Energetics of Photosynthetic Algal Growth: Influence of Intermittent Illumination in Short (40 s) Cycles

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A theory is developed to predict the growth rates of photosynthetic microbes in a photobiological reactor with light-sufficient zones and dark zones. The theory also predicts that in light-limited cultures the maintenance energy will increase in proportion to the duration of the dark period.

The theory was tested and quantitatively confirmed by means of a loop reactor which permitted light and dark periods to be varied with a total cycle time of 40 s. An axenic *Chlorella* culture grown at the maximum rate under light-sufficient conditions could continue growth at the maximum rate in the dark for 9.2 s. It was found that the maintenance energy was zero in the light, but endogenous metabolism of resting cells in the dark corresponded to a large maintenance energy of $8.8 \text{ kJ (g dry wt)}^{-1} \text{ h}^{-1}$. These results have much significance for the design of photobiological reactors.

INTRODUCTION

Light and dark zones in a photobiological reactor, with consequent intermittent energy supply to a photosynthetic culture in the reactor, may be unavoidable. Understanding the effects of intermittent illumination is therefore crucial to the design of photobiological reactors, which must be one of the main goals of microbiologists in the next decade in order to study cellular photosynthesis and to realize its great potential for harnessing solar energy.

Intermittent illumination of photosynthetic organisms is analogous to discontinuous feeding of a heterotrophic culture with its energy source. For instance, studies on methanol-oxidizing bacteria have shown that discontinuous feeding with methanol reduces the growth yield (Brooks & Meers, 1973). If light as the sole energy source were cut off, two situations may be envisaged: one is that the organisms have a sufficient internal energy reserve to permit continuation of synthesis and cell growth; secondly, intracellular energy reserves could be exhausted so that growth must cease. If the latter is the case, endogenous metabolism of cell constituents could be stimulated, as pointed out by Pirt (1975, p. 70) in his account of cell cultures at or near zero growth rate. Endogenous metabolism would result in a decrease in the biomass. The theoretical model advanced here is based on the biomass and energy balances of the processes.

Studies on photosynthetic metabolism have distinguished between the 'light reaction' and the 'dark reaction'. The 'light reaction' in green algal photosynthesis refers to the splitting of water to release O_2 , store reducing power and generate ATP; the 'dark reaction' refers to the fixation of CO_2 and the synthesis of biomass using the reducing power and ATP generated in the light reaction. Attempts have been made to determine how long the dark reaction can continue after the cells have been illuminated by a light flash. Results in this field were summarized by Phillips & Myers (1954). The flash times used were very short, ranging from

0.01 to 67 ms, and the dark reaction was observed to continue for up to 300 ms. Livanský (1979) developed, by an extremely complex mathematical analysis, a model to account for the influence of dark zones in a culture on the overall growth rate, but he gave no experimental test of his model. His one prediction, which differs from ours, is referred to in the Discussion of the present paper. In contrast to the short, light-dark cycles, diurnal illumination is an example of a long cycle of intermittent illumination. Quite probably the diurnal light-dark cycle differs qualitatively from short cycles because it would be long enough to permit within the cycle substantial variations in the amounts of enzymes and other structural components in the cells, whereas, by definition, short cycles are not long enough to cause a substantial change in cellular composition during the cycle. The present paper is concerned with short light and dark periods (cycle time about 40 s). Diurnal effects are beyond the scope of this paper.

For the experimental study of intermittent illumination effects, a novel technique was developed, based on the 'loop reactor', that is, a tubular reactor around which the culture is cycled. A part of the loop was blacked out to create a dark zone. The model and experimental technique developed allow the duration of the dark reaction in a growing photosynthetic culture to be determined definitively. The study also reveals important effects of intermittent illumination on the energetics of photosynthetic growth and the growth yields.

