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A Mathematical Model of Microalgal Growth in Light-Limited Chemostat Culture

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Abstract: A mathematical model for light-limited growth of a continuous microalgal culture is proposed. Average light intensity inside the photobioreactor was calculated, taking light attenuation by algae into account. The biomass absorption coefficient was determined by means of two new parameters, the pigment-free biomass, Y_B, and the total pigment absorption, Y_p (absorption coefficient normalized to total pigment content). The model reproduced the steady states reached and the dynamic behavior of the system when the dilution rate was changed.

Key words: microalga, growth model, light-limitation.

NOTATION

C	Biomass concentration (mg dm^{-3} or g m^{-3})
D	Dilution rate (h^{-1})
I, $I(C, X_p)$	Mean light intensity (quanta cm^{-2} s^{-2})
I_k	Constant representing the affinity of algae to light (quanta cm^{-2} s^{-1})
I_0	Incident light intensity on culture surface (quanta cm^{-2} s^{-1})
$I(p)$, $I(S, C)$	Light intensity at given point inside the photobioreactor (quanta cm^{-2} s^{-1})
K_a	Biomass absorption coefficient (m^2 g^{-1} biomass)
m	Specific maintenance rate (h^{-1})
p	Length of light path inside the photobioreactor (m)
R	Vessel radius (m)
S	Distance from vessel surface to an internal point (m)
X_p	Mass fraction of total pigment content
Y_b	Absorption coefficient normalized to pigment-free biomass (m^2 g^{-1})
Y'_p	Absorption coefficient normalized to total pigment content (m^2 g^{-1})
μ	Specific growth rate (h^{-1})
μ_{max}	Maximum specific growth rate (h^{-1})
ϕ	Angle of incidence of the light path

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1 INTRODUCTION

Economic and scientific interest in microalgae is increasing as they are considered to be potential sources of highly valuable products in the pharmaceutical and dietetics industry. With this objective, closed microalgae culture systems are reaching the same level of technology as other fermentation systems, especially since the light required to support photoautotrophic growth is an additional factor. Algal biomass production is generally limited by light and cultivation of microalgae is faced with problems of light attenuation and the light harvesting capacity.[1] In well-mixed dense cultures of microalgae, where light attenuation takes place a few centimeters below the culture surface and light distribution within the photobioreactor is not homogeneous, average light intensity must be considered, as the algae adapt.[2,3]

In this context, the study of the light-limited kinetics has made modelling light distribution inside the culture necessary.[4] One parameter strongly affecting the calculation of the real average light intensity is the biomass absorption coefficient, which is based on Lambert–Beer's law. Estimation of the coefficient depends on both chlorophyll *a* content,[1,15] and on other pigments and light-absorbing molecules.[6] Therefore, this coefficient must be determined accurately.

In the present work, a model of light-limited growth of microalgae in a chemostat culture which takes into account light distribution in the vessel is presented.

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2 EXPERIMENTAL

2.1 Organism

The microalga used was an isolate (labelled II-4) selected from among 42 isolates from a single strain of *Isochrysis galbana* in a phenotypic selection program carried out in our laboratory to select a strain rich in eicosapentaenoic acid (EPA).[7,8]

2.2 Growth conditions

Cultures were performed in a 5-dm^3 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 were as described by Molina *et al.*[9] Medium nutrients were provided in excess.

The cultures were constantly illuminated with four Osram Dulux EL (20 W) fluorescent lamps arranged around the culture vessel. Incident light intensity on the culture surface was measured with a Biospherical Instruments laboratory Quantum Scalar Irradiance Meter (QSL-100).

All growth experiments were carried out at a temperature of 20°C. The air supply was sterilized by filtration through 0·22 μm Millipore filters at a rate of 1·5 $dm^3\ min^{-1}$ with agitation at 150 rev min^{-1}. The pH was maintained constant at pH 8 by pure CO_2 injection.

