Control of the Microalgae Photosynthetic Growth in a Torus Photobioreactor

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Abstract: The microalgae have the ability to use CO_2 as carbon source and, together with the solar energy, to biosynthesize various components, generating O_2 . They have a huge potential in various industrial applications such as the production of therapeutic and industrial metabolites, biofuels and environmental applications. The aim of this paper is to control the photosynthetic growth of the microalga *Chlamydomonas reinhardtii*, in a continuous torus shape photobioreactor. The control strategy was to maintain the biomass concentration constant into the photobioreactor using the dilution rate as control variable. The main disturbance was the incident light flux. Two control laws were designed and analyzed in simulation, to wit a linear control algorithm and a nonlinear one. The linear controller was synthesized in a PI structure which was further tested in simulation at variable setpoints and incident light fluxes. The nonlinear controller was conceived in order to minimize the inconveniences encountered at the PI controller. The simulation conditions were identical for both controller types.

Keywords: Chlamydomonas reinhardtii, photosynthetic growth control, linear controller, linearizant control, biomass growth process

1. INTRODUCTION

The Earth's atmosphere before Life was rich in CO_2 and CH_4 . In these hostile conditions the first microorganisms – cyanobacteria – emerged 3.5 billion years ago. For eons, they were the sole photosynthesizers which contributed with oxygen to the reducing and anaerobic atmosphere. The first eukaryote appeared only 1.5 - 2.2 billion years ago, so far no living organism ever grew larger than a single cell [1]. The first photosynthetic eukaryotes – microalgae – played an essential role in the formation of the actual breathable atmosphere. The microalge have the ability to use CO_2 as carbon source and, together with the solar energy, to biosynthesize various components for the cell, at the same time generating O_2 as a residue. The photosynthesis process underpins all these by starting with the tool for harvesting light, the chlorophyll.

The microalgae stirred up the interest of scientists and industrialists due to their huge potential. Species like *Arthrospira* and *Chlorella* are widely used in human and animal nutrition [2]. These organisms are also used in various applications such as the production of therapeutic and industrial metabolites as the long chain polyunsaturated fatty acids, pigments, polycarbohydrates, vitamins or various biological active compounds [3]. However, the microalgae can be also used in environmental applications due to their capability to fix the carbon dioxide and certain heavy metals during growth – wastewater treatment and the greenhouse gases reduction – and to produce energy without emission of greenhouse gases – biofuels production [4]. The researches for new technologies will allow us to use and produce a clean and renewable energy just like the one recently approached – hydrogen production with microalgae [5].

Most of the recent research in microalgal culturing has been carried out in photobioreactors with external light supplies, designed as either tubular reactors, flat panel reactors, or column reactors with large surface areas, short internal paths, and small dark zones [6].

The control of photosynthetic growth processes is generally difficult to realize because of the nonlinear and time-varying nature of the systems. The slow response and the lack of suitable on-line sensors able to read the most important state variables are also obstacles for an accurate control.

The aim of this paper is to synthesize and test in simulation two control algorithms based on the Fouchard *et al.*, model [7]. After a brief introduction, the second part describes the photobioreactor and the model used for this work. The third part is dedicated to results and discussion on the control algorithms followed by conclusions and references.

2. PHOTOBIOREACTOR DESCRIPTION AND MODELING

The photobioreactor modeled in this work (Fig. 1) has one enlightened surface of torus shape where the light falls perpendicularly. In this way the light per volume ratio is higher. The reactor is of only 4 cm wide giving thus a working volume of 1 L. The culture homogenization is provided through a marine impeller. The photobioreactor possess a complete loop of common sensors and automations for microalgae culture (pH, temperature, nutrients, dissolved O_2), and allows an accurate control of the injected and collected gas (O_2 , H_2 , N_2 , CO_2). The batch and the continuous mode are both suitable for this type of reactor [8].

