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Abstract:

The multitime-scale nature residing in the processes concerning the growth of microalgae is presented as a singularly perturbed problem. In order to provide the convincing simulation results, the model of photosynthesis and photoinhibition in microalgae based on the mechanistic description in the form of the so called photosynthetic factory (PSF model) is chosen. Two complementary reductions to both slow and fast dynamics are used to derive the performance index, being the integral average of the activated state of PSF model. Afterward, for a periodic piecewise constant input, so-called light-dark cycles, the performance index is calculated in the explicit form from the non-reduced PSF model. The objective is to show graphically the limits of the order reduction by singular perturbation method.

Keywords:
Photosynthesis, microalgae, photoinhibition, flashing light effect, model of photosynthetic factory, singular perturbations, bilinear system

1 Introduction

Biotechnology with microalgae and photo-bioreactor (PBR) design is nowadays regaining attention thanks to emerging projects of algal biofuels and CO_2 sequestration. Nevertheless, there neither exist reliable methods nor software for modelling, simulation and control of PBR. This is based on fact that modelling in a predictive way the photosynthetic response in the three-dimensional flow field seems today unrealistic, because the global response depends on numerous interacting intracellular reactions, with various time-scales.

Till nowadays, the most important information about the photosynthetic production of some microalgae species resides in the measurement of the coupling between photosynthesis and irradiance (being a controlled input), in form of the steady-state light response curve (so-called *P–I curve*), which represents the microbial kinetics. There are two most common type of microbial kinetics, i.e. *Monod* and *Haldane* type kinetics [18], see Fig. 1, when the first one is usually chosen either due to the small levels of irradiance in microalgal culture or due to the fast measurements (photoinhibition needs at least some minutes to be detected).
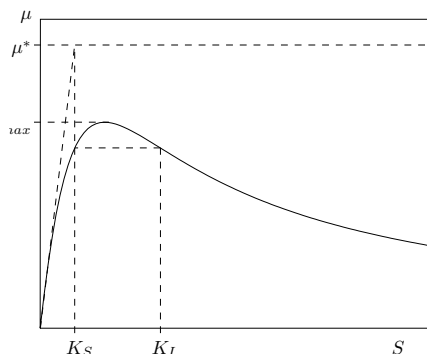

Figure 1: Steady-state production curve of *Haldane* type or *Substrate inhibition kinetics*. The physiological meaning of parameters μ^*, K_S (saturation constant), K_I (inhibition constant) is clearly shown. Note that for $K_I \to \infty$, the production curve changes to *Monod* kinetics.

Hence, if we see that even in the laboratory experiments both dynamic and photoinhibition effects are often neglected, the same occurs in modelling and simulation of photobioreactors, despite the fact that PBR operating under high irradiance, permitting the photoinhibition of the cell culture, belong to intensively studied topics of microalgal biotechnology, see e.g. [11] and references within there.

Moreover, there is another important phenomenon, which occurs under periodic intermittent light condition, the so-called flashing light effect, urgently demanding some dynamical model describing the photosynthetic productivity under fluctuating light condition. One of such adequate dynamical models of microorganism growth is, as we know, the three-state phenomenological model of photosynthetic factory – PSF model [3, 4].

In our previous papers [14, 17, 16] we studied the PSF model behavior and the techniques for its parameter estimation as well. The purpose of this paper is to continue in

our work [15], where the reduction to slow dynamics was used in order to derive the optimal feedback control. We aim to present the analysis and an illustrative example of the two-time-scale phenomena of PSF model and namely, two alternative order reductions of this dynamical system under periodic excitation. For a piecewise constant (intermittent) periodic input, see Fig. 2, the corresponding boundary value problem is solved analytically for the reduced problem and for the non-reduced as well. The resulting performance index, being an integral average of the activate state of PSF model, is then compared with the results computed using both slow and fast reduction.
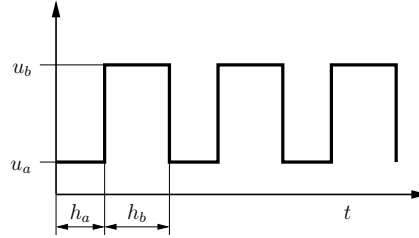

Figure 2: Schematic presentation of the periodic intermittent input signal $u(t)$: u_a stands for irradiance during time period h_a, u_b is irradiance level during period h_b. Total cycle period is $h = h_a + h_b$.