THEORY

Biomass balance in light-sufficient cultures

The loop reactor with culture recycle used for photosynthetic algal growth is represented diagrammatically in Fig. 1. The theoretical model of the intermittent light effects involves the following assumptions. The culture growth is not limited by nutrients in the medium. The culture flows turbulently along the tubular reactor. The cycle time in the loop is small enough to make the biomass concentration (x) practically constant throughout the reactor, that is, the increase in biomass concentration in one cycle, $\Delta x \ll x$. The reactor has a total volume V ; a fraction α of the total volume is illuminated. It is postulated that, as a result of storage of light energy by the organisms in the illuminated zone, CO_2 fixation and growth may continue in the dark for a period. This 'dark growth zone' is designated as a fraction β of the whole culture volume. The remainder of the dark zone in which growth is arrested (the 'dark rest zone') will be a fraction $\{1 - (\alpha + \beta)\}$ of the culture volume. Also, it is assumed that growth restarts without lag when the organisms pass from the dark to the light zone.

Let the resting cells in the dark have a specific endogenous metabolic rate a_m . This factor a_m can be equated with the 'specific maintenance rate' (Pirt, 1975) which is related to the notional maintenance coefficient (m_r) of the resting cells by the relation $a_m = m_r Y_G$, where Y_G is the true growth yield from light (Pirt, 1975, p. 68).

The biomass balance for the loop reactor is

$$\text{Net growth} = \text{Growth in light zone} + \text{Growth in dark zone} - \text{Biomass decrease through endogenous metabolism in dark rest zone}$$

For a very small time interval Δt , this balance is

$$\mu_o x V \Delta t = \mu x \alpha V \Delta t + \mu x \beta V \Delta t - m_r Y_G x V \{1 - (\alpha + \beta)\} \Delta t \quad (1)$$

where μ_o is the 'observed specific growth rate', which would be obtained from a plot of x against time (t), and μ is the specific growth rate in the growth zone (light or dark). From equation (1) we obtain

$$\mu_o = \mu(\alpha + \beta) - m_r Y_G \{1 - (\alpha + \beta)\} \quad (2)$$

The second term on the right-hand side of equation (2) represents the biomass loss due to the

Fig. 1. Model of a loop reactor for photosynthetic algal growth.

endogenous metabolism of the *resting* cells. If this endogenous metabolism term is small compared with the growth, so that it can be neglected, and the specific growth rate (μ) has its maximum value (μ_m), then equation (2) reduces to

$$\mu_o/\mu_m = \alpha + \beta \quad (3)$$

Experimentally the condition for equation (3) should be obtained if the culture is both energy- and nutrient-sufficient in the growth zones. Thus the presence of a dark growth zone can be tested for by a plot of μ_o/μ_m against α . This plot should be a straight line with an intercept β on the ordinate when $\alpha = 0$.

Energetics of light-limited growth

Let the incident light intensity be I_0 , the fraction of photosynthetically available radiation be ϕ , the total surface area of the culture be A , and the total culture volume be V , and other symbols as before. The energy balance will be

$$\text{Light energy absorbed} = \text{Energy required for growth} + \text{Energy required for maintenance}$$

where the term 'maintenance' covers functions other than growth. With light-limited growth it is assumed that all the photosynthetically available radiation incident on the culture is absorbed so that the energy balance becomes

$$\phi I_0 A = \mu_o x V / Y_G + m x V \quad (4)$$

hence,

$$\phi I_0 A / x V = \mu_o / Y_G + m \quad (5)$$

where the term on the left-hand side is the specific light absorption rate (q). So far it has been assumed that maintenance energy is consumed at a constant specific rate (m) throughout the growth and rest zones. However, whereas the light source can supply the maintenance energy required in the illuminated zone, endogenous metabolism must be the source of maintenance energy in the dark rest zone. The maintenance energies of the growing and resting cells may not be equal. Indeed, the evidence of studies on microbial behaviour at zero or near zero growth rate (Pirt, 1975, p. 70) shows that turnover of protein and degradation of cell constituents is stimulated when growth ceases. With the postulate that the maintenance energy in the growth zone is insignificant compared with that in the dark rest zone (m_r) the energy balance becomes

