ELSEVIER

Evaluation of photosynthetic efficiency in microalgal cultures using averaged irradiance

E. Molina Grima, F. García Camacho, J. A. Sánchez Pérez, F. G. Acién Fernández, and J. M. Fernández Sevilla

Departamento de Ingeniería Química, Facultad de Ciencias Experimentales, Universidad de Almería, E–04071, Almeria, Spain

The growth yield of the PUFA-producing marine microalga Isochrysis galbana *grown in light-limited continuous operation was measured under a wide variety of conditions of incident irradiance (I_o) and dilution rates (D). The experiments were conducted under laboratory conditions at 20°C with continuous light.* D *ranged from 0.0024–0.0410 h^{-1} at three values of I_o (820; 1,620; and 3,270 $\mu E\ m^{-2}\ s^{-1}$) close to those found in outdoor cultures. A maximum efficiency of $\Psi_{max} = 0.616\ g\ E^{-1}$ was obtained at $I_o = 820\ \mu E\ m^{-2}\ s^{-1}$ and D = 0.030 h^{-1}, and the maximum capacity of the biomass to metabolize light was found to be 13.1 $\mu E\ g^{-1}\ s^{-1}$. Above this value, a significant drop in system efficiency was observed. A new approach based on averaged irradiance is used to assess the photon flux absorbed by the biomass. The biomass productivity to be expected in an outdoor tubular photobioreactor growing* I. galbana *was predicted from efficiency data obtained in laboratory cultures and was found to be in agreement with the actual value.* © *1997 Elsevier Science Inc.*

Keywords: Quantum yield; average irradiance; light absorption; photobioreactor; photosynthetic efficiency

Introduction

Nowadays, microalga mass production is a commercially attractive source of high value chemicals and biomass, but for the most part has not been economically successful. In view of the highly efficient basic process of photosynthesis as measured by O_2 generation as a function of the quantum flux absorbed,[1] a system designer might expect to attain yields close to 2×10^{-2} g kJ^{-1}.[2] According to the results reported by some authors,[3–5] some fast-growing strains such as *Chlorella* can in fact yield 20% of the PAR available; nevertheless, the biomass obtained from these strains is of little interest since it contains none of the products of current interest, namely PUFAs, pigments, and antioxidants on which microalgal system designers now focus their attention. For the production of fine chemicals usually synthesized by more sensitive strains, however, growth yield data in the literature is, even in the best case, rather lower than the theoretical maximum.

In an attempt to find a technically reliable quantum yield coefficient, several workers have measured the biomass generation rate instead of oxygen generation. The coefficient thus obtained could well be expected to provide a good estimate of productivities as a function of the incident irradiance and, thereby, for photobioreactor design; nevertheless, the yields obtained in laboratory experiments are still barely attained in commercial photobioreactors. This is attributable to the difference between commercial operating conditions and laboratory experiments which are usually conducted with diluted cultures under low light so that optical homogeneity and photolimiting conditions can be assumed. In this way, the conditions are identical for all cells and the growth yield measured can be considered a property of the microorganism which is independent of the culture system; however, optical homogeneity is attained at the expense of a great loss (usually higher than 80%)[6] of light supplied at solar irradiance intensities; thus, such data are of little technical interest, since the conditions under which they were obtained differ widely from mass production systems where light gradients are established. There is therefore a need for a laboratory growth yield coefficient in dense cultures at high irradiances that could be useful for the design of outdoor systems for the cultivation of specific strains.

In the present work, a new procedure for calculating

Address reprint requests to Dr. J. M. F. Sevilla, University of Almeria, Depto. de Ingenieria Quimica, E–04071, Almeria, Spain
Received 16 May 1996; revised 4 February 1997; accepted 4 March 1997

655 Avenue of the Americas, New York, NY 10010

0141-0229/97/$17.00
PII S0141-0229(97)00012-4

growth yields in microalga mass cultures at high incident irradiances is proposed based on laboratory continuous cultures. Additional results obtained in an outdoor closed tubular photobioreactor allows comparison to see to what extent laboratory data can be used for the analysis of external systems. The availability of a reliable quantum yield coefficient for outdoor conditions, together with the maximum quantum capacity would supply a basis for rational photobioreactor design, as well as parameters for the evaluation of the efficiency of a system in harvesting energy and converting it into biomass.

Materials and methods

Organism

The microalga used was an isolate labeled ALII–4 (CCAP n° 927/15 Oban, Scotland) selected from among 42 of a single strain of *I. galbana* in a phenotypic selection program performed in our laboratory to select an EPA-rich strain.[7]

Growth conditions

Cultures were performed in a cylindrical (di = 0.17 m) 5-l computer-controlled fermentor (New Brunswick Scientific Bioflo III). The culture vessel and head plate were sterilized by autoclaving at 120°C for 60 min. The culture medium and sterilization processes are described by Molina Grima *et al.*[8]

To obtain high irradiances while maintaining perfect temperature control, our group designed an illuminating device composed of 16 Osram Dulux EL (20 W) fluorescent lamps with a cylindrical reflector for high irradiance arranged around the culture vessel. Incident irradiance on the culture surface and center of the vessel was measured with a Biospherical Instruments Laboratory Quantum Scalar Irradiance Meter QSL–100 (San Diego, CA). The different incident irradiances, I_o, tested were achieved by varying the number of lamps on over the experiment.