2.3 Analytical methods

Chlorophylls were measured according to the method of Hansmann.[10] Carotenoid determination was carried out as described by Whyte.[11]

2.4 Biomass absorption coefficient (K_a)

The K_a of the *I. galbana* culture was determined by measuring the absorbance of six cultures with different pigment contents at 12 biomass concentrations between 50 and 1100 mg dm^{-3}. Light intensity in the center of a cylindrical vessel (4·9 cm diameter) illuminated from all directions was measured with the QSL-100 instrument for each biomass concentration analyzed. Absorbance was calculated using Lambert–Beer's law. The absorption coefficients were calculated by dividing the slopes of lines of absorbance over biomass concentration by the vessel radius.

$$\ln\left[\frac{I_0}{I}\right] = K_a RC \qquad (1)$$

3 RESULTS AND DISCUSSION

Continuous cultures of microalgae are characterized by the steady states produced by the photosynthetic activity. Carbon dioxide and light availability determine biomass concentration at any dilution rate, when other nutrients are in excess. In a chemostat, the relationship between dilution rate (or specific growth rate) and the steady-state biomass concentration resulting from mutual shading in light-limited growth is well-known.[3,12] In a previous study, a combination of carbon and light limitations at dilution rates from 0·006 h^{-1} to 0·0377 h^{-1} were found to give rise to biomass concentrations and productivities ranging from 525 mg dm^{-3} to 144 mg dm^{-3} and from 3·15 mg $dm^{-3}\ h^{-1}$ to 7·43 mg $dm^{-3}\ h^{-1}$, respectively.[3]

A new set of experiments preventing carbon limitation with the pH fixed at 8 by CO_2 injection and with dilution rates ranging from 0·0024 h^{-1} to 0·0377 h^{-1} has been performed. The results indicated an increase in biomass concentration and productivity (Fig. 1). The maximum biomass concentration achieved was 1024 mg dm^{-3} and the maximum productivity was 13·2 mg $dm^{-3}\ h^{-1}$ at $D = 0{\cdot}0024\ h^{-1}$ and $D = 0{\cdot}029\ h^{-1}$, respectively. The limiting factor within the dilution rate range examined was probably light availability.

The difference between the steady-state biomass concentrations in the two sets of experiments diminishes at increasing dilution rates. Therefore, the limiting factor has less influence as growth rate increases. Thus, at $D = 0{\cdot}035\ h^{-1}$ and $0{\cdot}0377\ h^{-1}$, close to the maximum specific growth rate (wash-out) where a small change in D gives considerable variation in biomass concentration, the steady-state biomass concentration was approximately the same in both cases. In this state, there was continuous exponential growth and the algae grew without limitation by external conditions.

Differences in biomass productivity between pH-uncontrolled and pH-controlled experiments increased with D up to $D = 0{\cdot}029\ h^{-1}$ and, analogous with biomass concentration, productivity was similar at the highest dilution rate. Therefore, by means of CO_2 injection, biomass productivity was approximately double that obtained when atmospheric CO_2 was used. This additional supply of carbon also had an effect on biomass composition as the C/N ratio in the culture medium increased.

3.1 Light distribution

In dense cultures, the gradient of light varies along the radius of the culture vessel because of light attenuation. Furthermore, as physiological adaptation of the algae seems to respond to the average light intensity to which they are exposed,[2,3] this must be calculated. In general, light intensity at a given point inside the photobioreactor depends on the path length of light to this point, p, biomass concentration, C, and on light absorption by the algae, that is to say, the biomass absorption coefficient, K_a. Assuming that the light attenuation obeys Lambert–Beer's Law then:

