A study on simultaneous photolimitation and photoinhibition in dense microalgal cultures taking into account incident and averaged irradiances

E. Molina Grima, J.M. Fernández Sevilla *, J.A. Sánchez Pérez, F. García Camacho

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

Received 24 March 1995; revised 18 September 1995; accepted 19 September 1995

Abstract

From chemostat cultures of the marine microalga Isochrysis galbana (CCAP 927/15) simultaneous photolimitation and photoinhibition was observed. The extent of each phenomenon depends on the light gradient inside the culture and therefore on the incident irradiance. Variations in biomass concentration and average irradiance inside the culture with dilution rate at three incident irradiances, $I_o$, were studied (from $I_o = 820$ to $3270 \, \mu E \, m^{-2} \, s^{-1}$). At $I_o$ above $1630 \, \mu E \, m^{-2} \, s^{-1}$ a photoinhibition effect was observed, although the specific growth rate remained a hyperbolic function of average irradiance regardless of incident irradiance. To calculate average irradiance, a three-dimensional irradiance distribution model for cylindrical geometry is proposed, improving the estimation of the irradiance field inside culture with regard to other methods used up to now since the variations in illumination along the vertical axis are considered. Lastly, a new approach to model simultaneous photolimitation and photoinhibition is proposed by considering that specific growth rate is related to average irradiance and that parameters representing the cell adaptability to light are a function of the maximum irradiation at which cells are exposed, that is, the incident irradiance.

Keywords: Microalgae; Photoinhibition; Light distribution; Growth modelling; Isochrysis galbana; Photobioreactor

1. Introduction

Microalga-based processes have been receiving increasing interest, due to some of their key characteristics, such as their use of solar light as the main energy source and their ecological nature. Although for the production of some substances, microalga-based processes are unbeatable, because certain molecules cannot be synthesized by other organisms (Richmond, 1990; De La Noue and De Pauw, 1988; Bajpai and Bajpai, 1993), system performance in general may be greatly improved, as can easily be inferred from the quantum yield figures in the literature (Lee and Erickson, 1987; Pirt, 1986; Sukenik et al., 1987).

The large investment required to set up a large-scale process, coupled with the long wait for returns, make microalga-based processes a risky enterprise. Since one of the reasons why such large installations are needed is the generally low biomass concentrations attained, much attention has been given the
growth-limiting factors of microalga. Among them, the availability of light is the fundamental problem in photoautotrophic microalga cultivation, since the supply of any other nutrient but light and CO₂ is simply a matter of adding a few dissolved salts to the culture medium. Even for gas nutrients (CO₂) the problem is well defined, and the main goals are minimizing losses and optimizing economies for the enhancement of productivity.

Light must be continuously supplied to the culture because radiative energy may not be accumulated. In dense cultures, light availability can only be modified in a limited way through manipulation of the light path, the incident irradiance, the amount of mixing and the biomass concentration. Considered as a substrate, light has others peculiarities in the way in which it is distributed within the culture and absorbed by the microorganisms. Perhaps the greatest controversy is over what happens in dense cultures in which microalga cells move along strong light gradients and are thus exposed to varying irradiances. There is no general agreement on how growth or photosynthetic rate respond under rapidly varying irradiance. Nevertheless, it is consistently admitted that it is influenced by total irradiance and by how often and to what extent variations in irradiance occur (Terry, 1986). This is included in a more general concept often referred to as ‘light regime’ (Richmond, 1990), and in how growth is related to changes in irradiance within a dense culture is still not well defined.

Photoinhibition is also a matter of discussion (Vonshak et al., 1994). This effect is felt extensively in outdoor cultures and some authors have proposed that a degree of shading can even improve productivity in certain systems (Richmond and Quiang, 1994). Many attempts to study and characterize this phenomenon have been made and some growth models have been successfully proposed for non-dense systems (Talbot et al., 1991), but it is still not clear what happens in dense cultures where dark zones and photoinhibited zones coexist within the same culture and it is difficult to distinguish whether cells are light-limited or light-inhibited (Vonshak, 1993).

In this report, a study of the effect of light in a photosynthetic culture is presented. Geometric distribution of light (radiation field) in a dense microalgal culture as well as its relationship to the growth rate are analyzed from the point of view of the average irradiance concept. This theory is then applied to a case study in which there is photoinhibition.

