Growth Kinetics of Oscillatoria agardhii Gomont in Continuous Culture, Limited in its Growth by the Light Energy Supply

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Growth efficiency (c) and specific maintenance rate constant (μₘ) were determined in continuous cultures of the cyanobacterium Oscillatoria agardhii Gomont, in light energy-limiting conditions, according to the formula µ = cg - μₘ in which qₑ is the specific light energy uptake rate. Values of the efficiency factor c varied with irradiance from 0.23 at 0.5 W m⁻² to 0.05 at 40 W m⁻². The specific maintenance rate constant was 0.001 h⁻¹. The culture pH value influenced c and μₘ. A diurnal light/dark cycle with a 16 h photoperiod did not affect either of the two parameters, μₘ and c. The relationship between growth rate and light energy uptake rate, embodied in the above formula, was also valid for nitrogen (nitrate)-limited cultures.

INTRODUCTION

When light limits the growth of phototrophic micro-organisms, the rate at which the light energy is taken up is directly proportional to the rate of cell synthesis (Tamiya et al., 1953; Gons & Mur, 1975; Van Liere & Mur, 1978), that is:

\[
\frac{dx}{dt} \frac{1}{x} = -\frac{dE}{dt} \frac{1}{x}
\]  

where \( \frac{dx}{dt} \frac{1}{x} \) is the specific growth rate µ; \( \frac{dE}{dt} \frac{1}{x} \) is the specific light energy uptake rate qₑ, and \( Y_E \) is a proportionality factor under a prescribed set of conditions for the conversion of radiant energy into chemical energy. This factor has been called the yield value. Equation (1) can thus be written as:

\[
\mu = Y_E q_E
\]

The specific light energy uptake rate (qₑ) affects cell synthesis. However, part of the light energy actually consumed is used for "maintenance" functions, which are neglected in the equations above. These functions are, for example, the maintenance of solute gradients across membranes, maintenance of cell integrity and turnover of macromolecular components of the cell (Pirt, 1965). Thus:

\[
q_E^{(actual)} = q_E^{(growth)} + q_E^{(maintenance)}
\]

Dividing by µ, and as \( q_E/µ = 1/Y_E \), then

\[
\frac{1}{Y_E} = \frac{1}{Y_E^{growth}} + \frac{q_E^{(maintenance)}}{µ}
\]  

This is the 'light energy analogue' of the formula derived by Pirt (1965) for nutrient-limited growth of heterotrophic micro-organisms. \( Y_E^{growth} \) is the maximum yield value on light energy.
(or 'true' yield value), the incremental increase in specific light energy uptake rate required to support an incremental increase in growth rate, corrected for maintenance losses. Gons & Mur (1975) have rewritten equation (4), to describe algal growth, as:

\[ \mu = q_e c - \mu_e \]  

in which \( \mu_e = Y_{growth} \times q_e \) (maintenance) (Powell, 1967) and \( c \) (called the efficiency factor) is identical to \( Y_{growth} \). If the biomass \( x \) is expressed in energetic terms, like irradiance, then \( c \) is an efficiency factor for energy conversion to biomass. For reasons of comparability we also use the notation \( c \). If \( c \) and \( \mu_e \) have constant values for fixed environmental conditions, then equation (5) should give a linear relationship between the specific growth rate and the specific light energy uptake rate \( q_e \). The intercept with the ordinate will be \( \mu_e \) upon extrapolation, and the slope will be \( c \). Such a linear relationship has been found with phototrophically growing green algae (Gons, 1977).

Without any direct knowledge of the mechanism, one can say that \( q_e \) (maintenance) is the rate at which light energy is being consumed in maintenance processes; at the same time, \( \mu_e \) is the amount by which the growth rate is reduced below a potential maximum because of this diversion of energy.

