The Photosynthetic Efficiency of Chlorella Biomass Growth with Reference to Solar Energy Utilisation

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The photosynthetic efficiency (PE) of a growing algal culture was determined from the growth yield (Y), that is, biomass produced/light absorbed and the calorific value of the biomass (k); PE = k/Y. To obtain the maximum photosynthetic efficiency the algae were grown in light-limited chemostat cultures in urea-mineral salts media plus CO₂ and steady-states were obtained at different specific growth rates. With a given light input the biomass output rate was independent of the specific growth rate up to at least 70% of the maximum specific growth rate. The photosynthetic efficiency was independent of the incident light intensity over the range studied, 5.3–21.3 W m⁻². The light source had a spectral range of 400–700 nm and its mean wavelength was assumed to be 575 nm. The values of the maximum growth yields (Yc, g dry weight kJ⁻¹) were 0.0153 for the Sorokin Chlorella strain 211/8k and 0.0206 for a newly selected mixed culture MA003 which consisted of an alga and three species of heterotrophic bacteria. The maintenance energy (m) of the mixed culture MA003 was in the range 0–0.32 kJ g⁻¹ dry weight h⁻¹ and the specific maintenance rate (m Yc) was in the range 0–0.0066 h⁻¹. In Chlorella strain 211/8k the maximum PE was 34.7% which corresponds to a quantum demand (n) of 6.6 per O₂ molecule evolved. In the mixed culture MA003 the maximum PE was 46.8% with 95% confidence limits, 42.7–51.5. This PE value corresponds to a quantum demand (n) of 4.8 per O₂ molecule evolved. These results call in question the current model of photosynthesis which predicts that the maximum PE with absorbed light of mean wavelength 575 nm should not exceed 29% and the minimum quantum demand, n = 8. From our results with culture MA003 it is deduced that the maximum practicable storage of total solar energy by algal biomass growth in vitro is 18%.

1. Introduction

The efficiency of photosynthesis as a means of storing energy by biomass growth is of crucial importance in the harnessing of solar energy. It also has much significance in determining the mechanism of photosynthesis. The process of photosynthetic biomass production can be represented as:

\[ a\text{CO}_2 + b\text{H}_2\text{O} + c\text{NH}_3 + \text{minerals} + nhv = \text{biomass} \quad (a\text{C-mol}) + \text{O}_2 \]  

where 1 C-mol of biomass contains 1 mol carbon, hv is one quantum of light of frequency v, h is Planck’s constant and n is the quantum demand for evolution of 1 mol O₂. In this reaction the value of a is < 1. The photosynthetic efficiency (PE) of biomass growth is defined by energy stored in biomass/light energy absorbed. However, despite its fundamental importance, determination of the maximum possible value of this PE parameter has been rarely attempted. In general, the maximum possible PE has been estimated from the classical representation of photosynthesis as:

\[ \text{CO}_2 + \text{H}_2\text{O} + nhv = \frac{1}{6} \text{C}_6\text{H}_{12}\text{O}_6 + \text{O}_2 \]  

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Clearly reaction (2) is an oversimplification since it ignores nitrogen assimilation and the formation of protein, nucleic acids and lipids in addition to carbohydrate. The PE of reaction (1) would be given by energy of carbohydrate formed/light energy absorbed, however, reaction (1) is hypothetical and it is not possible to measure carbohydrate production. Instead the PE has been based on measurements of the light absorbed and the O₂ evolved in resting, that is, non-growing algal cells, and assuming stoichiometry between O₂ evolution and carbohydrate production given by reaction (2). Unfortunately, estimates of the PE by this resting-cell O₂ evolution have been highly variable giving values for the quantum demand n ranging from about 4–12, which has naturally lead to much controversy. Out of the range of values for n found by the measurement of O₂ evolution from resting cells, the value n=8 has been selected as the minimum value largely because it accords with the current model of photosynthesis where it is assumed that two photons are required for the transfer of one electron from water to the production of the reduced pyridine nucleotide, NADPH₂. The continuing uncertainty about the PE is shown by the recent proposal of Chain and Arnon that n = 12; a value based on measurement of the ATP produced by illumination of isolated spinach chloroplasts.