2. Materials and methods

2.1. Organism

The microalga used was an isolate of the marine microalga Isochrysis galbana labeled ALII-4, CCAP No. 927/15 Oban, Scotland (Molina Grima et al., 1994a).

2.2. Growth conditions

Cultures were grown in a 5-dm³ computer-controlled reactor (New Brunswick Scientific Bioflo III, Edison, NJ, USA). 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. (1992).

The cultures were constantly illuminated with up to 16 Osram Dulux EL (20 W) fluorescent lamps with a cylindrical reflector arranged around the culture vessel for high irradiance. 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, USA). The different incident irradiances, I₀, tested were achieved by varying the number of lamps turned on or off. The homogeneity in the light distribution inside the reactor vessel was checked by direct measurement. Strong irradiance conditions were given special importance, because these must be withstood by outdoor cultures, so incident irradiance ranged from 820 µE m⁻² s⁻¹ to 3270 µE m⁻² s⁻¹.

The dilution rate was fixed in each experiment 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 dm³ min⁻¹ with agitation at 150 rev min⁻¹. Nutrient saturation was checked by supplying successively increasing nutrient concentrations during continuous growth, observing no significant changes in the steady-state biomass when the nutrient concentration was doubled. pH was maintained
constant at 8 by automatic pure CO₂ injection. Steady state was maintained for at least 4 days to assure constant biomass composition.

2.3. Analytical methods

Biomass concentration expressed as dry weight was measured as described by Molina Grima et al. (1992). The total pigment content was obtained by summing chlorophyll (a, c) and carotenoid mass fractions. Chlorophylls were measured according to the method of Hansmann (1973). Carotenoid determination was as described by Whyte (1987). The biomass absorption coefficient, \( K_a \), of the \( I. galbana \) culture as a function of the total pigment content was determined as described by Molina Grima et al. (1994b). Data presented are averaged over the steady-state period.

Numeric procedures were carried out with the Mathematica v2.1 software package (Wolfram, 1991). Statistical and regression procedures were performed by the Statgraphics v5 software package (Fry, 1993).

3. Theoretical background

Steady states in nutrient-saturated chemostat cultures are characterized by the light-limited growth that is a consequence of self-shading by the microalgae when biomass concentration in the broth becomes too dense for enough light to get through (Molina Grima et al., 1993).

In dense cultures, the rays of light that penetrate the culture surface from outside the vessel are rapidly attenuated, as predicted by the Lamber-Beer law. Thus, inside the culture vessel light gradients are established so that at any given point different irradiances \( I_p \) are found. In an ideally mixed reactor, the average irradiance, \( I_{av} \), is the mean of the \( I_p \) found in every single element of volume that makes up the total culture. This may be defined by:

\[
I_{av} = \frac{1}{V_T} \sum_i^n \frac{V_i I_{p_i}}{V_i} = \frac{1}{V_T} \sum_i^n V_i I_{p_i}
\]

3.1. Radiation field

In order to calculate \( I_{av} \), it is first necessary to know what the radiation field inside the culture is, as a function giving the irradiance at any point inside the vessel, represented by the vector of its position, \( \vec{r}, I_p(\vec{r}) \). It makes no difference whether the radiation field is given by a tabulated set of data obtained by experiment (in this case, Eq. 1 may be used straightforward to calculate \( I_{av} \)) or by an analytical function arrived at by considering the physics of light attenuation by the microalgal biomass. Since to obtain \( I_p \) data in every experimental run would be extremely complicated, the latter option is much more convenient, especially when it is necessary to express the radiation field as a function both of the biomass concentration, and its pigment content, which not only greatly influences the biomass absorption coefficient (Molina Grima et al., 1994b), but is also the only option when a mathematical model for an irradiance-growth rate relationship is desired.

