

Model for Photosynthesis and Photoinhibition: Parameter Identification Based on the Harmonic Irradiation O_2 Response Measurement

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Abstract—A method for parameter identification of a model describing the growth of the algae is presented. The method is based on the description in the form of the so-called photosynthetic factory. The experimental data are gained by measuring the steady-state photosynthetic production when the input of the photosynthetic factory (light intensity) is a harmonic signal. Estimation of parameters is based on a sufficient number of experiments compared with simulated data via the least-squares technique. As the input signal is harmonic and the dynamics of the unforced system is exponentially stable, the resulting asymptotical steady-state trajectory of the photosynthetic factory is also periodic and can be computed via determining an appropriate center manifold graph by solving the corresponding first-order partial differential equation. The latter is performed by the finite-element method. The application of the proposed method is demonstrated on an example using real experimental data.

Index Terms—Biological system modeling, identification, least-squares method, nonlinear systems.

I. INTRODUCTION

A DYNAMIC model of the photosynthetic production in microalgal culture is of fundamental importance for photobioreactor design and process optimization. In the area of algal biotechnology, the photosynthetic microorganisms growth modeling has long been regarded as a well-defined discipline consisting of the adequate coupling between photosynthesis and irradiance resulting in the light response curve which represents the microbial kinetics; see, e.g., the so-called *Haldane* type kinetics in Fig. 1 [5], [15].

However, several dynamic phenomena, e.g., flashing light enhancement, cannot be explained by a simple kinetic relation. Thereafter, the interconnection between the steady-state kinetic model and the dynamic one is often artificial [16]. The main difficulty in considering the dynamic behavior of the photosynthetic processes (i.e., light and dark reactions, photoinhibition, and photoacclimation) consists of different time scales.

Manuscript received January 22, 2007; revised August 17, 2007. This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic under Grant MŠMT MSM 600 766 58 08 and by the Czech Science Foundation under Grant GA ČR 102/05/0903, Grant 102/05/0011, and Grant 102/07/P413.

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Digital Object Identifier 10.1109/TAC.2007.911345

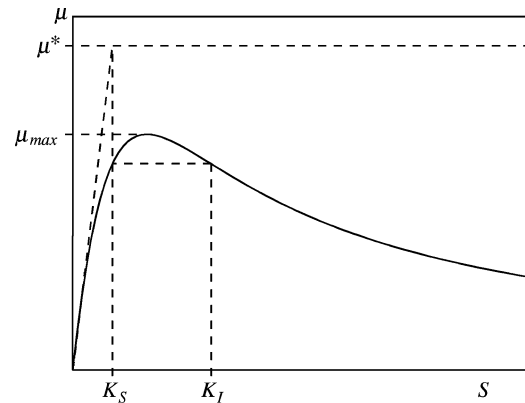


Fig. 1. Steady-state production curve of *Haldane* type or *Substrate inhibition* kinetics. The governing relation is (using the most usual notation in biotechnological literature): $\mu = mu^*S/(K_S + S + S^2/K_I)$, where μ is specific growth rate (defined as $\mu := \dot{c}_x/c_x$, where c_x is the cell density) and S is a limiting substrate, and μ^* , K_S , and K_I are model constants. Maximum occurs at $S = \sqrt{K_S K_I}$, when $\mu_{\max} = (\mu^*/2)\sqrt{K_S/K_I + 1}$. Note that, for $K_I \rightarrow \infty$, the production curve changes to *Monod* kinetics.

While the characteristic time of microalgal growth (e.g., doubling time) is of the order of hours, the photosynthetic light and dark reactions occur in milliseconds. Moreover, another relevant process defining the light regime in the microalgal culture, i.e., the mean period of light/dark cycles induced by algal suspension flow in a photobioreactor, is generally in seconds.

As we possess some experiment-based knowledge of relevant processes, we can formulate the basic model behavior and further determine the model structure and the number of model parameters. In our case, when looking for a model of photosynthesis and photoinhibition in microalgae, we can measure the steady-state behavior (i.e., so-called *P-I* curve, see Fig. 1), and the behavior under intermittent light regime, the so-called *flashing light experiments* [10], [11], [16]. The qualitative results are as follows:

- 1) steady-state kinetics is of *Haldane* type;
- 2) microalgal culture in suspension has the so-called *light integration* property, i.e., as the light/dark cycle frequency is going to infinity, the value of the resulting production rate (e.g., oxygen evolution rate) in the microalgal culture goes to a certain limit value, which depends on average irradiance in the culture only.