$$I(p) = I_0 \exp(-pK_a C) \qquad (2)$$

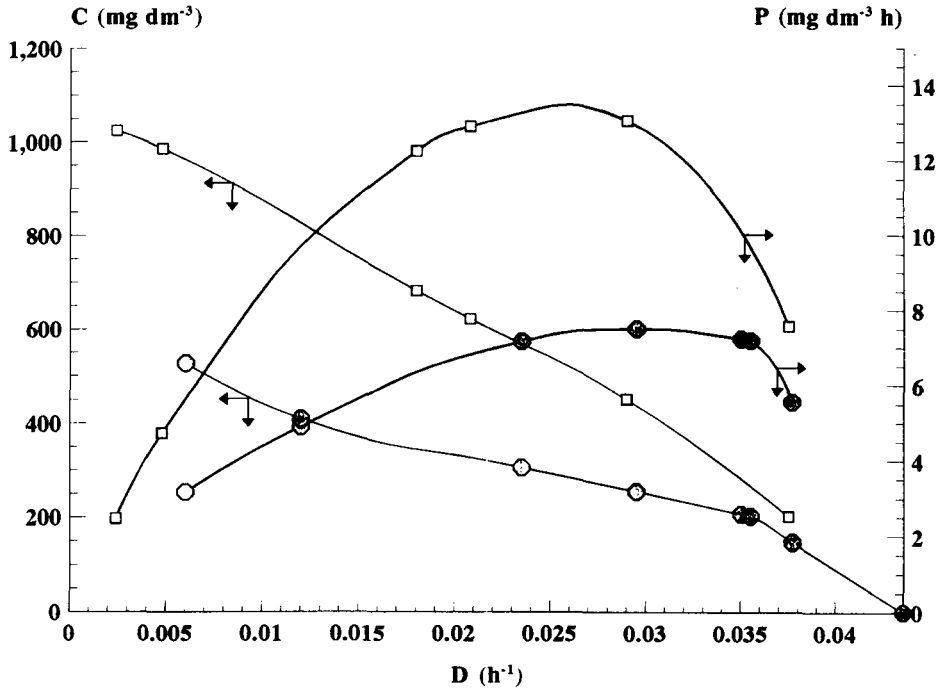

Fig. 1. Effect of dilution rate, D, on steady-state biomass concentration of *Isochrysis galbana* and productivity, P, when grown in light-limited chemostat culture: —○— pH-uncontrolled; —□— pH-controlled.

Using this law, Evers[4] described the distribution of light for a cylindrical vessel illuminated evenly from all sides and obtained the following expression for light intensity at a point inside the culture:

$$I(S, C) = \frac{I_0}{\pi} \int_0^{\pi} \exp\{-K_a C[(R - S)\cos\phi + [R^2 - (R - S)^2 \sin^2\phi]^{0.5}]\} \quad (3)$$

3.2 Absorption coefficient

The attenuation of light through the culture depends mainly on the algal absorption coefficient, K_a. Previously, K_a has been seen to be constant in each species, although several authors have reported that K_a strongly depended on the pigment content of the biomass.[1,5] Even for natural phytoplankton populations which usually occur several orders of magnitude more diluted than in mass culture, a constant value for the product of $K_a C$ has been assumed (usually when C is expressed as chlorophyll concentration).[1,3] In mass cultures, in which C is usually expressed in biomass dry weight and varies considerably, there is little rationale for assuming a constant value of $K_a C$.

As a new approach, light attenuation in six cultures of the same strain of *I. galbana* grown under different light conditions has been measured, with different pigment contents, in order to calculate the variation in the absorption coefficient. The results obtained are shown in Fig. 2 and details are given in Table 1. As the pigment content was lowered, K_a decreased by up to 100%. By plotting K_a as a function of pigment content, X_p (Fig. 3), a linear relationship is obtained, making application of a linear regression possible, and giving the equation:

$$K_a = Y'_p X_p + Y_b \quad (4)$$

TABLE 1
Influence of the Pigment Content of Microalgal Biomass on the Light Absorption Coefficient, K_a

	Mass fraction of total pigments ($X_p \times 10^2$)					
	·29	*2·07*	*2·09*	*2·33*	*3·51*	*3·65*
Chlorophyll *a* (mass fraction 10^2)	0·79	1·22	1·31	1·52	2·09	2·12
Chlorophyll *c* (mass fraction 10^2)	0·16	0·34	0·35	0·39	0·58	0·56
Carotenoids (mass fraction 10^2)	0·34	0·53	0·44	0·42	0·85	0·87
$K_a \times 10^2$ ($m^2\ g^{-1}$ biomass)	3·755	5·898	5·898	6·743	7·959	8·163
r^2	0·9996	0·9999	0·9999	0·9995	0·9992	0·9986