The problem of radiation field modeling inside photoreactors has been the object of great interest in the past years (Alfano et al., 1986) and can be approached with different methods of increasing complexity. A classical approach is the bi-dimensional model adopted by Evers (1991), which is a border case of the partially diffuse model proposed by Matsuura and Smith (1970). It is implemented assuming that the total irradiance that reaches a given point inside the culture can be calculated by totaling the contributions coming in from all directions. This may be done by means of the following expression for an evenly illuminated cylindrical culture vessel:

\[
I(S,C) = \frac{I_0}{\pi} \int_0^\pi \exp \left( -K_a C_b \left[ (R-S) \cos \phi + \left( R^2 - (R-S)^2 \sin^2 \phi \right)^{0.5} \right] \right) d\phi
\]

where \( K_a \) is the biomass absorption coefficient, \( C_b \) stands for the biomass concentration, \( S \) is the distance from the vessel surface to the point for which the irradiance is being totaled and \( \phi \) is the angle of penetration of the light. In a previous study (Molina Grima et al., 1994b), the radiation field model proposed by Evers was successful in interpreting both
steady-state results and the dynamics of the transitory stages of an Isochrysis galbana chemostat culture. Nevertheless, the experimental measurement of strong variations in light along the vertical axis, suggests the consideration of more rigorous models.

Taking into account the light source used (cool fluorescent lamps with a cylindrical reflector, that may in no way be considered point emitters), a three-dimensional, totally diffused incidence model is the next step. A three-dimensional light distribution model has thus been considered.

To represent the radiation field found inside the system used in this study, the following model proposed by Zolner and Williams (1971) was used to calculate $I_p(R)$. The three-dimensional diffused nature of the model leads to rather complex mathematical expressions. In the development of the model, two concentric cylinders were considered (Fig. 1). The inner cylinder takes the place of a culture vessel with a given radius, $R$. The outer, with radius $R_s$, represents a fictitious radiation source of uniform intensity. Every element of the source is assumed to emit radiation uniformly in all spatial directions. The cylindrical coordinates $z_0$ and $r_0$ define a point inside the culture. The angular coordinate may be omitted because of the symmetry (evenly illuminated cylinder). The coordinates $z$ and $\phi$ define, relative to $\rho(r_0, z_0)$, a point on a fictitious light-emitting surface and are used for integration. The geometrical relationships used are shown in Fig. 2. The following equations give the radiation field (Alfano et al., 1986).

$$I_p(r_0, z_0) = \int_0^{z_0} \int_0^{2\pi} e^{-K_s C_0 \rho(r_0, z, \phi)} dI_0(z, \phi)$$

$$+ \int_H^{z_0} \int_0^{2\pi} e^{-K_s C_0 \rho(r_0, z, \phi)} dI_0(z, \phi)$$

(3)

$$dI_0(z, \phi) = \frac{I_0 R_s d\phi dz}{R_s^2 + r_0^2 + z^2 - 2 R_s r_0 \cos(\phi)}$$

(4)

The model has two adjustable variables that depend on the reactor geometry, $R_s$ and $I_o$. One takes into account the power of the lamp, $I_0$, and is determined by direct measurement on the reactor wall. The other, $R_s$, is the distance from the center
of the vessel at which the fictitious lamp is placed. It governs the axial distribution of the radiation field and can be adjusted to the value that best fits the experimental data of the case considered. When \( R_s \to \infty \), the model is reduced to that used by Evers and the radiation profile along the vertical axis is flat. In this study, it has been assumed that the other limiting condition is \( R_s = R \) (the emitting surface is on the culture vessel surface) so that the two border cases can be compared \( (R_s \to \infty \) and \( R_s = R \)). Otherwise, the calculations that must be performed to obtain \( P \) (the optical path along which the light is attenuated by the biomass) become too complex to handle through all the subsequent integration processes.

\[
R_s = R \quad L = P(r_0, z, \phi) = \sqrt{R^2 - 2r_0 R \cos(\phi)} + r_0^2 + z^2
\]  

(5)

In Fig. 3 the radial profiles obtained by calculating the radiation field inside the culture with both models, Eq. 2 and Eqs. 3 to 5, are compared and confronted with experimental data, the more complex model being found to more accurately resemble real \( I_p \) than the two-dimensional one.

Another point to be stressed in this comparison of the two models against the experimental data is that good results were obtained despite having neglected the effect of scattered light. In some studies (Yokota et al., 1991) it has been argued reasonably that the calculation of light attenuation considering absorption only with the Lambert-Beer's law overestimates the attenuation. Nevertheless, it may be said of Fig. 3 that, for this system and microalga strain, both the accuracy and trend of the model are quite acceptable. This could be because the extinction coefficient was calculated under conditions closely resembling actual reactor operation and, therefore, probably includes the effect of light scattering.