$$\phi I_0 A = \mu_o x V / Y_G + m_r x V \{1 - (\alpha + \beta)\} \quad (6)$$

Table 1. *Symbols, definitions and units*

Symbol	Definition	Unit
A	total surface area of culture	m^2
a	specific maintenance rate	h^{-1}
a_m	specific endogenous metabolic rate	h^{-1}
I_0	incident light intensity	$W\ m^{-2}$
I_T	transmitted light intensity	$W\ m^{-2}$
m_r	maintenance coefficient of resting cells	$J\ (g\ dry\ wt)^{-1}\ h^{-1}$
m'	apparent maintenance coefficient of culture	$J\ (g\ dry\ wt)^{-1}\ h^{-1}$
q	specific light absorption rate	$J\ (g\ dry\ wt)^{-1}\ h^{-1}$
t	time	$h\ or\ s$
Δt	a small time interval	$h\ or\ s$
V	total culture volume	m^3
x	biomass concentration	$g\ dry\ wt\ l^{-1}$
Δx	increase in biomass concentration	$g\ dry\ wt\ l^{-1}$
Y_G	true growth yield from light	$g\ dry\ wt\ J^{-1}$
Y	actual growth yield	$g\ dry\ wt\ J^{-1}$
α	light zone, a fraction of the total volume illuminated	—
β	dark growth zone, a fraction of the total culture volume where cells continue to grow in dark	—
δ	dark rest zone, a fraction of the total culture volume where cell growth is arrested	—
μ	specific growth rate in the growth zone (light or dark)	h^{-1}
μ_m	maximum specific growth rate	h^{-1}
μ_o	observed specific growth rate	h^{-1}
ϕ	fraction of photosynthetically available radiation	—

hence,
$$\phi I_0 A / xV = \mu_o / Y_G + m' \quad (7)$$

where
$$m' = m_r \{1 - (\alpha + \beta)\} \quad (8)$$

Another postulate made is that when growth is light-limited in the illuminated zone, there is no storage of energy in the cells, so that growth cannot continue in the dark zone, that is, $\beta = 0$, hence equation (8) simplifies to

$$m' = m_r - \alpha m_r \quad (9)$$

or, substituting $\alpha = 1 - \delta$, where δ is the dark fraction of the culture we obtain

$$m' = \delta m_r \quad (10)$$

Relation (10) can be tested experimentally by determining m' as a function of δ .

Another possibility is that the maintenance coefficient (m) could vary with the specific growth rate. An expression suggested by Neijssel & Tempest (1976) for such an effect in heterotrophic growth is $m = m_o(1 + k\mu)$, where k is a constant, positive or negative according to whether m increases or decreases with μ . Such an effect would lead to variation in both the m' and the Y_G factors of equation (7). The complexities of such an effect are beyond the scope of this analysis.

Experimental tests of these theoretical models are described below. The definitions and units of the various symbols used are listed in Table 1.

METHODS

Cultures. Two algal cultures were used: one was a pure culture of *Chlorella vulgaris* 211/8k (Sorokin strain) obtained from the Culture Centre of Algae and Protozoa, Cambridge; the second was a defined consortium MA003 which consisted of a *Chlorella*-like alga A003 and three heterotrophic bacterial species (Pirt *et al.*, 1980).

Fig. 2. Diagrammatic representation of the loop reactor: (1) gas outlet and condenser; (2) nutrient input; (3) degasser; (4) sampling point; (5) peristaltic pump; (6) CO₂ input; (7) temperature sensor; (8) temperature controller and fan heater. The arrows indicate the direction of flow.

The cultures were grown photosynthetically on slopes of medium A9 plus agar (2%, w/v) under 5% CO₂ in air at 37 °C. The stock cultures were stored at ambient temperature (20–30 °C) in the light.

Culture medium. Chemically defined medium A9 had the following composition (amounts in g l⁻¹): KH₂PO₄, 2.63; CaCl₂·2H₂O, 0.08; MgSO₄·7H₂O, 3.0; FeCl₃·6H₂O, 0.096; MnSO₄·4H₂O, 0.016; ZnSO₄·7H₂O, 0.018; CuSO₄·5H₂O, 0.004; Na₂B₄O₇·10H₂O, 0.035; NaVO₃, 0.005; NiSO₄·7H₂O, 0.0008; Na₂EDTA·2H₂O, 0.313; urea, 4.0. The pH was 6.7. The medium was made up in four separate parts: (a) KH₂PO₄ adjusted to pH 6.7 with NaOH, (b) CaCl₂ and MgSO₄, (c) urea, and (d) other salts plus EDTA adjusted to pH 6.7 with NaOH. Each stock solution was prepared and autoclaved separately at 121 °C for 15 min and then mixed with the others after cooling.

