Dynamic Optimization of Biomass Productivity in Continuous Cultures of Microalgae *Isochrysis galbana* through Modulation of the Light Intensity

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Abstract: This paper presents a possible approach for the dynamic optimization of biomass productivity in continuous cultures of microalgae, using light intensity as the manipulated variable. Extremum seeking control is applied as the real-time optimization algorithm to evaluate feasibility. Two different models of microalgae growth (of different complexities) were used in this study, with parameters representative of the *Isochyris galbana* specie. Results showed relatively good performances of the optimization procedure despite parameter variations and the presence of measurement noise.

Keywords: microalgae, continuous culture, real-time optimization, photo-acclimation, photo-inhibition.

1. INTRODUCTION

Our understanding of the photosynthesis process, along with our ability to efficiently exploit its properties, is at the core of any advance towards the successful industrialization of algal bioprocesses. Algae are expected to play a key role in making our world a sustainable place to live again: replacing fossil fuels with renewable alternatives (Chisti, 2007; Lam and Lee, 2012; Demirbas, 2010), capturing CO_2 and mitigating climate change (Lam et Lee, 2012; Demirbas, 2010), even providing proteins and other nutrients for animal feed and human food supplements (Chisti, 2007; Becker, 2007; Mata et al., 2010; Lam and Lee, 2012) as well as other high-value molecules of pharmaceutical interest (Chisti, 2007; Mata et al., 2010).

Continuous mode of production (chemostat) is interesting for microalgae for different reasons: first, it is a powerful tool for studying the cell growth kinetics and physiological behaviors (Ethier et al., 2011), on which much research still remains to be done, and second, it allows prolonged stable production of biomass, possibly at maximum productivity (Gojkovic et al., 2013). For example, (Sforza et al., 2013) produced 2 distinct strains of microalgae for over 100 days in continuous mode, with a constant biochemical composition and photosynthetic behavior. Similar observations are found in (Ferreira et al., 2008) and (Kaspar et al., 2014). Heterotrophic cultures also show similar properties: (Ethier et al., 2011) used continuous cultures to characterize DHA productivity, and (Gojkovic et al., 2013), selenomethionine (SeMet) production.

This paper will focus on photosynthetic aspects of continuous (autotrophic) productions of algal biomass, for which detailed rigorous models were recently made available accounting for photo-inhibition and photo-acclimation (Bernard et al., 2009; Bernard, 2011). These phenomena drive many physiological responses of the algae, impacting product formation kinetics, hence the importance of a good control over their influence.

Here, only biomass productivity will be considered, as a first stage of development. Real-time optimization of this quantity will be conducted using the extremum-seeking control (ESC) algorithm, with light intensity as the manipulated variable. To the best of the author's knowledge, there has apparently been no report of the application of dynamic optimization to light intensity in photobioreactors: there are however several static optimization studies, (e.g., Hallenbeck et al., 2014), one databased feed-forward (without feedback correction) approach to batch cultures in (Kandilian et al., 2014) and one trajectory tracking approach for the light-to-algae ratio to minimize the light requirements in batch productions of a desired biomass quantity (Tebbani et al., 2014).

The objective of this paper is therefore to investigate the possibility of adjusting the light intensity online in order to continuously track the optimal biomass productivity and to achieve this in an almost model-free setup, in the spirit of the extremum seeking control approach popularized by (Ariyur and Krstić, 2003). In addition, robustness of the ESC scheme is assessed against plant-model "mismatch" (regarding the model knowledge that is used at the parameter tuning step) and measurement noise. The paper is organized as follows: first, the general principles for the models are presented and discussed, and the main parameters are given. The ESC implementations (and the tuning approach) are then presented and tested in various situations including parameter variations and in the presence of measurement noise.

2. MODELS OF MICROALGAE GROWTH

2.1 Generalities

The dynamic model used in this study is borrowed from the excellent survey (Bernard, 2011), and describes the culture of *Isochrysis galbana* in a continuous photobioreactor under time-varying light conditions. The keystone of this model is

the classical Droop model (Droop, 1968), supplemented by appropriate kinetics accounting for photo-acclimation, photo-inhibition, as well as light absorption and diffusion.