Hence, a simple dynamic model describing the cell growth [given by the equations (3)–(4)] is introduced below. This model takes the form of the so-called bilinear system being linear in the state for fixed input and linear in input when the state is fixed, but

nonlinear in total. In our case, the state x has three components representing three states of a photosynthetic unit, while a single scalar input u represents the irradiance in the culture.

The bilinear system, having the inherent property of Lipschitz dependence of trajectory on input with respect to certain weak type norm of input space [2]–[4], satisfy both of the above-mentioned requirements 1) and 2); see [13] for interpreting and proving the above light integration property using functional analysis tools. Moreover, the widely cited bilinear model of photosynthetic factory (PSF), proposed by Eilers and Peeters in 1988 [6], has proved to be an effective mean to model microalgal growth in a lumped-parameter system under both constant [6] and dynamic light regime [7]. The early fruit of all of these modeling efforts is the excellent qualitative compliance of the simulation results with the so-called flashing-light experiments (the experimental measurements of photosynthesis in intermittent light); see [11], [13], and [16]. The important consequence of this fact is that the studied dynamic model of photosynthesis based on the PSF model is sensitive to a characteristic time scale of microalgal cell transport from the light to dark zone and vice versa, enabling further extension of the PSF model into a distributed-parameter system (e.g., aiming to optimize photobioreactor design parameters and operating conditions).

Nevertheless, there are only a few quite limited results on the identification of the model parameters [17]. Moreover, the 95% confidence interval for PSF model parameters published in [17] shows some problems which are faced when identifying PSF model parameters.

Therefore, the main goal of this paper is to develop a new methodology of identification of PSF model parameters. It is based on computing a nonstationary asymptotical steady-state response to various harmonic input signals and comparing them with appropriate experimental data from the 400-ml laboratory photobioreactor (see Fig. 2). Computation of the asymptotic response is possible by adding a harmonic generator to our model and finding a center manifold of the resulting extended model by solving the corresponding partial differential equation (PDE), which is done by the finite-element method (FEM). This uses the fact that the unforced model is an asymptotically stable linear system and further develops ideas presented in [9].

This paper is organized as follows. Section II describes the dynamical model of photosynthesis and redefines its parameters, showing how a part of them can be determined from the Haldane-type curve. Section III—the main contribution of this paper—presents a new methodology for identification of the remaining parameters. It is based on the measurement of the harmonic excitation response. Section IV collects particular identification results for specific experimental data. Section V draws conclusions and gives some outlooks for future research.

II. DYNAMIC MODEL OF PHOTOSYNTHESIS AND ITS REPARAMETERIZATION

The authors of the paper [6] originally worked with probabilities that a hypothetical PSF is in one of the three states R , A , or B : p_R represented the probability that the PSF is in the resting state R , p_A the probability that the PSF is in the activated state

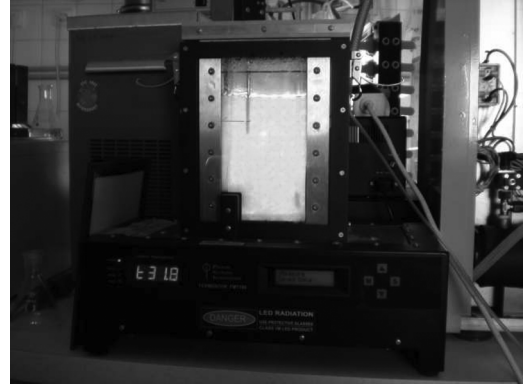


Fig. 2. Photobioreactor FMT 150, made by Photon Systems Instruments, Czech Republic. FMT 150 is a unique combination of the cultivator and monitoring device enabling a dynamic regulation of operating conditions (i.e., light, temperature, and gas composition) according to a user-defined protocol. The growth of the cultures is monitored continuously by measuring the optical density, and the instantaneous physiological state of the culture is measured by the chlorophyll fluorescence quantum yield.