Dilution rate was fixed using a programmable peristaltic pump. The temperature was set at 20°C. The air supply was sterilized by filtration through 0.22 μm Millipore filters at a rate of 1.5 l min^{-1} with agitation at 150 rpm. Nutrient saturation was previously checked by supplying successively increasing nutrient concentrations of the culture medium during continuous growth and observing no significant changes in the steady-state biomass concentration when the nutrient concentration described by Molina *et al.*[8] was doubled. pH was maintained constant at 8 by injecting pure CO_2 with the air supply.

Analytical methods

Biomass concentration was followed by measuring optical density of the culture at 530 nm[8] to monitor the occurrence of a steady state and then checked by dry weight. Chlorophylls were measured according to the method of Hansmann.[9] Carotenoid determination was as described by Whyte.[10]

Background

Quantum yield, Ψ_E, is defined in microalgal cultures as the amount of biomass generated by the unit of radiation (usually photon mole or Einstein) absorbed by the culture. Since it represents the ratio of biomass generation to absorbed photon flux, it can be calculated by the expression:

$$\Psi_E = \frac{P_b}{F_{vol}} \qquad (1)$$

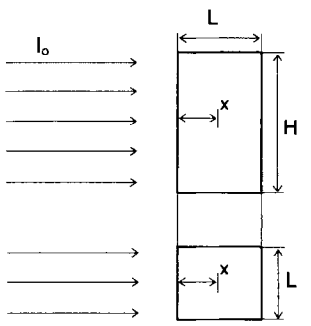

Figure 1 Schematic drawing of unidirectional collimated light flux in a parallelepipedal vessel

where P_b stands for the volumetric biomass productivity and F_{vol} for the photon flux absorbed in the volume unit. This coefficient can be converted to energy units, commonly noted Ψ_{kJ}, by taking into account the average energy of the light used (kJ E^{-1}).

The bioenergetic yield, Ψ, quantifies the percent of light energy that is converted to chemical energy[11] and can be calculated as the product of Ψ_{kJ} by the biomass combustion heat, Q_b.

$$\Psi = \Psi_{KJ} \cdot Q_h \qquad (2)$$

Since P_b in a steady-state continuous culture can be calculated as the product of biomass concentration, C_b, and dilution rate, D, any of the growth yields defined by Eqs. (1) and (2) can be readily evaluated as long as the absorbed photon flux can be found.

Absorbed photon flux

Assessment of the photon flux absorbed is a question of major importance to be tackled prior to photosynthetic efficiency. This is a complicated question, especially when it must be evaluated in geometries other than flat parallelepipedal reactors. Many factors such as light scattering effects may lead to a misevaluation of the absorbed flux and in every case they need to be evaluated by direct measurement of the light leaving the reactor vessel.[15]

In this sense, it must be pointed out that the light absorbed can be evaluated by integrating the local volumetric rate of energy absorption (LVREA)[16] into the total reactor volume. This integral can be readily obtained, as shown later on, from the extinction coefficient of biomass, K_a, and the average irradiance, I_{av}. This latter parameter can be defined for any given geometry by:

$$I_{av} = \frac{\sum_{i=1}^{n} V_i \cdot I_{Pi}}{\sum_{i=1}^{n} V_i} = \frac{\sum_{i=1}^{n} V_i \cdot I_{Pi}}{V_T} \qquad (3)$$

where V_i represents a volume element in which the local irradiance, I_{pi}, can be considered constant and V_T stands for the total reactor volume.

In the simple case of a parallelepipedal vessel evenly illuminated by a collimated beam, (*Figure 1*), the average irradiance defined in Eq. (3) can be obtained as an analytical function.

Since the attenuation of any given light ray traveling through the medium can be calculated by Lambert-Beer's law, the irradiance in any inner point separated distance x from the wall is obtained as

$$I_p(x) = I_o \cdot EXP(-x \cdot C_b \cdot K_a) \qquad (4)$$

Thus, the average irradiance corresponding to the whole vessel volume is

$$I_{av} = \frac{\int_V I_p(x) \cdot dV}{V} = \frac{\int_0^L I_o \cdot EXP(-K_a C_b x) \cdot H \cdot L \cdot dx}{H \cdot L^2}$$

$$= \frac{I_o}{L \cdot K_a \cdot C_b} \cdot (1 - EXP[-L \cdot K_a \cdot C_b]) \quad (5)$$

The same equation can also be worked out from the following photon flux balance:

$$\begin{pmatrix}\text{Incoming}\\ \text{flux}\end{pmatrix} = \begin{pmatrix}\text{Absorbed}\\ \text{flux}\end{pmatrix} + \begin{pmatrix}\text{Outgoing}\\ \text{flux}\end{pmatrix} \quad (6)$$

