a bicarbonate-rich inorganic medium, the pH became higher as the carbonate ion was used for growth, and finally became growth-limiting. Fig. 3 shows the relation of μ to pH, indicating that μ is scarcely affected by pH in the range between 8.5 and 10.5. The time course of growth with regulation of the pH by adding 1 N hydrochloric acid is illustrated in Fig. 4. These results imply that there is no growth inhibitor in the culture and that the cessation of growth in cultures without pH regulation is caused only because the pH became too high. Fig. 5 shows the accumulation of biomass in a culture with a 10 *l* jar fermenter (diameter: 20 cm), in which the pH was regulated by supplying carbon dioxide to the culture. In this instance, the cell

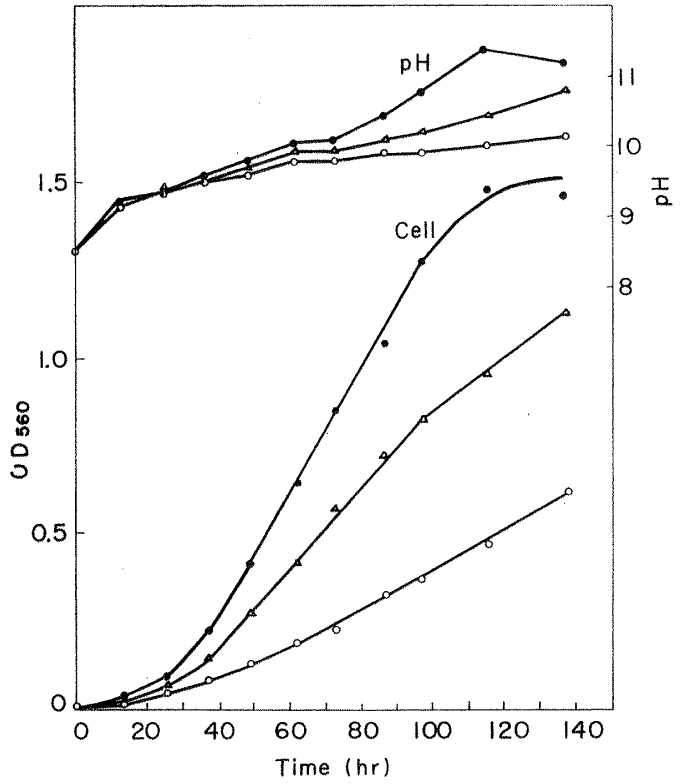

Fig. 2. Time courses of batch cultures (*S. platensis*)
Light intensity: ●—● 30 Klux;
△—△ 15 Klux;
○—○ 7.5 Klux.

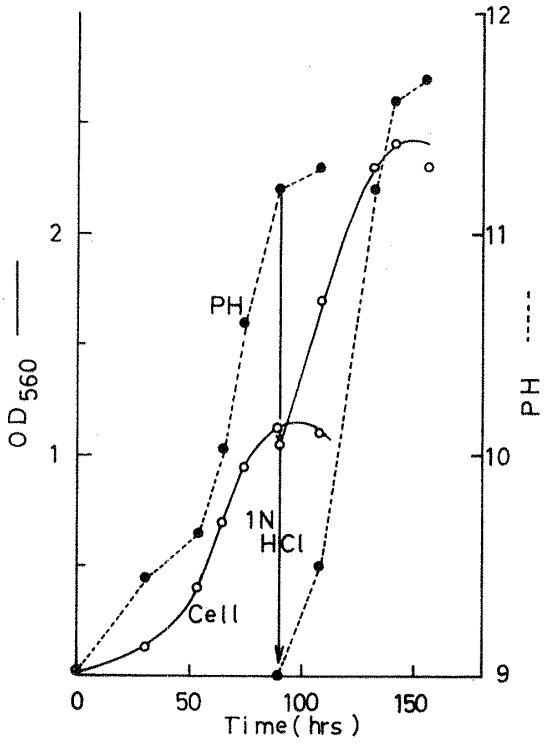

Fig. 4. Enhanced growth due to pH adjustment. At the time indicated by the arrow, 1 N HCl solution was added to lower the pH.