The microorganism modeled was the microalga *Chlamydomonas reinhardtii* (wt 137c strain from the Chlamydomonas Genetic Center, Duke University, Durham, USA) [7].

The models used at microalgae culturing are as many as the objectives for which they were developed and as complex as their utility. As commonly applied in photobioreactor modeling, they can describe the kinetics of the photosynthetic growth coupled with the light transfer inside the culture, which needs to be modeled as soon as the light is absorbed by cells. Accurate formulation of such a coupling, to correctly consider its influence on the process, is a problem on its own [7].

In an optimal system where no factors limit the growth, the rate of photosynthesis and productivity is determined by the light availability [9].



Fig. 1. Schematic representation of the photobioreactor operating system in turbidostat mode [7]

Various works were performed on photosynthetic growth modeling [10], [11]. The specific growth rate (μ) increases along with the increasing irradiance, reaching a maximum value, μ_{max} . Further increase in irradiance may inhibit growth – a phenomenon known as photoinhibition. Although this phenomenon is well documented, it has often been disregarded [12], [13].

Fouchard *et al.* [7] proposed a model which describes the three stages which precede the hydrogen production under sulfur limitation conditions.

The model retained

The model underlying the work presented in this paper is the model proposed by Fouchard *et al.* [7]. It introduces a continuous formulation to describe the progressive transition from oxygenic growth to anoxia in order to obtain biohydrogen. The model is expected to be independent of the case under study, with corresponding parameters estimated from individual sets of experiments. This condition is important for optimizing the culture conditions and to investigate new protocols for biohydrogen and biomass production [7].

According to Fouchard *et al.* the process is divided in three phases: photosynthetic growth, sulfur deprivation and hydrogen production. For the mathematical simulation of the process it was considered only the first phase in order to demonstrate that the model, with the parameters already identified [7], can be also used in biomass production processes (independent of hydrogen production conditions).

Because light is a limiting factor in the model formulation must be introduced the coupling between a radiative model [14] and a Haldane one to represent lightdependent photosynthetic growth kinetics [15]. The light transfer inside the culture – irradiance G – is dependent of the photobioreactor geometry. The torus-shaped photobioreactor [14], [15] under study (Fig. 1) enables the one-dimensional hypothesis to be applied – light attenuation occurring in only one direction namely the depth of culture *z* which is perpendicular to the illuminated surface.

The two-flux model can then be used and the following formulation of irradiance (Eq.1) distribution can be employed as follows:

$$G(z) = 2q_0 \frac{\left(1+\alpha\right)e^{\delta(L-z)} - \left(1-\alpha\right)e^{-\delta(L-z)}}{\left(1+\alpha\right)^2 e^{\delta L} - \left(1-\alpha\right)^2 e^{-\delta L}}$$
(1)

where $\delta = X \sqrt{E_a (E_a + 2bE_s)}$ is the two-flux extinction coefficient, and $\alpha = \sqrt{(E_a)/(E_a + 2bE_s)}$ is the linear scattering modulus. E_a and E_s are the mass absorption and the mass scattering coefficients (m²·kg⁻¹), and *b* is the backward scattering fraction (dimensionless). q_0 represents the hemispherical incident light flux. *X* represents the biomass concentration inside the photobioreactor (kg·m⁻³) and *L* represents the depth of the photobioreactor (m).

Light dependency is represented by a photosynthetic growth model with an inhibitory term [16] to characterize the small decrease of growth rate that can be observed for high irradiance (photoinhibition):

$$\mu_G = \mu_{\max} \frac{G}{K_I + G + \frac{G^2}{K_{II}}}$$
(2)

where K_I is the half-saturation constant and K_{II} the inhibition constant.

