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A simple equation to quantify the effect of frequency of light/dark cycles on the photosynthetic response of microalgae under intermittent light

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ABSTRACT

Accurately assessing the influence of light/dark changes on the productivity of microalgal cultures is currently a complicated task that requires the use of a set of differential equations. A simple, algebraic equation that allows quantifying the effect of the frequency of light on the photosynthetic response of microalgae under varying light is presented and evaluated. The equation was derived from a particular case of the mechanistic model proposed by Camacho Rubio et al. (2003) [1]. The algebraic equation is demonstrated in this work to be a rapid method that allows characterizing the dynamic photosynthetic response of *Muriellopsis* sp. The parameters obtained using this method ($P_{max} = 8.22{\cdot}10^{-7}$ mol O_2 g^{-1} s^{-1}, $\alpha = 1.82{\cdot}10^{-4}$ mol photon m^{-2} s^{-1}, $\beta = 15.3$ s^{-1}, $\kappa = 0.0402$, at 25 °C, where P_{max} is the maximum rate of photosynthesis, α is the saturation constant, β is the characteristic frequency and κ is a shape factor of the photosynthesis-irradiance curve) are very close to the values obtained using the original model proposed by Camacho Rubio et al., which is based on differential equations. The proposed new equation requires a reduced set of experiments that can be carried out under easy-to-attain conditions (continuous light and short flashes), and a straightforward, subsequent calculation of the dynamic parameters of photosynthesis. It can be used as a time-saving, efficient method to optimize the light regime for microalgal production.

1. Introduction

In the last few years, microalgae have raised great expectations as a possible source for sustainable biofuel production [2,3]. As photoautotrophs, microalgae rely only on sunlight to produce biomass, while simultaneously taking up CO_2 from the atmosphere, thus ameliorating the concern for global warming. Microalgae have the potential for very rapid growth and thus high biomass productivity that, on an areal basis, can be several times higher than the most productive crops. Mainly for these reasons, microalgae have been the object of intense research by agents such as oil companies, which have regarded microalgae cultures as a potential feedstock for the fuel industry.

In spite of all of these advantages, at present the general agreement is that there are significant hurdles in the basic science that will prevent the imminent realization of microalgae-derived oils [4]. However, industry and researchers have gained substantial understanding on the problems that need to be dealt with in order to bring about microalgae biofuel in the future. One of the conclusions is that, after extensive research, none of the wild strains that naturally thrive on CO_2 and sunlight or those available from culture collections can provide a level of performance that is sufficient for commercial oil production. Simple modifications of these available strains have also proved to be insufficient for raising performance up to economic levels.

The basic problem is the lack of microalgal strains that can reproduce quickly enough while yielding large concentrations of oils. Currently available strains with high lipid content have low growth rates or are unable to grow in dense cultures, leading to low oil productivity. For instance, Chisti [2], in a pioneering work, assumed lipid contents of up to 70% d.wt. in order to estimate the potential of microalgae as biofuel producers. These figures have been revealed unrealistic, especially when coupled with biomass productivities that have only been observed in strains with much lower lipid content.

It is clear that overcoming these difficulties will require more comprehensive changes at a metabolic level for the microalgae to accumulate higher amounts of lipids while maintaining high biomass productivity. Moreover, these microalgae will have to be able to grow fast in dense cultures, which is a must in order to attain a high areal productivity and to achieve an efficient use of light. In dense cultures the distribution of light is heterogeneous and the microalgae moving between light and dark zones have more difficulties in sustaining growth than under continuous illumination [5]. Actually, selecting potential overproducing species under continuous light conditions can

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Abbreviations

a	total concentration of PSUs, mol g^{-1}
a^*	concentration of activated PSUs, mol g^{-1}
C_b	biomass concentration, g m^{-3}
D	dilution rate, h^{-1}
D_{opt}	optimal dilution rate, h^{-1}
I	irradiance, mol $m^{-2} s^{-1} \equiv E\, m^{-2} s^{-1}$
I_{av}	average irradiance, mol $m^{-2} s^{-1} \equiv E\, m^{-2} s^{-1}$
Io	external or incident irradiance, mol $m^{-2} s^{-1} \equiv E\, m^{-2} s^{-1}$
k	proportionality constant defined by Eq. (3), –
k_a	absorption or extinction coefficient of the PSUs, m^2 mol^{-1} or m^2 g^{-1}
K_a	absorption or extinction coefficient of the biomass, m^2 mol^{-1} or m^2 g^{-1}
K_s^*	concentration of activated PSUs for which $P = P_{max}/2$, mol g^{-1}
L	optical path, m
m	maintenance rate, mol $g^{-1} s^{-1}$
P	rate of photosynthesis for intermittent illumination, mol $g^{-1} s^{-1}$
P_{cont}	rate of photosynthesis for continuous illumination, mol $g^{-1} s^{-1}$
P_{max}	maximum rate of photosynthesis, mol $g^{-1} s^{-1}$
PSF	photosynthesis factory, –
PSU	photosynthesis unit, –
R	radius of a cylindrical photobioreactor, m
r	rate of consumption of photochemical energy, mol $g^{-1} s^{-1}$
r_m^*	maximum rate of energy consumption, mol $g^{-1} s^{-1}$
t	time, s
t_c	cycle time, duration of the light/dark cycle, s
t_d	cycle time, duration of the dark phase, s
t_c	cycle time, duration of the light phase, s
x^*	fraction of functional activated PSUs, –
x_2	fraction of activated PSFs, –
x_e^*	steady-state value of x^* for continuous illumination, –
x_{min}^*	minimum value of x^* under light-dark cycling, –