In retrospect, microbiologists can conclude that the use of resting cells to determine the bioenergetics of growing cells is not generally valid. PE is an expression of the growth yield from the energy source, light. Growth yield (Y) is defined by the biomass formed/light absorbed, and if k is the calorific value of the biomass we have PE = kY. The principles of bioenergetics show that the growth yield will be a function of the growth rate of the cells. From these principles the light requirement of a growing culture of cells is expected to conform with the relation:

\[ q = \frac{\mu}{Y_G} + m \]  

where q is the specific rate of light utilisation, µ is specific growth rate, Y_G is the maximum growth yield and m is the maintenance energy coefficient. Maximum growth yield is to be expected only when the energy source is growth-limiting. The actual growth yield (Y) should vary with the specific growth rate (µ) according to the relation:

\[ \frac{1}{Y} = \frac{1}{Y_G} + \frac{m}{\mu} \]  

It is now difficult to attach much meaning to the O₂ evolution rates in resting cell suspensions where µ = 0 and where cell degradation through turnover of cell components is stimulated. The maximum theoretical PE of a growing culture is given by k Y_G; the nearness of approach to this maximum value will depend on the values of m and µ. The value of Y will depend on the degree of reduction of the N source used. Generally nitrate has been used instead of ammonia for algal growth, this will decrease Y with respect to that found with NH₃ because of the need for extra reducing equivalents produced by light to reduce nitrate to NH₃; the reduction in PE would be of the order of 20% of its value. The maximum PE deduced from the classical representation of photosynthesis (reaction 2) is 29%. This assumes that n = 8 and the mean wavelength of the light is 575 nm so that 1 einstein = 208 kJ. Attempts to determine the maximum possible PE during growth have been almost totally neglected. Wassink et al. found PE = 24% for growth of the green alga Chlorella; for growth of the blue-green alga Anacystis nidulans the PE was 30%, for growth of the blue green alga Spirulina the PE was 12%. In all these attempts the N source was nitrate, hence a PE value about 20% higher should be possible with NH₃ instead of the nitrate; this would give a maximum PE of 36% for Anacystis nidulans. However, none of these workers took into account the maintenance energy although Aiba and Ogawa did consider the growth-rate effect. In two cases, there is no evidence that the workers aimed to achieve light-limited growth throughout the culture and the non-linearity of the q vs. µ plot [equation (3)] found by Aiba and Ogawa suggests that the culture was not light-limited over the whole growth rate range.

There is clearly a need for more rigorous determinations of the PE of biomass growth. Our aim
in this paper is to present such data. In order to obtain the data we have used chemostat culture to vary the specific growth rate of algal cultures and determined the maintenance energy effects.

2. Methods

2.1. Culture
Two types of culture were used. One type was a pure culture of Chlorella strain 211/8k (Sorokin strain) from the Culture Centre for Algae and Protozoa, Cambridge. The other type was a defined mixed culture designated MA003, which consisted of an algal species with three heterotrophic bacterial species.\textsuperscript{14} We selected this culture from a London pond by successive subculture in illuminated urea–CO\textsubscript{2}–salts medium at 37°C (310 K). The algal and bacterial species in MA003 were each isolated in pure culture and MA003 was obtained by reconstitution from the isolated pure cultures. The alga species in MA003 was designated A003; it was similar to Chlorella spp. in morphology and light absorption spectrum. The interactions between the algal and bacterial species in culture MA003 have been briefly reported.\textsuperscript{14}

The cultures were maintained on the urea–salts–agar medium A8 described previously.\textsuperscript{15}

2.2. Chemostat culture
Two types of continuous-flow chemostat culture apparatus were used. One type was similar to that used by Watts Pirt and Pirt.\textsuperscript{15} The culture vessel\textsuperscript{16} was scaled up to a nominal capacity of 200 cm\textsuperscript{3}. This vessel was illuminated and operated as described previously.\textsuperscript{15} In some experiments stated below the vessel and lamps were placed in a white Perspex light box to increase the light intensity at the surface of the culture.

We also used a miniloop culture vessel,\textsuperscript{13} which had a much greater surface:volume ratio than the stirred vessel and consequently permitted higher algal mass densities. The miniloop was operated as a chemostat as described previously.\textsuperscript{13}

The dilution rate $D$ (h\textsuperscript{-1}), is defined as culture flow rate/culture volume. In the steady state, the specific growth rate ($\mu$) = $D$.

2.3. Illumination
The stirred vessel was illuminated with one or two circular fluorescent lamps (Phillips, warm white, 32 W each). The miniloop cultures were illuminated with one or two fluorescent strip lamps (Phillips, warm white, 20 W each).