The specific growth rate expressed in this form (Eq.2) enable us to determine the local photosynthetic response

 $\mu_G(G(z))$. However in our simulation there was used the average photosynthetic response $\langle \mu_G \rangle$ calculated all over the reactor's volume, obtained by integrating local photosynthetic responses (Eq.3).

$$\left\langle \mu_{G} \right\rangle = \frac{1}{L} \cdot f(\mathcal{Q}) \cdot \int_{0}^{L} \mu_{G}(G(z)) dz$$
 (3)

The mass balance equation (Eq.4) was used to illustrate the evolution of biomass concentration X(t):

$$\frac{dX}{dt} = \left\langle r_X \right\rangle - DX \tag{4}$$

In this equation D (h⁻¹) is the dilution rate and $\langle r_X \rangle$ is the volumetric rate which is detailed in [7].

The connection between biomass and sulfur concentrations (sulfur limitation representing the protocol for biohydrogen production) is given by f(Q) function which represents the intracellular sulfur quota Q influence on the photosynthetic activity. It is inserted as a factor in volumetric rate $\langle r_X \rangle$, which can take values from 0 to 1. In the case of non-limiting intracellular sulfur quota, the specific growth rate is only related to light limitation – f(Q) = 1.

In the following section which regards the control of the photosynthetic growth process the sulfur substrate was considered unlimited and uninhibitory, therefore it does not influences the biomass concentration.

3. CONTROLLER DESIGN AND TEST IN SIMULATION

The biomass concentration is one of the most important variables, which needs to be controlled although it is the desired output or not. The biomass concentration values can be acquired through sampling or on-line measurements based on principles like cytometry or optical density. Currently, the most used on-line measurement of the biomass is the turbidity which represents a cheap and fast solution. However, the turbidity sensors need to be periodically calibrated and often cleaned in place. The turbidity is a nondiscriminative measurement which includes all suspended solids, and aggregate formation can induce erroneous measurements.

Classical manners to control the biomass production in continuous photobioreactor are the turbidostat and the chemostat. In these cases the biomass X or the substrate S are kept constant through control variables such as the dilution rate (D) or/and the incident light flux (q_0) – to optimize the production system and to avoid the photoinhibition in early growth phases (Fig. 2).



Fig. 2. General control structure of the micro-algae growth process

The control algorithms designed in this work considered only dilution rate D as control variable while the incident light flux was kept to a constant value.

Before choosing a proper solution for a controller, the biological system was linearized around a stationary steady state (nominal operating point). Having as target the control of the biomass X(t) through a command on the dilution D, it was determined the optimal dilution (D_o) which corresponds to the maximal performance. The performance of the system was determined by computing the performance index I (Eq.5) which represents the total amount of biomass obtained at the output of the photobioreactor after a given period of time t.

$$I = D \cdot V \cdot \int_{0}^{t} X dt = F \cdot \int_{0}^{t} X dt$$
(5)

where V is the photobioreactor volume (1L) and F is the flow rate (L/h).

Fig. 3 shows the variation of the total amount of biomass produced after 5 days of cultivation in accordance with the dilution rate. The biomass amount (expressed in grams) was determined at four different values of the incident light flux (q_0) : 110, 350, 650 and 1100 µmol photon·m²·s.



Fig. 3. The performance index variation at different light intensities

As it can be seen in Fig. 3 the optimal dilution takes values between 0.05 and 0.07 h^{-1} , on the given incident

light flux interval. For further linearization there were considered the following nominal values: $q_0=110 \ \mu \text{mol}$ photon·m²·s involving $D_o = 0.07 \ \text{h}^{-1}$. Considering the proposed values, the steady state value of the biomass was determined and the linearization was made around that point. The steady state value of the biomass was determined as being 0.18 g/L.

To linearize the system, the mass balance equation of the biomass was reconstructed in *Simulink*[®]. The linearization was made using the linmod function of *Matlab*[®] ver. 7.9 which returned the following transfer function (Eq. 6):

$$H(s) = \frac{-4.286}{s + 0.846} \tag{6}$$

Because the transfer function denominator is a first degree equation the most suitable controller for this case is a PI (proportional-integral) one.

