

## OPTIMAL CONTROL FROM INOCULATION ON A CONTINUOUS MICROALGAE CULTURE

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The present work is centred on the problem of biomass productivity optimization of a culture of microalgae *Spirulina maxima*. The mathematical tools consisted of necessary and sufficient conditions for optimal control coming from the celebrated Pontryagin's Maximum Principle (PMP) as well as the Bellman's Principle of Optimality, respectively. It is shown that the optimal dilution rate turns to be a bang-singular-bang control. It turns out that, the experimental results are in accordance to the optimal mathematical findings.

*Keywords:* optimal control, microalgae, singular arc, dilution rate

*Classification:* 93C95, 90C46

### 1. INTRODUCTION

Microalgae production is nowadays achieving great impact in industry, investigation and social life, thanks to its importance in the global carbon cycle, nutritional benefits, capability to produce oil (bio-fuels), pigments and the ability to remove contamination in residual waters [14, 20] and [10]. Because a lot of microalgae cells are needed to develop any of the issues mentioned earlier, finding optimal conditions under which microalgae maximize its growth is one of the fundamental topics analysed in several disciplines. The use of microalgae at industrial or commercial levels is limited by production cost and time, hence the optimization of the growth process might have an economic positive impact.

It is known that the specific growth rate of microalgae is strongly influenced by experimental conditions (temperature, pH, light irradiance, etc.) and nutrients concentration (mainly carbon source) [8]. However, very often, the only quantities that can be considered as control variables are the irradiance and the dilution rate feeding a given microalgae culture. In this vein, the problem of the optimal microalgae growth of the so-called photosynthetic factory (PSF) was considered in [5] and [19], where an analytical solution to maximize the photosynthetic production rate by manipulating the irradiance is given.

Actually, many works focused on the study of the growth dynamics based in nutrients concentrations, specially focused in finding the best control policies (dilution rate) aiming to get optimal biomass production [6, 8, 9] and [17]. However, in general, the inoculation time is not taken into account (period where biotechnologist operates continuous cultures as fed batch cultures), considering it as a dead time for biomass production. In this work, on the basis of a mass-balance model with a simple but extensively used Monod’s rate of growth, the optimal dilution rate starting from microorganism inoculation is analysed.

The control objective is the maximization of a performance index that depends just on the biomass productivity and the experimentation time. It turns out that the optimal solution is in the form of the so-called bang-singular-bang control. It is shown that, for an experimental time large enough, the optimal steady state dilution rate commonly used by biotechnologist can be reached.

The rest of the manuscript is organized as follows. The preliminaries about the celebrated Monod’s mathematical model and the optimal dilution rate computed of the stationary state of the microorganisms culture are recalled in Section 2. Section 3 starts with the problem formulation by defining the performance index to be optimized, then the Pontryagin’s maximum principle and the Belmman’s optimality principle are applied to find necessary and sufficient optimality conditions, respectively. The experimental setup is briefly described in Section 4. In turn, Section 5 is devoted to the results, both in simulation and real-time, which are based on the complementarity of Pontriagny’s and Bellman’s approaches, the latest allows to determine the initial conditions for the Lagrangian system from which the bang-singular-bang nature of the optimal control was deduced. Finally, the conclusions are given in Section 6.

## 2. MATHEMATICAL MODEL

We consider a homogeneous system and the two-dimensional model

$$x(t) = \begin{bmatrix} x_1(t) \\ x_2(t) \end{bmatrix} =: \begin{bmatrix} \text{Biomass Concentration} \\ \text{Nutrients Concentration} \end{bmatrix};$$

subject to the equation

$$\dot{x}(t) = F(x(t), u(t));$$

that, after a mass conservation analysis, can be rewritten as an equation that consist of two parts, a non linear and a bilinear part, i. e.

$$\begin{cases} \dot{x}(t) = f(x(t)) - x(t)u(t) + Bu(t) \\ x(t = t_0) = x_0, \quad u(t) \in [0, u^+] \end{cases} \tag{1}$$

$$f(x(t)) = \begin{bmatrix} \mu(x(t))x_1(t) \\ -Y\mu(x(t))x_1(t) \end{bmatrix}, \quad \mu(x(t)) = \mu_{\max} \frac{x_2(t)}{K+x_2(t)},$$

$$B = \begin{bmatrix} 0 \\ S \end{bmatrix}, \quad \mu_{\max}, Y, S, K \in \mathfrak{R}_+ \setminus \{0\},$$

where  $\mu_{\max}$ ,  $K$  and  $S$  are experimental constants referring to the maximal growth rate, the inhibition constant and the concentration of nutrients in the inflow, respectively. The Monod's formulation is assumed for the rate of growth and is denoted by  $\mu(\cdot)$ . In turn,  $u(t)$  will denote the dilution rate (control input), restricted due to physical constraint (mechanical pumping capability).