A , and p_B the probability that the PSF is in the inhibited state B . The PSF can only be in one of these states, so

$$p_R + p_A + p_B = 1. \quad (1)$$

Later on, Eilers and Peeters [7] abandoned the “probabilistic” definition of PSF states and interpreted the state variables of a PSF model as the molar fractions of phytoplankton cells in the rested state (x_1), the activated state (x_2), and the inhibited state (x_3); the same notation is also used in [17], [18], i.e.,

$$\begin{bmatrix} p_R \\ p_A \\ p_B \end{bmatrix} := \begin{bmatrix} x_1 \\ x_2 \\ x_3 \end{bmatrix} := x. \quad (2)$$

The possible transitions among the states indicated schematically in Fig. 3 are supposed to be linear with respect to the irradiance u giving the following state space model of the PSF:

$$\dot{x} = [A + uB]x \quad (3)$$

$$A = \begin{bmatrix} 0 & \gamma & \delta \\ 0 & -\gamma & 0 \\ 0 & 0 & -\delta \end{bmatrix} \quad (4)$$

$$B = \begin{bmatrix} -\alpha & 0 & 0 \\ \alpha & -\beta & 0 \\ 0 & \beta & 0 \end{bmatrix}.$$

Further, taking into account (1), only two state variables, say x_2 and x_3 , need to be evaluated giving the following dynamics of the activated and inhibited states:

$$\begin{bmatrix} \dot{x}_2 \\ \dot{x}_3 \end{bmatrix} = \begin{bmatrix} -\gamma & 0 \\ 0 & -\delta \end{bmatrix} \begin{bmatrix} x_2 \\ x_3 \end{bmatrix} \quad (5)$$

$$+ u \begin{bmatrix} -(\alpha + \beta) & -\alpha \\ \beta & 0 \end{bmatrix} \begin{bmatrix} x_2 \\ x_3 \end{bmatrix} + u \begin{bmatrix} \alpha \\ 0 \end{bmatrix}. \quad (6)$$

Here, α , β , γ , and δ are again the rate constants of the PSF model and $u(t)$ is the known scalar input function. It is assumed that $u(t)$ is at least piecewise continuous.

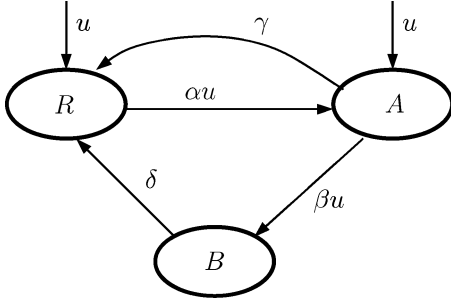


Fig. 3. Scheme of the states and the transition rates of the PSF—Eilers and Peeters PSF model. Three states of the photosynthetic factory are: R , the resting state, A , the activated state, and B , the inhibited state. The transition rates are: αu , βu , γ , and δ (unit: s^{-1}). The input variable u is the irradiance (unit: $\mu E \cdot m^{-2} s^{-1}$).

For a given constant input, i.e., the constant irradiance u , the relation (3) and (4) and consequently the relation (5) and (6) are systems of linear differential equations with constant coefficients and can be solved explicitly by classical means. Many authors, e.g., Eilers and Peeters in [6], Zonneveld in [19], and Hang in [8], restrict themselves to the steady-state solution, when a constant irradiance is maintained long enough so that the PSF states no longer change. More specifically, according to [6], the specific growth rate of the photosynthetic production μ at the steady state $x_{2ss}(u)$ is proportional to the number of transitions from the resting to the activated state, i.e.,

$$\mu = \kappa \gamma x_{2ss} = \frac{\kappa \gamma \delta \alpha u}{\alpha \beta u^2 + \delta(\alpha + \beta)u + \gamma \delta} \quad (7)$$

where κ is a new dimensionless constant that is one more parameter to be identified later on. In other words, (7) gives the relation between the irradiance u and the production (growth) rate μ at the steady state. The value of the constant irradiance to maximize growth rate is

$$u_{opt} = \sqrt{\frac{\gamma \delta}{\alpha \beta}}. \quad (8)$$

Further, the relation between traditional model constants of Haldane-type kinetics introduced by Fig. 1 and the parameters of PSF model is

$$\begin{aligned} \mu^* &= \kappa \gamma \frac{\alpha}{\alpha + \beta} \\ K_S &= \frac{\gamma}{\alpha + \beta} \\ K_I &= \frac{\delta(\alpha + \beta)}{\alpha \beta}. \end{aligned} \quad (9)$$

Notice that the role of the substrate S of the traditional Haldane-type model at Fig. 1 is equivalent to the role of the irradiance u in the PSF model (5) and (6).

Three model constants μ^* , K_S , K_I can be determined from Haldane curve, see, e.g., [6], therefore the parameters α , β , and γ are easily obtained from (9). In other words, to determine all five (α , β , γ , δ , and κ) of the PSF model, more conditions are needed to determine the remaining two parameters.