3.1. The PI control of the biomass growth process

Having a stable system (negative root of the denominator) and a linear transfer function the first step was to synthesize the control architecture of the system (Fig. 4).



Fig. 4. The PI control structure for the biomass growth process

$$H(s) = K_P\left(1 + \frac{1}{T_i s}\right) \tag{7}$$

where K_P is the proportional gain and T_i is the integral time setting of the controller.

The values of the coefficients were determined through graphical tuning.

The next step implied was to implement the linear controller into the nonlinear model and for this the following control (Eq. 8) was used:

$$D(t) = D_o + K_P \cdot e(t) + \frac{K_P}{T_i} \int_0^t e(t)$$
(8)

e(t) represents the error: biomass measured value subtracted from the setpoint.

Even if the controller is initialized with steady state values and the optimal dilution is inherent, its addition in the control variable formulation has an overshoot limiting effect of the integral term (Fig. 5). Besides, an antiwindup structure implemented on the nonlinear model had a further intense reducing effect of the overshoot (Fig. 5).



Fig. 5. The cumulated effect of the anti-windup structure and D_o (for $K_P = 1$ and $T_i = 0.5$)

It is well known that the shorter the integral time (T_i) is, the more often the proportional correction is repeated, and thus the integral contribution is more effective. If the system allows a higher integral time (T_i) , the integral contribution will be more limited and the controller will "hit" faster and more accurately the setpoint. In Fig. 6 there are rendered the evolution of the biomass concentration and the control variable profile. The setpoint was chosen in the vicinity of the steady state (0.2 g/L).



Fig. 6. Biomass concentration in a PI controlled system $(K_P = 1, T_i = 5)$ and the control variable of the system

In fig. 6 one can observe that the biomass reaches the setpoint value in less than 1 day with no overshoot, and chimes with the technological reality. The control variable is a smooth one which stabilizes at a steady value, namely the optimal dilution D_o .

The system must also have the ability to track different setpoints. Fig. 7 illustrates the biomass evolution (at setpoints other that the steady state one) and the control variable (dilution rate) in constant light flux (110 μ mol photon·m²·s). The simulation was realized for a 20 days period conferring 5 days for every chosen setpoint: 0.2 g/L, 0.35 g/L, 0.5 g/L and back to 0.2 g/L.

As it can be observed in Fig. 7 the biomass reaches the desired setpoint after more than 2 days which is considered to be a very slow response.



Fig. 7. Biomass concentration at different setpoints ($K_P = 1$, $T_i = 1$) and dilution rate needed to reach the setpoint

Another important variable of the system is the incident light flux whose variation substantially influences the biomass concentration. Hereinafter (Fig. 8) a variable incident light flux was imposed, the simulation being made for the same period of 20 days. On the first three days the photosynthetic growth deployed at 110 μ mol photon·m²·s, on the next 5 days the light flux was raised at 250 μ mol photon·m²·s, after another 5 days heightened at 350 μ mol photon·m²·s and on the last 8 days dropped back to 110 μ mol photon·m²·s.



Fig. 8. Biomass concentration at different incident light fluxes ($K_P = 1$, $T_i = 1$) – solid line, dilution rate for tracking the setpoint – dotted line and setpoint – thin dotted line

As it can be observed in Fig. 8 the controller reaches the setpoint (0.4 g/L) after more than 2 days, imposing an error of over 10%.

3.2. The Linearizing control of the biomass growth process

A more accurate method to track the setpoint of a biological system is the linearizing control which gives withal a faster response.

As it is well known, the linearizing control is a non-linear one designed to achieve a linear closed loop which is unconditionally stable no matter the operating point [17].

