Preprints of the 2012 20th Mediterranean Conference on
Control & Automation (MED), Barcelona, Spain, July 3-6,
2012

Interaction between time scales in microalgae based processes

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Abstract— **Microalgae are often seen as a potential biofuel producer. In order to predict achievable productivities in the so called raceway culturing system, the dynamics of photosynthesis has to be taken into account. In particular, the dynamical effect of inhibition by an excess of light (photoinhibition) must be represented. We propose a model considering both photosynthesis and growth dynamics. This model involves three different time scales. We study the response of this model to fluctuating light with different frequencies by slow fast approximations. Therefore, we identify three different regimes for which a simplified expression for the model can be derived. These expressions give a hint on productivity improvement which can be expected by stimulating photosynthesis with a faster hydrodynamics.**

I. INTRODUCTION

Microalgae have received a specific attention in the framework of biodiesel production and renewable energy generation since a decade. Their high actual photosynthetic yield compared to terrestrial plants (whose growth is limited by CO_2 availability and access to nutrients) lead to large potential algal biomass productions which is orders of magnitudes higher than biofuel from field crops [27].

After a nitrogen limitation, this biomass can reach a very high lipid content (up to 60% of dry weight [17]). These possibilities have led some authors to consider that microalgae could be one of the main biofuel producers in the future [14], [7]. Moreover, the ability of microalgae to fix CO_2 in a controlled way opens up applications in mitigation systems [1], [18]. Microalgal biofuel production systems could therefore be associated with industrial powerplants with a high CO_2 production. In the same spirit, microalgae could be used to consume inorganic nitrogen and phosphorus, and improve wastewater treatment technology.

These advantages put microalgae in a good position for renewable energy production at large scale [7]. In the coming years there might be large scale industrial plants to produce microalgae. However, the culture of algae is not straightforward and suffers from many limitations [21], [5]. Among them, the paradox of microalgae is that their growth is often reduced because of an excess of light which inhibits the photosynthesis process. As a consequence, productivity is often under its optimal value. Better understanding photoinhibition and therefore improving growth efficiency of the algae is therefore a key issue. The dynamics of photoinhibition has been described by models [10], [11], [13]. However, its effect on the long term, especially in concordance with the light signal induced by succession of high and low light [20] has not been investigated. Here we propose a new model which couples Han photoinhibition dynamical model with Droop's model [8] to account for the interaction between photoinhibition mechanisms (at the scale of the second) and the growth process (at the scale of the day). Droop's model [8], [9] has been widely studied and proved to accurately reproduce situations of nitrogen limitations [9], [23], [4]. The Droop model has been validated for the prediction of microalgal growth in the case of nitrogen limitation for biodiesel production [16].

In order to better understand the photoinhibition effect, we derive an analytical approximation of the Droop-Han model depending on the Light Dark (LD) frequency. It highlights the dependency of productivity with respect to light frequency. The analytical expression is also used to calibrate the model with experimental data and we use it to determine the physiological parameters of the model. Further, the approximation can be a basis for a simple description of the photosynthesis response to variability in light signals, i.e. as experimented by the microalgal cells in turbulent hydrodynamical regimes. For each frequency of light variation, the model predicts productivity in response to light (named PI-response) with a shape comparable to the shape of the model of Haldane [19]. The predicted productivity is strongly increased for faster light fluctuation, which coincides well with the findings of [6]. Further, it is remarkable that the characteristic shape of the dependency of productivity to fluctuation frequency can be easily utilized to verify the model experimentally.

This paper is organized as follows. In a first part, we recall the Droop model. Then we introduce the light influence in this model. In a third part we propose analytic approximations of the model for the whole frequency range. In the last part, the applicability of the model and the approximations are discussed.