As the steady-state behavior is completely characterized by the parameters α , β , and γ , additional conditions taking advantage of some time-dependent measurements should be developed.

Before doing so, let us first conveniently reparameterize parameters involved in the problem. For this purpose, let us introduce new parameters derived from the steady-state production curve as follows:

$$\begin{aligned} p_1 &:= \sqrt{K_S K_I} = \sqrt{\frac{\gamma \delta}{\alpha \beta}} = u_{opt} \\ p_2 &:= \sqrt{\frac{K_S}{K_I}} = \sqrt{\frac{\alpha \beta \gamma}{\delta}} \frac{1}{\alpha + \beta} \\ p_3 &:= \mu^* \sqrt{\frac{K_I}{K_S}} = \kappa \gamma \sqrt{\frac{\alpha \delta}{\beta \gamma}} \\ p_4 &= \alpha u_{opt} = \gamma \sqrt{\frac{\delta / \gamma}{\beta / \alpha}} \\ p_5 &= \frac{\beta}{\alpha}. \end{aligned} \quad (10)$$

Note that only p_2 and p_5 are dimensionless, p_3 and p_4 are in s^{-1} , and the units for p_1 are those of irradiance, i.e., $\mu E \cdot m^{-2} s^{-1}$. Taking into account $\sqrt{\delta / \gamma} = (p_2(1 + \beta / \alpha))^{-1} \sqrt{\beta / \alpha}$ and introducing a new dimensionless input u^* defined as follows:

$$u^* = u / u_{opt} \quad (12)$$

[see (8)] the system (5) and (6) takes the reparameterized form

$$\begin{bmatrix} \dot{x}_2 \\ \dot{x}_3 \end{bmatrix} = [A + C u^*] \begin{bmatrix} x_2 \\ x_3 \end{bmatrix} + B u^* \quad (13)$$

$$A = p_4 \begin{bmatrix} -p_2(1 + p_5) & 0 \\ 0 & -\frac{p_5}{p_2(1 + p_5)} \end{bmatrix} \quad (14)$$

$$B = p_4 \begin{bmatrix} 1 \\ 0 \end{bmatrix} \quad (14)$$

$$C = p_4 \begin{bmatrix} -(1 + p_5) & -1 \\ p_5 & 0 \end{bmatrix}. \quad (15)$$

Again, all three parameters p_1 , p_2 , and p_3 are equivalent to steady-state behavior of the PSF. The remaining two parameters p_4 and p_5 basically correspond to the time constants of two interconnected processes (i.e., photosynthetic light and dark reactions and photoinhibition). As a consequence, they might be determined from dynamic measurements only. In the sequel, those measurements are of two kinds: 1) step input response and b) quasi-steady-state response to a harmonic forcing. It will be shown later on that, due to the presence of the fast and the slow dynamics, the step response is able to determine slow-dynamics time constant only. To determine the remaining parameter, the harmonic input response is used. This is the main contribution of this paper.

Summarizing, the above reparameterization will be used for the identification as follows:

- the use of the fixed-point steady-state measurements which lead to the determination of parameters p_1 , p_2 , and p_3 ;
- the dynamic measurements to determine the remaining parameters p_4 , and p_5 .

III. DYNAMIC BEHAVIOR-BASED IDENTIFICATION

As already shown, the steady-state measurements only suffice to determine the parameters p_1, p_2 , and p_3 while the time response of the plant to a suitable input signal is to be analyzed to determine the parameters p_4 and p_5 . The corresponding method is the main contribution of this paper and is described in this paper below.

All considerations here are based on the reparameterized description of the PSF system by (13)–(15).

According to [7], [17], [18], the relation (7) can be extended to the case when the state x_2 reaches its quasi-steady state (e.g., for the periodic input with the period T [13]). Then, the measured production rate of photosynthetic oxygen satisfies the following equation:

$$\mu = \frac{\kappa\gamma}{T} \int_0^T x_2 dt. \quad (16)$$

In other words, (16) gives the relation between the time integral of a hypothetical state of the PSF model and the measurable quantity μ . In such a way, this integral may be computed based on experimental measurements and then compared with the model-predicted value, thereby giving the basic framework for future identification methodology.

Therefore, the basic idea of our identification method is to analyze the time-dependent responses of the PSF system under suitable inputs, predict the corresponding value of μ , and, by comparing with its measured value, identify the remaining parameters.