So far it has been accepted that the maintenance rate is constant and independent of growth rate. The fact that a linear relationship generally has been found between the specific rate of light energy uptake (or specific rate of substrate consumption in heterotrophic growth) and specific growth rate supports this conclusion, but does not prove it to be so. However, as the light energy uptake rate (or substrate consumption) increases linearly with growth rate then, should the maintenance rate vary, it would probably do so as a linear function of the growth rate (Neijssel & Tempest, 1976). If we assume this, then the constant \( \mu_e \) should be amended to \( \mu_e (1 + p \mu) \), in which \( p \) is the proportionality constant between maintenance rate and growth rate; thus equation (5) becomes:

\[ \mu = c' q_e + \mu_e (1 + p \mu) \]  

or rearranging:

\[ \mu = \left( \frac{c'}{1 + p \mu} \right) q_e - \frac{\mu_e}{1 + p \mu} \]  

The relationship between specific growth rate and specific light energy uptake rate will still be linear. If \( p = 0 \), equation (7) reduces to equation (5). If \( p > 0 \) then, for example, \( \mu_e / (1 + p \mu) < \mu_e \) so one would underestimate the value of \( \mu_e \) and \( c \). Whatever the realistic values of these constants may be, the function of \( \mu \) versus \( q_e \) is mathematically a valid description of the growth of phototrophic organisms under conditions that are limited by the supply of light energy.

In this paper, the growth kinetics of the cyanobacterium Oscillatoria agardhii Gomont, limited in its growth by the light energy supply, are reported. The experiments were performed in various types of continuous cultures. The results are discussed in relation to other published data.

**METHODS**

Oscillatoria agardhii Gomont was isolated from a shallow eutrophic lake, the Veluwemeer, by the Rijksinstituut voor Drinkwatervoorziening, Holland. The red strain of O. agardhii Gom var. CYA 18 was isolated from Lake Gersjøen, Norway, and supplied by the Norsk Institutt for Vannforskning, Oslo. Both cultures were free from contaminant algae and other cyanobacteria. The number of contaminant bacteria in the cultures was counted regularly and the amount present generally was so low (<0.5 % dry wt) that the results would not be significantly affected.

Cultivation and measurements. The continuous culture vessel and the mineral salt medium used were described previously (Van Liere et al., 1977; Van Liere & Mur, 1978). Incident irradiance and average irradiance were measured according to methods described by Van Liere et al. (1978) and Loogman & Van Liere (1978). The energy that left the culture vessel, again as a function of incident irradiance and biomass, is given
Light energy-limited cyanobacterial growth

Specific growth rate (h⁻¹) Incident irradiance (W m⁻²)

Fig. 1. Biomass concentration of *Oscillatoria agardhii* Gomont during light energy-limited steady states. Incident irradiance values were (W m⁻²): ○, 0.5; ●, 1.5; □, 6; ■, 13; △, 25; ▲, 40. The intercepts with the abscissa are not steady state values; they indicate the maximum specific growth rate ($\mu_m$) at an average irradiance as indicated (Van Liere *et al.*, 1978). The intercepts with the ordinate denote the biomass that is measured when the pump is switched off and a stationary phase is reached.

Fig. 2. Specific light energy uptake rate of an *Oscillatoria agardhii* Gomont population at zero growth rate.

by Loogman & Van Liere (1978). Subtraction of incoming irradiance and 'lost' irradiance gives (multiplied by the irradiated area) the energy absorbed by the culture. If the biomass is expressed in J l⁻¹ [by measuring the heat of combustion of freeze-dried material in a Phillipson Microbomb Calorimeter (Gentry Instruments) and correcting this value for oxidation into nitrate], one can calculate $q_E$ as follows:

$$q_E = \frac{(\text{d}E/\text{d}t)}{(l/x)}$$

is absorbed energy (J h⁻¹) divided by the biomass in the culture vessel (J).

In the case of a diurnal light/dark cycle (16 h photoperiod), $\mu$ and $c$ were derived on a 24 h production basis.