It is assumed that the solution volume in the biological reactor is kept constant during all the operation time, that is to say, there is a proportional relation between the inflow and the dilution rate. In turn, the productivity is defined as the amount of biomass in the outflow given by the formula,

$$P(x(t), u(t)) = x_1(t) u(t) =: \text{Productivity.} \quad (2)$$

It is well known that if a constant input is applied long time enough ( $u(t) = u_{ss}$ ), system (1) becomes stable and reaches a steady biomass concentration that depends only in the dilution rate [1, 15], i. e.

$$x_{1ss}(u_{ss}) = Y^{-1} \left( S - \frac{K u_{ss}}{\mu_{\max} - u_{ss}} \right),$$

in such a manner that the productivity can be understood as a function exclusively of the dilution rate, namely

$$P(x_{1ss}(u_{ss}), u_{ss}) = P(u_{ss}) = Y^{-1} \left( S - \frac{K u_{ss}}{\mu_{\max} - u_{ss}} \right) u_{ss}. \quad (3)$$

Notice that, the productivity function (3) is a convex function, so it can be easily verified that

$$\max_{u_D \in [0, u_D^+]} \{P(u_{ss})\} = P(u_{ss}^{opt}),$$

where

$$u_{ss}^{opt} = \min \left\{ \mu_{\max} \cdot \left( 1 - \sqrt{\frac{K}{K+S}} \right), u^+ \right\}. \quad (4)$$

This result is widely used, but only considers the system after reaching a steady state condition and consequently transient phenomena are omitted. Such situation motivates the following problem formulation looking for the optimal control starting from micro-organism inoculation.

### 3. PROBLEM FORMULATION

For definiteness, the functional cost when seeking for the maximization of biomass productivity is equivalent to the minimization of the following non-linear cost function,

$$J(t_0, x_0; u(\cdot)) = - \int_{t_0}^{t_f} L(x(t), u(t)) dt, \quad (5)$$

subject to the system dynamics (1), where  $L(x(t), u(t))$  is proposed following the natural definition of the productivity equation (2)

$$L(x(t), u(t)) = x_1(t) u(t),$$

here  $t_f$  is a given fixed end time and the control is restricted to the following values,

$$u(t) \in [0, u^+].$$

### 3.1. Pontryagin's Maximum Principle

Pontryagin's Maximum Principle (PMP) provides necessary conditions for optimality (for further details check [12, 16]). The Hamiltonian equation is given by

$$H(\lambda(t), x(t), u(t)) = x_1(t) u(t) + \langle \lambda(t), f(x(t)) - x(t) u(t) + Bu(t) \rangle \quad (6)$$

where the dynamic of the co-state vector  $\lambda(t) \in \mathbb{R}^2$  is

$$\dot{\lambda}(t) = g(x(t), \lambda(t)) + \lambda(t) u(t) - \bar{B}u(t), \quad \lambda(t_f) = [0 \ 0]^T \quad (7)$$

with

$$g(x(t), \lambda(t)) = \begin{bmatrix} (Y\lambda_2(t) - \lambda_1(t)) \mu(x(t)) \\ (Y\lambda_2(t) - \lambda_1(t)) \left( x_1(t) \frac{\mu_{\max} K}{(K+x_2(t))^2} \right) \end{bmatrix},$$

$$\bar{B} = \begin{bmatrix} 1 \\ 0 \end{bmatrix}.$$

If there exists an optimal dilution rate  $u^{opt}$  that minimizes the cost functional (5), then it must satisfy

$$H(\lambda(t), x^{opt}(t), u^{opt}) = \max_{u(t) \in [0, u^+]} \{H(\lambda(t), x(t), u(t))\} \quad (8)$$

after removing all terms of equation (6) where the control input  $u(\cdot)$  does not appear explicitly, we have that it is enough to search the maximum over the following function

$$\max_{u(t) \in [0, u^+]} \{[x_1(t) + \langle \lambda(t), B - x(t) \rangle] u(t)\}.$$

At this point, a particular kind of optimization problems has been reached in which the equation system and the cost functional, despite the nonlinearity on the states, are linear with respect to the control input. At first sight PMP seems to point a bang-bang control, but the optimal control may contain intervals where the applied control is not on the boundary of admissible control, this control is named singular control [11, 13]. Let us define the following switching function

$$h(x(t), \lambda(t)) := x_1(t) + \langle \lambda(t), B - x(t) \rangle \quad (9)$$

one has the following lemma.