2.2 Droop model

Microalgae have the ability to store nutrient *s* into a so-called internal quota *q*, which is further used for biomass *x* growth. Nutrient uptake $\rho(s)$ can be described by a Michaelis-Menten type kinetics

$$\rho(s) = \rho_{\max} \frac{s}{K_s + s} \tag{1}$$

whereas the growth rate can be modelled by Droop kinetics (Droop, 1968):

$$\mu(q) = \mu_{\max}\left(1 - \frac{q_{\min}}{q}\right)$$
(2)

In these expressions, K_S is a half-saturation constant, q_{\min} is the quota threshold below which no growth is possible, and ρ_{\max} and μ_{\max} are the maximum uptake and growth rates, respectively. Mass balances in the chemostat lead to the following set of ordinary differential equations

$$\begin{aligned} \dot{x} &= \mu(q)x - Dx \\ \dot{s} &= -\rho(s)x + D(s_{in} - s) \\ \dot{q} &= \rho(s) - \mu(q)q \end{aligned} \tag{3}$$

where s_{in} represents the concentration of inorganic substrate (nitrogen) in the inlet flow and *D* the dilution rate. The Droop model has been used in a large variety of applications and is a widely accepted model. Under time-varying light conditions, however, it has to be extended to include photo-acclimation as well as photo-inhibition. In photobioreactors operating at high cell density, the phenomena of light absorption and diffusion also have to be taken into account.

2.3 Photo-acclimation and photo-inhibition

Model extensions are necessary to describe the influence of time-varying light exposition of the microalgae. Such model extensions should however remain relatively simple so as to avoid introducing an excessive number of parameters, which would be very difficult to extract from experimental data and would make the model of little use for control applications. The idea is to modulate the maximal growth rate $\mu_{max}(I, \theta)$ as a function of the light intensity *I* and the chlorophyll to biomass ratio $\theta = Chl / x$, to account for two mechanisms: (1) photo-acclimation, the adaptation of chlorophyll synthesis to light intensity and (2) photo-inhibition, leading to a reduced growth at higher light intensities (Eilers and Peeters, 1988; Eilers and Peeters, 1993), for example:

$$\mu_{\max}(I,\theta) = \overline{\mu} \frac{I}{K_{sI} + I + \frac{I^2}{K_{iI}}}$$
(4)

In this expression, the parameter K_{sI} is a function of the chlorophyll to biomass ratio in the following way:

$$K_{sI} = \frac{K_{sI}^*}{\theta} \tag{5}$$

Chlorophyll is considered to be proportional to the particulate nitrogen (Laws and Bannister, 1980):

$$Chl = \gamma (I^*) x q \tag{6}$$

with a coefficient

$$\gamma(I^{*}) = \gamma_{\max} \frac{k_{I^{*}}}{k_{I^{*}} + I^{*}}$$
(7)

being an empirical function of I^* representing the irradiance at which microalgae are acclimated following the dynamics:

$$\dot{I}^* = \delta \mu(q, I, \theta) (\bar{I} - I^*)$$
(8)

In this latter expression, δ is the adaptation rate and \overline{I} is the average irradiance along the culture volume.

This average irradiance can be computed quite easily for the case of flat panel photobioreactors (length L), where the light attenuation due to the biomass can be modelled using a Beer-Lambert law (Bernard et al., 2009) and approximated as:

$$\bar{I} = I \frac{K_g}{K_g + (a Chl + b)L}$$
⁽⁹⁾

Where K_g , *a* and *b* are parameters for this "workable" model (Bernard et al., 2009). Rather than directly introducing this expression into the Haldane law describing the dependency of the maximum growth rate on light, it is preferable to use an averaged value of the maximum growth rate leading to a modified expression (Bernard et al., 2009):

$$\mu_{\max}(I,\theta) = \overline{\mu} \frac{\overline{I}}{K_{sI} + \overline{I} + \left(\frac{\overline{I}^2}{K_{iI}}\right)} e^{Ch/L/2}$$
(10)

An alternative form (arctangent expression) for this function of the growth rate, presented in (Bernard, 2011), will also be of interest later in this paper (with same parameters K_{sI} , K_{il}).