The loop reactor. The tubular loop reactor is depicted in Fig. 2. It consisted of 12 Pyrex tubes, each about 25 cm long and of 0.7 cm bore, connected by Pyrex and silicone rubber U-bends. The tubing was laid horizontally and the end was connected to a vertical tube (2.9 cm bore) which acted as a degasser. The culture volume was about 280 ml; this was checked when necessary with a measuring cylinder. The culture was recycled by means of a peristaltic pump (MHRE 200; Watson Marlow, Falmouth, Cornwall). The recycle rate was 420 ml min⁻¹, which gave rise to a Reynolds number of 1000, that is, about the lower limit for turbulent flow (Brown, 1961). The cycle time was 40 s. CO₂ (5% in air) was injected at 15 ml min⁻¹ into the reactor through a hypodermic needle installed downstream from the pump. The temperature of the culture was maintained at 37 °C by means of a fan heater operated by a temperature controller (Pirt *et al.*, 1979). The culture maintained a pH of 6.5 ± 0.1.

Illumination was provided by four horizontal warm white fluorescent tubes (20 W, Philips) placed below the reactor tubes and parallel to them. The average light intensity at the surface of the reactor was 11 W m⁻². The tubular reactor was placed under a white polyfoam cover to facilitate both maximum light input to the culture and the temperature control. When necessary, some of the tubes were covered by thick black paper to produce a dark zone in the reactor. The illuminated area was taken to be the entire inner surface of the tubes, that is, $2\pi rl$ where r is the inner radius and l the length.

Adherence of cells to the walls of the tubular reactor (wall growth) did not occur under the conditions used.

Analytical methods. The turbidity (A) of the culture was measured in a Unicam SP500 spectrophotometer at a wavelength of 680 nm with a 1 cm light path. At this wavelength the light absorption of the algal cells was maximal.

The specific growth rate (μ) and maximum specific growth rate (μ_m) of the batch culture (Pirt, 1975, p. 22) of algae in the loop reactor were determined by measuring the increase in turbidity (A_{680}) at short time intervals in light-limited and light-sufficient culture. The contribution of the bacteria to the turbidity was negligible at 680 nm.

The algal biomass (x) was estimated from the culture turbidity (A_{680}). The ratio of turbidity to algal biomass remained fairly constant up to 40 g biomass l⁻¹ at all growth rates tested (0.02–0.235 h⁻¹). An A_{680} unit corresponded to 0.092 g dry biomass l⁻¹ for *Chlorella* 211/8k, and 0.124 g dry biomass l⁻¹ for consortium MA003.

RESULTS

Light-sufficient growth

For growth to be light-sufficient the available light energy must exceed the maximum light required for growth, that is, $\phi I_0 A > \mu_m x V / Y$, where Y is the actual growth yield.

Fig. 3

Fig. 4

Fig. 3. Exponential growth curves of light-sufficient batch cultures of *Chlorella* 211/8k at various light fractions (α).

Fig. 4. Exponential growth curves of light-sufficient batch cultures of consortium MA003 at various light fractions (α).

Fig. 5

Fig. 5. Plots of the ratio of the observed specific growth rate to the maximum specific growth rate (μ_o/μ_m) as a function of the light fraction (α) for *Chlorella* 211/8k (○) and consortium MA003 (●) in batch culture.

Fig. 6

Fig. 6. Examples of the linear increase in turbidity (A_{680}) with time for light-limited batch cultures of *Chlorella* 211/8k at $\alpha = 0.405$ (○) and consortium MA003 at $\alpha = 0.6$ (●). Each curve is for a different biomass concentration.

Accordingly, cultures were set up in the loop reactor with the aim of satisfying the above condition for light-sufficient growth. The growth curves of some of these cultures are shown in Figs 3 and 4. When the cultures were illuminated along their entire length ($\alpha = 1$) the maximum specific growth rate of *Chlorella* 211/8k was 0.235 h^{-1} and of consortium MA003, 0.170 h^{-1} . To test equation (3) a dark zone was introduced along part of the reactor length. The ratio of the observed specific growth rate (μ_o) to the maximum value (μ_m) is plotted against the light fraction (α) for cultures of *Chlorella* 211/8k in Fig. 5. These data show that $\mu_o/\mu_m = 1$ until the light fraction (α) was less than 0.77. At lower α values μ_o/μ_m

Fig. 7

Fig. 7. Specific light absorption rate (q) as a function of the observed growth rate (μ_o) in light-limited batch cultures of *Chlorella* 211/8k.