Our paper is organized as follows. Section 2 presents the dynamical model of the microalgal growth in detail and derives its reduction to both slow and fast manifold. Simulation experiments are collected in Section 3 while the final section draws some conclusions and outlooks for further research.

2 Dynamical model of photosynthesis and photoinhibition in microalgae

Microalgal growth is modelled based on the following experimental observations: **(i)** the steady state kinetics is of *Haldane* type or *Substrate inhibition kinetics* [13]; **(ii)** the microalgal culture in suspension has the so-called *light integration* property [19, 13], i.e. as the light/dark cycle frequency, [6], is going to infinity, the value of the resulting production rate (e.g. oxygen evolution rate) goes to a certain limit value, which depends on the average irradiance only [14]. These features are best comprised by the dynamical model, called as the **model of photosynthetic factory**, described further in detail.

2.1 Model of photosynthetic factory – PSF model

The dynamical **model of photosynthetic factory – PSF model**, see Fig. 3, has been thoroughly studied in the biotechnological literature [10, 22]. Although relatively simple, it describes three phenomena occurring simultaneously in three largely separated time-scales:

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(i) cell growth, (ii) photoinhibition, and (iii) photosynthetic light and dark reactions. The state vector y of the PSF model is three dimensional, namely, $y = (y_R, y_A, y_B)^\top$, where y_R represents the probability that PSF is in the resting state R, y_A the probability that PSF is in the activated state A, and y_B the probability that PSF is in the inhibited state B. The PSF can only be in one of these states, so:

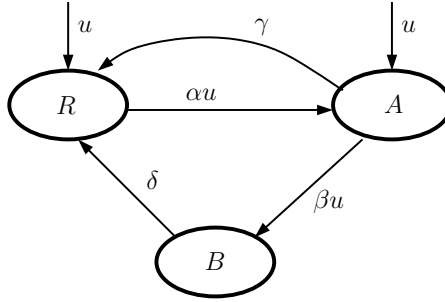$$y_R + y_A + y_B = 1 \ . \tag{1}$$

Figure 3: States and transition rates of the photosynthetic factory – Eilers and Peeters's PSF model.

The possible transitions among states are supposed to be of zero or first order respective to the irradiance $I(t)$. Hence from the PSF model, schematically depicted in Fig. 3, it directly follows that

$$\begin{bmatrix} \dot{y_R} \\ \dot{y_A} \\ \dot{y_B} \end{bmatrix} = \begin{bmatrix} 0 & \gamma & \delta \\ 0 & -\gamma & 0 \\ 0 & 0 & -\delta \end{bmatrix} \begin{bmatrix} y_R \\ y_A \\ y_B \end{bmatrix} + I(t) \begin{bmatrix} -\alpha & 0 & 0 \\ \alpha & -\beta & 0 \\ 0 & \beta & 0 \end{bmatrix} \begin{bmatrix} y_R \\ y_A \\ y_B \end{bmatrix} \tag{2}$$

For given values of the model parameters α, β, γ, δ and the input variable, i.e. the irradiance $I(t)$, the ODE system (2) can be solved either by numerical methods or by asymptotic methods, cf. [8]. For the special case of the periodic piecewise constant input, the state trajectories were calculated explicitly in [14].

The PSF model has to be completed by an equation connecting the hypothetical states of PSF model with some quantity related to the cell growth. This quantity is the specific growth rate μ.[3] According to [3, 22], the rate of photosynthetic production is proportional (there is a dimensionless constant κ) to the number of transitions from the activated to the resting state:

$$\frac{\mathrm{d}}{\mathrm{d}t} c_x = \kappa \ \gamma \ y_A(t) \ c_x \ . \tag{3}$$

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[3] $\mu := \dot{c_x}/c_x$, c_x stands for microbial cell concentration. The notation used is the most usual in biotechnological literature, cf. [2].

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Considering that the value of $\kappa \cdot \gamma$ is of order 10^{-4}, cf. [22], and $y_A(t)$ is periodic with period h, cf. [14] for more details, we have the following relation for the specific growth rate μ, according to [8]:

$$\mu = \frac{\kappa\gamma}{h} \int_0^h y_A(t)\mathrm{d}t \ . \tag{4}$$

Equation (4) reveals the reason why PSF model can successfully simulate the microalgae growth in high-frequency fluctuating light conditions: the growth is described through the "fast" state y_A, hence we reach the sensitivity to high-frequency inputs, see e.g. flashing light experiments [13]. As we know, this highly required sensitivity is not reached in any other model describing microalgae growth, see e.g. [12] where the time step for the numerical integration of the ODE describing the microalgae cell concentration has to be set to one hour...