The light intensity at the culture vessel surface ($I_o$) was the average of the values at a number of points determined by means of a silicon photodiode (United Detector Technology, Santa Monica, California). The calibration of the photodiode, for the light sources used, was verified with a thermophile (Kipp solarimeter). The photodiode measured all the light in the spectral range 400–700 nm, that is, the range available for photosynthesis. It was verified by means of light filters that practically all (97\%) of the light measured was in the spectral range 400–750 nm. In the calculation of the photosynthetic efficiency in the light-limited cultures it was assumed that all the incident light as measured by the photodiode was utilised by the culture. In the stirred vessel the surface area ($a$) illuminated was taken to be the top and side surfaces and the ratio of $a: V$ where $V$= culture volume was 0.61 cm\textsuperscript{-1}. The same photosynthetic efficiencies were obtained in two vessels of very different geometries and we assume the geometric effect was nil. Since the miniloop was illuminated only on one side, the area illuminated was taken to be half the total surface area and the value of $a: V$ was 6.1 cm\textsuperscript{-1}.

2.4. Culture media and culture conditions
For the stirred vessel the urea–inorganic salts medium described by Watts Pirt and Pirt\textsuperscript{15} was used, except that the urea concentration was increased to 0.60 g litre\textsuperscript{-1} and the NaVO\textsubscript{3} concentration was $0.05 \times 10^{-8}$ g litre\textsuperscript{-1}. The culture pH value was maintained at 6.5–6.7 and the temperature
was 37°C (310 K). The gas supply was 5% CO₂ in air, supplied at 40 cm³ min⁻¹. The culture evaporation was negligible (<1%).

For the miniloop vessel the culture medium constituents were at four-fold the strength of the medium of Watts Pirt and Pirt¹⁵ except that the urea was at 5 g litre⁻¹ and the NaVO₃ at 0.2 × 10⁻³ g litre⁻¹. The gas supply was humidified 10% CO₂ in air at a flow rate of 150 cm³ min⁻¹. Otherwise the conditions were the same as those for the stirred vessel. The heating lamp required for the temperature control was covered with aluminium foil so that it did not contribute to the illumination.

2.5. Analytical methods

The total biomass was determined by dry weight measurements. The biomass was dried at 105°C (378 K). The bacterial biomass in the mixed culture MA003 was determined from viable counts of the bacterial population on nutrient agar plates. The nutrient agar consisted of PI medium¹⁶ solidified with agar (2% w/v). These plates were incubated at 37°C in the dark. The bacterial dry weight was related to the viable count by determinations on pure cultures of each of the three bacterial species grown in medium PI of Pirt¹⁸ with glucose (10 g litre⁻¹) as the C substrate. The conversion factor was 10⁹ bacteria = 1.14 mg dry weight.

The C, H, N and ash contents of the dry biomass of culture MA003 were determined in the micro-analysis laboratory of Queen Elizabeth College. The oxygen content of the ash-free biomass was determined by the difference after subtraction of the C, H and N contents. The calorific value of the MA003 dry biomass was determined by the method of Miller and Payne.¹⁰ Since the nitrogen source was urea, the calorific value was corrected for the combustion of the NH₂ content of the biomass by subtraction of 24.7 J mg⁻¹ N.¹⁷
3. Results

Light-limited chemostat cultures of both *Chlorella* 211/8k and the mixed culture MA003 were set up. Steady-state conditions were obtained with different dilution rates. By means of the critical dilution rate method it was found that the maximum specific growth rate ($\mu_m$) of *Chlorella* 211/8k was 0.22 h$^{-1}$ and of MA003 was 0.14 h$^{-1}$. Wall growth was negligible for the duration of each culture (1–2 months). The raw data obtained with both stirred vessel and miniloop cultures are shown in Table 1.