If the total photon flux absorbed is noted as F_T, and the surface crossed by light rays S and L denotes the vessel optical path, then the incoming flux is given by $I_o \cdot S$, the outgoing by $S \cdot I_o \cdot Exp[-K_a C_b L]$ and, thus, F_T can be obtained as the following difference:

$$F_T = S \cdot I_o \cdot (1 - Exp[-K_a C_b L]) \quad (7)$$

The volumetric photon flux absorbed, F_{vol}, can be readily obtained dividing Eq. (7) by the vessel volume ($V = L \cdot S$):

$$F_{vol} = \frac{F_T}{V} = \frac{I_o}{L} \cdot (1 - Exp[-K_a C_b L]) \quad (8)$$

The comparison of Eqs. (5) and (8) helps envision the connection existing between I_{av}, the optical properties of the medium, K_a, and the absorbed photon flux:

$$\frac{F_{vol}}{I_{av}} = \frac{\frac{I_o}{L} \cdot (1 - EXP[-L \cdot K_a \cdot C_b])}{\frac{I_o}{L \cdot K_a \cdot C_b} \cdot (1 - EXP[-L \cdot K_a \cdot C_b])} = K_a \cdot C_b \quad (9)$$

Thus, photon flux absorbed throughout the reactor volume may be obtained from I_{av}, both on a biomass unit basis or on a culture volume basis using the following equations:

$$F_{ab} = I_{av} \cdot K_a \quad (10)$$

$$F_{vol} = I_{av} \cdot K_a \cdot C_b \quad (11)$$

The calculation of the photon flux absorbed by this procedure has several advantages. It is independent of the system geometry once I_{av} is known, and can thus be used in any type of reactor as long as I_{av} can be determinate by any means. Photon flux losses due to the reflection of light off the reactor walls that cannot be neglected[15] are easily obviated by measuring the irradiance inside the culture, thus only the radiation that penetrates the vessel is taken into account for the determination of I_{av}.

Basically, Eq. (10) agrees with the expression obtained by Cornet *et al.*[14] for the volumetric rate of energy absorbed using the integro-differential equation of radiative transfer in its integrated form. This assures that Eqs. (10) and (11) are valid for any geometry and whether scattering takes place or not as long as only the light absorption coefficient (Ea in Cornet's nomenclature) is used.

On the other hand, the validity of Eqs. (10) and (11) for the cylindrical geometry can easily be proven by considering it as a collection of parallelepipeds of a differential thickness as shown in *Figure 2*.

The average irradiance in any volume element can then be obtained by Eq. (5) since each parallelepiped has a different light path given by p as defined in *Figure 2;* thus, using the definition of I_{av} given in Eq. (3), this can be calculated remembering that the differential volume corresponding to each element is $dV = H\ p\ dx$. This can be integrated into:

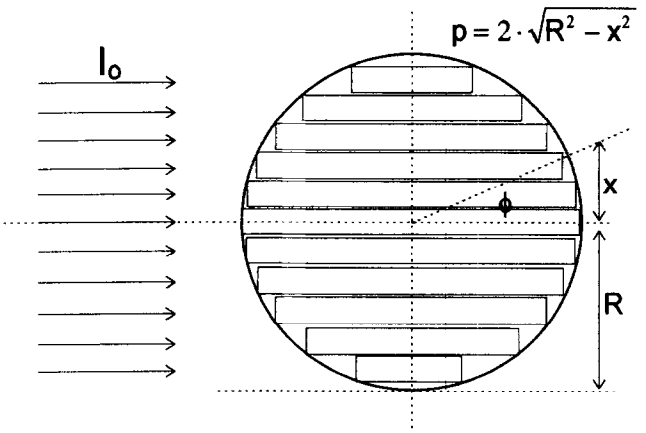

Figure 2 Calculation of the average irradiance in a cylindrical system illuminated by unidirectional parallel flux considering the cylinder as a collection of differential parallelepipeds

$$I_{av} = \frac{2 \cdot I_o}{R \cdot K_a \cdot C_b \cdot \pi} \cdot \left(1 - \int_0^{\pi/2} Cos\ (\phi) \cdot EXP\ [-2 \cdot R \cdot K_a \cdot C_b \cdot Cos\ (\phi)] \cdot d\phi\right) \quad (12)$$

which is also valid in the case of an evenly illuminated cylinder since this flux can be considered to be made up of n different beams arriving from different directions as shown in *Figure 3.*

If the radiation arrives uniformly from all directions, the average irradiance every component produces is given by Eq. (5) resulting in I_{av}/n. Since the irradiance is an additive property [Eq. (3)], the total irradiance is $n \cdot I_{av}/n = I_{av}$; thus, Eq. (12) is also valid for any case of plane-parallel flux which is corroborated by the total agreement in the results that are obtained with Eq. (12) and by integrating the model for irradiance distribution in an evenly illuminated cylindrical vessel given by Evers[17] as proposed by Molina Grima *et al.*[18] which is:

$$I_{av}(C_b, K_a) = \frac{I_o}{R^2 \pi} \int_0^R \int_0^{\pi} Exp\ \{-K_a \cdot C_b \cdot [r \cdot Cos\ (\phi) + \sqrt{R^2 - r^2\ Sin^2\ (\phi)}]\} r dr \cdot d\phi \quad (13)$$

where R represents the vessel radius and r and ϕ are the integration variables. The coincidence of the two equations means that the same average irradiance is caused by a given incident photon flux regardless of the direction of distribution it comes from.