One can think about different kinds of input signal (the light intensity applied to the algae). The most promising idea appear to be those adopted from control engineering: to subject the photosynthetic system to excitation of a variety of harmonic signals of different frequencies, amplitudes, and phases. Before considering harmonic forcing, let us briefly show some disadvantages of more straightforward approach based on studying the **time transition curve** to the steady fixed point corresponding to some constant input.

A. Constant Input Signal

Using more adequate control engineering terminology, the response to step input is being used here. The constant input response is easier to implement and predict; nevertheless, it does not enable to fully identify all parameters. The reason is that one should use the time curve of the transition process, which is decaying to the appropriate equilibrium point. As the system dynamics has both very fast and slow components, the only hope is to disregard a short time period when the fast component of system dynamics is vanishing to recover its slow component. As a consequence, despite the variety of constant inputs used, only the product $p_4 p_5$ can be determined.

To be more specific, let us realize that the system (13) is a stiff system. This has the following consequence: there is a relation $x_2 = Rx_3 + L$ which is established very rapidly, no matter what the initial conditions are. (To be precise, the state converges to the set described by the relation very quickly.) This convergence is practically unmeasurable. Further convergence to the steady

state is then much slower, and one may think of measuring it and comparing with the predicted one. Moreover, the state converges to the steady state without breaking the relation stated above. The set described by this relation is called *the slow manifold*. It will play an important role in future considerations.

Assume that a constant light intensity \hat{u} was applied to the system (13). As this system is stable, the states approach a constant vector $(\hat{x}_2, \hat{x}_3)^T$ in the limit. Denote by ξ_2, ξ_3 the following differences:

$$(\xi_2(t), \xi_3(t))^T = (x_2(t), x_3(t))^T - (\hat{x}_2, \hat{x}_3)^T.$$

Substituting this equality into the system (13), one can see that the variables ξ_i obey the following equation:

$$\frac{d}{dt} \begin{pmatrix} \xi_2 \\ \xi_3 \end{pmatrix} = p_4 \begin{pmatrix} -(1+p_5)(p_2 + \hat{u}) & -\hat{u} \\ p_5 \hat{u} & -\frac{p_5}{p_2(1+p_5)} \end{pmatrix} \begin{pmatrix} \xi_2 \\ \xi_3 \end{pmatrix}. \quad (17)$$

Let us multiply the first equation of the system (13) by the constant p_5 . Its typical value is known to be approximately 1/1000 [7], [17]. Therefore, one gets the following singularly perturbed system:

$$\frac{d}{dt} \begin{pmatrix} p_5 \xi_2 \\ \xi_3 \end{pmatrix} = p_4 p_5 \begin{pmatrix} -(1+p_5)(p_2 + \hat{u}) & -\hat{u} \\ \hat{u} & -\frac{1}{p_2(1+p_5)} \end{pmatrix} \begin{pmatrix} \xi_2 \\ \xi_3 \end{pmatrix}. \quad (18)$$

The left-hand term of the first equation can be replaced by zero. One gets the following algebraic equation

$$(p_2 + \hat{u})\xi_2 = -\hat{u}\xi_3. \quad (19)$$

This relation between the variables is established very rapidly. It actually describes the slow manifold indicated above.

The second equation in (18) and the relation (19) yield together the differential equation describing the evolution of the state ξ_2 in the manifold

$$\begin{aligned} \dot{\xi}_2 &= \frac{-\hat{u}}{p_2 + \hat{u}} \dot{\xi}_3 \\ &= -p_4 p_5 \left(\frac{\hat{u}^2}{p_2 + \hat{u}} + \frac{1}{p_2} \right) \xi_2 \\ &= - \left(p_4 p_5 \frac{\hat{u}^2 + \hat{u}/p_2 + 1}{p_2 + \hat{u}} \right) \xi_2. \end{aligned} \quad (20)$$

The last quantity in parenthesis should be equal to the coefficient of exponential decay of x_2 . This coefficient can be experimentally determined from the integral of x_2 measurements; nevertheless, one can now easily see the most important drawback, due to the term $p_4 p_5$ in (20), that only this product of parameters p_4 and p_5 could be determined from the constant inputs-based identification. Therefore, some additional kind of input is needed, namely the harmonic persistent input introduced in the following subsection.