To account for the dark periods (which occur as microalgae move through darker spots inside the photobioreactor), it is also necessary to include a down regulation of the substrate uptake (Lehman et al., 1975):

$$\rho(s,q) = \rho_{\max} \frac{s}{K_s + s} \left(1 - \frac{q}{q_{\max}}\right)$$
(11)

The full model can thus be summarized as follows:

$$\dot{x} = \mu(q, I, \theta)x - Dx - Rx$$

$$\dot{s} = -\rho(s, q)x + D(s_{in} - s) \qquad (12)$$

$$\dot{q} = \rho(s, q) - \mu(q, I, \theta)q$$

$$\dot{I}^* = \delta \mu(q, I, \theta)(\bar{I} - I^*)$$

where a respiration term R was also introduced in the balance equation for the biomass (Bernard, 2011).

2.4 Parameters

The model parameters were obtained for *Isochrysis galbana* in (Bernard, 2011) and are provided in Table 1 for the reader.

Symbol	Value (units)	Symbol	Value (units)
$ ho_{ m max}$	$0.073 \text{ gN} \cdot \text{gC}^{-1} \cdot d^{-1}$	K_{iI}	295 $\mu E \cdot m^{-2} \cdot s^{-1}$
K_S	$0.0012 \text{ gN} \cdot \text{m}^{-3}$	k_{I^*}	$63 \ \mu E \cdot m^{-2} \cdot s^{-1}$
Kg	$1.25 \ \mu E \cdot m^{-2} \ s^{-1}$	δ	1 (no units)
$q_{ m max}$	0.250 gN·gC ⁻¹	K_{sI}^{*}	$1.4 \ \mu E \cdot m^{-2} \ s^{-1}$
q_{\min}	0.050 gN·gC ⁻¹	а	$16.2 \text{ m}^2 \cdot \text{gChl}^{-1}$
γ _{max}	0.57 gChl·gN ⁻¹	b	$0.087 \ m^{-1}$
$\overline{\mu}$	$1.7 d^{-1}$	R	$0.0081 d^{-1}$

Table 1. Model parameters

3. EXTREMUM SEEKING IMPLEMENTATION

The real-time volumetric biomass productivity in continuous bioreactors is the product between the biomass concentration X (grams per volume) and the dilution rate D (days d):

$$P\left(g \operatorname{Vol}^{-1} d^{-1}\right) = D\left(d^{-1}\right) \times X\left(g \operatorname{Vol}^{-1}\right)$$
(13)

Real-time measurements of the biomass concentration can be reasonably achieved with OD correlations (Deschênes et al., 2012; Havlik et al., 2013) while D is a manipulated variable. Thus, the real-time availability of the optimization criterion P is possible in these situations.

In our particular context, it is assumed that the dilution rate D depends on external process conditions or constraints that are beyond remaining actuators for reactor operation and control. This leaves the task to other actuation variables, such as light intensity, to optimize the uptake of nutrients from the effluent stream and/or the production of biomass. For a fixed value of D (non-zero), the latter can also be maximized through acting on the biomass concentration alone: from (13), maximizing X also maximizes P in this situation. The two possibilities will be considered as the variable y(t) in the (perturbation-based) ESC optimization scheme (Fig. 1) used throughout the paper.



Fig. 1. Perturbation-based ESC scheme used in this paper.

3.1 Tuning of the perturbation-based ESC

The ESC tuning was done following (Deschênes et al., 2012) and (Deschênes, 2013): a step-response test was conducted to obtain an approximation of the natural system dynamics. This information was then used for the determination of the dither signal fundamental frequency, based on desired performances and robustness considerations. The amplitude of this signal is fixed based on a trade-off between sufficient excitation on the output and the variation of the input over its possible range. A second-order Butterworth high-pass filter is used, with its parameters based on the system's dynamics and the choice of the dither signal frequency (Deschênes, 2013). Integrator gain k is last obtained, by a trial-and-error approach. Although the present study is entirely in simulation, this general procedure has been used successfully in the past with a real microalgae bioprocess (Deschênes et al., 2012) for pH optimization.