**Lemma 3.1.** A singular control exists over the interval where the switching function  $h(t)$  and its consecutive time derivatives, until the control variable appears explicitly, will vanish. In which case, the control policy given by

$$u_{singular}^{opt} = \mu_{\max} \left( \frac{x_2^2 - 2Yx_1x_2 - \frac{Yx_1x_2^2}{S-x_2}}{2x_2^2 + 2(K-S)x_2 - 2KS} \right), \quad (10)$$

is the optimal singular control associated with functional (5) and Hamiltonian system (6).

*Proof.* It is clear that any control  $u(\tau)$  such that  $h(\tau) = 0$  for the interval  $t_1 < \tau < t_2$  will satisfy the maximum principle. In order to find the singular control, following the synthesis procedure presented in [11], the equalities given by,

$$\frac{d^i}{dt^i} (h(x(t), \lambda(t))) = 0, \quad (11)$$

must be verified for all  $i = 0, 1, 2, \dots$  until the control input appears explicitly. One has first, for  $i = 0$ ,

$$h(x, \lambda) = (1 - \lambda_1)x_1 + \lambda_2(S - x_2),$$

which implies

$$\lambda_2 = -\frac{(1 - \lambda_1)x_1}{S - x_2}.$$

In turn, the first time derivative provides

$$\dot{h} = \frac{\mu_{\max}x_1x_2^2 + K\mu_{\max}x_1(x_2 + \lambda_1x_2 - Yx_1 - S\lambda_1 + Y\lambda_1x_1)}{(K + x_2)^2} = 0,$$

which implies

$$\lambda_1 = \frac{KYx_1 - Kx_2 - x_2^2}{Kx_2 + KYx_1 - KS}.$$

Finally, at the second time derivative the control appears explicitly allowing to get the following singular control

$$\ddot{h} = -(2x_2^2 + 2(K-S)x_2 - 2KS)u + \mu_{\max}x_2^2 + \dots - 2\mu_{\max}Yx_1x_2 - \frac{\mu_{\max}Yx_1x_2^2}{S-x_2} = 0.$$

that is to say,

$$u_{singular}^{opt} = \frac{\mu_{\max}x_2^2 - 2\mu_{\max}Yx_1x_2 - \frac{\mu_{\max}Yx_1x_2^2}{S-x_2}}{(2x_2^2 + 2(K-S)x_2 - 2KS)}.$$

□

Using the previous lemma, we have that the optimal control is given by

$$u^{opt} = \begin{cases} u^+ & \text{if } h(\cdot) > 0 \\ 0 & \text{if } h(\cdot) < 0 \\ u_{singular}^{opt} & \text{if } h(\cdot) = 0 \end{cases}.$$

As known [18], the optimal control existence is now subject to finding the initial conditions of the  $\lambda$ -system that satisfies the equation (7).

### 3.2. Bellman’s Optimality Principle

The Bellman’s Optimality Principle provides sufficient conditions for optimality [2], where the Hamilton–Jacobi–Bellman equation

$$-\frac{\partial}{\partial t}V(t, x) = \inf_{u \in [0, u^+]} \left\{ L(x, u) + \left\langle \frac{\partial}{\partial x}V(t, x), F(x, u) \right\rangle \right\} \tag{12}$$

must be verified for all  $t \in [t_0, t_f)$  and all  $x \in \mathbb{R}^2$ , where the value function  $V(\cdot)$  is defined as

$$\begin{aligned} V(t_0, x_0) &:= \inf_{u(\cdot) \in [0, u^+]} J(t_0, x_0; u(\cdot)) \\ V(t_f, x(t_f)) &= 0. \end{aligned}$$

Given the properties of the specific problem we are studying (such as a finite experiment time, bounded control and bounded states), a search of the solution in the discretization of the problem was chosen.