II. THE DROOP MODEL

The Droop model has been proven to appropriately represent the effect of macronutrients, such as nitrogen on the growth rate of microalgae [9]. The Droop model is known to predict a unique equilibrium in the interior domain, if the culturing conditions (i.e. influent concentration of nitrogen s_{in} and dilution rate D) are kept constant [15], [3]. In contrast to the simpler Monod Model, Droop considers a dependence of growth on the intracellular nitrogen concentration or quota q. This defines nutrient uptake and growth as uncoupled

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process. In the following, the differential equations define a modified version of Droop's model (as in [2]) representing the evolution of the biomass (x) with an inorganic nitrogen substrate s.

$$(D)\begin{cases}\dot{s} = Ds_{in} - \rho(s)\cdot(1-q/Q_l)x - Ds \\ \dot{q} = \rho(s)\cdot(1-q/Q_l) - \mu(q)q \\ \dot{x} = \mu(q)x - Dx\end{cases} \tag{1}$$

In this model the absorption rate $\rho(s)$ and growth rate $\mu(q)$ are generally taken as Michaelis-Menten and Droop functions:

$$\rho(s) = \rho_0 \frac{s}{s+K_s} \tag{2}$$

$$\mu(q) = \mu_0(1-\frac{Q_0}{q}) \tag{3}$$

where K_s is the half saturation constant for substrate uptake and Q_0 the minimal cell quota. At the quota Q_0, the growth rate equals 0. In eq. 1, a factor $(1-q/Q_l)$ is multiplied with the term for nitrogen uptake in order to limit the internal quota to the interval

$$Q_0 \leq q \leq Q_m \leq Q_l \tag{4}$$

Q_m is the maximum cell quota which is obtained in conditions with no nutrient limitation. As a direct consequence, the growth rate is also bounded:

$$0 \leq \mu(q) < \mu_m = \mu_0(1-Q_0/Q_m) \tag{5}$$

where μ_m is the maximum growth rate reached in non limiting conditions. The parameters of the Droop model can be derived well with growth experiments under nutrient limiting conditions [2].

The Droop model has been widely studied [15], [3], [25] and validated [9], [23], [4], [25]. However, it cannot directly be used in the case of photobioreactors or raceways since it does not account for light intensity, and especially for the fast variation of light due to cell advection in a very turbid medium.

III. INTEGRATION OF THE FAST PSU TIME SCALE

A. Model presentation

At fast time scales (<1h), the dynamics of the photosynthetic process have to be taken into account. On a molecular scale three elementary components are involved in the photosynthetic process [24], that are two photosystem types (denoted PSI and PSII) linked by the electron transfer chain. The photosynthetic production is triggered by the simultaneous photoexcitation of both photocenters. Han [12] assumes in his model the activation of the second photosystem (PSII) to be the limiting factor in photosynthetic productivity. It identifies photodamage to and recovery of the PSII as a main factor of photoinhibition in the photosynthetic process. The PSII is assumed to be always in one of the three states: open (A), closed (B), or inhibited (C). Open Photosynthetic Units (PSU) may be excited by a photon, trigger the photosynthetic process, and turn to the closed state. The turnover rate for this process is the product of the PSII's cross section σ and the light intensity I. A closed PSU may return to an open state at the rate $\frac{1}{\tau}$, whereas τ represents the turnover time of the electron transfer chain. If facing excessive radiation, a closed PSU can also be destroyed by incident light. The rate for photoinhibition processes is defined by the product $k_d\sigma I$, being k_d the PSII's damage parameter. Inhibited PSUs can be repaired by chemical processes in the cell at a constant rate k_r. The equations for the three states consequently are:

$$\frac{dA}{dt} = -I\sigma A + \frac{B}{\tau} \tag{6}$$

$$\frac{dB}{dt} = I\sigma A - \frac{B}{\tau} + k_r C - k_d\sigma I B \tag{7}$$

$$\frac{dC}{dt} = -k_r C + k_d \sigma I B \tag{8}$$

A, B and C are the relative frequencies of the respective states, their sum is 1. Consequently, B can be replaced by $1-A-C$ and the System can be described completely by:

$$\frac{dA}{dt} = -I\sigma A + \frac{1-A-C}{\tau} \tag{9}$$

$$\frac{dC}{dt} = -k_r C + k_d\sigma I(1-A-C) \tag{10}$$

To couple this model with the Droop model, we consider that growth rate results from the product of the total cross section of the cell PSUs in open state (resulting thus from their number and size) and light. In line with the Droop model, we assume that the PSUs number and size is related to the internal nitrogen quota with a Droop relationship. In that spirit, the Droop relationship can be seen as a factor describing the total cross section of all PSUs. As a consequence, the total cross section of open PSUs is proportional to $\sigma(1-\frac{q_0}{q})A$. The growth rate in terms of inorganic carbon fixation rate is then proportional to the product of the light intensity I with the total cross section of the open photocenters.