Greek letters

α	saturation constant defined by Eq. (4), mol $m^{-2} s^{-1} \equiv E\, m^{-2} s^{-1}$
β	characteristic frequency defined by Eq. (4), Hz ≡ s^{-1}
$\beta_{0.05}$	characteristic frequency for φ = 0.1, Hz ≡ s^{-1}
$\beta_{0.1}$	characteristic frequency for φ = 0.05, Hz ≡ s^{-1}
γ	specific rate constant of PSF deactivation, s^{-1}
κ	shape factor of the *P-I* curve defined by Eq. (4), –
μ	specific growth rate, h^{-1}
μ_{max}	maximum specific growth rate, h^{-1}
ν	light/dark frequency, Hz ≡ s^{-1}
τ	dimensionless time ($=t\nu$), –
φ	duty cycle, illuminated fraction of the cycle ($=I_{av}/I_o$), –

be severely misleading when up-scaling to commercial size. Enhancing the mixing degree in photobioreactors and adequately quantifying its effect on the frequency of light/dark cycles is one key factor [6], but in order to properly assess the growth potential of a particular microalgal strain in dense cultures it is necessary to take into account the dynamics of photosynthesis to attain a "light-integrating" situation as described by Terry [7]. The characterization of the dynamic photosynthetic response is most comprehensively attained with the use of dynamic photosynthesis models, such as those proposed by Camacho Rubio et al. [1] or Eilers and Peeters [8]. The use of such models, together with the fluid-dynamics characterization of the culture system, allows estimating the photosynthetic response of a microalgal strain in a real culture system and thus is a more reliable evaluation [9]. The downside is that the use of such dynamic models requires knowing their characteristic parameters, which is usually time-consuming.

This work presents a simple, algebraic equation that allows the quantification of the effect of frequency of varying light on the photosynthetic response of microalgae and thus can be used as a growth model that explicitly takes the frequency of light variations into account. As a demonstration, the equation is used to devise a simplified method to obtain the characteristic parameters of this dynamic model of photosynthesis for a given microalgal strain and thus, a full characterization of its growth potential in dense cultures. The photosynthetic parameters thus obtained also provide some insight regarding the influence of biochemical profiles of microorganisms on growth rate, and thus allow hinting which characteristics should be sought to ensure a high growth potential.

2. Materials and methods

2.1. Microorganism

The microorganism used to obtain the photosynthesis-irradiance (*P-I*) data was *Muriellopsis* sp. (CCAG). The culture medium was prepared according to Arnon et al. [10], and the microorganism was grown in a photobioreactor operated under semi-continuous mode. This setup provided algal biomass in an optimal steady-state condition. All *P-I* experiments presented in this work were performed in a tank (a detailed description of the tank may be found in Brindley et al. [11]) containing diluted samples of the steady-state culture.

2.2. Experimental materials

The photobioreactor used to culture the microorganism was a 1.8-l bubble column in which air was sparged from the bottom of the column at 0.2 v v^{-1} min^{-1} and carbon dioxide was injected into the air flow on demand using pH control (pH = 8). Three fluorescent lamps placed around the column produced a 12 h/12 h light/dark cycle. Irradiance inside the photobioreactor was measured by placing a quantum scalar irradiance sensor (QSL-100; Biospherical Instruments, San Diego, CA) at the center of the column containing cell-free culture medium. Thus, the maximum irradiance measured in the photobioreactor was 1000 μE $m^{-2} s^{-1}$. The temperature of the culture was maintained at 25 °C and the average biomass concentration was 3 g l^{-1}, with a dilution rate of ca. 0.2 d^{-1}.

2.3. Photosynthetic activity measurements

The data presented in this work are the photosynthetic responses to well-defined light regimes of the microalgae studied. The photosynthetic activity was derived from dissolved O_2 evolution measurements, as described in detail by Brindley et al. [11].

For each experiment, samples were removed from the photobioreactor only during the light period (from 5.00 a.m. to 5.00 p.m.) and, in particular, during the interval from 10.00 a.m. to 3.00 p.m. because the measurements from this period were verified to be the most reproducible in terms of specific photosynthetic response and biomass concentration [11,12].

To carry out the *P-I* experiments, samples were removed from the photobioreactor and divided into 2- and 7-ml aliquots. The 7-ml aliquots were placed in the tank and diluted by a factor of 30 with culture medium, thus avoiding mutual shading of cells during oxygen evolution