3.2. Average irradiance

Once an analytical expression for the irradiance field has been found, Eq. 1 can be now used to calculate an average irradiance translating the summations in Eq. 1 into integrals for a cylindrical vessel.

\[
I_{av} = \frac{\int_0^H \int_0^R \int_0^{2\pi} I_p(r_0, z_0) r_0 d\phi_0 dr_0 dz_0}{\int_0^H \int_0^R \int_0^{2\pi} r_0 d\phi_0 dr_0 dz_0} = \frac{2\pi \int_0^H \int_0^R I_p(r_0, z_0) r_0 dr_0 dz_0}{V_T}
\]  

(6)

The average irradiance obtained in this way is then used to calculate the parameters of a growth model by correlating these \( I_{av} \) with the specific growth rate, \( \mu \). This means that \( \mu \) is assumed to be a function of \( I_{av} \) and that energy uptake depends on this average value. This is not equivalent to calculating growth rate as a function of the local irradiance at every point of the vessel and then averaging for the whole culture volume. Evers (1991) considers the former inadequate, as it makes it difficult to give the model parameters a physiological meaning. Nonetheless, it is also unrealistic to assume that a microalgae moving along a light gradient can adapt its growth rate quickly enough to the varying conditions taking place in an ideally mixed stirred tank to always grow at the \( \mu \) given by the instantaneous irradiance it is exposed to.

This last point must be given some consideration. The two situations previously described are border cases that should not be rejected or accepted a priori based on qualitative considerations. The question is which of the two magnitudes, \( I_p \) or \( \mu \), is to be
averaged. This is quite an important point, because when growth rate is a saturating function of irradiance the global growth rates obtained by averaging \( I_p \) are always higher than those obtained with \( \mu \), as shown by Terry (1986).

Cellular growth is a multi-phased process too complicated for the rate to change in under a few seconds, which averaging \( \mu \) would require. But beyond this qualitative consideration there is experimental evidence that the inertia of growth is too great to be changed by short variations in the light regime, since the photosynthetic cell seems to be able to accumulate a certain amount of the energy and reducing power, making it possible for photosynthesis to continue for short periods when irradiation is dimmed or interrupted. This has been investigated with both flashing light (Philiph and Myers, 1954) and cycling of the photosynthetic microorganisms through a tubular loop reactor having both light and dark zones (Lee and Pirt, 1981). Philiph and Myers show that a 1 ms 24 W m\(^{-2}\) pulse of PAR could sustain Chlorella growth in the dark for 20 ms. Pirt (1986) concludes that growing Chlorella cells become fully charged with sufficient energy and reducing power to maintain growth if they are exposed to 20 W m\(^{-2}\) for 0.52 s at least every 9 s. A more exhaustive study was carried out by Terry (1986), who used a flashing light and optically thin cultures to relate the photosynthetic rates to the frequency and duration of the cycles. He showed that with high frequency cycles, the photosynthetic rate depended on average irradiance, while for low frequency cy-
cles it must be calculated by averaging instantaneous photosynthetic rates over exposure time.

In the present study, Eq. 6 will be used.

4. Results

When operating as a light-limited chemostat, the steady-state biomass concentration attained, \( C_b \), is determined by the imposed dilution rate, \( D \), as the limiting growth factor is light availability, which is limited by self-shading of the cells in dense cultures. Thus, high dilution rates must be supported by fast-growing cells whose illumination requirements can only be met at low biomass concentrations, and low dilution rates give rise to cultures with growth severely limited by self shading. In this situation, if the irradiance supplied to the system is increased at a fixed \( D \), light availability inside the culture increases. But this method of overcoming light limitation cannot be exploited indefinitely. This is clearly shown in Table 1, where the characteristics of the steady states achieved at different dilution rates for three irradiance levels are displayed.

When experiments with different \( I_o \), at the same dilution rates are compared, steady-state biomass concentration is found to increase when incident irradiance is raised from 820 to 1630 \( \mu \)E m\(^{-2}\) s\(^{-1}\) as would be expected from self-shaded light-limited growth. Nevertheless, this behavior is not maintained when \( I_o \) is then raised to 3270 \( \mu \)E m\(^{-2}\) s\(^{-1}\), where \( C_b \) decreases at all \( D \) tested instead of continuing to increase as expected.