For both models, the step-response was of the first-order type with a time constant of approximately 1 day (rough estimate). Relative to this value of τ , different choices can be made for the dither excitation frequency ω : three will be considered for this paper: $1/\tau$, $5/\tau$ and $10/\tau$. The dither signal amplitude was set to $a = 8 \ \mu \text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (on a 0-120 $\mu \text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ variation range). The best values for *k* were found to be 20 ($\omega = 1 \ \text{rad} \cdot d^{-1}$), 100 ($\omega = 5 \ \text{rad} \cdot d^{-1}$) and 400 ($\omega = 10 \ \text{rad} \cdot d^{-1}$).

4. PERFORMANCE RESULTS

4.1 Influence of the model

Models from (Bernard et al., 2009) and (Bernard, 2011) were implemented and compared in open-loop ($I = 60 \ \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and with the ESC ($D = 0.35 \ d^{-1}$), with D and I set at realistic values for this algae. Fig. 2 shows the results for $\omega = 1 \ \text{rad} \cdot d^{-1}$ with the ESC, where differences are rather small between the two models. For $\omega = 5$ and 10 rad $\cdot d^{-1}$ as the dither excitation frequency, the results are hardly distinguishable (not shown).



Fig. 2. Model comparison in open-loop and with ESC.

One important thing that can be noticed in Fig. 2 is that as the output converges, the input continues to evolve through time. This is a consequence of the photo-acclimation phenomenon, which would be rather complex and lengthy to analyse here, but simply put, the optimum varies with the acclimated light intensity I^* . Looking at the maximal growth rate function (Fig. 3) for two distinct values of I^* (and associated boundary values for θ as $q = q_{\min}$ and q_{\max}) illustrates the situation: the optimum for \overline{I} is always slightly higher than I^* (e.g. between 70 and 90 $\mu \text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ for $I^* = 70 \ \mu \text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, and between 170 and 210 $\mu \text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ for $I^* = 130 \ \mu \text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), and this constantly pushes the input light intensity towards higher values. Also, as the acclimated light intensity becomes higher, the maximal growth rate decreases (as Fig. 3 also shows).



Fig. 3. Theoretical mapping between the maximal growth rate and average light intensity inside the photobioreactor.

Consequences of this are that the optimum has to be tracked continuously, and as it goes, biomass productivity eventually decreases. Despite these theoretical issues, our results for this microalga (parameter values) showed no significant reduction in productivity over 100 days, a longest practical duration for microalgae cultivation: at this stage, other phenomena such as accumulation of biofilm or dead cells have other undesirable effects that force the process to stop.

Increasing the fundamental frequency of the dither signal also improves the convergence rate (Fig. 4). Note that all further results including this one will use the more recent model in (Bernard, 2011), which is more realistic in terms of physical interpretation.

4.2 Influence of the measured variable (X vs P)

As discussed earlier, optimization of the biomass productivity could well be done by optimizing the biomass concentration, the dilution rate *D* being an independent variable. The tuning of the ESC (following the same methodology as with *P* as the output variable) has to be modified to adjust integrator gain *k* to 5, 80 and 180 for $\omega = 1$, 5 and 10 rad· d^{-1} respectively. Fig. 5 shows a comparison between the two situations. Using *X* as the output variable has little impact for the most part: indeed, step perturbations on *D* show similar responses on the output, and most convergence rates are similar. Convergence is faster however for $\omega = 5 \operatorname{rad} \cdot d^{-1}$ with *X* as the output variable.

4.3 Dilution rate variations (nonlinear map)

Variations in the dilution rate affect the nonlinear mapping between I and X (or P). To that end, the sensitivity of the

ESC implementations is tested (Fig. 6), using X as the measured variable for the optimization and for the three different choices of ω .