Once a method for assessing I_{av} from the biomass concentration, C_b, extinction coefficient, K_a and incident irradiance, I_o, is set up, the photon flux for any situation can be easily calculated without direct measurement of the irradiance.

Results

Table 1 shows the results obtained in a nutrient-saturated continuous culture at each dilution rate and incident irradiance. Every pair of operating conditions (D, I_o) gave rise to a steady state, the occurrence of which was determined by monitoring the biomass concentration until it was found to be constant for at least 4 days. K_a was then determined as a

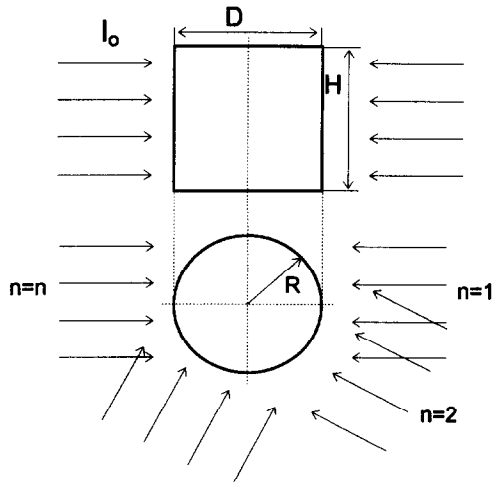

Figure 3 Hypothetical schematic drawing of a cylindrical system with diffuse flux by *n* beams

function of total pigment as described by Molina Grima *et al.*[18] The average irradiance was calculated using Eq. (12) as a basis for the assessment of the absorbed photon flux.

Table 1 Biomass generation results and the corresponding bioenergetic parameters calculated for the steady-state conditions obtained in each experiment

I_o ($\mu E\ m^{-2}\ s^{-1}$)	D (h^{-1})	P_b ($g\ l^{-1}\ h^{-1}$)	F_{ab} ($\mu E\ g^{-1}\ s^{-1}$)	Ψ (%)
820	0.0025	0.0064	6.33	3.25
820	0.0049	0.0083	6.76	4.22
820	0.0121	0.0128	8.00	6.53
820	0.0181	0.0152	9.18	7.85
820	0.0181	0.0150	8.76	8.22
820	0.0211	0.0154	10.08	8.13
820	0.0231	0.0154	10.65	8.32
820	0.0241	0.0166	12.05	7.62
820	0.0291	0.0148	13.18	8.22
820	0.0381	0.0085	21.61	6.39
1620	0.0059	0.0205	6.62	4.81
1620	0.0124	0.0231	10.29	5.17
1620	0.0192	0.0251	11.59	6.53
1620	0.0237	0.0242	16.44	5.51
1620	0.0239	0.0246	16.09	5.67
1620	0.0241	0.0246	16.26	5.65
1620	0.0245	0.0244	16.69	5.58
1620	0.0307	0.0250	20.01	5.68
1620	0.0356	0.0205	29.30	4.43
1620	0.0406	0.0082	57.10	2.56
3270	0.0059	0.0183	16.52	1.93
3270	0.0121	0.0214	24.78	2.11
3270	0.0182	0.0192	40.83	1.77
3270	0.0231	0.0165	56.95	1.55
3270	0.0239	0.0156	65.51	1.39
3270	0.0235	0.0169	56.87	1.58
3270	0.0242	0.0165	62.41	1.48
3270	0.0306	0.0050	98.34	1.15
2050	0.0235	0.0196	12.31	2.07
2440	0.0234	0.0178	30.62	2.11
2440	0.0236	0.0182	32.12	2.05

The specific growth rate, μ, was determined from the dilution rate, D, by

$$\mu = D + m \tag{14}$$

where m is the maintenance energy which is assumed to be a constant at $m = 0.00385\ h^{-1}$ as determined by Molina Grima *et al.*[7] for the same strain and culture system. As can be appreciated in *Table 1*, m is a small value but significant when compared to D especially in the experiments at low growth rate.