2.2 Re-parametrization of the PSF model

In order to apply the singular perturbation method, we shall use the re-parametrization firstly introduced in [17]:

$$q_1 := \sqrt{\frac{\gamma\delta}{\alpha\beta}} \ , \quad q_2 := \sqrt{\frac{\alpha\beta\gamma}{\delta(\alpha+\beta)^2}} \ , \quad q_3 := \kappa\gamma\sqrt{\frac{\alpha\delta}{\beta\gamma}} \quad q_4 := \alpha q_1 \ , \quad q_5 := \beta/\alpha.$$

Consequently, the PSF model has the following form:

$$\dot{y} = \left[\mathcal{A} + u(t)\mathcal{B}\right] y \ , \tag{5}$$

$$\mathcal{A} = q_4 \begin{bmatrix} 0 & q_2(1+q_5) & \frac{q_5}{q_2(1+q_5)} \\ 0 & -q_2(1+q_5) & 0 \\ 0 & 0 & -\frac{q_5}{q_2(1+q_5)} \end{bmatrix} \ , \quad \mathcal{B} = q_4 \begin{bmatrix} -1 & 0 & 0 \\ 1 & -q_5 & 0 \\ 0 & q_5 & 0 \end{bmatrix} \ . \tag{6}$$

The single scalar input $u(t)$, representing the dimensionless irradiance in the culture, is defined as $u := I/q_1$, where I is the non-scaled irradiance (units: $\mu\mathrm{E}\ \mathrm{m}^{-2}\ \mathrm{s}^{-1}$). It is assumed that $u(t)$ is at least piecewise continuous. In other words, the PSF model is the so-called bilinear controlled system whose inherent property is the so-called light integration capacity [13], i.e. due to the *Lipschitz dependence of trajectories on control*, cf. [1] and references within there, as the frequency of fluctuating light is going to infinity, the value of resulting production rate (specific growth rate μ) goes to a certain limit value, which depends on the average irradiance only [14].

Let us see that $q_1 = I_{opt}$ (I_{opt} maximizes μ, see Fig. 1 and Remark 1), q_2, q_5 are dimensionless, q_3, q_4 are in s^{-1}. The reasoning for such choices arises from the utility to separate the steady state PSF model behavior (parameters q_1, q_2, q_3) from the PSF model dynamics (the *fast* rate $q_4 := \alpha I_{opt}$ and the *slow* rate $q_4 q_5 := \beta I_{opt}$), for more details cf. [17, 16].

For the constant input signal (irradiance $u \geq 0$) the ODE system (5) is linear and its system matrix $\mathcal{A} + u\mathcal{B}$ has three distinct eigenvalues. Two eigenvalues are negative

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(λ_F, λ_S), and the third is zero (its corresponding eigenvector is the globally stable steady state solution of (5)). In the sequel, we will need the steady state values of states y_A and y_B:

$$y_{A_{ss}}(u) = \frac{u}{q_2(1 + q_5)(u^2 + u/q_2 + 1)} \, , \tag{7}$$

$$y_{B_{ss}}(u) = \frac{u^2}{u^2 + u/q_2 + 1} \, . \tag{8}$$

Remark 1: Notice that the parameter q_5 quantifies the separation between the fast and slow dynamic; $q_5 \approx 10^{-4}$, based on [22].[4] Moreover, the PSF model steady state behavior corresponds to *Haldane* type kinetics (or so-called *Substrate inhibition kinetics*), see Fig. 1: $\mu = \frac{\mu^* \, S}{K_S + S + S^2/K_I}$, where S is irradiance, i.e. limiting substrate for photosynthetic microorganism, and μ^*, K_S, K_I are model constants. The connection between PSF model and *Haldane kinetics* could be described as follows: $\mu^* = q_2 q_3$, $K_S = q_1 q_2$, and $K_I = \frac{q_1}{q_2}$. For the constant value of irradiance which maximizes the steady-state growth rate, i.e. $I_{opt} := q_1 = \sqrt{K_S K_I}$, we have $\mu(I_{opt}) := \mu_{max} = \frac{\mu^*}{2\sqrt{K_S/K_I} + 1} = \frac{q_2 q_3}{2q_2 + 1}$.