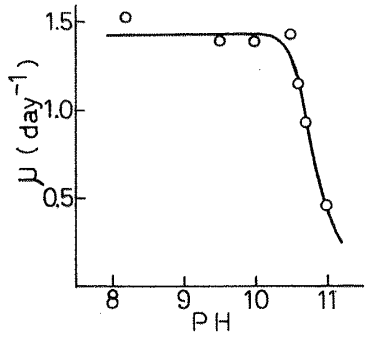

Fig. 3. Effect of pH on the specific growth rate of *S. platensis*.

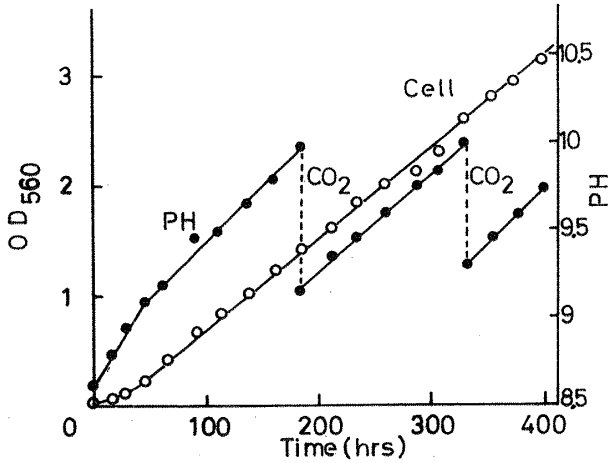

Fig. 5. Accumulation of *S. platensis* cells in cultures with pH-regulation by CO_2.
Conditions: 30 Klux; gently agitated culture in a 10 *l* jar fermenter (ϕ=20 cm).

concentration reached about 4.2 g/*l*. The effect of temperature on the specific growth rate (μ) in the exponential growth phase is shown in Fig. 6, indicating that the optimum temperature range is 35−37°C.

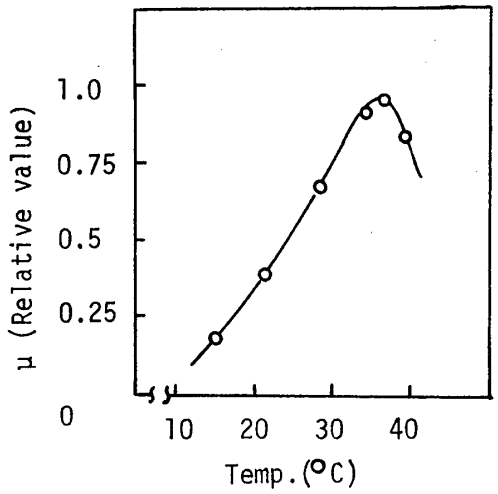

Fig. 6. Effect of temperature on the specific growth rate.

2. Relation between the growth rate and light intensity First, conformity to Beer's law was tested with a suspension of *S. platensis* cells. This result is shown in Fig. 7; the plot of $\ln I_0/I$ *vs.* cell concentration was a straight line. From the slope, the extinction coefficient (a) is computed to be 1.58 ($g^{-1} \cdot l \cdot cm^{-1}$). The dependence of the specific growth rate upon the intensity of incident light is shown in Fig. 8 by a Lineweaver-Burk's plot. A very dilute, exponentially growing cell suspension (40 ml in a 70 ml Roux bottle) was used in these experiments. From the results, the Monod-type equation[10] (1) was deduced to express μ as a