RESULTS

Pipes & Koutsoyannis (1962) and Gons (1977) showed that during steady state growth under light energy-limiting conditions there should be an inverse relationship between the culture biomass concentration and the specific growth rate, assuming the energy absorbed by the population remains constant. Experiments were carried out at several incident irradiance values. At each incident irradiance, the growth rate was varied by altering the dilution rate. The cultures were normally so dense that the energy absorbed was close to the irradiated energy. The results of these experiments are presented in Fig. 1. The intercepts with the abscissa are not steady state values; they indicate the maximum specific growth rate ($\mu_m$) at an average irradiance as indicated (Van Liere *et al.*, 1978). The intercepts with the ordinate denote the biomass that is measured when the pump is switched off and a stationary phase is reached. When the specific light energy uptake rate ($q_E$) in this stationary phase was plotted against the incident irradiance, which equalled the energy absorbed by the population, a linear relationship was found (Fig. 2). This indicated that $q_E$ (maintenance) at zero growth rate is dependent on the incident irradiance, which makes the Pirt equation (4) inappropriate for our purposes. Figure 3 shows the observed relation between $\mu$ and $q_E$. The value of $c$ was found to be constant over a wide range of growth rates, but dependent on the incident irradiance (Fig. 3 and Table 1). With an incident irradiance of 25 and 40 W m⁻², a marked
decrease in the efficiency factor was evident at high growth rates. In Fig. 1 it can be seen that the biomass concentration decreased rapidly with growth rate, so a high average irradiance occurred at high growth rate and high incident irradiance. In fact when a decreased value of $c$ was found, an average irradiance of approximately 10 W m\(^{-2}\) had been exceeded. The $\mu_e$ value was constant over the measured incident irradiance at low growth rates (Fig. 3 and Table 1). The $\mu_e$ value was thus easier to apply to a description of the growth kinetics of this cyanobacterium than $q_h$(maintenance), which changed with irradiance. The relationship between specific light energy uptake rate and specific growth rate was linear (Fig. 3). The slope of the line was dependent on the incident irradiance. The efficiency factor did not change with average irradiance. Thus, it seems that *O. agardhii* adjusts to the highest irradiance experienced in the culture vessel, i.e. that close to the vessel wall.

To determine whether pH influenced $c$ and $\mu_e$, a series of steady state cultures growing at pH 9.0 were analysed (Fig. 4). Both $c$ and $\mu_e$ increased with pH. Further, since in nature photoautotrophic organisms are exposed to a diurnal light/dark rhythm, a study was undertaken to test whether this influenced $c$ and $\mu_e$. A diurnal light/dark cycle was imposed on two steady state cultures in which the pH was held constant (at 8.0). The results showed there was no significant influence on these parameters, compared with continuous illumination.

The influence of another limitation on the growth kinetics, as specified by equation (5), was studied using nitrogen(nitrate)-limited chemostat cultures. The results (Table 2) are compared with data from an energy-limited culture. A decrease in $c$ with incident irradiance was found, similar to that which occurred during limitation by the light energy supply. The value of $c$, however, was much lower than that with light energy-limited conditions. Energy-spilling processes (fluorescence, for example) presumably occur at a higher rate under conditions of light energy excess. However, there was no significant difference in the $\mu_e$ value. These findings suggest that the equation $\mu = c q_h - \mu_e$ is also valid for nitrogen(nitrate)-limited chemostat conditions.
Table 1. Comparison of the measured data with published data for green algae and cyanobacteria, according to \( \mu = cq_E - \mu_e \)