B. Harmonic Input Signal

As already indicated, this approach takes advantage of the fact that forcing an asymptotically stable system by a harmonic signal generates nonstationary periodic steady-state nonlinear oscillation of its state. Clear advantage here is their steady-state

character: after some initial transition, these oscillations are a simple static nonlinear image of the forcing ones, they do not depend on an initial state, and they can go on forever. In such a way, together with input constantly varying throughout wide range of values, they provide a rich set of predicted data to be compared with measured ones. Moreover, the above static nonlinear function is the solution of a certain PDE that depends on frequency only, but is the same for all amplitudes and phases of the appropriate harmonic inputs.

More specifically, both components of the system dynamics can be excited throughout the time interval $(0, +\infty)$ if the frequency of the harmonic signal is properly chosen. Moreover, the response of the plant tends to a “periodic steady state.” In this state, the influence of the initial conditions is negligible, and the response is only dependent on the harmonic input signal. The response lies on a certain center manifold. Thus, the well-known center-manifold theory can be applied to determine the response.

To do so, one assumes that the input signal is generated by an external autonomous dynamic system. This system is usually called in control theory as the exosystem [9], and it will be defined later on.

The input of the system—the intensity of the light—is a harmonic signal with amplitude $K = (u(0)/u_{\text{opt}}) > 0$ and angular frequency ω . Moreover, a constant value is added so that intensity varies between zero and $2K$ as

$$u^*(t) = K(1 - \cos \omega t). \quad (21)$$

This signal is generated as follows. First, define the matrix S by

$$S = \begin{pmatrix} 0 & \omega \\ -\omega & 0 \end{pmatrix} \quad (22)$$

and the vector $w(t)$ by $w(t) = (\sin \omega t, \cos \omega t)^\top$. The function u^* is then generated by the so-called exosystem

$$\dot{w} = Sw, \quad w(0) = (0, 1)^\top. \quad (23)$$

The output of the exosystem is equal to the input of the controlled system. This signal describes also the light intensity that is applied to the photobioreactor. For future purposes, let us define also

$$\mathbf{u}(w) = K(1 - w_2).$$

This together with the definition of the variable w implies that $\mathbf{u}(w(t)) = u^*(t)$.

Some further assumptions must be done before the algorithm is introduced. First, one assumes that all trajectories of the exosystem lie in a bounded connected open set $0 \in W \subset R^2$. This poses a restriction on the amplitudes of the light intensity. This input of the controlled system generates the state $x(t)$ of this system. The influence of the initial conditions decays with time due to stability of the system. Thus, the state of the system tends to a periodic function. According to the center-manifold theorem [1], there exists a function $\mathbf{x} : W \rightarrow R^n$ so that $x(t) \rightarrow \mathbf{x}(w(t))$ for $t \rightarrow +\infty$. If the harmonic input signal

is generated by the system (23), the following holds in the limit case:

$$\dot{x}(t) = \nabla \mathbf{x}(w(t)) \dot{w}(t) = \nabla \mathbf{x}(w(t)) Sw(t), \quad \nabla \mathbf{x} := \begin{bmatrix} \nabla \mathbf{x}_1 \\ \vdots \\ \nabla \mathbf{x}_n \end{bmatrix}.$$

On the other hand, if the system is described by (13), one has the following:

$$\begin{aligned} \dot{x}(t) &= Ax(t) + Bu^*(t) + u^*(t)Cx(t) \\ &= A\mathbf{x}(w(t)) + B\mathbf{u}(w(t)) + \mathbf{u}(w(t))C\mathbf{x}(w(t)). \end{aligned} \quad (24)$$

As the previous equalities hold for every t , one arrives at the equation of the above-mentioned center manifold

$$\nabla \mathbf{x}(w)Sw = A\mathbf{x}(w) + B\mathbf{u}(w) + \mathbf{u}(w)C\mathbf{x}(w) \quad (25)$$

for every $w \in W$.

Use of this equation is advantageous since the value of the output in the limit case is available immediately after the solution of (25) for a set of initial conditions (i.e., for a set of magnitudes of the amplitude of the light intensity). Moreover, the periodic operation is used to gain the experimental data.

The function $\mathbf{x}(w)$ depends on the coefficients of the matrices A, B, C , i.e., it depends on the parameters p_2, p_4, p_5 . Concerning the fact that the parameters p_1, p_2, p_3 can be determined from the steady-state measurements (the measurements where the light intensity is the constant one), the only interesting dependence is the relation between the parameters p_4, p_5 and the solution.

The values of these parameters will be determined using a least-squares technique. The average values of the variable x_2 are measured on the real system. The system is excited by the signal u^* as described in (21). The angular frequency ω is kept fixed during a single measurement. On the other hand, one makes a series of measurements with various angular frequencies.