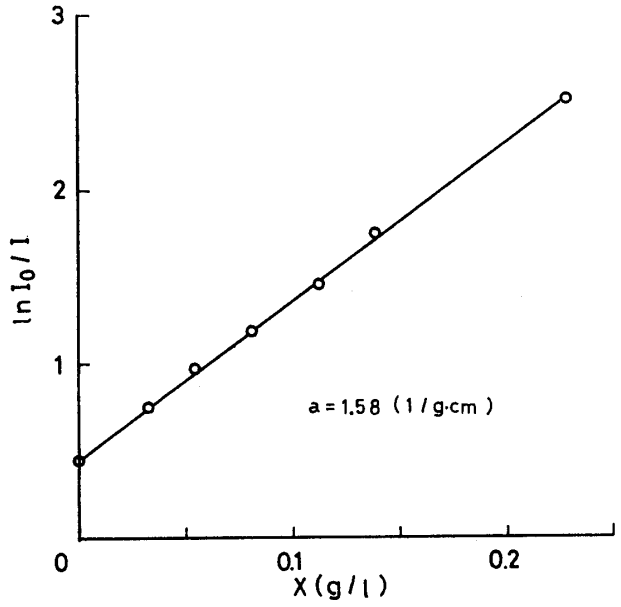

Fig. 7. Relation between cell concentration and optical density.
I_0: Intensity of incident light.
I: Intensity of light transmitted through cell suspensions having a thickness of 5 cm.

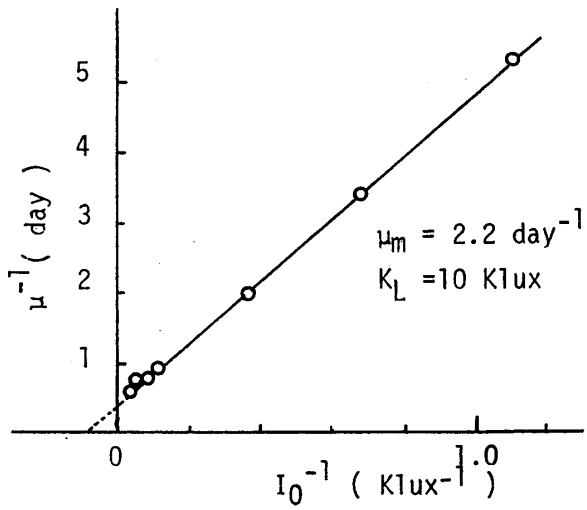

Fig. 8. Lineweaver-Burk's plot showing the relation between the specific growth rate (μ) in the logarithmic phase and the intensity of incident light (I_0).

function of I_0. The μ_m-value was estimated to be 2.2 day^{-1} and the saturation constant (K_L) for the light intensity to be 10 (klux), which seems to be much higher than that calculated from the data reported with *Chlorella*. Next, Eq. (5) was tested for fit with the experimental results. As shown in Fig. 9, the value of G_L calculated by Eq. (5) became apparently smaller than the experimental value as I_0 was increased. As a result, the value of K_L calculated by Eq. (6) decreased as I_0 was increased (Table 1). Such a tendency was perceived in the case of *Chlorella*, for which the K_L-values were calculated based on Tamiya's data for the linear-phase growth rate.[5]

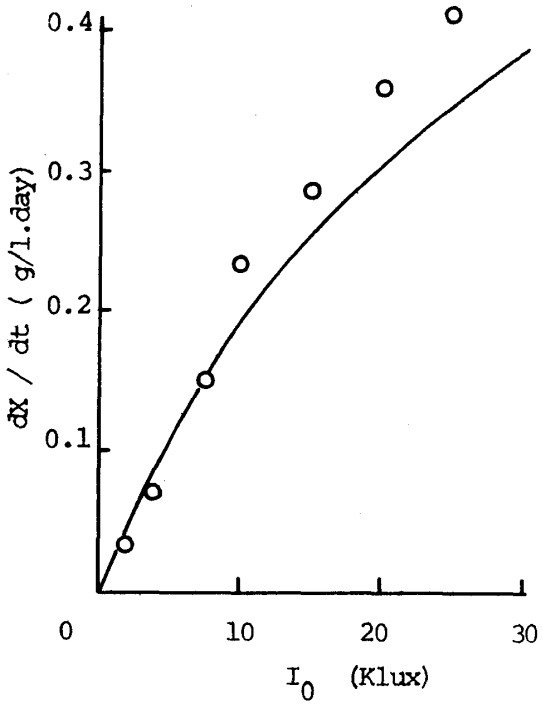

Fig. 9. Comparison of the plot of Eq. (5) with the experimental results.
Plot of Eq. (5): ——;
experimental values: ○.
a=1.58 $l/g\cdot$cm μ_m=2.2 day^{-1}
K_L=10 Klux h=5.0 cm