It is evident from the results presented that a photoinhibition phenomenon appears, as at the same \( D \), \( C_b \) decreased with an increase in \( I_o \). This is better appreciated in the steady-state biomass productivities, \( P_b \), obtained at each \( I_o \) tested. The maximum biomass productivity of 22.1 mg l\(^{-1}\) h\(^{-1}\) is attained at \( I_o = 1630 \) \( \mu \)E m\(^{-2}\) s\(^{-1}\) and \( D = 0.030 \) h\(^{-1}\). Series B productivities are in every case higher than those observed for series A and C. The rise in productivity from series A to B is obviously attributable to the greater light availability in series B. On the other hand, in series C, the negative effects of photoinhibition override the benefits of the greater light availability at all the dilution rates and biomass concentrations tested. The harmfully high irradiance used in series C is observed at every \( D \), but becomes more notorious at high dilution rates that give rise to low steady-state biomass concentrations and thus to optically thin cultures that are more affected by the photoinhibition.

The results presented are of major interest since outdoor cultures may be exposed to irradiances even higher than those in these experiments. In this sense, the laboratory limitations are evident in the obtention of growth models, especially considering that the effects of photoinhibition were only clearly observed under rather strong irradiances (3270 \( \mu \)E m\(^{-2}\) s\(^{-1}\)), several fold greater that those usually used to work out the indoor growth model parameters (e.g., Talbot et al., 1991; Bannister, 1979; Jensen and Knutsen, 1993; Sukenik et al., 1987).

5. Discussion

In a previous work (Molina Grima et al., 1994b), the relationship between specific growth rate, \( \mu \), and average irradiance, \( I_{av} \), was adjusted to a hyperbolic expression. By balancing steady-state material to a chemostat and introducing a maintenance factor, \( m \) = 0.00385 h\(^{-1}\) previously measured by Molina Grima et al. (1993), the following expression was obtained:

\[
D = \mu - m \cdot \frac{I_{av}^n}{I_{k}^n + I_{av}^n} - m \quad (7)
\]

where \( n \) is a factor taking into account the abruptness of the \( \mu \) vs. \( I_{av} \) curve in the transition from low to high irradiance conditions, analogous to Bannister's 'shape parameter' (Bannister, 1979). \( I_k \) is a constant representing the affinity microalgae have for light. Quantitatively, \( I_k \) is the \( I_{av} \) which gives rise to half the maximum growth rate.

In a light-limited chemostat with unchanging dilution rate, and increase in the incident irradiance, \( I_o \), an increase may be expected in the steady-state biomass concentration and/or pigment content, so that the optical thickness of the culture increases until the \( I_{av} \) leads to a \( \mu \) in equilibrium with the imposed \( D \).

The dramatic increase in the \( I_{av} \) (see Table 1) calculated for the experiments carried out at 3270
\[ \frac{\mu}{\mu_{\text{max}}} = \frac{I}{I_{\text{max}}} \]

\[ \mu = \frac{I}{I_{\text{max}}} \text{EXP}\left[1 - \frac{I}{I_{\text{max}}}\right] \]

\[ \mu = \frac{I}{I_{\text{max}}} \left[1 - \text{EXP}\left(-\frac{I}{I_{\text{max}}}\right)\right] \]

\[ \mu = \frac{\mu_{\text{max}} I}{K_s + I + \frac{I^2}{K_1}} \]

where Eq. 8 is the equation proposed by Tamiya et al. (1953), Eq. 9 is the equation proposed by Steele (1977), Eq. 10 is the exponential model (van Oorschot, 1955), and Eq. 11 is the Aiba equation (Aiba, 1982). Those models were usually used successfully when experiments were done either in batch or turbidostat mode with optically thin cultures, so that self-shading effects could be neglected and all the cells were considered to be exposed to the incident irradiance. Nevertheless, none of the above equations was able to correlate the chemostat data of the experiments presented in this study. This is clearly shown in Table 2, where the results of fitting each of the experimental series separately by nonlinear regression show that none of the models is satisfactory, since different \( I_o \) lead to different parameters and thus there is a lack of generality. Nevertheless, it must be pointed out that Eq. 7 gives the bests fits for the three individual series and is the only one which offers certain generality by rendering similar values for \( \mu_{\text{max}} \) in different conditions of incident irradiance.

The changes obtained in the model parameters must be connected with the incident irradiance, as this is the only operating variable that was changed from one experimental series to another. In this sense, a particularly significant aspect of the results presented that must be stressed is the fact that, although high incident irradiances caused a decrease in the steady-state biomass concentration, no one of the experiments resulted in a total wash-out of the culture, a new steady state was always reached, indicating that the growth inhibiting factor cannot be only \( I_{\text{av}} \), since any degree of photoinhibition would always lead to the total wash-out of the system.