### Table 1. Raw data to determine the maximum photosynthetic efficiency of light-limited algal growth

<table>
<thead>
<tr>
<th>Dilution rate $D$ (h$^{-1}$)</th>
<th>Total dry biomass (g litre$^{-1}$)</th>
<th>Bacterial dry weight (g litre$^{-1}$)</th>
<th>Incident light intensity $I_a$ (W m$^{-2}$)</th>
<th>$I_a/v^a$ (kJ litre$^{-1}$ h$^{-1}$)</th>
<th>Growth yield $Y$ [g dry weight (kJ)$^{-1}$]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chlorella 211/8k in stirred vessel in light box</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0300</td>
<td>1.180</td>
<td>—</td>
<td>11.9</td>
<td>2.91$^b$</td>
<td>0.01216</td>
</tr>
<tr>
<td>0.0720</td>
<td>0.550</td>
<td>—</td>
<td>11.9</td>
<td>2.91</td>
<td>0.01360</td>
</tr>
<tr>
<td>0.0950</td>
<td>0.450</td>
<td>—</td>
<td>11.9</td>
<td>2.91</td>
<td>0.01469</td>
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<tr>
<td>0.1020</td>
<td>0.420</td>
<td>—</td>
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<td>2.91</td>
<td>0.01472</td>
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<tr>
<td>0.1600</td>
<td>0.240</td>
<td>—</td>
<td>11.9</td>
<td>2.91</td>
<td>0.01319</td>
</tr>
<tr>
<td>0.0392</td>
<td>1.650</td>
<td>—</td>
<td>11.9</td>
<td>3.24$^e$</td>
<td>0.02003</td>
</tr>
<tr>
<td>0.0934</td>
<td>0.618</td>
<td>—</td>
<td>11.9</td>
<td>3.24</td>
<td>0.01781</td>
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<tr>
<td>0.1596</td>
<td>0.352</td>
<td>—</td>
<td>11.9</td>
<td>3.24</td>
<td>0.01734</td>
</tr>
<tr>
<td>0.0300</td>
<td>2.720</td>
<td>—</td>
<td>21.1</td>
<td>5.15$^b$</td>
<td>0.01584</td>
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<tr>
<td>0.0720</td>
<td>1.140</td>
<td>—</td>
<td>21.1</td>
<td>5.15</td>
<td>0.01593</td>
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<tr>
<td>0.1600</td>
<td>0.510</td>
<td>—</td>
<td>21.1</td>
<td>5.15</td>
<td>0.01584</td>
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<tr>
<td>0.0402</td>
<td>2.592</td>
<td>—</td>
<td>21.1</td>
<td>5.74$^e$</td>
<td>0.01816</td>
</tr>
<tr>
<td>0.0934</td>
<td>1.106</td>
<td>—</td>
<td>21.1</td>
<td>5.74</td>
<td>0.01834</td>
</tr>
<tr>
<td>0.1508</td>
<td>0.619</td>
<td>—</td>
<td>21.1</td>
<td>5.74</td>
<td>0.01627</td>
</tr>
<tr>
<td>0.2249</td>
<td>0.423</td>
<td>—</td>
<td>21.1</td>
<td>5.74</td>
<td>0.01657</td>
</tr>
<tr>
<td><strong>Mixed culture MA003 in stirred vessel without light box</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0325</td>
<td>0.669</td>
<td>0.141</td>
<td>5.3</td>
<td>1.14</td>
<td>0.01907</td>
</tr>
<tr>
<td>0.0550</td>
<td>0.379</td>
<td>0.043</td>
<td>5.3</td>
<td>1.14</td>
<td>0.01828</td>
</tr>
<tr>
<td>0.0704</td>
<td>0.309</td>
<td>0.039</td>
<td>5.3</td>
<td>1.14</td>
<td>0.01908</td>
</tr>
<tr>
<td>0.0288</td>
<td>2.235</td>
<td>0.575</td>
<td>9.5</td>
<td>2.72</td>
<td>0.02366</td>
</tr>
<tr>
<td>0.0570</td>
<td>1.037</td>
<td>0.173</td>
<td>9.5</td>
<td>2.72</td>
<td>0.02173</td>
</tr>
<tr>
<td>0.0802</td>
<td>0.810</td>
<td>0.069</td>
<td>5.5</td>
<td>2.72</td>
<td>0.02388</td>
</tr>
<tr>
<td>0.1100</td>
<td>0.504</td>
<td>0.042</td>
<td>9.5</td>
<td>2.72</td>
<td>0.02038</td>
</tr>
<tr>
<td><strong>Mixed culture MA003 in miniloop</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0235</td>
<td>28.278</td>
<td>6.086</td>
<td>15.5</td>
<td>33.87</td>
<td>0.01963</td>
</tr>
<tr>
<td>0.0631</td>
<td>12.411</td>
<td>3.046</td>
<td>15.5</td>
<td>33.87</td>
<td>0.02311</td>
</tr>
<tr>
<td>0.0265</td>
<td>36.770</td>
<td>10.204</td>
<td>21.3</td>
<td>46.57</td>
<td>0.02092</td>
</tr>
<tr>
<td>0.0467</td>
<td>19.460</td>
<td>4.482</td>
<td>21.3</td>
<td>46.57</td>
<td>0.01951</td>
</tr>
<tr>
<td>0.0671</td>
<td>14.140</td>
<td>2.109</td>
<td>21.3</td>
<td>46.57</td>
<td>0.02037</td>
</tr>
<tr>
<td>0.0849</td>
<td>11.020</td>
<td>0.912</td>
<td>21.3</td>
<td>46.57</td>
<td>0.02009</td>
</tr>
<tr>
<td>0.1038</td>
<td>9.530</td>
<td>1.024</td>
<td>21.3</td>
<td>46.57</td>
<td>0.02124</td>
</tr>
</tbody>
</table>