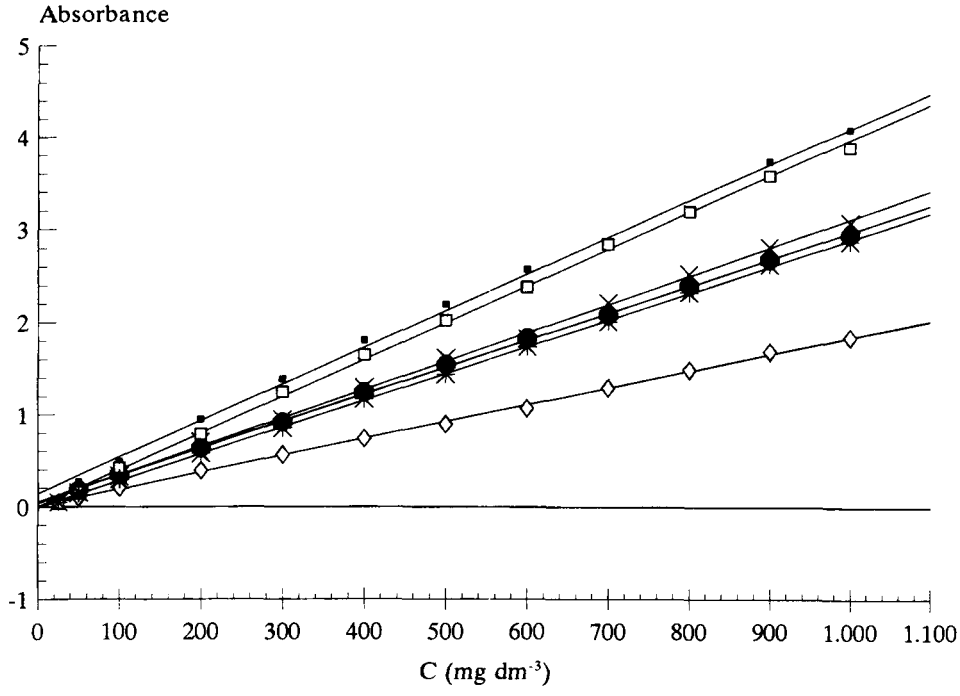

Fig. 2. Influence of biomass concentration of *Isochrysis galbana* with different pigment composition on culture absorbance. ✳ 2·074; —□— 3·50; —■— 3·65; —●— 2·09; ✳ 2·33; —◇— $1{\cdot}28X_p \cdot 10^2$.