$$\mu(I,A,q) = \mu_m(1-\frac{q_0}{q})\cdot\sigma I A \tag{11}$$

Where μ_m is a parameter such that $\mu_m\sigma I_0 A = \mu_0$ for a constant light I_0, and the associated fraction of open states A.

In the following considerations, only $2T$-periodic light is studied. Assuming state periodicity, remark that:

$$\int_{2T}\dot{A} = -\int_{2T} I\sigma A + \int_{2T}\frac{B}{\tau} = 0 \tag{12}$$

$$\Rightarrow \overline{IA}\sigma = \bar{B}/\tau \tag{13}$$

We define with $P(I,T)$ the average fraction of the photosynthetic productivity related to photosystems activity:

$$P(I,T) := \bar{B} = \overline{IA}\sigma\tau \tag{14}$$

Its value stays bounded between 0 and 1. In the following section, we will give a number of different approximations (denoted P_i) to analytically compute the function P in a special frequency domain.

IV. Model analysis

A. Motivation

In this section, we investigate the mathematical behaviour of the Droop-Han model under periodic forcing of light. It is worth noting that this model has a cascade structure, and that the Han part, associated to a faster time scale, is independent of the Droop model variables. As a consequence, we will first focus on the response of the Han model to a periodic light regime.

The Han model integrates two different time scales. The fast time scale (open/closed dynamics) is in the range of seconds and is related to the process of regular photosynthetic production. The slow time scale results from the inhibition processes (as defined by Han) and is in the range of minutes. The dynamics of nitrogen uptake and carbon accumulation according to the Droop model adds one more time scale of the day to the growth model. The model is thus a slow/fast system which can be approximated using singular perturbation theory. Based on this approach we will derive approximate solutions in the case of a periodic, rectangular light signal.

We expect that analytical solutions may better highlight the dependency of growth rate to LD cycle frequency. Moreover, we will also use the obtained relationships to identify parameters by fitting a simple analytical expression.

B. Slow-Fast-Approximation

We rewrite the Han model as a system with slow fast time scales:

$$\frac{dA}{dt} = -(\sigma I + 1/\tau)A + (1-C)/\tau \qquad (15)$$

$$\frac{dC}{dt} = k_d(-(k_r/k_d + \sigma I)C + \sigma I(1-A)) \qquad (16)$$

Indeed, using an exemplary parameter set (according to [28]):

$$k_r = 4.8 \cdot 10^{-4} s^{-1} \; k_d = 2.99 \cdot 10^{-4}$$

$$1/\tau = 0.1460 s^{-1} \; \sigma = 0.0019 m^2/(\mu mol)$$

$$I \in [0, 2000] \mu mol \; s^{-1} m^{-2}$$

System (16) has then a slow fast dynamics, with a ratio of the two time scales given by k_d. The dynamic of C is more than a factor 1000 slower that the dynamics of A. Although the considered parameters depend on the species, this key time scale property remains qualitatively true for any species due to the nature of the involved physiological processes.