Discussion

The validity of the procedure proposed to assess the absorbed photon flux was checked using data published by Lee *et al.*[19] obtained in a flat parallelepipedal vessel operating as a turbidostat. In those experiments, the light absorbed is calculated as the difference between the incoming and outgoing photon flux and then the efficiency is obtained from the following expression:

$$\psi = \frac{D \cdot \sigma_b \cdot \gamma_b \cdot q_o}{12 \cdot \left(\frac{I_a \cdot A}{C_b \cdot V}\right)} \tag{15}$$

where D is the dilution rate, σ_b the carbon fraction of biomass, γ_b the degree of reduction, q_o represents the energetic content equivalent of electrons of organic carbon (113 kJ eq^{-1} e^-), I_a the energy absorbed, A is the illuminated surface, V stands for the culture volume, and C_b for biomass concentration.

Since data for the extinction coefficient ($K_a = 0.18\ m^2\ g^{-1}$) are provided in the work and the average irradiances are also calculated, it may be compared with the procedure proposed in Eq. (11). In *Figure 4*, the original efficiencies (Ψ) and the recalculated (Ψ') are compared with Eq. (11) using their own data for I_{av} and K_a. As shown, both values turn out to be quite similar and the few discrepancies found can be attributed to the excessive simplification. Lee *et al.*[19] calculate I_{av} using an arithmethic mean instead of the real

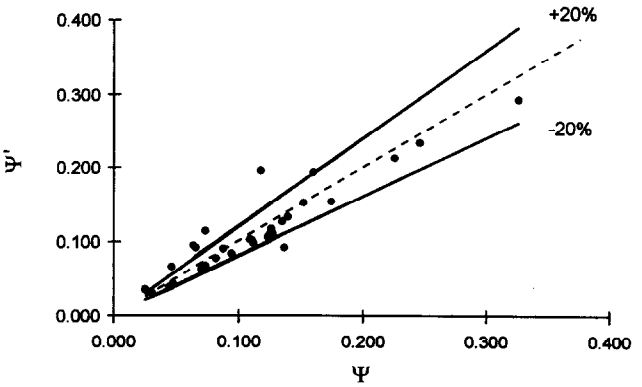

Figure 4 Evaluation of the procedure for calculating absorbed photon flux using the data published by Lee et al.[19] (1987). The efficiencies (Ψ) displayed are as calculated by the author and calculated by Eqs. (3) and (4) from the authors own data on I_{av} and K_a

Table 2 Combustion enthalpy of *Isochrysis galbana* AlII–4 from biochemical profile

Substance	Enthalpy[b] (kJ g^{-1})	Content (% p.s.)		Contribution to total enthalpy (kJ g^{-1})	
		High enthalpy	Low enthalpy	High enthalpy	Low enthalpy
Protein	17.5	15	25	2.62	4.36
Lipids	38.7	35	12	13.54	4.64
Glucids	16.6	45[a]	53[a]	7.48	8.81
Ash	0	5	10	0	0
Total	–			23.65	17.81

[a]Estimated by difference
[b]Lehninger[2]

exponential profile. Furthermore, the influence of pigment content, which plays a major role in K_a, is ignored.[18]

Once Eq. (3) is accepted as a reliable procedure for assessing the absorbed photon flux, Ψ_E can be readily evaluated using Eq. (1), and Ψ with Eq. (2) as long as a value for Q_b can be found. This can be estimated considering the average biochemical profile of the biomass as described in *Table 2*; thus, 21.4 kJ g^{-1} can be taken as a mean value for Q_b which is in agreement with the 21.0 kJ g^{-1} used by Aiba,[6] the 22.2 kJ g^{-1} from Payne,[20] and the 25.1 kJ g^{-1} proposed by Ogawa *et al.*[21]

The combustion heat can also be deduced from the stochiometry of the biomass ($C_1H_bO_cN_d$) using the expression of Lee *et al.*[19]

$$(\sigma_b \cdot \gamma_b \cdot q_o)/12 = Q_b$$

where all the parameters but the degree of biomass reduction (γ_b) are known. This can be calculated from the expression:

$$\gamma_b = 4 + b - 2 \cdot c - 3 \cdot d[eq(e^-)atgC^{-1})] \qquad (16)$$

Table 3 shows the results of applying Eqs. (6) and (7) to the data on biomass composition reported by several authors.

From this data, a mean value of 20.8 kJ g^{-1} can be drawn for Q_b, which is coherent with the 21.4 kJ g^{-1} proposed for *I. galbana* taking into account its biochemical profile. This was the value used to calculate the bioenergetic yield.

Aiba[6] reports the yield coefficients of a number of different strains and culture conditions. The efficiencies presented there are higher in most cases than those obtained in this work, which can again be attributed to the ideal conditions of low optical density and moderate irradiance under those which experiments were run. The low biomass concentration during the experiments seriously limits the technical interest of the data, since such diluted cultures are extremely inefficient in retaining light while cells in dense cultures are exposed to a very different light regime[22] and thus no extrapolations can be made.

The limitations of the *I. galbana* biomass in metabolizing the light harvested are evident in *Figure 5* where the quantum yield obtained is plotted against the specific photon flux absorbed (F_{ab}, expressed on a biomass unit basis). A maximum Ψ_E of around 10 μE m^{-2} s^{-1} can be seen for the two light-limited series performed at 820 and 1,620 μE · m^{-2} s^{-1}.