Values of the parameters are gained from the measured data as follows. Having an initial guess of the parameters, one can compute the solution of (25) and then evaluate the average value of the variable $x_2(t)$. Let us now turn our attention to the real system. As supposed before, the corresponding value x_2 approaches the periodic steady state. If the initial guess of the unknown parameters was equal to the true values of the real system, then all of the measured quantities would be equal. If this is not the case, the initial guess must be adjusted to decrease the discrepancy between the measurements and values predicted by the model. To do this, a “measure of discrepancy” has to be defined.

The measured quantity is mathematically defined as

$$f(\mathbf{x}) = \frac{1}{T} \int_0^T x_2(w(t)) dt.$$

Let \mathcal{C} be a closed trajectory of the exosystem in the state space. Equation (13) was derived so that the norm of the state of the

exosystem is normalized, i.e., $\|\dot{w}\| = \omega$. A straightforward calculation (recall $\omega = 2\pi/T$) leads then to the formula

$$\begin{aligned} \int_{\mathcal{C}} \mathbf{x}_2(w) ds &= \int_0^T \mathbf{x}_2(w(t)) \|\dot{w}(t)\| dt \\ &= \omega \int_0^T \mathbf{x}_2(w(t)) dt \\ &= 2\pi f(\mathbf{x}). \end{aligned}$$

(The symbol $\int_{\mathcal{C}} \dots ds$ denotes the curve integral along the curve \mathcal{C} .) Assume that there are N measurements of the quantity $f(\mathbf{x})$. These are denoted by the symbols y_1, \dots, y_N , the corresponding values of angular frequency and amplitude are $\omega_1, \dots, \omega_N$, respectively, K_1, \dots, K_N . Let the symbol $\mathbf{x}_{p,K,\omega}$ define the solution of (25) with the amplitude of the state of the exosystem equal to K , its angular frequency ω and parameters being $p = [p_4, p_5]$. Then, one can define the functional J by

$$J(p) = \sum_{i=1}^N (y_i - f(\mathbf{x}_{p,K,\omega_i}))^2. \quad (26)$$

During the iteration process, one changes the values of the parameters p_i so that the value of the above functional decreases.

The optimization of the functional equation (26) is a rather delicate topic itself. The reason for this is that this functional is in general nonconvex. However, from the point of view of this study, this topic is also a technical matter. Indeed, the main contribution of this paper is the method of how to compute efficiently, using the center-manifold PDE and a particular parameter estimate, the corresponding algae production prediction and the discrepancy. The optimization of the discrepancy is then optimization of some function of a finite number of parameters which is, in a sense, a quite standard procedure. Thus, a more detailed description of the optimization algorithm is omitted.

IV. EXPERIMENTAL RESULTS

The method was applied to the real data obtained from the measurement of the integral average of the quantity x_2 . The data used for the parameter identification were measured on the algae *Chlorella vulgaris*. This algae were grown in glass vessels and then exposed to the modulated light in the photobioreactor FMT 150 (see Fig. 2).

The periodic signal—the light intensity—was supplied to the algae. The light intensity was as described above with amplitude of the harmonic signal equal to $K = 1$. The period T of this signal was changed as described below. The measured values are summarized in the following table:

T	0.001	0.005	0.01	0.05	0.1	1
x_{2av}	0.52	0.49	0.47	0.41	0.34	0.27

It is important at this stage to point out that these experimental data have an illustrative and testing purpose only. To determine the coefficients p_4 and p_5 of (13) with a sufficient precision, one

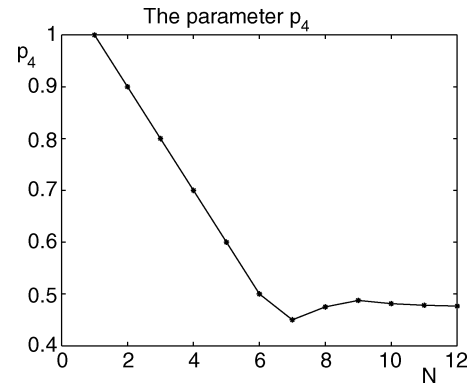


Fig. 4. Change in the parameter p_4 .