2.3 Slow reduction of the ODE system (5)

As stated above, the PSF model operates in three time scales. In the slowest time-scale, for the cell growth, the governing equation is (3), resp. (4). Two other time-scales are comprised in the system (5). Taking into account the condition (1), and preferring the states y_A, y_B (due to their measurability[5]), we further analyze only two following differential equations:

$$\frac{d}{dt} y_A = q_4 \left[-q_2(1 + q_5)y_A + u\left(1 - (1 + q_5)y_A - y_B\right) \right] \, , \tag{9}$$

$$\frac{d}{dt} y_B = q_4 \, q_5 \left[-\frac{y_B}{q_2(1 + q_5)} + uy_A \right] \, . \tag{10}$$

Now wee see that the above equation (9) contains coefficients that are two order higher than those of (10). To make advantage of that, one can reduce the dynamics to the one dimensional one using the singular perturbation approach with respect to the small parameter $q_5 \approx 10^{-4}$ [20]. This is done in the following way. First, introduce a new "faster" time scale $\tau = q_5^{-1} t$, so that the system (5) takes the form

$$\frac{q_5}{q_4} \frac{d}{d\tau} \begin{bmatrix} y_A \\ y_B \end{bmatrix} = \begin{bmatrix} -q_2(1 + q_5) & 0 \\ 0 & -\frac{q_5}{q_2(1+q_5)} \end{bmatrix} \begin{bmatrix} y_A \\ y_B \end{bmatrix}$$
$$+ u \begin{bmatrix} -(1 + q_5) & -1 \\ q_5 & 0 \end{bmatrix} \begin{bmatrix} y_A \\ y_B \end{bmatrix} + u \begin{bmatrix} 1 \\ 0 \end{bmatrix} \, . \tag{11}$$

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[4]For the microalga *Porphyridium* sp., on basis of parameters α, β, γ, δ, κ, cf. [22], we have calculated: $q_1 = 250.106 \, \mu E \, m^{-2}$, $q_2 = 0.301591$, $q_3 = 0.176498e - 3 \, s^{-1}$, $q_4 = 0.483955 \, s^{-1}$, $q_5 = 0.298966e - 3$.

[5]Equation (3) describes the connection of y_A with μ a measurable quantity; y_B can be estimated via chlorophyll fluorescence measurement, cf. [11, 22].

Now, after dividing the second equation by q_5 one obtains the standard form of the singularly perturbed system [7] with respect to the small parameter q_5:

$$q_5 \frac{d}{d\tau} y_A = g(y_A, y_B, u(t), q_5) , \tag{12}$$

$$\frac{d}{d\tau} y_B = f(y_A, y_B, u(t), q_5) , \tag{13}$$

where q_5 is further allowed to be decreasing until zero, and

$$g(y_A, y_B, u(t), q_5) := q_4 \left[-(u + q_2)(1 + q_5)y_A + u(1 - y_B) \right] ,$$
$$f(y_A, y_B, u(t), q_5) := q_4 \left[-\frac{y_B}{q_2(1 + q_5)} + u y_A \right] .$$

This system thanks to the properties of its right hand side clearly satisfies the sufficient condition for the convergence of the singular perturbation[6] [7, 20]. One can therefore take the limit $q_5 \to 0$ in (11) to obtain

$$y_A{}^S = (1 - y_B{}^S) \frac{u}{u + q_2} , \tag{14}$$

$$\frac{d}{d\tau} y_B{}^S = q_4 \frac{u^2}{u + q_2} \left[1 - \frac{y_B{}^S}{y_{Bss}(u)} \right] , \tag{15}$$

where the upper index "S" aims to avoid confusion with notation for the non-reduced system (5). The above relation (14), no matter what the initial conditions are, is quickly satisfied with great precision. Further convergence to the steady state along the dynamics (15) without breaking the relation (14) is then much slower. The set of all states satisfying (14) is called the **slow manifold** while the relation (15) is called the **slow dynamics**. Often, for simplicity, these relations are called the **slow reduction**.

2.4 Complementary reduction of the ODE system (5)

Notice, that there are basically two options, how to reduce the ODE system (5). The first one, the slow reduction, was considered in the previous subsection. Nevertheless, in the presence of high-frequency inputs, this approach leads to unsatisfactorily results, cf. Fig. 4.