The considered light signal is a consecutive series of Light-Dark periods with a length of $2T$. For sake of simplicity, the analytical computation has been carried out for light and dark periods of equal length. The outcome of this computation will support a simple measurement protocol in order to determine the model parameters from the growth rates under different fluctuation frequencies.

$$I(t) = \begin{cases} I_0, & \text{for } 0 < \text{t} < \text{T} \\ 0, & \text{for T} < \text{t} < 2\text{T} \end{cases} \qquad (17)$$

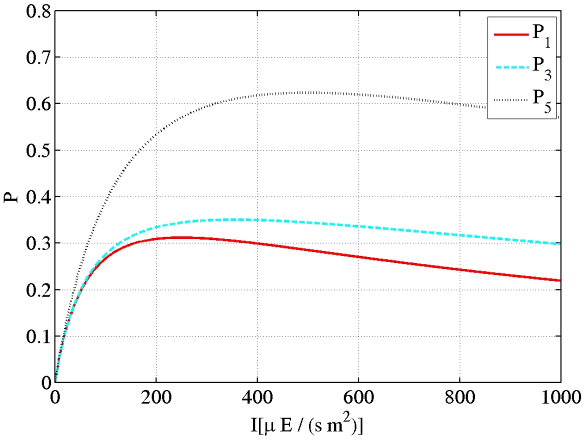

Fig. 1. PI-curves resulting for the approximations P_1 (red), P_3 (dashed cyan) and P_5 (dashed black)

LD cycles are a simple representation of the light signals occurring during mixing processes. The simplicity of the light signal allows an analytical integration of the system (which would also be possible for non equal light and dark durations, at the expense of additional computation). To integrate the model for different frequencies of the LD signal, two domains of the light variation frequency have to be distinguished: *slow* frequencies with $T > 10 \cdot \tau \approx 100s$, and *fast* frequencies with $T \leq 100s$. In the following paragraphs, we give the steady state solution of the Han model for continuous illumination and the approximations for the low and the high frequency assuming domains, as they are shown in figures 1 and 4. All approximations consider a forced periodic state of the system.

1) Steady State solution: For very low light frequencies ($T >> 10 \cdot 1/k_d \approx 10^5 s$), the Han model can reach its steady state. Consequently, A can be computed with respect to the long period where I is maintained constant at its value I_0 [10], and finally, $P(I,T)$ can be approximated by P_1:

$$P_1 = \frac{\sigma I_0 \tau}{2 \cdot (1 + \tau\sigma I_0 + k_d/k_r \tau(\sigma I_0)^2)} \qquad (18)$$

Note that, during the dark phase, productivity is 0.

2) Low frequencies: For low frequencies, the time for the fraction A to reach its equilibrium is too short compared to the cycle length, and A is assumed to hardly change. Only the dynamics of C has to be considered; A is considered to stay in an equilibrium distribution. Considering these properties, the following analytical approximation can be deduced:

$$P_2 = \left(1 + \frac{\delta_I}{k_r}\frac{1}{T}\frac{(1-\gamma(0))(1-\gamma(I_0))}{1-\gamma(0)\gamma(I_0)}\right) \cdot P_1 \qquad (19)$$

with

$$\delta_I = \frac{k_d \tau \frac{(\sigma I_0)^2}{1+\tau\sigma I_0}}{k_r + \frac{(\sigma I_0)^2}{1+\tau\sigma I_0}} \qquad (20)$$

$$\gamma(I) = e^{-(k_r + k_d \frac{(\sigma I)^2}{1+\tau\sigma I})T} \qquad (21)$$

Using the rule of de l'Hôpital, the limits for $T \to 0$ and $T \to \infty$ can be computed.

$$\lim_{T\to 0} P_2 = P_3 = \frac{\sigma I_0 \tau}{(2 + 2\tau\sigma I_0 + k_d/k_r \tau(\sigma I_0)^2)} \quad (22)$$

$$\lim_{T\to\infty} P_2 = P_1 \quad (23)$$

$$(24)$$

The limit for long periodicities is the steady state solution. That is not surprising and supports the coherence of the model. The limit for high frequencies delivers an increased productivity of the system for faster signals. However, this solution does not remain valid for values $T \leq 10{\cdot}\tau \approx 68,5s$, since the assumptions for the dynamics of A do not hold. P_3 and P_1 are shown on Figure 1.