This *Figure* is in agreement with the 10.56 μE g^{-1} s^{-1} at 20°C supplied by Simmer *et al.*[23] for the "biomass quantum capacity" which represents the highest photon flux a biomass unit can transform into new biomass. This is an important parameter for system design since it enables calculation of the minimum biomass concentration necessary to metabolize a given photon flux.

Attention has also been given to the possible relationship between the specific growth rate and the quantum yield. In *Figure 6*, the influence of operation variables on system efficiency is shown by plotting Ψ_E against the specific growth rate μ at the three irradiance levels tested.

Figure 6 shows how the lower the incident irradiance is, the higher the photosynthetic efficiency results. This becomes dramatically reduced in photoinhibiting conditions (3,270 μE m^{-2} s^{-1}) at all the D tested. On the other hand, the experiments under light-limiting conditions show a wide plateau in the efficiency from which it can be deduced that growth rate (and hence D in a steady-state continuous culture) is not a critical variable for high efficiency. The experiments conducted at 820 μE m^{-2} s^{-1} give rise to quantum yields that can be considered constant within the experimental error in a D range between 0.020 and 0.035

Table 3 Estimation of combustion heat based on the elemental biomass composition

Reference	Stochiometry	σ_b	γ_b	Q_b (kJ g^{-1})
Aiba[25] (*S. platensis*)	$C_1H_{1.67}O_{0.44}N_{0.2}$	0.510	4.19	20.12
Oswatz[26] (Wastewater)	$C_1H_{1.69}O_{0.42}N_{0.15}$	0.533	4.40	22.08
Cornet *et al.*[12] (*S. platensis*)	$C_1H_{1.65}O_{0.531}N_{0.17}$	0.510	4.22	20.26

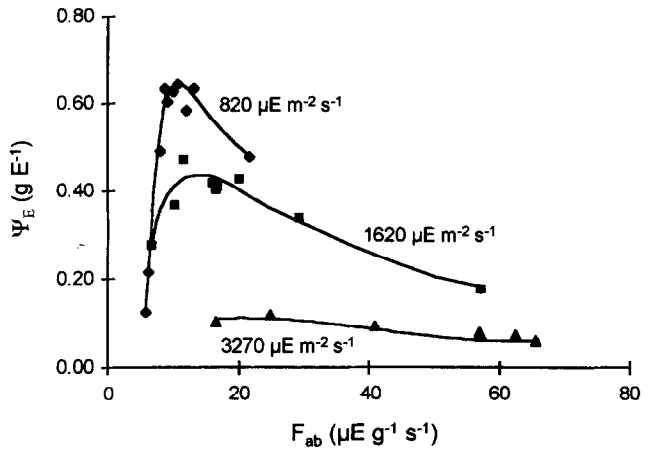

Figure 5 Quantum yield as a function of the specific absorbed photon flux for the three experimental series performed at 820; 1,620; and 3,270 μE m^{-2} s^{-1}

h^{-1}, thus, this value could be accepted as the maximum growth yield for *I. galbana* AL–II4:

$$\Psi_E = 0.619 \text{ g E}^{-1} = 3.64 \cdot 10^{-3} \text{ g kJ}^{-1}$$

Application to outdoor systems

In a wide study comparing many different species, culture conditions and light sources, Aiba[6] found Ψ_{kJ} to be independent of light composition, keeping its value within a narrow margin of under 3.5% variation. This suggests that comparison to other conditions is possible.

One of the reasons for obtaining indoor growth coefficients is their possible utility in assisting in technical outdoor system design, especially in finding the minimum biomass concentration necessary to make the most out of a given photon flux and the maximum productivities[6] that should be expected from a given culture system, strain, and geographic position.

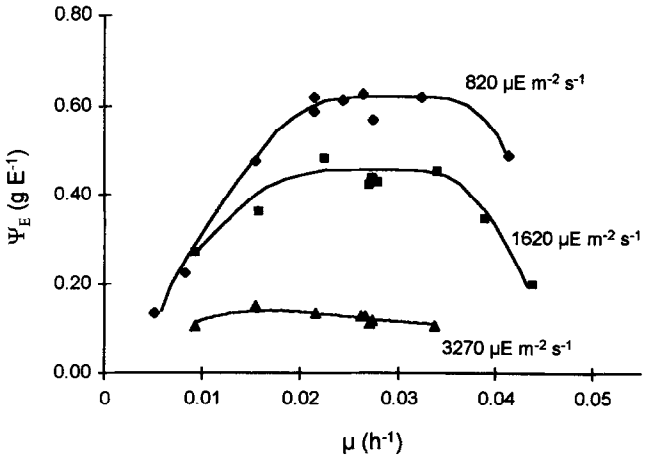

Figure 6 Quantum yield as a function of the specific growth rate for the three experimental series performed at 820; 1,620; and 3,270 μE m^{-2} s^{-1}