Results show that as frequency ω is increased, the algorithm becomes less sensitive to variations in the static map relation. All tunings remain stable as *D* decreases, but slower choices of ω become impractical at some point (open-loop results are sometimes better). However, as $D < 0.1 d^{-1}$, little is gained by even trying to optimize the biomass productivity, as it is quite low in that range and the open-loop situation shows a similar behaviour as most implementation results (not shown).



Fig. 4. Influence of the dither signal fundamental frequency on the convergence rate.



Fig. 5. Comparison between *P* and *X* as the output variable for the optimization routine ($D = 0.35 d^{-1}$).

4.4 Parameter sensitivity

Model parameters were varied to evaluate the sensitivity of the algorithm to uncertainties at this level. Parameters tied to the light influence (K_{il}, K_{sI}^* , γ_{max}, k_{I^*} and δ) and the maximum growth rate $\overline{\mu}$ were tested for this evaluation. Parameters K_{il} , K_{sI}^* , k_{I^*} and δ were varied by ±50%, while γ_{max} and $\overline{\mu}$ were varied by +50 % and -20% only, as -50% variations for these variables completely disrupts the behaviour of the model in the conditions tested. Most have a significant influence in this range, except δ , which effect is essentially negligible. Results for K_{sI} (Fig. 7), K_{iI} (Fig. 8) and $\overline{\mu}$ (Fig. 9) are provided with $\omega = 5 \operatorname{rad} \cdot d^{-1}$ only (plus the open-loop case) as situations with other dither frequencies are also affected similarly. It is also important to note that all situations retain stability despite the importance of the parameter variations. In practice, the ESC parameters would be retuned in such situations (to maintain performance) after re-identification. Nonetheless, this shows the very good robustness of these ESC implementations.



Fig. 6. ESC implementations for different values of D.

4.5 Noise sensitivity

Noise sensitivity is a most important aspect to evaluate before implementing a control algorithm on real systems, especially biological systems where there are often high uncertainties on the measurements or time-varying behaviors. Here, situations of white noise (Fig. 10) and low-pass filtered noise (Fig. 11) were tested. Results show that the ESC is more sensitive to noise for high values of ω (which induce fast convergence rates), and that it is quite tolerant to it for lower values of ω . In the white noise scenario, variances shown in Fig. 10 are the worst case that can be tolerated for each case: the figures also include the open-loop case (same noise of same variance) to clearly show the level of output (measurement) noise. As one could think of (low-pass) filtering the biomass measurement to reduce the noise, this in fact does not help the situation, as it simultaneously filters the dither frequency contents needed by the ESC to allow convergence. Other means of removing random noise could be investigated, however. Fig. 11 shows the results for low-pass filtered white noise, where similar conclusions are drawn: noise contents at the same frequencies as the dither signal should be of lesser amplitude than those originating from it.



Fig. 7. Parameter sensitivity results for K_{sl}^{*} ($\omega = 5 \operatorname{rad} d^{-1}$).



Fig. 8. Parameter sensitivity results for K_{il} ($\omega = 5 \operatorname{rad} d^{-1}$).



Fig. 9. Parameter sensitivity results for $\overline{\mu}$ ($\omega = 5 \operatorname{rad} d^{-1}$).



Fig. 10. White noise sensitivity results.

4.6 Wrap-up of design considerations

Design considerations should include the noise level and its frequency contents, performance requirements and sensitivity to the nonlinear input-output relations. For this case, selecting higher dither frequencies will increase speed of convergence (performances) and reduce the ESC's sensitivity to variations in the nonlinear map (section 4.3). It however increases its sensitivity to measurement noise.

5. CONCLUSIONS

This study has investigated the use of ESC for optimizing the light intensity input to a photobioreactor in real-time so as to increase biomass productivity in continuous operation mode. The approach appears quite successful as it is possible to tune the parameters of the ESC with little prior knowledge on the process, mostly based on a step-response experiment, and to achieve significant productivity improvements. Furthermore, the ESC is quite robust to relatively large process parametric variations as well as reasonable measurement noise levels. Further research entails testing the approach in real (physical) experiments, as well as investigating more filtering options and online parameter adaptation.



Fig. 11. Low-pass filtered noise sensitivity results.

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