3) High frequencies: For high frequencies, another approximation for the integrated model can be found.

$$P_4 = \frac{\sigma I_0}{2} \frac{\eta - \theta + \tau\delta\theta}{\eta^2 + \frac{k_d(\sigma I_0)^2}{2k_r}(\eta - \theta)} \quad (25)$$

$$\eta = (\sigma I_0 + 1/\tau) \quad (26)$$

$$\theta = \frac{1}{T} \frac{(1 - \alpha(0))(1 - \alpha(I_0))}{1 - \alpha(I_0)\alpha(0)} \quad (27)$$

$$\alpha(I) := e^{-(1/\tau + \sigma I)T} \quad (28)$$

This solution can be obtained by utilizing the assumption $\dot{C} = 0$. Taking the formula to the limits for $T \to 0$ and $T \to \infty$, the expression turns to:

$$\lim_{T\to 0} P_4 = P_5 = \frac{\tau\sigma\frac{I_0}{2}}{(1 + \tau\sigma\frac{I_0}{2} + k_d/k_r\tau(\sigma\frac{I_0}{2})^2)} \quad (29)$$

$$\lim_{T\to\infty} P_4 = P_3 \quad (30)$$

The limit for low frequencies coincides with the limit for high frequencies of the formulation P_2 in equation (19). The limit for high frequencies is the steady state solution for continuous illumination with the mean intensity $\overline{I} = I_0/2$. P_5 is presented in fig. 1.

C. Dynamics of the Droop Variables

For sake of simplicity, in this study, we will only consider the case where nitrogen is not limiting. As a consequence nitrogen absorption rate ($\hat{\rho}$) is maximum. The dynamics of the biomass and of the nitrogen in the medium will not be discussed here. Only the equation for q will be considered in order to focus on the growth rate $\mu(I, A, q)$. Based on these considerations, q can be resolved to its equilibrium value Q_{eq}:

$$Q_{eq}(P) = \frac{(\hat{\rho} - \mu_m P(I,T))Q_l}{2\hat{\rho}} + \sqrt{\left(\frac{(\hat{\rho} - \mu_m P(I,T))Q_l}{2\hat{\rho}}\right)^2 + \frac{q_0 \mu_m P(I,T) Q_l}{\hat{\rho}}} \quad (31)$$

The growth rate can then be computed as follows:

$$\mu = \mu_m \left(1 - \frac{q_0}{Q_{eq}(P(I,T))}\right) \cdot P(I,T)/\tau \quad (32)$$

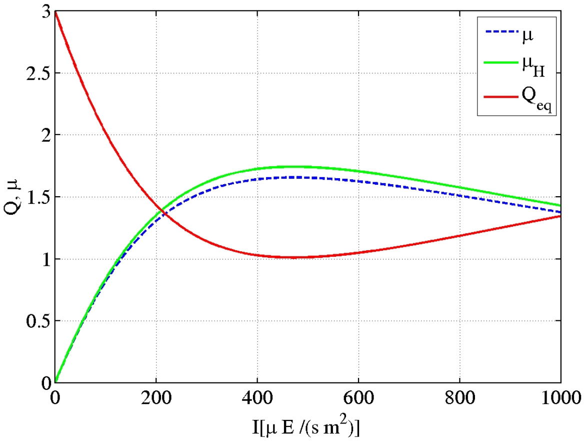

Fig. 2. Values of Q_{eq} (red) in respect to irradiance at steady state based on P_1 (eq. 31). Growth rate μ (dashed blue) is compared to the growth rate μ_H (green) assuming constant intracellular nitrogen (32).

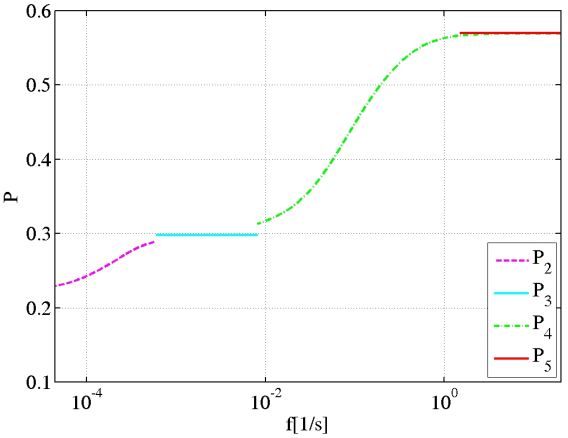

Fig. 3. Approximations P_2 (purple, dashed), P_3 (cyan), P_4 (green, dashed), P_5 (red) against frequency on a logarithmic scale