Published data were recalculated from experiments with continuous cultures in light energy-limited conditions. Numbers in parentheses denote 95% confidence intervals.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Temp. (°C)</th>
<th>pH</th>
<th>Incident irradiance (W m(^{-2}))</th>
<th>( c )</th>
<th>( \mu_e ) (h(^{-1}))</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oscillatoria agardhii Gom. (Veluwe-meer isolate)</td>
<td>20</td>
<td>8-0</td>
<td>0-5</td>
<td>0-23 (0-07)</td>
<td>0-001 (0-003)</td>
<td>This paper</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>8-0</td>
<td>1-5</td>
<td>0-15 (0-02)</td>
<td>0-001 (0-002)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>8-0</td>
<td>6</td>
<td>0-12 (0-01)</td>
<td>0-001 (0-001)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>8-0</td>
<td>13</td>
<td>0-10 (0-01)</td>
<td>0-001 (0-002)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>8-0</td>
<td>25</td>
<td>0-09 (0-03)</td>
<td>0-001 (0-002)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>8-0</td>
<td>40</td>
<td>0-05</td>
<td>0-002</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>8-0</td>
<td>6†</td>
<td>0-12</td>
<td>0-001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>9-0</td>
<td>8</td>
<td>0-19</td>
<td>0-004</td>
<td></td>
</tr>
<tr>
<td>Oscillatoria agardhii Gom. var. (Gersjøen isolate)</td>
<td>20</td>
<td>8-0</td>
<td>6</td>
<td>0-09</td>
<td>&lt; 0-001</td>
<td>This paper</td>
</tr>
<tr>
<td>Spirulina platensis</td>
<td>35</td>
<td>9-4/9-8</td>
<td>19</td>
<td>0-10</td>
<td>0-002</td>
<td>Aiba &amp; Ogawa (1977)</td>
</tr>
<tr>
<td>Scenedesmus protuberans Fritsch*</td>
<td>20</td>
<td>7-3/9-8</td>
<td>12†</td>
<td>0-18</td>
<td>0-008</td>
<td>Gons &amp; Mur (1978) (recalculated)</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>7-3/9-8</td>
<td>38†</td>
<td>0-13</td>
<td>0-014</td>
<td></td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>7-3/9-8</td>
<td>38†</td>
<td>0-13</td>
<td>0-008</td>
<td>J. G. Loogman (pers. comm.)</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>8-0</td>
<td>36</td>
<td>0-12</td>
<td>0-001</td>
<td></td>
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<tr>
<td>Scenedesmus obliquus</td>
<td>25</td>
<td>10-3</td>
<td>30</td>
<td>0-04</td>
<td>0-009</td>
<td>Oswald (1970)</td>
</tr>
<tr>
<td>Chlorella sorokiana Tx 71105</td>
<td>35</td>
<td>—</td>
<td><em>approx. full sunlight</em></td>
<td>0-08</td>
<td>0-015</td>
<td>Myers (1970)</td>
</tr>
<tr>
<td>Chlorella ellipsoidea</td>
<td>25</td>
<td>—</td>
<td><em>approx. full sunlight</em></td>
<td>0-08</td>
<td>0-012</td>
<td>Myers (1970)</td>
</tr>
<tr>
<td>Chlorella pyrenoidosa (Emerson strain)†</td>
<td>25</td>
<td>~10</td>
<td>30</td>
<td>0-13</td>
<td>0-008</td>
<td>Oswald (1970)</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>~10</td>
<td>30</td>
<td>0-08</td>
<td>0-007</td>
<td></td>
</tr>
<tr>
<td>Chlamydomonas agloformis†</td>
<td>25</td>
<td>~10</td>
<td>30</td>
<td>0-03</td>
<td>0-010</td>
<td>Oswald (1970)</td>
</tr>
</tbody>
</table>

* Nitrogen source, urea. † Growth medium, domestic sewage. ‡ Measured in a diurnal light/dark cycle.

Table 2. Comparison of the ‘energy balance’ of Oscillatoria agardhii grown in conditions limited by light energy supply with those limited by nitrogen(nitrate) supply

<table>
<thead>
<tr>
<th>Incident irradiance (W m(^{-2}))</th>
<th>Nitrogen(nitrate) limitation</th>
<th>Light energy limitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Integer</td>
<td>( c )</td>
<td>( \mu_e ) (h(^{-1}))</td>
</tr>
<tr>
<td>2-3</td>
<td>0-10</td>
<td>0-001</td>
</tr>
<tr>
<td>3-6</td>
<td>0-07</td>
<td>0-001</td>
</tr>
<tr>
<td>4-8*</td>
<td>0-08</td>
<td>0-004</td>
</tr>
</tbody>
</table>

* Only two steady states were measured, which made extrapolation to \( \mu_e \) less accurate.

DISCUSSION

The efficiency factor \( c \) is influenced by any process which leads either to a change in the efficiency with which radiant energy is converted into chemically stored energy, or to a loss of this stored energy. Such processes include:

(a) Efficiency of light energy trapping by the photosystems. With optimum conditions some 15% of the light energy trapped is lost as heat or fluorescence (Papageorgiou, 1975).
In dense algal cultures this might be less, because re-emitted light can be trapped by other cells. Gons (1977) estimated these losses to be approximately 10%.

(b) Uncoupling of growth and energy generation. This could lead to a loss of about 10\%, if the level of uncoupling is comparable with that found in bacteria (Gons, 1977).

(c) Photoinactivation of ribulosebisphosphate carboxylase. This seems to occur only when a high irradiance of light in the blue spectral region is applied (Codd & Stewart, 1978). However, it could be that slight inhibition occurs at lower irradiance.

(d) Photorespiration (light dependent oxygen uptake and concomitant carbon dioxide release). This is enhanced by high oxygen tensions caused by a high photosynthetic production at high irradiance. Gons (1977) suggested that photorespiration is initiated when a critical intracellular oxygen concentration is exceeded. The critical tension depends on the ability of the cells to excrete rapidly the oxygen produced. This transport is regulated by the difference in oxygen tension in the cell and at the cell surface, and by the cell surface/volume ratio.

(e) Photo-oxidation of the pigments. This will occur only at very high irradiance. Chlorophyll \(a\) is converted into phaeophytin \(a\) and loses its photosynthetic functions.