Fig. 8

Fig. 8. Specific light absorption rate (q) as a function of the observed growth rate (μ_o) in light-limited batch cultures of consortium MA003.

was a linear function of α as predicted by the model in equation (3). The intercept on the ordinate gives $\beta = 0.23$, that is, the maximum fraction of the culture in which 'dark growth' could occur. Since the cycle time in the loop reactor was 40 s, the maximum duration of growth in the dark is deduced to be $0.23 \times 40 = 9.2$ s. When the light fraction (α) exceeded 0.77, μ_o was equal to μ_m , which indicated that growth then extended to the full length of the culture system.

The plot of μ_o/μ_m against α for consortium MA003 is also shown in Fig. 5. Again the plot was linear in accordance with equation (3). The value of the intercept β was 0.09; thus the duration of the dark growth was $0.09 \times 40 = 3.6$ s.

Light-limited growth

Light-limited cultures were set up. The onset of light-limited growth is shown by the linearity of the increase in turbidity with time. However, if there is a finite maintenance energy, the rate of increase in turbidity will decrease with increasing biomass concentration. This is illustrated by the light-limited growth curves shown in Fig. 6. The observed specific growth rate of the culture (μ_o) was calculated at different biomass concentrations (x) from the ratio of σ/x , where σ was the slope of the light-limited growth curve (Fig. 6). Plots of the specific light absorption rates ($q = \phi I_o A/xV$) against the observed specific growth rates (μ_o) with different light fractions (α) are given in Figs 7 and 8. Both the axenic *Chlorella* 211/8k (Fig. 7) and the consortium MA003 (Fig. 8) showed a linear relation of q and μ_o for each α . The slope of the plot is $1/Y_G$ and the intercept on the ordinate gives the value of the apparent maintenance energy coefficient which is m' according to the model equation (7).

For *Chlorella* 211/8k (Fig. 7) with the light fraction (α) above 0.5 the value of Y_G was constant and independent of α . The mean and 95% confidence limits of Y_G were $1.54 \times 10^{-5} \pm 0.15 \times 10^{-5}$ g dry wt J⁻¹. The value of Y_G decreased significantly only when the light fraction was decreased to 0.405. One possible explanation is that when the light fraction of a culture decreases below a critical value for a particular photosynthetic organism, the maintenance energy or endogenous metabolism become dependent on both the light fraction and the specific growth rate.

Fig. 9. Apparent maintenance coefficient (m') as a function of the dark fraction (δ) in light-limited batch cultures of *Chlorella* 211/8k.

The model equation (10) showing the effect of the light fraction on the maintenance coefficient (m') was tested by plotting m' against the dark fraction (δ) as shown in Fig. 9. The plot for *Chlorella* 211/8k shows that when $\delta = 0$, the value of m' is practically zero, which is in agreement with the model postulates that for light-limited growth there is no growth in the dark zone ($\beta = 0$) and that the maintenance energy or endogenous metabolism is significant only in the dark zone. The maximum value of the maintenance coefficient (m') was $8.8 \text{ kJ (g dry wt)}^{-1} \text{ h}^{-1}$.

With light-limited growth of the consortium MA003, increase in the dark fraction from 0 to 0.39 was associated with an increase in the slope of the q versus μ_0 plot (Fig. 8) which means that there was a large reduction in the apparent maximum growth yield (Y_G), from 2.1×10^{-5} to $1.15 \times 10^{-5} \text{ g dry wt J}^{-1}$. Also, Fig. 8 shows that the intercept on the ordinate, which corresponds to the maintenance coefficient, increased with increase in the dark fraction. The variation in both Y_G and the maintenance coefficient for the consortium MA003 could mean that the maintenance term depended on both the specific growth rate and the light fraction.

DISCUSSION

The loop reactor with light and dark zones is a novel means for the study of intermittent illumination in a photosynthetic culture. Previous workers on the problem, e.g. Kok (1953) and Phillips & Myers (1954), used instead a flashing light, which seems less convenient for cycles of several seconds or longer duration. The new theoretical model of intermittent light effects proposed here has the virtues of simplicity and that it predicts important effects which can be tested experimentally. This model and the experimental technique have, for the first time, permitted definitive measurement of the duration of the dark growth reaction when the cultures are light-sufficient.