Therefore, the following complementary approach is further developed, cf. [5]. Let us change the variable y_A as follows:

$$y_D := y_A - (1 - y_B) \frac{u}{u + q_2} . \tag{16}$$

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[6]Recall, that this condition geometrically means that the slow manifold given by (14) satisfies certain stability properties, namely, $\dot{y}_A < 0$ for $y_A > (1 - y_B)\frac{u}{u+q_2}$ and $\dot{y}_A > 0$ for $y_A < (1 - y_B)\frac{u}{u+q_2}$, which is indeed obviously the case here.

The slow manifold can now be identified as the set of pairs (y_D, y_B) such that $y_D = 0$. In the new variables, (12, 13) are represented by the system

$$q_5 \frac{d}{d\tau} y_D = g_\circ(y_D, y_B, u(t), q_5) , \tag{17}$$

$$\frac{d}{d\tau} y_B = f_\circ(y_D, y_B, u(t), q_5) , \tag{18}$$

where

$$g_\circ := q_4 \left[-(1 + q_5)(u + q_2) y_D + q_5 u (1 - y_B) \right] + q_5 \frac{u}{u + q_2} f_\circ ,$$

$$f_\circ := q_4 \left[-\frac{y_B}{q_2(1 + q_5)} + u y_D + \frac{u^2}{u + q_2}(1 - y_B) \right] .$$

Let us change the time scale back to the real "slow" time variable t:

$$\frac{d}{dt} y_D = g_\circ(y_D, y_B, u(t), q_5) , \tag{19}$$

$$\frac{d}{dt} y_B = q_5 f_\circ(y_D, y_B, u(t), q_5) . \tag{20}$$

The above ODE system (19, 20) has an equilibrium point for $y_D = 0$ and $q_5 = 0$. Furthermore, the behavior of the system (19, 20) at $q_5 = 0$ is characterized by the only one ODE

$$\frac{d}{dt} y_D = g_\circ(y_D, y_B, u(t), 0) = -q_4 (u + q_2) y_D , \tag{21}$$

and the "slow" variable y_B can be regarded as a constant.

The two systems (14, 15) and (21), which are defined on two different time axis, represent two limit behaviors of the original system (5). The purpose of the singular perturbation approach is to infer the asymptotic properties of the non-reduce system from the solution of both complementary reduced systems (14, 15) and (21). To illustrate this fact, we present a case study in the following section.

3 Case study

Two complementary reductions to both slow and fast dynamics were presented in the preceding section. In the sequel, for the special although common conditions of the periodic intermittent input, see Fig. 2, the state trajectories will be calculated in the explicit form for each of the three PSF model formulations. Let us remark that the periodic input is very common in biological systems. It causes the periodic or cyclic behavior. The proof that there exists a unique periodic solution is performed in detail in [10] for the continuous periodic inputs, and in our work [14] for the periodic piecewise constant input signal $u(t)$, where during time period h_a the irradiance is u_a, and u_b is irradiance level during period h_b, see Fig. 2. Total cycle period is then $h = h_a + h_b$.

From the biotechnological point of view, the long term cultivation of microalgae culture in continuous mode is of utmost importance. Hence, we supposed that the quasi-steady state is reached, and as the most important measure of productivity the specific growth rate μ is chosen. Consequently, according to (4), we calculate the integral average of the activated state of PSF model over one period h, being the performance index J. Afterwards, for a periodic piecewise constant input, J is calculated in the explicit form for:

- non-reduced PSF model (5) – J_{NR},

- slow reduction of PSF model (14, 15) – J_{SR},

- "fast" reduction of PSF model derived further from (21) – J_{FR}.

Let us denote the average irradiance as $u_{av} := \frac{1}{h} \int_0^h u(t) \mathrm{d}t$. Then the "fast" reduction of the ODE system (5) in terms of states y_A and y_B has finally the following form

$$\dot{y_A}^F = -q_4 \left[u(t) + q_2 \right] y_A{}^F + q_4 u(t) \left[1 - y_{B_{ss}}(u_{av}) \right] \; , \tag{22}$$

where the upper index "F" aims to avoid confusion with notation for the non-reduced model (5), and as the constant value of the "slow" state variables $y_{B_{ss}}(u_{av})$ was taken.

Remark 2: Roughly speaking, we have just applied the theorem of *Lipschitz dependence of trajectories on control* [1, 14, 16] supposing that the period of light cycles h is "sufficiently small" for "averaging" of y_B but not so small for averaging y_A.