Table 1. Calculation of K_L by Eq. (6) based on the experimental data.

a) *S. platensis*		b) *Chlorella**	
I_0	K_L	I_0	K_L
2	18.0	0.8	4.95
4	16.4	2	3.65
7.5	11.0	5	3.35
10	8.2	10	2.84
15	9.2	25	1.96
20	8.5	50	1.51
25	6.2		

* from Tamiya's data[5]

As to the causes of the inconsistency of Eq. (6), the following points merit consideration.

(1) In the agitated cultures, individual cells receive a light intensity fluctuating rapidly between I_0 and $I \cong 0$ in the linear growth phase, and the response of μ of individual cells to the fluctuating light intensity might not be quick enough to be in accord with Eq. (I) at any moment.

(2) Not only the intensity, but also the composition of the light from the tungsten lamp used in this investigation may change as it passes through the cell suspension; individual cells receiving a fluctuating intensity and composition of light might be subject to an Emerson effect of a complicated nature.

(3) The composition of cell pigment might be changed in response to the light intensity received by growing cells.

Other factors, including the effect of scattered light, may also be involved.

Concerning hypothesis (1), we have made some trial calculations to estimate how the fluctuating light intensity affects μ when the fluctuation occurs according to a function of $\sin t$ and there is a monomolecular response of μ to the changed intensity of light. The result is that the light fluctuation is remarkably moderated as it is expressed in the form of μ-fluctuation if the 90% response time is not more than ten seconds, which is considered to be a reasonable assumption for the lag time.[11] Therefore, we assume that hypothesis (1) does not explain the cause of the inconsistency of Eq. (6). The results of light-dark phasing experiments are also inconsistent with hypothesis (1). As to hypothesis (2), a set of experiments with monochromatic light would be useful for testing its validity. For testing hypothesis (3), detailed biochemical studies together with growth experiments using varying light intensities are being performed; unpublished data details will be reported later.

References

1) Ogawa, T., Terui, G.: *J. Ferment. Technol.*, **48**, 361 (1970).
2) Clement, G., Giddey, C., Menzi, R.: *J. Sci. Food and Agric.*, **18**, 497 (1967).
3) Leonard, J.: Presented at the Spirulina Conference, Södergarn, Sweden, June (1967).
4) Tamiya, H.: *Synchrony in Cell Division and Growth.* Edited by E. Zeuthen, pp. 247–305, John Wiley and Sons, New York (1964).
5) Tamiya, H., Hase, E., Shibata, K., Mituya, A., Iwamura, T., Sasa, T.: *Algal Culture from Laboratory to Pilot Plant*, (Edited by J. S. Burlew), pp. 204–232, Carnegie Institution of Washington Publication 600 (1953).
6) Rabe, A. E., Benoit, R. J.: *Biotechnol. Bioeng.*, **4**, 377 (1962).
7) Ragonese, F. P., Williams, J. A.: *Biotechnol. Bioeng.*, **10**, 83 (1968).
8) Shelef, G., Oswald, W. J., Golueke, C. G.: *Proc. 4th Symposium on Continuous Culture of Microorganisms*, (Prague) pp. 601 (1968).
9) Myers, J.: *J. Ferment. Technol.*, **44**, 344 (1966).
10) Monod, J.: *Ann. Inst. Pasteur*, **79**, 390 (1950).
11) Kamen, M. D.: *Primary Process in Photosynthesis*, Academic Press, (1963).

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