Molina Grima *et al.*[24] in an outdoor culture of *I. galbana* ALII–4 obtained a maximum productivity of 0.54 g l^{-1} d^{-1} with a daily mean photon flux of 96 E m^{-2} d^{-1}. Cultures were grown in a closed tubular photobioreactor with the following specifications:

* D = 0.034 h^{-1}
* Biomass concentration in steady state, C_b = 1.61 g l^{-1}
* Reactor volume, V = 50 l
* Dimensions; internal diameter r_i = 2.6 cm; external diameter r_e = 3.0 cm
* Tube length, L_g = 80.8 m

For this experiment, the solar irradiance throughout the day was used to calculate an average (integrated over a day) value of Ψ_E. This was obtained assigning to each time of the day a value of Ψ_E estimated for D = 0.034 h^{-1} and interpolating for each instantaneous incident irradiance the laboratory data at 820; 1,620; and 3,270 μE m^{-2} s^{-1} (at D = 0.034 h^{-1}. Ψ_E resulted 0.603 g E^{-1} at 820 μE m^{-2} s^{-1}, 0.428 g E^{-1} at 1,620 μE m^{-2} s^{-1} and 0.087 g E^{-1} at 3,270 at μE m^{-2} s^{-1}). For irradiances below 820 μE m^{-2} s^{-1}, a Ψ_E of 0.603 g E^{-1} was used, and for irradiances above 3,270 μE m^{-2} s^{-1}, Ψ_E was considered 0.087 g E^{-1}. This procedure rendered an integrated Ψ_E for the outdoor culture of 0.231 g E^{-1}. The global photon flux incident on the cross section of the reactor tube was obtained as the product of the average daily photon flux (96 E m^{-2} d^{-1}) and the cross section of the surface of the reactor tubes (2.09 m^2) for a value of 200.6 E d^{-1}. The maximum biomass productivity can then be readily evaluated by means of Eq. (1) resulting in 46.3 g d^{-1}.

The value obtained is somewhat higher, although still in agreement with the actual 27.0 g d^{-1} reported by Molina Grima *et al.*;[24] nevertheless, this discrepancy may be due to the average growth yield Ψ_E obtained for outdoor conditions still being overestimated, since all the daytime values corresponding to incident irradiances over 3,270 μE m^{-2} s^{-1} were assigned the value measured at this irradiance (0.087 g E^{-1}) which was the highest experimental value used; thus, if data for a wider range of conditions obtained indoors were available, a more exact quantum yield obtained by this same procedure could be expected and, hence, enhance the capacity to predict values of maximum productivity for a given system, strain, and incident irradiance conditions.

Acknowledgments

This research was supported by the Comisión Interministerial de Ciencia Y Tecnología (CICYT; BIO–91–0652; Spain) and Plan Andaluz de Investigación II, Junta de Andalucia.

List of symbols

A	Area of illuminated surface (m^2)
C_b	Biomass concentration (g m^{-3} or mg l^{-1})
D	Dilution rate (h^{-1})
F	Photon flux (μE s^{-1})
F_{ab}	Photon flux absorbed by the biomass unit (μE g^{-1} s^{-1})
F_T	Total absorbed photon flux (μE s^{-1})

F_{vol}	Photon flux absorbed in the volume unit ($\mu E\ m^{-3}\ s^{-1}$)
I_a	Absorbed light energy (Lee *et al.*[19] notation)
I_{av}	Average irradiance inside a culture bulk ($\mu E\ m^{-2}\ s^{-1}$)
I_o	Incident irradiance ($\mu E\ m^{-2}\ s^{-1}$)
I_p	Local irradiance ($\mu E\ m^{-2}\ s^{-1}$)
K_a	Extinction coefficient ($g\ m^{-2}$)
L	Light path (m)
Lg	Tube length in the tubular reactor (m)
P_b	Biomass productivity ($g\ m^{-3}\ h^{-1}$)
Q_b	Biomass combustion enthalpy ($kJ\ g^{-1}$)
q_o	Energetic content of an equivalent of electrons [113 kJ (eq. e^-)$^{-1}$]
R	Radius (m)
r_e	External radius (m)
r_i	Internal radius (m)
V	Volume (m^{-3})
V_i	Volume element (m^3)
V_T	Total volume (m^3)
x	Distance from the vessel surface to a inner point (m)
Ψ	Bioenergetic yield coefficient (%)
Ψ'	Bioenergetic coefficient from Lee *et al.*[19] recalculated with Eq. (1) (%)
Ψ_E	Quantum yield ($g\ E^{-1}$)
Ψ_{kJ}	Energetic yield ($g\ kJ^{-1}$)
γ_b	Biomass reduction grade ($eq.e^-\ eq.C^{-1}$)
σ_b	Carbon content of biomass (mass fraction)
μ	Specific growth rate (h^{-1})