Let us consider the general model (Eq.9) of a biotechnological process:

$$\frac{d\xi}{dt} = K\varphi(\xi) - D\xi + F - Q \tag{9}$$

with ξ - state vector (dim(ξ)=N), φ - reaction rate vector (dim(φ)=M), F – flow rate and Q – gaseous product. The objective is to control a scalar output, *y*, which is a linear combination of state variables (Eq.10) that can be measured in the process.

$$y = \sum_{k=1}^{N} c_k \xi_k = C^T \xi$$
⁽¹⁰⁾

Any of the dilution rate D or the flow rate F can be considered as control inputs denoted by u. The control objective of the system is to track a reference signal $y^*(t)$. The principle of the linearizing control is to design a control law which is a multivariable nonlinear function of ξ , y^* , F and Q so that the tracking error is given by a prespecified stable linear model (reference model).

The linearizing control design procedure consists in three steps, as follows:

1. Establishing a model for variable y (a δ^{th} order differential equation – where δ is called relative degree).

$$\frac{d^{\delta} y}{dt^{\delta}} = f_0(t) + u(t)f_1(t) \tag{11}$$

2. Selecting a stable linear reference model of the tracking error (Eq.12):

$$\sum_{k=0}^{\delta} \lambda_{\delta-k} \frac{d^{k}}{dt^{k}} \Big[y^{*}(t) - y(t) \Big] = 0, \quad \lambda_{0} = 1$$
(12)

The coefficients $\lambda_{\delta k}$ are chosen so that the differential equation (Eq.11) to be stable.

3. Calculus of the control law u(t) so that the I/O model (Eq.11) exactly matches the reference model (Eq.12):

$$u(t) = \frac{1}{f_1(t)} \left[-f_0(t) + \sum_{k=0}^{\delta - 1} \lambda_{\delta - k} \frac{d^k}{dt^k} \left[y^* - y \right] + \frac{d^{\delta} y^*}{dt^{\delta}} \right]$$
(12)

In the case of the microalgae photosynthetic growth process the model order can be considered equal to 1 (the nutrient concentration is considered unlimited), this means that the relative degree δ is also equal to 1. The structure of the linearizing control system is represented in Fig. 9.



Fig. 9. Linearizing control structure for the biomass growth process

The following linearizing control was determined (Eq.13) according to the rules presented above (the dimensionless coefficient λ_I was chose so that the system reaches the maximal specific growth rate):

$$D = \frac{r_x - \lambda_1 \cdot e}{24 \cdot X} \tag{13}$$



Fig. 10. Biomass concentration in a linearizing control system and the control variable of the system (λ_1 =5)



Fig. 11. Biomass concentration at different setpoints and dilution rate needed to reach the setpoint (λ_1 =5)



Fig. 12. Biomass concentration at different incident light fluxes – solid line, dilution rate for tracking the setpoint – dotted line and setpoint – thin dotted line (λ_1 =5)

The simulation was made in the exact same conditions as the ones imposed for the linear PI controller.

In Fig. 10 it can be observed that the setpoint is accurately tracked by the nonlinear control, the system stabilizing itself at the same optimal dilution as the PI controller. However these details will be later validated in practice. The real difference between the two control laws can be observed in Fig. 11 and 12. The nonlinear control is not designed around a steady state value and, as a consequence, it can work better at various setpoints giving a faster and better response for a similar control variable. Moreover, the results for incident light flux variations are far better than the ones obtained with the PI controller in the same conditions.

4. CONCLUSIONS

The linear PI controller gives satisfactory results in what regards the photosynthetic growth process, but the disadvantage is that it only works well around the steady state for which it was tuned. The results could be improved by designing a nonlinear PI controller able to adapt itself at a wider range of setpoints and light intensities.

Instead the linearizing control gives better results, being independent of a steady state value and easy to adjust, but it is more complex and requires a validated model and knowledge on the specific growth rate (μ). Its implementation compels the use of a process computer, whereas the PI structure is easier to implement on industrial scale.

The further objective is to validate both control laws on laboratory scale. Additional work will be done on the nonlinear linearizing controller implementation which will be coupled with a software sensor that gives the online estimation of the specific growth rate from gas measurement (O_2 , CO_2).