For *Chlorella* 211/8k the duration of the dark growth was 9.2 s when the specific growth rate was at its maximum value ($\mu_m = 0.235 \text{ h}^{-1}$). Hence, there must have been enough energy stored in the light-sufficient period to permit the biomass to increase by the fraction $\mu_m \Delta t$ where Δt is the dark growth period; this fraction was 6.0×10^{-4} for *Chlorella* 211/8k. It would be of interest to know the nature of the stored energy and reducing power which permit the continuation of biomass synthesis from CO_2 in the dark; reduced NADP, ATP and a proton-motive force could be among the products involved.

Whereas we deduced that $\mu_0/\mu_m = \alpha + \beta$ (equation 3), and verified this experimentally for light-sufficient cultures, the theoretical model of Livanský (1979) led to the conclusion, in our notation, that μ_0/μ_m approximately equals α^2 , provided α is not much less than unity. Livanský's model was not verified experimentally. Also, his model did not take account of the duration of the dark reaction or the effects on the growth yields.

Light-limited growth is important because it permits maximum utilization of the light source. However, light-limited growth will cause reduction of the specific growth rate; under

this condition the maintenance energy requirement could cause a significant loss of energy. An important prediction of the model for the light-limited condition is that the maintenance energy will increase in proportion to the dark fraction of the culture. This prediction was quantitatively verified by the experimental measurements for light-limited cultures of *Chlorella* 211/8k. Its maintenance coefficient was zero in the light and rose to $8.8 \times 10^3 \text{ J (g dry wt)}^{-1} \text{ h}^{-1}$ for resting cells in the dark. If the maintenance coefficient is expressed as a specific maintenance rate ($a = m' Y_G$), the maximum value obtained for *Chlorella* 211/8k (when $a = 0$) is $8.8 \times 10^3 \times 1.54 \times 10^{-5} \text{ h}^{-1}$, that is, 0.136 h^{-1} , which is more than half the maximum specific growth rate. Thus the endogenous metabolism rate in the dark zone was extremely high.

The dark response of the consortium MA003 was qualitatively similar to that of the axenic *Chlorella* 211/8k; however, the data, which are fewer, indicated that both Y_G and the maintenance energy were affected at a higher light fraction than was the case with *Chlorella* 211/8k.

These findings have an important implication for deep algal cultures in which the light penetration is relatively small. The turbidity of the culture is given by $\log(I_0/I_T)$, where I_0 is the incident light intensity and I_T is the transmitted light intensity. If the turbidity were 10 for a culture 1 cm deep, this means that 99% of the incident light would be absorbed or scattered in the 10^{-2} cm layer of culture next to the tube wall exposed to the light. This means that 97% of the culture would be effectively shaded by the outer 3% of the culture, assuming that the tube is illuminated over half its surface. Therefore, if the culture were light-limited, it should be imperative for the cells to move periodically into the thin illuminated layer next to the wall of the tube in order to pick up light energy. Turbulence would permit the cells to pick up light in this way and, in work to be reported elsewhere, we have found that the growth yield dropped dramatically if the Reynolds number in the tube fell below 1000, that is, below the minimum for turbulent flow. Calculations of the mean time taken for cells to cycle from one side of a tube to the other in turbulent flow have been made by A. P. Ippen, quoted by Arthur D. Little Inc. (1953). These data suggest that, in our loop reactor with tubing of 0.7 cm bore, the mean cycle time would be of the order 0.5 s. If the cycle time became of the order of 40 s, through low turbulence or increase in the reactor depth, then the intermittent illumination effects reported here, that is, increase in maintenance energy and consequent reduction of yield, could occur even in the illuminated section. Lack of attention to this effect could lead to large discrepancies between the growth yields found in different laboratories using different types and sizes of photoreactor with varying degrees of turbulence. For example, in the pilot plant of Mituya *et al.* (1953) the dark fraction (sump plus tower) was 0.6 and the cycle time was 2 min. Such a large dark fraction, our model predicts, will cause a large endogenous metabolism (maintenance) effect and result in a substantial drop in the efficiency of biomass production.

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