$^a$ a/v = ratio of illuminated area (a) to culture volume, v.
$^b$ In stirred vessel A.
$^c$ In stirred vessel B.
Plots (Figure 1) of biomass output rate ($Dx$, where $x =$ biomass concentration) during steady-states vs. $D$ show that, for a given light intensity, $Dx$ was virtually constant. This is the result to be expected if the photosynthetic efficiency is constant and the amount of unabsorbed light is negligible as we have assumed. Figure 2 shows that the mean biomass output rate increased linearly with incident light energy litre$^{-1}$ and the line seemed to pass through the origin, which again indicates that the amount of light unabsorbed at all light intensities was immeasurably small. Also the photosynthetic efficiency was independent of both the amount of light energy and the intensity of the light incident on the culture surface.

![Graph showing biomass output rate vs. incident light energy](image)

**Figure 2.** Steady-state biomass output rate as a function of incident light energy per unit volume ($I_a \, a/V$) in chemostat culture: (a), *Chlorella 211/8k*; (b), mixed culture MA003.

The plots of specific light absorption rate ($q$) vs. specific growth rate ($\mu$) in steady states are given in Figure 3. In each case, for the axenic culture of *Chlorella 211/8k* and for the mixed culture MA003 there was a linear relation between $q$ and $\mu$. Figure 3(b), which shows the graph of $q$ vs. $\mu$ for the algal component of culture MA003, was obtained by subtracting the bacterial dry weight from the total dry weight. Again a linear relation was obtained with the same slope as that for the total biomass. It follows from equation (3) that the slope of the graph of $q$ vs. $\mu$ is $1/Y_0$. The intercept on the ordinate gives the value of the maintenance energy coefficient ($m$). The value of $m$ (see Table 2) did not differ significantly from zero, either for the *Chlorella 211/8k* strain or for the total biomass in the mixed culture MA003. However, for the algal strain alone, in the mixed culture there was a significant intercept on the ordinate. To account for the maintenance energy term of the algal component of the culture MA003 we postulate that there is an algal product,
Figure 3. Specific light absorption rate \( q = \frac{I_{sa}}{V_x} \) as a function of specific growth rate \( \mu \) in steady states of chemostat cultures. (a), *Chlorella* 211/8k in the stirred vessel; (b), mixed culture MA003: ○, △, △ (line A) for total biomass (algal + bacterial); ○, △, △, △ (line B) for algal biomass only (total biomass-bacterial mass).

Produced at a constant rate, which acts as the energy source for the growth of the heterotrophic bacteria in MA003. The nature of this algal product is unknown.

The value of the maximum possible growth yield \( (Y_\mu) \) for each culture is shown in Table 2. The product \( k Y_\mu \), where \( k \) is the calorific value of the biomass gave the maximum photosynthetic efficiency. The calorific value given in Table 2 was obtained only for the mixed culture MA003 and the value for the *Chlorella* 211/8k biomass was assumed to be the same.