Euler discretization of the general system (1), gives

$$x[k + 1] = x[k] + \Delta t \begin{bmatrix} \mu(x[k])x_1[k] - x_1[k]u[k] \\ -Y\mu(x[k])x_1[k] + (S - x_2[k])u[k] \end{bmatrix} \tag{13}$$

where  $t > 0$  is the discretization step small enough so that the discrete system (13) approximates close enough the continuous system by piecewise constant functions. In turn, the discretized cost functional is given by

$$J(t_0, x_0; u(\cdot)) = -\Delta t \sum_{k=0}^{T-1} x_1[k]u[k]$$

with the number of discrete states  $T$  defined by the formula

$$T = \frac{t_f}{\Delta t};$$

where  $\Delta t$  is selected arbitrarily such that  $T$  is an integer. The value function is naturally modified to

$$\begin{aligned} V(m, x[m]) &= \inf_{u(\cdot) \in [0, u^+]} \left\{ -\Delta t \sum_{k=m}^{T-1} x_1[k]u[k] \right\} \\ &= \inf_{u(\cdot) \in [0, u^+]} \left\{ -\Delta tx_1[m]u[m] - \Delta t \sum_{k=m+1}^{T-1} x_1[k]u[k] \right\}. \end{aligned}$$

Notice that, for a given input signal  $u[k]$ , the transition from one state  $x[k]$  to  $x[k + 1]$  has a related cost and the final cost is calculated as the sum of all partial costs of transitions steps for  $k \in \{0, 1, \dots, T - 1\}$ .

Using Bellman’s Principle of Optimality, one has

$$V(m, x[m]) = \inf_{u(\cdot) \in [0, u^+]} \{ \Delta tx_1[m]u[m] + \dots + V(m + 1, x[m + 1]) \};$$

and the optimal cost from time  $m$  and an admissible state  $x[m]$ , is equivalent to the minimum possible value of the operation cost from time  $m$  to time  $m+1$  plus the optimal cost from time  $m+1$ , and so on. Such procedure makes possible to find recursively the backward sequence  $\{V(T-i, x(T-i))\}$ ,  $i = \{1, \dots, T\}$ .

Now, from the physical restrictions over the states and control of the system (1), one has

$$x_i[k] \in [x_{i,\min}, x_{i,\max}], \quad u[k] \in [u_{\min}, u_{\max}],$$

where the minimum admissible values can be set to zero, since by the nature of the system, the variables cannot take negative values. The maximum value is taken arbitrarily large, but as is intuitively obvious, it suffices to consider the maximum values found in previous experiments.

In order to deal with the discretization of the admissible states and control, let us define the set

$$\begin{aligned} \chi_{x_i} &:= \{x_i^{(1)}, x_i^{(2)}, \dots, x_i^{(p)}\}; \quad x_i^{(j)} \in x_i[k]; \quad i = 1, 2; \quad j = 1, \dots, p; \\ \chi_u &:= \{u^{(1)}, u^{(2)}, \dots, u^{(q)}\}, \quad u^{(j)} \in u[k], \quad j = 1, \dots, q; \end{aligned}$$

where

$$\begin{aligned} x_{i,\min} &= x_i^{(1)} < x_i^{(2)} < \dots < x_i^{(p)} = x_{i,\max} \\ x_i^{(i+1)} - x_i^{(i)} &= \Delta x_i = \text{const}_{x_i}; \end{aligned}$$

and

$$\begin{aligned} u_{\min} &= u^{(1)} < u^{(2)} < \dots < u^{(q)} = u_{\max} \\ u^{(j+1)} - u^{(j)} &= \Delta u = \text{const}_u. \end{aligned}$$

The number of elements in each set is given by the formula

$$\begin{aligned} \text{card}(\chi_{x_i}) &= p = \frac{x_{i,\max} - x_{i,\min}}{\Delta x_i} + 1, \\ \text{card}(\chi_u) &= q = \frac{u_{\max} - u_{\min}}{\Delta u} + 1, \end{aligned}$$

it must be noticed that a smaller discretization step  $\Delta x_i$  or  $\Delta u$  will imply a better numerical approximation to the real solution, but will increase computation time. The flow diagram for obtaining a discrete approximation of the value function is proposed in [12].

#### 4. EXPERIMENTAL SETUP

In this section a brief account of the experimental set up is given. For the growth of microorganisms, all experiments were carried out with a modified medium Zarrouk, which is well known to be quite adequate and is widely accepted in the literature of the subject, [7]. All the experiments were carried out in triplicate for statistical consistency. Further details on *Spirulina maxima* cultures can be found in [3, 4, 7]. The way the kinetic parameter are typically adjusted is recalled here for completeness, such standard topic can be found in [1]. This section may be skipped by the readers interested just in optimal control.