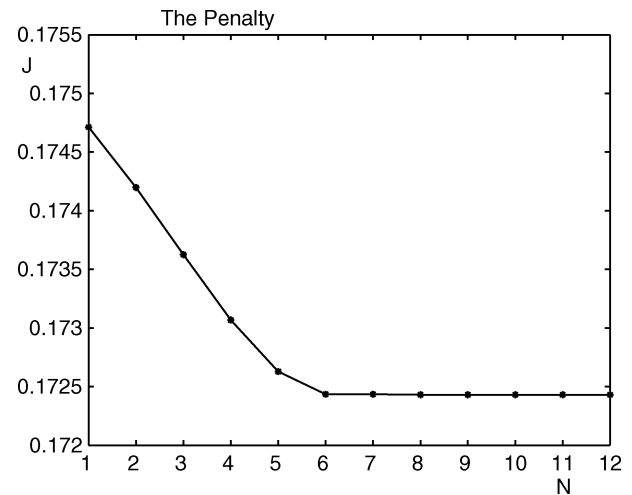


Fig. 5. Decrease of the penalty.

has to take a much more extensive amount of measured data. Moreover, for the sake of simplicity, we restricted ourselves to the case of determining the value of the constant p_4 ; the product of the parameters $p_4 p_5$ was set equal to $1/8000$, which is the value probably very close to the true one, as it corresponds to the time constant of 30 min of the exponential decay of x_2 under constant irradiance $u = u_{opt}$, see (20). Optimization of both parameters would be possible if a sufficient amount of data is available and a more sophisticated method for numerical optimization is implemented. (The method used to obtain the simulations in this paper was the one-dimensional gradient-based optimization.) It is worth pointing out that the shape of the involved expressions (the penalty functional and the center manifold equation) allows us to find equations for the derivatives of the minimized quantity with respect to the parameters. Hence, application of a gradient-based method is straightforward.

To avoid oscillations around a minimum that can sometimes occur during numerical optimization, a rather conservative setting of the numerical optimization method was used. However, this resulted in a slow speed of the algorithm. Thus, it was stopped before a near optimum was found. The change of the parameter p_4 can be seen in Fig. 4. Fig. 5 demonstrates how the penalty [(26)] decreases. One can see that the decrease of the value of the penalty slows down. Further changes of the parameter p_4 do not cause a significant decrease of the penalty—in

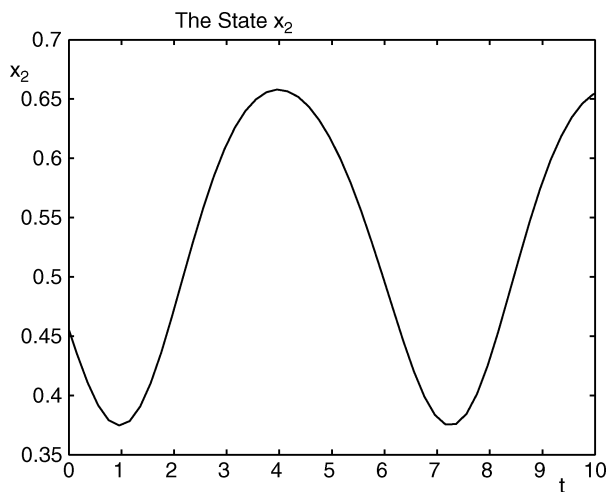


Fig. 6. State x_2 .

other words, the model already fits the given data quite well. Indeed, one has to realize that penalty does not have in the real case an optimum at zero, as the corresponding experimental measurements may carry the error. The seemingly minor penalty change from 0.17455 to 0.1725 is caused by the fact that the initial parameter approximation was taken already as the best available estimates known from literature. Therefore, Fig. 5 shows convincingly that those best available estimates were further improved (note the clear distinction between the penalty decrease up to the fifth iteration and its constant value after the sixth iteration). The periodic state of the model, if forced by the described input signal, can be seen in Fig. 6. Here, the evolution of the state x_2 is depicted. (This state describes the oxygen production rate. It is exactly the quantity that was used to gain the data for the parameter identification.) This figure was obtained using the formula (24) through the computed functions $\mathbf{x}(w)$ and $\mathbf{u}(w)$.

V. CONCLUSION

In this paper, a novel methodology for nonlinear identification based on the prediction of the response to harmonic forcing of a nonlinear system via its input channel was introduced. Its main purpose was to identify the model of microalgal growth that may further serve for optimizing photosynthetic production of real biotechnological plants.

Our future goals are related to the further experimental verification of the presented approach: 1) to test our method of the parameter estimation of PSF model parameters in the other microalgal species and 2) to study the behavior of PSF model parameters in a far time horizon, which corresponds to the photoacclimation process. For both purposes, more extensive experiments will be prepared, encouraged by the results presented in this paper.

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