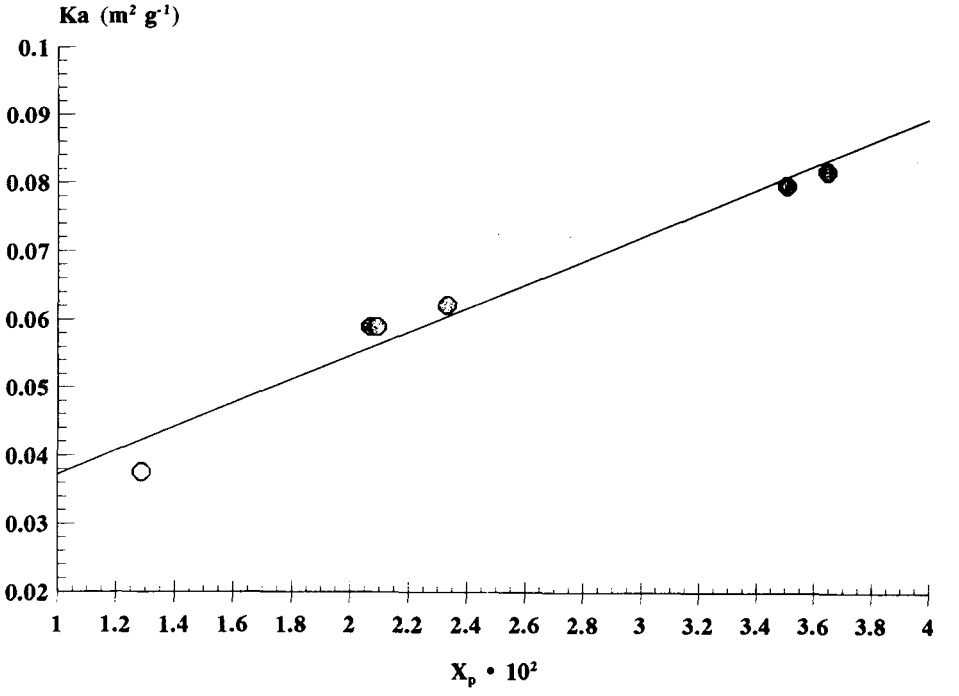

Fig. 3. Variation in biomass extension coefficient, K_a, with total pigment mass fraction, X_p, of *Isochrysis galbana*.

where $Y_b = 0{\cdot}0199\ m^2\ g^{-1}$ (biomass) and $Y'_p = 1{\cdot}7356\ m^2\ g^{-1}$ (pigments); $r^2 = 0{\cdot}967$.

Therefore, the overall absorption coefficient was not constant and may also be considered as the result of two contributing factors, pigment-free biomass, Y_b, and pigment absorption, Y'_p (absorption coefficient normalized to total pigment content). The contribution of Y_b to biomass absorption coefficient, K_a, varied from 47% to 24% for X_p values of $1{\cdot}29 \times 10^{-2}$ and $3{\cdot}65 \times 10^{-2}$, respectively.

The extinction coefficient normalized to chlorophyll *a*, K'_a is commonly used instead of Y'_p. Such an assumption considers attenuation of light in the culture as exclusively dependent on chlorophyll *a* concentration, ignoring the

contribution of the various accessory pigments and other light-absorbing molecules.[6] However, with eqn (4), two specific species parameters are obtained, Y'_p and Y_b, which justify the interspecific variability of K'_a. In agreement with Sathyendranath *et al.*,[14] more than 95% of this variability may thus be explained by variations in pigment composition and, to a lesser extent, by the effects of particle size.

3.3 Mean light intensity

Equation (3) gives the light intensity at a point inside the culture at a distance, S, from the surface. To obtain the average light intensity for the whole of the photobioreactor, eqn (3) is integrated between $0 < S \leqslant R$, obtaining the following expression:

$$I(C, X_p) = \frac{I_0}{\pi R} \int_0^R \int_0^\pi \exp\{-K_a C[(R - S)\cos\phi + [R^2 - (R - S)^2 \sin^2\phi]^{0.5}]\}\, d\phi\, dS \qquad (5)$$

where the mean light intensity is now a function of the biomass concentration and pigment content, bearing in mind the relationship between K_a and X_p (eqn 4). In Fig. 4, a three-dimensional graph represents the surface (I, C, X_p) on which experimental points are located.

3.4 Growth modelling

Several models have been suggested to describe the relationship between specific growth rate and light intensity. In this study, the hyperbolic model was modified by an exponent, n, similar to that of Moser[15] for nutrient-limited cultures and analogous to Bannister's 'shape parameter' describing the abruptness of the transition from weakly-illuminated to strongly-illuminated regions:[16]

$$\mu = \frac{\mu_{max} I^n}{I^n + I_k^n} \qquad (6)$$

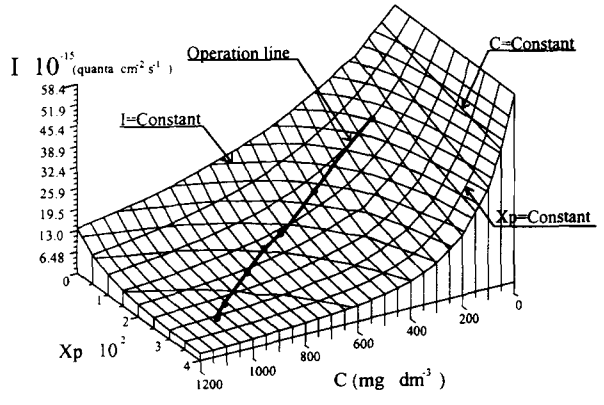

Fig. 4. Operation line localization and experimental data on possible response surface (I, X_p, C) of system.