(f) The composition of the growth medium, one of the most important components being the source of nitrogen. More energy per mol N is required for the reduction and assimilation of nitrate than for ammonia. A decrease in the yield value of about 15\% has been reported by Wesselius (1973) for the green alga \textit{Scenedesmus} when growing with either ammonium nitrate or potassium nitrate.

In our experiments, we found the highest \(c\) value at an incident irradiance of 0.5 W m\(^{-2}\). At this low irradiance uncoupling and photorespiration will be minimal. When the incident energy is increased, the value of \(c\) decreases. This might be due to an increase in the rate of one or more of the above-mentioned processes. If the rate increases linearly with \(q_E\), this would explain a biphasic linear function. Eventually the slope has to decrease, because \(\mu_m\) will be reached and the growth rate cannot increase any further as the light energy uptake rate is increased further.

On extrapolation, the value of \(\mu_E\) was found to be constant. The \(\mu_m\) value for \textit{O. agardhii} was 0.036 h\(^{-1}\) (Van Liere \textit{et al.}, 1978). This means that, if the maintenance energy does not vary with growth rate, the cells have to overcome a loss of 3\% energetic efficiency due to population maintenance. Neijssel & Tempest (1976) pointed out that the maintenance energy could vary with growth rate. It is, however, difficult to see how this could explain the change in the slope of the curve observed at 25 and 40 W m\(^{-2}\). If this was due to a higher maintenance energy rate, then, at the same efficiency of growth, \(\mu_m\) should increase 30- to 40-fold. Some 80 to 100\% of the energy would then be used in maintenance processes. It is therefore more likely that \(p\) (equation 7) is small, giving a slight underestimation of \(\mu_m\) and \(c\). We cannot exclude, however, the possibility that a combination of changes in \(\mu_m\) and \(c\) gives rise to the changing slope. More conclusions can be drawn when the processes that cause these changes have been studied in more detail.

On a theoretical basis, starting from cell composition and metabolic pathways, \(c\) can be calculated (Gons, 1977). For growth on nitrate, a theoretical value of 0.19 to 0.26 could be calculated for \textit{O. agardhii}; this was not significantly different from that derived for green algae (Gons, 1977). The highest value found for \(c\) for \textit{O. agardhii} was 0.23 (Table 1) at an incident irradiance of 0.5 W m\(^{-2}\), which agrees with the theoretical value.

There seem to be very few published accounts of algal or cyanobacterial growth in continuous culture under conditions of limited light energy supply. Oswald (1970), Myers (1970) and Aiba & Ogawa (1977) measured the net yield on energy, \(Y_E = (\mu x)/(dE/dt)\). However, only Aiba & Ogawa (1977) measured the light energy escaping from the culture system, which means that the other authors underestimated their \(Y_E\) values. Gons (1977) measured the efficiency factor in the same way as described in this paper, and only he paid attention to maintenance energy. The results of calculations based on published data are presented.
in Table 1. Two phenomena immediately attract attention. First, the calculated efficiency factor varied greatly. However, the incident irradiance, pH and temperature varied also, while most authors worked only at one incident irradiance. We have shown in this paper that $c$ is dependent on incident irradiance, a fact that has been overlooked by most authors. The decreasing efficiency factor with increasing incident irradiance has also been found by Gons & Mur (1978). Second, there appeared to be an order of magnitude difference in the extrapolated $\mu$, value between cyanobacteria (0.001 to 0.004 h$^{-1}$) and green algae (0.008 to 0.015 h$^{-1}$). These differences can be correlated with the difference in cellular structure between the eukaryotic green algae and the prokaryotic cyanobacteria.

The differences in $\mu$ and $c$ noted above will have strict implications for the growth kinetics of these organisms. At low irradiance, cyanobacteria can exert a higher net growth yield ($Y_n$), which results in a higher growth rate, as compared with green algae. An example has been given by comparing the growth kinetics of the green alga Scenedesmus protuberans and O. agaradhi (Van Liere et al., 1978), and their succession, with light energy supply as competing factor (Mur et al., 1977).

Gerard de Groot provided the data on nitrogen(nitrate) limitation, Inger Johanna Johnsen and Hans C. Utkilen did the experiments with the red strain. We wish to thank Uka Dijkstra for technical assistance and David W. Tempest for his support with the theory and linguistic help.

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