The functions for Q_{eq} and μ are represented in figure 2. High light conditions lead to a lowered Q and therefore a slight reduction of the growth rate, compared to the photosynthetic efficiency only considered with the Han model. Further, the influence of the steady state value Q_{eq} turns to a constant factor for intermediate and high productivities. Therefore, in the case of non limiting nitrogen, it can often be disregarded when estimating the parameters defining the dynamics of the photosynthesis.

D. Parameter identification

From measurements of substrate, biomass and intracellular nitrogen under different conditions, the key parameters of Droop's model can be identified [2]. As it has been discussed, the limited nitrogen quota fluctuation due to light (when nitrogen is not limiting) hardly influences growth rate. The dynamics of the photosynthetic productivity is thus mainly driven by the photosynthetic activity and the term $P(I, T)$.

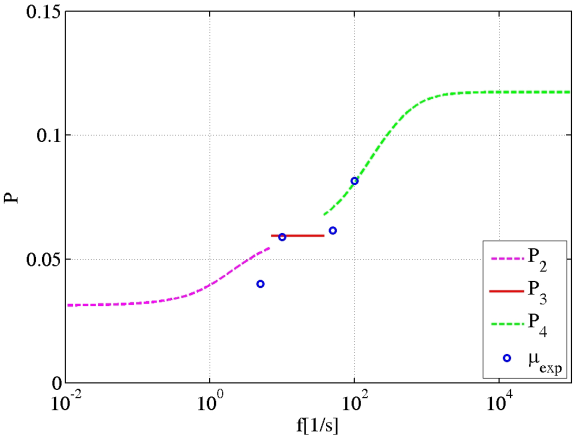

Fig. 4. Comparison of the results of Vejrazka et al. [26] (blue circles) with the approximations from our model (continuous lines)

Assuming now that the growth rate response for continuous illumination $P_1(I)$ (Haldane curve) is available, two parameters can be determined. The parameters g_1 and g_2 can be estimated by a least square fit of the function P_1 normalized by the value of its maximum:

$$P_1 = \frac{\sigma I_0 \tau}{2 \cdot (1 + g_1 I_0 + g_2 I_0^2)} \tag{33}$$

with

$$\begin{aligned} g_1 &= \tau\sigma && (34) \\ g_2 &= k_d/k_r \tau \sigma^2 && (35) \\ & && (36) \end{aligned}$$

Now, we can use the PI-curves for intermediate frequencies $P_3(I)$, and for continuous illumination to compute the following quantity:

$$F_1(I) = I \cdot \left(\frac{1}{P_1} - \frac{1}{P_3} \right) = \frac{1.5}{\sigma} + 1.5\tau I \tag{37}$$

F_1, is a linear function of I, and a linear regression will provide the values for τ and σ.

As a consequence, σ, τ and $\frac{k_d}{k_r}$ can be determined by measuring P_1 and P_3 only.

To get the absolute values of k_d and k_r, a possible approach is to use the productivity response $P_2(I, T)$ as a function of T. Since the system has only one remaining degree of freedom, a least-square fitting can provide this value.

With the proposed method, measuring the growth rate response for various irradiances and LD frequencies, leads to the identification of all the model parameters.

E. Validation of the Droop-Han Model

The presented results for the model are only valid for the periodic state of the system. Acclimation processes are not included yet in the model, but the approach proposed by [2] could be used to take photoacclimation into account. As a consequence, only data for acclimated microalgae should be taken into account, which means that long term experiments are mandatory to validate our model. Further, it is necessary to use diluted cultures. This should guaranty a homogeneous light regime and avoid additional light fluctuation at the scale of the cell due to transport in a turbid medium with strong light gradient. Due to these experimental restrictions, appropriate data are scarce. In recent experiments, Vejrazka et al. [26] provided data for the growth of *Chlamydomonas reinhardtii* in LD cycles at a LD ratio of 0.1.