Assuming perfext mixing and also taking into account that part of the absorbed energy can be utilized for algal maintenance, the following expression is obtained by means of a steady state material balance in a chemostat in which μ is expressed by eqn. (6):

$$D = \frac{\mu_{max} I^n}{I^n + I_k^n} - m \qquad (7)$$

The experimentally determined specific maintenance rates in the above equation was 0·00385 h^{-1}.[3]

As average light intensity and, consequently, the specific growth rate, depend on C and X_p the same steady state (D, C) could be reached with different (C, X_p). However, a univocal relationship between D and C was found here. The same steady-state biomass concentration was reached when a given dilution rate was used, regardless of time or growth status. Thus, as incident light and vessel geometry remained constant, a relationship between steady-state biomass concentration and pigment content must exist. So, for any given value of C, the solution of eqn (5) will result in an unique value for I. With the present system and conditions, this relationship may be expressed by the following polynomial:

$$\left.\begin{aligned} X_p &= 1{\cdot}12 \times 10^{-2} - 8{\cdot}6 \times 10^{-6} C + 2{\cdot}6 \times 10^{-6} C^2 \\ r^2 &= 0{\cdot}992 \end{aligned}\right\} \qquad (8)$$

Therefore, by introducing eqn (8) in $I = I(C, X_p)$, the function $I = I(C)$ is obtained. This is termed the operation line which, in Fig. 4, represents the geometric space where all possible steady-state points are located. The operation line is also the projection of eqn (8) on the (I, C, X_p) response surface (see Fig. 4).

Starting with C, X_p and D in the experiment, (D, I) is obtained by application of eqnas (5) and (8), which when adjusted by non-linear regression to eqn (7), give the values for the parameters and the regression coefficient is:

$$\mu_{max} = 0{\cdot}046\ h^{-1};\ I_k = 9{\cdot}67 \times 10^{15}\ \text{quanta cm}^{-2};$$
$$n = 1{\cdot}7;\quad r^2 = 0{\cdot}9975$$

The fit can be judged by the regression coefficient. Furthermore, μ_{max} and I_k provide reasonable estimates of the maximum specific growth rate and saturation constant, as commonly used in the Monod model. Experimental data and theoretical values obtained with eqn (7) are shown in Fig. 5.

3.5 Dynamic behavior

The model also explains the dynamics in transitory states when dilution rate is changed during the development of the culture from a first to a second steady state. In non-steady state, biomass change may be expressed with regard to time by balacing mass in the chemostat, thus:

$$\frac{dC}{dt} = C(\mu - m - D) = C\left(\frac{\mu_{max} I^n}{I^n + I_k^n} - m - D\right) \qquad (9)$$