3.1 Simulation results

Computer simulations were performed within MAPLE. Two most relevant results are shown in Fig. 4 and Fig. 5. In the y-axis of both figures is the integral average of the activated state of PSF model over one period h. In the x-axis is the natural logarithm of the cycle period h. The irradiance level u_a was set to 0 and $h_a = h_b$. The average value of irradiance was $u_{av} = 1$, i.e. $u_b = 2$. Such an excitation is in biotechnological literature called as light/dark cycles with light-to-dark ratio $1 : 1$, cf. [13].

Remark 3: Recall, that a constant control $u \equiv 1$ is optimal among all possible constant controls in the following sense. The PSF model is for any constant fixed input a linear system having constant coefficients and constant non-homogeneity. Moreover, it can be easily shown to be asymptotically stable. In the re-parametrization introduced in this paper, $u \equiv 1$ maximizes the value of the steady state of y_A, thereby maximizing also its integral being the value $y_{A_{ss}}(1) := \frac{1}{2q_2+1} \approx 0.62$.

One can observe in Figure 4 that $\tilde{h}$ is the lower curve for the long periods, corresponding to the result based on slow reduction of PSF model (14, 15), close to the exact solution (upper bold curve).

Simulations based on "fast" reduction of PSF model derived from (21) are shown on Figure 5. There is the left range of periods h, where we can see the curve corresponding to the reduced system close to the exact solution (lower bold curve). Consequently, its use is justified for the "short" light-dark cycles.

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Further numerous simulations were performed, for various light-to-dark ratio, average irradiances and system parameters (even those biologically unrealistic). All simulations confirm the general conclusions based on singular perturbation methods.
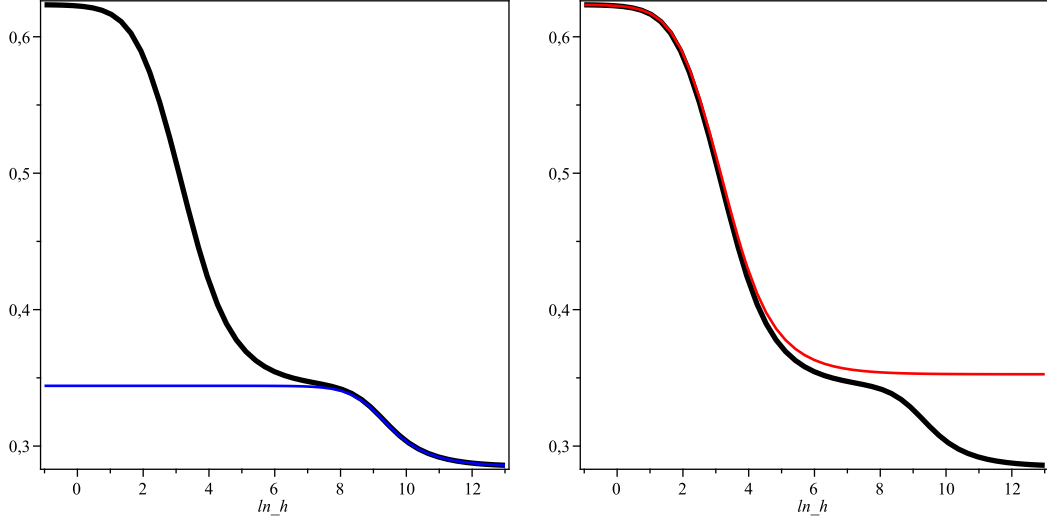

Figure 4: On the left: Integral average of the activated state y_A depending on period h of light-dark cycles (in logarithmic scale) for the slow reduction (in blue) and the exact solution (upper bold curve).

Figure 5: On the right: Integral average of the activated state y_A depending on period h of light-dark cycles (in logarithmic scale) for the fast reduction (in red) and the exact solution (lower bold curve).

4 Conclusion

The problem of the microalgal growth modelling using the dynamical model of photosynthetic factory (PSF model) was considered here and treated as singularly perturbed problem by its reduction to both slow and fast dynamics. The biotechnological relevance of the presented study is clear: in the presence of unmeasurable disturbances (either of biological or external nature) the reduced model offers a viable mean of real time optimization of operating conditions, while the off-line (or *open-loop*) optimal solution is useless. In the special case of periodic intermittent control, both reduction were compared with the exact solution of the non-reduced problem, showing their limits. Important lesson here is that the fast dynamics phenomena are not negligible and may play important role. This is in contradiction with a common intuition and deserves to be promoted. The outlooks for future research reside in developing necessary conditions to achieve a satisfactory approximation of the reduced solution.

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