<table>
<thead>
<tr>
<th>Algal species</th>
<th>( (Y_\mu) ) g(^{-1}) dry weight (kJ)</th>
<th>Maximum photosynthetic efficiency (( % )) (100 ( k Y_\mu ))(^a)</th>
<th>Maintenance coefficient (( m ) ( \times 10^{-3} ) kJ g(^{-1}) dry weight h(^{-1} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Chlorella</em> 211/8k</td>
<td>0.0153</td>
<td>34.7</td>
<td>20</td>
</tr>
<tr>
<td>MA003 (total biomass)</td>
<td>0.0206</td>
<td>46.8(^c)</td>
<td>30(^d)</td>
</tr>
<tr>
<td>MA003 (alga only)</td>
<td>0.0206</td>
<td>46.8</td>
<td>418</td>
</tr>
</tbody>
</table>

\( a \) \( k \)-calorific value of MA003 total biomass = 22.7 kJ g\(^{-1}\) dry weight determined by the method of Miller and Payne.\(^14\) Composition of MA003 total biomass: C = 48.6\%, H = 7.3\%, N = 8.1\%, ash = 8.0\% 1 C-mol biomass (MA003) = 25.6 g dry weight. The formula of the ash-free biomass is estimated to be \( \text{CH}_{1.80} \text{N}_{0.143} \text{O}_{0.428} \).

\( b \) 95\% confidence limits, 0.0188–0.0227.

\( c \) 95\% confidence limits, 42.7–51.5.

\( d \) 95\% confidence limits, 0–320.
4. Discussion

The overall process of photosynthetic biomass production for the mixed culture MA003 calculated from our data (Table 2) is found to be

$$0.81 \text{CO}_2 + 0.73 \text{H}_2\text{O} + 0.12 \text{NH}_3 + \text{minerals} + 4.8 \text{hv} \rightarrow \text{biomass} (0.81 \text{C-mol}) + \text{O}_2 \quad (5)$$

This balance was calculated from the biomass composition by assuming that the biomass was the sole carbon product and taking the mean value of 1 einstein to be 208 kJ ($\lambda=575 \text{ nm}$). In the case of *Chlorella 211/8k* the minimum quantum demand, $n=6.6$, compared with 4.8 for culture MA003. The ATP required for biomass synthesis ($Y_{ATP}$) from $\text{CO}_2 + \text{H}_2\text{O} + \text{NH}_3$ and mineral salts has been calculated to be 1 mol 6.5 g$^{-1}$ dry biomass. From this value of the $Y_{ATP}$, the number of ATP generated in photosynthesis can be estimated from the biomass yield. Thus the number of ATP required in reaction (5) is found to be 3.2.

The energy requirements for growth and maintenance of algae have been the subject of two recent theses. These authors, in contrast to ourselves, found that the plots of $q$ vs. $\mu$ were not linear. One possible reason for the non-linearity of the $q-\mu$ function is that light-limited growth was not maintained at the higher specific growth rates. The cultures of Gons and Liere could have been either Fe or $\text{CO}_2$-limited at the higher specific growth rates because of insufficient EDTA in the medium to prevent precipitation of Fe and by use of air alone as the $\text{CO}_2$ source. Also Liere found that the growth yield from light varied inversely with incident light intensity down to the lowest value, 0.5 W m$^{-2}$, whereas we found that the growth yield in light-limited cultures was independent of the incident light intensity up to at least 21 W m$^{-2}$. Another of our findings was that the biomass output rate in light-limited cultures was at the maximum over a wide range of specific growth rates and it did not show a maximum when $\mu \approx 0.5 \mu_m$ as other workers have found. Again this inconsistency may be because previous workers did not maintain light-limited growth over the whole growth-rate range. In order to compare the energy required for maintenance with that required for growth it is convenient to take the specific maintenance rate $m_{Y_G}$ (h$^{-1}$). For culture MA003 the value of $m_{Y_G}$ was in the range 0.0066 h$^{-1}$ (95% confidence limits) which is compatible with the value Gons found for *Scenedesmus* (i.e. about 0.007); Liere found $m_{Y_G}$ for *Oscillatoria* to be much smaller (i.e. 0.001 h$^{-1}$).

A mixed culture MA003 was by far the most efficient converter of light energy into biomass energy. The bacterial components of the culture appear to act as scavengers of products excreted by the algal component. This scavenging process could remove any niche for heterotrophic bacterial contaminants and thus make the cultures stable and resistant to bacterial contamination. Possibly also prokaryote contamination was restricted because $\text{Co}^{2+}$ ion was not added to the medium.

Our data on the growth of both culture MA003 and *Chlorella 211/8k* and probably the data of Goedheer and Hammans require the value of the quantum demand ($n$) to be <8, which is not consistent with the current model of photosynthesis. The value of $n$ found for culture MA003 is 4.8 and for *Chlorella 211/8k* is 6.6.