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REFERENCES

- [1] Seckbach J., "Algae and Cyanobacteria in Extreme Environments", Springer Press (2007)
- [2] Spolaore P, Joannis-Cassan C, Duran E, Isambert A, "Commercial applications of microalgae". *J Biosci Bioeng* (2006) 101:87–96
- [3] Greenwell H. C., Laurens L. M. L., Shields R. J., Lovitt R. W. and Flynn K. J., "Placing microalgae on the biofuels priority list: a review of the technological challenges". J. R. Soc. Interface (2010) 7, 703–726
- [4] Brennan L., Owende P., "Biofuels from microalgae

 A review of technologies for production, processing and extractions of biofuels and co

products", in press (2009) doi:10.1016/j.rser.2009.10.009

- [5] Fouchard S., Pruvost J., Degrenne B., Legrand J., "Investigation of H₂ production using the green microalga Chlamydomonas reinhardtii in a fully controlled photobioreactor fitted with on-line gas analysis", *International Journal Of Hydrogen Energy* 33 (2008) 3302 – 3310
- [6] Cornet J.F. and Dussap C.G., "A Simple and Reliable Formula for Assessment of Maximum Volumetric Productivities in Photobioreactors", *Biotechnol. Prog.* Vol. 25, No. 2 (2009) 424 – 435
- [7] Fouchard S., Pruvost J., Degrenne B., Titica M., Legrand J., "Kinetic Modeling of Light Limitation and Sulfur Deprivation Effects in the Induction of Hydrogen Production With Chlamydomonas reinhardtii: Part I. Model Development and Parameter Identification", *Biotechnology and Bioengineering*, Vol. 102, No. 1 (2009) 232 – 245
- [8] Fouchard S., Pruvost J., Legrand J., "Investigation of H2 production by microalgae in a fully-controlled photobioreactor", WHEC 16th/ 13-16 June 2006 – Lyon France
- [9] Aiba S., "Growth kinetics of photosynthetic microorganisms". *Adv. Biochem.* Eng. 23 (1982) 85– 156.
- [10] Molina Grima E., Fernandez Sevilla J.M., Sanchez Perez J.A., Garcia Camacho F., "A study on simultaneous photolimitation and photoinhibition in dense microalgal cultures taking into account incident and averaged irradiances" *J. Biotechnol.* 45 (1996) 59 – 69

- [11] Muller-Feuga A., "Growth as a function of rationing: a model applicable to fish and microalgae", *Journal of Experimental Marine Biology and Ecology*, 236 (1999) 1 – 13
- [12] Cornet J.-F., Dussap C.G., Gros J.B., "A simplified monodimensional approach for modelling coupling between radiant light transfer and growth kinetics in photobioreactors", *Chemical Engineering Science*, 50 (1995) 1489 – 1500
- [13] Carvalho A.P. and F.X. Malcata, "Kinetic modelling of the autotrophic growth of Pavlova lutheri: study of the combined influence of light and temperature", *Biotechnology Progress*, 19 (2003) 1128 – 1135
- [14] Pottier L, Pruvost J, Deremetz J, Cornet J, Legrand J, Dussap C. 2005. "A fully predictive model for one-dimensional light attenuation by Chlamydomonas reinhardtii in a torus photobioreactor". *Biotechnol Bioeng* 91 (2005) 569 582
- [15] Andrews J. "A mathematical model for the continuous culture of microorganisms utilizing inhibitory substrates". *Biotechnol Bioeng* 10 (1968) 707 – 723
- [16] Pruvost J, Pottier L, Legrand J. 2006. "Numerical investigation of hydrodynamic and mixing conditions in a torus photobioreactor". *Chem Eng Sc* 61 (2006) 4476 – 4489
- [17] Bastain G. and Dochain D., "On-line Estimation and Adaptive Control of Bioreactors", Elsevier Press (1990)