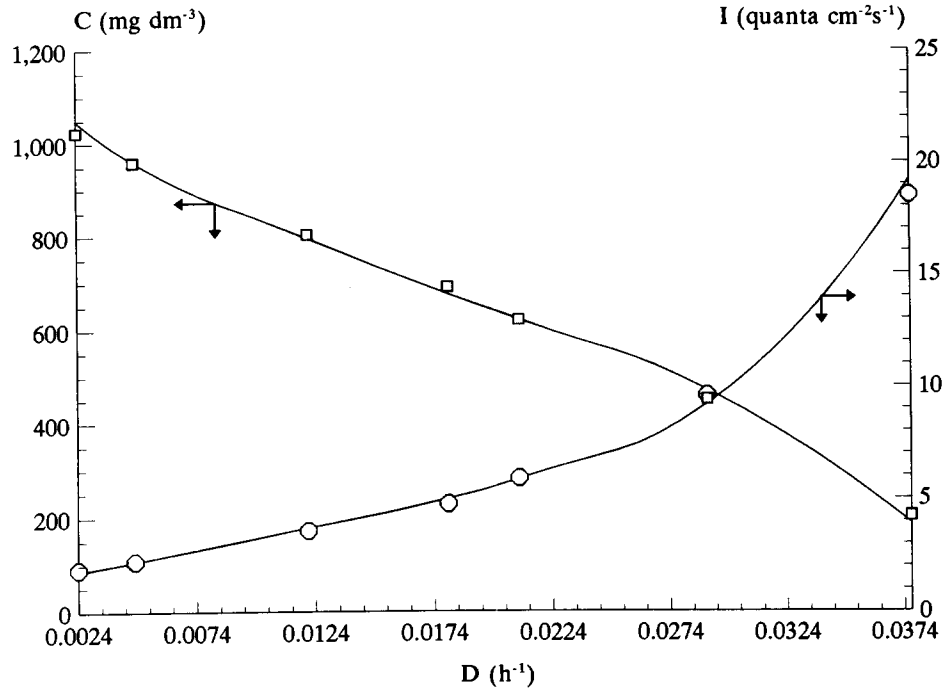

Fig. 5. Variation in biomass concentration, C, of *Isochrysis galbana* and mean light intensity, I, with dilution rate, D. Solid lines represent theoretical values.

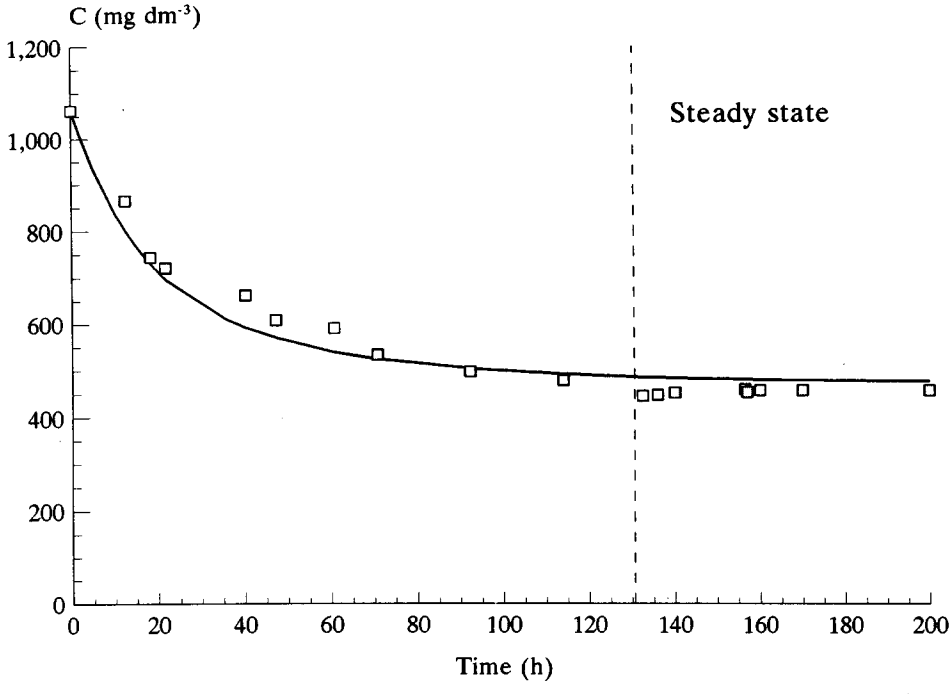

Fig. 6. Biomass concentration evolution with time for a change of dilution rate from $D = 0{\cdot}0024\ h^{-1}$ to $D = 0{\cdot}029\ h^{-1}$.

By applying the Runge–Kutta 4th order numeric and integration method, the theoretical values of C with time are obtained. As an illustration of a transitory state from $D = 0{\cdot}0024\ h^{-1}$ to $D = 0{\cdot}029\ h^{-1}$, Fig. 6 presents variations in C with time, with $\pm 10\%$ deviation. System behavior was seen to be similar to nutrient-limited chemostat cultures. That is, steady-state operation was only possible if any deviation from the dilution rate at stationary level was compensated for by a change in biomass concentration. In this way, transition time between two dilution rates can be predicted, as well as the biomass concentration reached at each steady state.

Thus, during transitions, eqn (8) can easily be used because the time scale of environmental changes seems to

be comparable to the time scale for biological response (i.e. under low light, cells tend to accumulate more photosynthetic pigments). Otherwise, the kinetics model should be expanded to include more components of the cell phase.

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