The current model requires that a minimum of two photons are required for the transport of each electron from water to the NADP level of redox potential ($E_h$). It follows from the photosynthetic efficiency of the MA003 culture that some electrons can be transported up the $E_h$ gradient to NADP with a single photon exciting each electron. The energy required per electron would be 1.24 eV whereas each photon with $\lambda=700 \text{ nm}$ has an energy of 1.8 eV. Such a high efficiency of energy transfer from a photon to a chlorophyll-like molecule is supported by recent observations. A possible modification of the photosynthesis model to account for the results is given in Figure 4. For the modified model it is assumed that the link between photosystems 1 and 2 provides a common electron transport pathway which permits electrons excited by light quanta to flow up the potential gradient; reverse flow down the gradient drives ATP production. Thus the overall quantum demand ($n$) could be the sum of three different demands (1) for the direct formation of NADPH$_2$ (one photon per electron), (2) for the conventional two-step route to NADPH$_2$ involving some reverse electron flow to produce ATP (two photons per electron) and (3) for cyclic phosphorylation (one
Photosynthetic efficiency of biomass growth

\[ 2\text{NADP} + 4\text{H}^+ + 4\text{e}^- \rightarrow 2\text{NADPH}_2 \]

\[ \text{ADP} \rightarrow \text{ATP} \]

\[ 2\text{H}_2\text{O} \rightarrow \text{O}_2 + 4\text{H}^+ + 4\text{e}^- \]

Figure 4. Revised model of electron transport pathways in green algal photosynthesis to account for quantum demands < 8 for evolution of 1 mol O₂. PSI and PS2 are photosystems 1 and 2, respectively. Route a–b–c is supposed to require 1 hv (via PS2); route a–b–d–b–c is supposed to require 2 hv (via PS2 and PSI); route b–c–e–d results in cyclic phosphorylation and requires 1 hv via PSI.

The minimum value of \( n \) to permit ATP generation for growth would be 5–6, for instance, three photons for route (1) plus two for route (2) \((n = 5)\) or three photons for route (1) plus two for route (2) and one for route (3) \((n = 6)\). The range of values we have found for \( n \) (4.8 for culture MA003 to 6.6 for Chlorella 211/8k) are, within the margin of error, consistent with a minimum \( n \) of 5–6 for growing cultures. It should be noted that in a non-growing cell suspension in which ATP is not required a value of \( n \) as low as 4 is possible for O₂ evolution, according to our model.

4.1. Maximum photobiological utilisation of solar energy

The maximum percentage conversion of solar energy to biomass free energy is obtained from the formula \([\text{PE} \times (\% ) \times (\text{PAR}) \times (\text{MC})]\) where PE is the fraction of the light absorbed which is stored in the biomass, PAR = 0.43 is the fraction of total solar energy which is available for photosynthesis (in the spectral range 400–700 nm), and MC is the fraction of energy available after correction for the maintenance energy or photorespiration. The reflectance is considered negligible if the culture system were an in vitro algal culture, in thin panels oriented perpendicular to the sun's rays. We found that the loss of energy due to maintenance effects in culture MA003 became significant...
when the culture was subjected to alternating light and dark periods of 12 h each. With such a night and day effect it was estimated that MC = 0.9 with $\mu = 0.06$ h$^{-1}$ in the daytime. Hence, the maximum possible conversion of total solar energy to biomass free energy with culture MA003 is estimated to be $46.8 \times 0.43 \times 0.9 = 18.1 \%$. This value is about three times the maximum previously predicted for conventional agriculture.$^{20,21}$ In comparison the photosynthetic efficiency of the USA maize crop is given as $1.26 \%$. An efficiency of $18.1 \%$ would correspond to a dry biomass output of 238 metric tons ha$^{-1}$ year$^{-1}$ in the UK where the mean insolation$^{27}$ is $8.9 \times 10^8$ Jm$^{-2}$ d$^{-1}$. Twice this output would be possible in the sunniest parts of the world. Occasionally it has been stated that algal cultures cannot fully utilise solar radiation at its highest intensity. This is probably a fallacy engendered by inadvertent limitation of growth by some factor other than the light intensity. The high photosynthetic efficiency of in vitro algal culture means that it should be the most efficient means for the photobiological utilisation of solar energy and comparable with photovoltaic cells and solar thermal energy for efficiency. This opens up the possibility of photobiological storage of solar energy by precision-engineered algal culture operated on any open space over land or sea.

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