References

1. Pirt, S. J. The thermodynamic efficiency (quantum demand) and dynamics of photosynthetic growth. *New Phytol.* 1986, **102,** 3–37
2. Lehninger, A. L. *Biochemistry* 2nd Ed. Worth Publishers, Inc., New York, 1985, 599–627
3. Kok, B. On the efficiency of *Chlorella* growth. *Acta Bot. Neerlandica* 1952, **1,** 445
4. Tamiya, H. Mass culture of algae. *A. Rev. Plant. Physiol.* 1957, **8,** 309–333
5. Myers, J. On the algae: Thoughts about physiology and measurements of efficiency. In: *Primary Productivity in the Sea* (Falkowski, P. G., Eds.). Plenum Press, New York, 1980, 1–15
6. Aiba, S. Growth kinetics of photosynthetic microorganisms. *Adv. Biochem. Eng.* 1982, **23,** 85–156
7. Molina Grima, E., Sánchez Pérez, J. A., García Camacho, F., García Sánchez, J. L., and López Alonso, D. *n*–3 PUFA productivity in chemostat cultures of microalgae. *Appl. Microbiol. Biotechnol.* 1993, **38,** 599–605
8. Molina Grima, E., Sánchez Pérez, J. A., García Sánchez, J. L., García Camacho, F., and López Alonso, D. EPA from *Isochrysis galbana*. Growth conditions and productivity. *Proc. Biochem.* 1992, **27,** 299–305
9. Hansmann, E. Pigment Analysis. In: *Handbook of Phycological Methods, Culture Methods and Growth Measurements* (Stein, J. R., (Ed.). Cambridge University Press, London, 1973, 359–368
10. Whyte, J. N. Biochemical composition and energy content of six species of phytoplankton used in mariculture of bivalves. *Aquaculture* 1987, **60,** 231–241
11. Lee, H. Y., Erickson, L. E., and Yang, S. S. The estimation of growth yield and maintenance parameters for photoautotrophic growth. *Biotechnol. Bioeng.* 1984, **26,** 926–935
12. Cornet, J. F., Dussap, C. G., Cluzel, P., and Dubertret, G. A structured model for simulation of cultures of the cynobacterium *Spirulina platensis* in photobioreactors II. Identification of kinetic parameters under light and mineral limitations. *Biotechnol. Bioeng.* 1992, **40,** 826–834
13. Cornet, J. F., Dussap, C. G., and Gros, J. B. Conversion of radiant energy in photobioreactors. *A.I.Ch.E.* 1994, **40**(6), 1055–1065
14. Cornet, J. F., Dussap, C. G., Gros, J. B., Binois, C., and Lasseur, C. A simplified monodimensional approach for modeling coupling between radiant light transfer and growth kinetics in photobioreactors. *Chem. Eng. Sci.* 1995, **50**(9), 1489–1500
15. Aiba, S. On the criticism of some measurements of photosynthetic efficiency. *Biotechnol. Bioeng.* 1983, **25,** 2775–2776
16. Alfano, O. M., Romero, R. L., and Cassano, A. E. Radiation field modelling in photoreactors. I. Homogeneous media. *Chem. Eng. Sci.* 1986, **41,** 421–444
17. Evers, E. G. A model for light-limited continuous cultures: Growth, shading, and maintenance. *Biotechnol. Bioeng.* 1991, **38,** 599–605
18. Molina Grima, E., García Camacho, F., Sánchez Pérez, J. A., Fernández Sevilla, J. M., Acién Fernández, F. G., and Contreras Gómez, A. A mathematical model of microalgal growth in light limited chemostat culture. *J. Chem. Technol. Biotechnol.* 1994, **61,** 167–173
19. Lee, H. Y. and Erickson, L. E. Theoretical and experimental yields for photoautotrophic, mixotrophic, and photoheterotrophic growth. *Biotechnol. Bioeng.* 1987, **29,** 476–481
20. Payne, W. J. Energy yields and growth of heterotrophs. *Ann. Rev. Microbiol.* 1970, **24,** 17–52
21. Ogawa, T., Fujii, T. and Aiba S. Prog. Rept. No. 101, Dept. Ferm. Technol., Osaka University, Osaka, Japan, 1978
22. Richmond, A. Large-scale microalgal culture and applications. *Prog. Phycol. Res.* 1990, **7,** 269–330
23. Simmer, J., Tichý, V., and Doucha, J. What kind of lamp for the cultivation of microalgae? *J. Appl. Phycol.* 1994, **6,** 309–313
24. Molina Grima, E., Sánchez Pérez, J. A., García Camacho, F., García Sánchez, J. L., Acién Fernández, F. G., and López Alonso, D. Outdoor culture of *Isochrysis galbana* ALII-4 in a closed tubular photobioreactor. *J. Biotechnol.* 1994, **37,** 159–166
25. Aiba, S. and Ogawa, T. Assessment of growth yield of a blue-green alga, *Spirulina platensis*, in axenic and continuous culture. *J. Gen. Microbiol.* 1977, **102,** 179–182
26. Oswald, J. A. Large-scale algal culture systems (engineering aspects). In: *Microalgal Biotechnology*. (Borowitzka, L. J. and Borowitzka, M. A., Eds.). Cambridge University Press, Cambridge, 1988, 357–395