The behavior observed, that is, the decrease in steady-state biomass concentration when irradiance is increased, could be explained by admitting that, due to the light gradients inside the culture, both light-limited growth and photoinhibiting conditions take place each in a different extent depending on the fixed \( I_o \). The degradation in microalgae capacity to harvest light has been attributed to a destruction of...
key components of the PSII photosystem (Samuelson et al., 1985). Jensen and Knutsen (1993) demonstrated that this is a reversible process in which degradation and regeneration of key components of the photosynthetic apparatus coexist and the available amount of functional photosynthetic pigments is the result of an equilibrium between that two processes. Bearing this in mind, photoinhibition can be explained by admitting that damage to the photosystem only occurs at very high irradiances that in our system are found only in a limited amount of the culture volume, while the regeneration process can take place throughout the culture. In this case, although with decreased efficiency for light harvesting, the biomass growth rate can continue to show hyperbolical relationship with $I_{av}$.

It would be quite useful to have a model that takes into account both the hyperbolic relationship of the growth rate and $I_{av}$ for a fixed $I_0$ as well as the influence of the latter on the model parameters. The expression presented below serves both these needs and is proposed bearing in mind the conditions described above:

$$
\mu = \frac{\mu_{max} f^2_{av} / I_0}{(I_k + (I_0 / K_1)^n_1 + f^2_{av} / I_0)}
$$

(12)

where the following analogies with Eq. 7 may be pointed out:

$$
I_k + \left( \frac{I_0}{K_1} \right)^{n_1} \approx I_{\text{apparent}}
$$

Apparent light affinity constant

$$
\frac{n_2}{I_0} \approx n_{\text{apparent}}
$$

Apparent 'reaction order'

This model contains the minimum parameters necessary to take into account the characteristics of the organism ($\mu_{max}$), the variation in growth rate, $\mu$, with the average irradiance inside the culture, $I_{av}$ (both its magnitude, $I_k$, and 'shape', $n_1$), and the photoinhibition effect, assumed to be mainly a function of the incident irradiance ($K_1$ to modulate the extent and, again, a 'shape' parameter, $n_2$).

In the model presented, only the essential variables (incident irradiance, $I_0$ and average irradiance, $I_{av}$, thus culture optical thickness) are included. All the other factors affecting microalga growth (such as the photosynthetic efficiency of the strain, maximum growth, geometrical characteristics of the photobioreactor, pigment content, etc.) are included in the model parameters and in the calculation of $I_{av}$.

To estimate the model parameters, a non-linear regression of all the data presented in series A, B and C was carried out using a value of 0.00385 h⁻¹ for $m$, with the following results:

- $\mu_{max} = 0.0444$ h⁻¹
- $I_k = 170.68$ $\mu$E m⁻² s⁻¹
- $K_1 = 2217.2$ $\mu$E m⁻² s⁻¹
- $n_1 = 12.8$
- $n_2 = 2728.8$ $\mu$E m⁻² s⁻¹
- $r^2 = 0.978$

The results of the regression analysis and representation of predicted and experimental values (Fig. 4) show that for this set of experimental data and within the range of incident irradiances analyzed, the model gives a reasonable approximation relating growth rate to average irradiance. The parameters obtained are coherent with earlier observations and statistically, highly significant, especially for $\mu_{max}$ and $I_k$ ($F$-ratio = 59.5 and 34.2, respectively) which means that the equation represents the phenomenon well.
6. Conclusions

The results presented in this work show that growth can simultaneously be photolimited and photoinhibited in a dense microalgal culture. This might be due to that photoinhibition is a reversible process (Jensen and Knutsen, 1993) so that degradation and degeneration of key components of the photosynthetic apparatus are in equilibrium. The extent of each phenomenon depends on the light gradient inside the culture and therefore on the incident irradiance.

The adaptation of the three-dimensional irradiance distribution model proposed by Zolner and Williams (1971) for photocatalysis improved the estimation of the irradiance field inside culture with regard to other methods used up to now. Therefore, it supplies a more accurate base for the calculation of the average irradiance since the variations in illumination along the vertical axis are considered.