Although, their experimental setup differs from the preconditions in the presented approach, Vejrazka's results show the same general structure as it can be seen in fig. 4. Their measurements show a strong increase in growth rate when increasing fluctuation frequency from 5 to 10 Hz, a very slight increase from 10 to 50 Hz and a second increase from 50 to 100 Hz. The "inflexion point"', which we denominated with P_3 can therefore be identified for this experiment between 10 and 50 Hz. The growth rate for 100 Hz is equal to the growth rate under continuous light with average light intensity. These results very well meet the predictions of the Droop-Han model and its analytical approximations for established periodic regimes. Nevertheless, data covering more variations of I_0 and T would allow a better model calibration and a more quantitative validation.

V. DISCUSSION

The Droop-Han Model accounts for the dynamic of the photosystem on one hand and for the nutrient limitation on the other hand. With the proposed analytical approximation, new insights about the model response to varying light signals could be obtained. For periodic Light-Dark cycles of different cycling frequency, the model predicts a characteristic dependency of growth rate to cycle frequency as shown in fig 4. The curve shows an increase in growth rate for low frequencies (below 10^{-3} Hz), a plateau or inflexion point for intermediate frequencies and a further increase for higher frequencies (above 10^{-2}Hz). This specific shape could also be found in the results of the experiments of Vejrazka et al. [26], which supports the validity of the model. The characteristic shape directly illustrates key properties of the Han model: the two gradients in growth rate correspond to the two time scales of the inhibition-repair process and the activation-relaxation process. For very slow light variations, growth is limited by strong inhibition during the light phase. With increasing frequency, the degree of inhibition turns to a constant equilibrium throughout each LD cycle. As a result, for increased frequency, there is less photoinhibition during the light phase, which results in an increased productivity of the photosystem. With further increasing frequency, the degree of photoinhibition remains constant, while the open-closed dynamics changes from two alternating states to a constant equilibrium value throughout the entire LD cycle. The constant equilibrium state leads to an increased productivity P_5 compared to the alternating state. P_5 matches the productivity of a constant illumination with the mean light intensity. The latter point coincides with the theoretical proof of Papaceck et al. [6].

The model properties have been derived by an analytical integration using a slow-fast approximation. The resulting formulas have a simple form compared to the exact solution of the Han model [6]. Based on these formulas, a measurement protocol for the deduction of the parameters of the model only from the dependency between growth rate to the cycle frequency T and the maximum light intensity I_0 can be derived. Concerning the physiological parameters describing the photosynthetic system, the proposed method for parameter identification varies fundamentally in complexity from other approaches, as proposed by [28] and [22]. Our approach is a simple procedure not implying a numerical integration of the system nor additional off line measurements. This simplifies the experimental protocol and the calculation. The simple analytical expressions allow for a better estimation of the approximation error.

The resulting formulas for the growth rate not only allow for the determination of the physiological parameters, they also show a characteristic dependency between signal frequency and growth rate which enables an easy experimental validation of the model. The shape of the curve explains the "Flashing Light Effect"' and shows that the increase of productivity with fluctuation frequency is not linear but shows a very characteristic saturation for intermediate frequencies. Only a weak increase in productivity can be expected with frequency augmentation in this domain. This insight can lead to important consequences for the design of raceways and photobioreactors. The dynamics of nitrogen assimilation and nutrient limited growth plays an inferior role under the conditions we were assuming.

The presented model is suitable to investigate the effect of the hydrodynamic regime in raceways on the growth rate of microalgae cultures due to photoinhibition. Regarding biofuel production with raceways and photobioreactors, the light variability on faster time scales is typically caused by mixing. The results from this study give important insights and are a first step in understanding the coupling of physical and biological models. With further research on the Droop-Han Model, the light intensity dynamics at the scale of the microalgae can be optimized by the design of the process and an increased productivity can be achieved using the same total light dose. Increasing efficiency in biomass production by optimized photobioreactors is an important step on the way to the industrial use of microalgae.

Acknowledgements: This work was carried out in the framework of the ARC Nautilus funded by INRIA.

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