Growth models based only in average irradiance or incident irradiance fail to interpret photoinhibition effects in chemostat cultures as the influence of the irradiance gradients that establish inside a dense culture are disregarded. These models predict a complete wash-out for chemostat cultures when photoinhibiting irradiance are reached. A new approach to model simultaneous photolimitation and photoinhibition is proposed by considering that specific growth rate is related to average irradiance and that parameters representing the cell adaptability to light are a function of the maximum irradiation at which cells are exposed, that is, the incident irradiance.

7. Nomenclature

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_b$</td>
<td>biomass concentration (mg l$^{-1}$ or g m$^{-3}$)</td>
</tr>
<tr>
<td>$D$</td>
<td>dilution rate (h$^{-1}$)</td>
</tr>
<tr>
<td>$I_{av}$</td>
<td>average irradiance inside the photobioreactor (µE m$^{-2}$ s$^{-1}$)</td>
</tr>
<tr>
<td>$I_k$</td>
<td>constant representing the affinity of cells to light (µE m$^{-2}$ s$^{-1}$)</td>
</tr>
<tr>
<td>$I_{max}$</td>
<td>maximum growth irradiance for the Steele and exponential models</td>
</tr>
<tr>
<td>$I_0$</td>
<td>incident irradiance, on culture surface (µE m$^{-2}$ s$^{-1}$)</td>
</tr>
<tr>
<td>$I_p$, $I_p(r)$</td>
<td>irradiance at a point inside the photobioreactor (µE m$^{-2}$ s$^{-1}$)</td>
</tr>
<tr>
<td>$K_a$</td>
<td>biomass absorption coefficient (m$^2$ g$^{-1}$ biomass)</td>
</tr>
<tr>
<td>$K_1$</td>
<td>fitting parameter in the light affinity term in Eq. 12</td>
</tr>
<tr>
<td>$K_s$</td>
<td>affinity constant in Eq. 11</td>
</tr>
<tr>
<td>$L$</td>
<td>total length of light path (m)</td>
</tr>
<tr>
<td>$m$</td>
<td>specific maintenance rate (h$^{-1}$)</td>
</tr>
<tr>
<td>$n_1$</td>
<td>fitting parameter in the light affinity term in Eq. 12</td>
</tr>
<tr>
<td>$n_2$</td>
<td>fitting parameter in the apparent reaction order term in Eq. 12</td>
</tr>
<tr>
<td>$P$</td>
<td>length of light path inside the photobioreactor (m)</td>
</tr>
<tr>
<td>$P_b$</td>
<td>biomass productivity (mg l$^{-1}$ h$^{-1}$)</td>
</tr>
<tr>
<td>$R$</td>
<td>photobioreactor vessel radius (m)</td>
</tr>
<tr>
<td>$R_s$</td>
<td>radius of the fictitious emitting surface (m)</td>
</tr>
<tr>
<td>$r_o$</td>
<td>radial coordinate defining a point inside the vessel</td>
</tr>
<tr>
<td>$S$</td>
<td>distance from vessel surface to an internal point (m)</td>
</tr>
<tr>
<td>$V_i$</td>
<td>volume element in the reactor</td>
</tr>
<tr>
<td>$V_T$</td>
<td>total photobioreactor volume</td>
</tr>
<tr>
<td>$X_p$</td>
<td>total pigment content (mass fraction)</td>
</tr>
<tr>
<td>$z$</td>
<td>axial coordinate defining a point height on the fictitious emitting surface used in the three-dimensional irradiance distribution model</td>
</tr>
<tr>
<td>$z_o$</td>
<td>axial coordinate defining a point inside the photobioreactor</td>
</tr>
<tr>
<td>$\phi$</td>
<td>angle of incidence of the light ray in the bidimensional model (radians)</td>
</tr>
<tr>
<td>$\varphi$</td>
<td>angle of incidence of the light ray in the three-dimensional model (radians)</td>
</tr>
<tr>
<td>$\mu$</td>
<td>specific growth rate (h$^{-1}$)</td>
</tr>
<tr>
<td>$\mu_{max}$</td>
<td>maximum specific growth rate (h$^{-1}$)</td>
</tr>
<tr>
<td>$\rho$, $\rho(r_o,z_o)$</td>
<td>point inside the culture vessel at an $r_o$ radial coordinate and $z_o$ height</td>
</tr>
</tbody>
</table>

References