#### 4.1. Materials and methods

*Spirulina maxima* (*Arthrospira*) is a native strain obtained from the Río de los Remedios, located in Ecatepec de Morelos, State of Mexico.

#### 4.2. Microorganism growth

Experiments were carried out in 1000 mL Erlenmeyer flask with 800 mL of modified Zarrouk medium and 10% of inoculum at exponential growth phase. All cultures were incubated at room temperature ( $28 \pm 2^{\circ}\text{C}$ ), continuous aeration 0.5 vvm, agitation 100 rpm, and continuous light of  $120 \mu\text{Em}^{-2}\text{s}^{-1}$ .

The microorganism growth was determined by quantification of chlorophyll “a” following the methodology of APHA (1997). The experiments were carried out by triplicate along 264 h for parameter estimation, while it took 336 h (14 days) for optimal control experiments.

#### 4.3. Analytical methods

Chlorophyll “a” content was determined in 5- ml samples were centrifuged at  $3,000 \times g$  x 1,000 for 5 min and heated in 90:10 (v/v) methanol:water for 3 min at  $80^{\circ}\text{C}$  in the dark and centrifuged as before. Supernatant absorbance was determined at 550 nm using a spectrophotometer (Velab 722-2000).

#### 4.4. Kinematic Parameters

In order to determine the kinematic parameters a series of batch growth experiments must be carried out at different levels of substrate concentration. In this work a Monod’s rate of growth was considered and is given by,

$$\mu(s) = \mu_{\max} \frac{x_2(t)}{K + x_2(t)},$$

the kinetic parameters  $\mu_{\max}$ ,  $K$  can be determined directly from the graphic of  $\frac{x_2}{\mu}$  with respect to  $x_2$  where,

$$\frac{x_2}{\mu} = \frac{K}{\mu_{\max}} + \frac{x_2}{\mu_{\max}}.$$

In our case, the determined parameter values were as follows;

$$\begin{aligned} \mu_{\max} &= 0.9170 \text{ [day}^{-1}\text{]} \\ K &= 5.4585 \text{ [nutrients]} \end{aligned}$$

and the yield parameter  $Y$  is

$$Y = \frac{\Delta s}{\Delta x} = 3.8165.$$

The previous procedure is a well known standard method in the biotechnology literature, further details can be found in [1].



#### 4.4.1. Experimental prototype

A continuous bioreactor was implemented in order to validate the results proposed in the previous section. The prototype consists of a tank containing the culture medium (Zarrouk) and the microorganism. The prototype has a pumping system able to feed variable flux at low speed to follow optimal control policies in an efficient way. Continuous agitation was maintained to have an uniform mixture of the microorganism and nutrients. The outflow is a mixture of microorganism and substrate allowing to keep a constant volume in the bioreactor.

It is worth noticing that the measured variable is just the absorbance (equivalently, the biomass concentration). Assuming the mathematical model and its parameters are reasonably consistent with experimental data, then it is possible to calculate the substrate in order to have registers of the two state variables for experimental validation.

### 5. RESULTS

In this section the simulation and experimental results are given. For such end, let us summarize the considered parameter values for the *Spirulina Maxima* microalgae as follows

$$\mu_{\max} = 0.9170, \quad K = 5.4585, \quad Y = 3.8164, \quad S = 8;$$

with initial conditions

$$x_0 = \begin{bmatrix} 0.113 \\ 6.125 \end{bmatrix} \quad (14)$$

where the admissible discrete control and states are restricted to the following values,

$$x_1 \in [0, 1.5], \quad x_2 \in [0, 8], \quad u \in [0, 1], \quad \Delta t = 0.01.$$

As mentioned before, we are considering bounded control and bounded states together with a finite experiment time. The final time ( $t_f$ ) was fixed at 14 days, taking into account that in batch experiments, the microorganism growth needs between 4 to 5 days to reach the steady state [3].

**Remark 5.1.** PMP provides a high speed and low robustness method for finding optimal control, while numeric solution using the Bellman's Optimal Principle is a low speed high robustness method for finding optimal control. Since obtaining initial conditions for the  $\lambda$ -system (given in eq. (7)) is a hard work, even when trying the well known method of constructing the backward system, the numerical solution for the HJB equation was firstly solved.

#### 5.1. Bellman's Optimality Principle

Let the discretization of the states of the system be given by

$$\begin{aligned} \chi_{x_1} &= \{0, 0.05, \dots, 1.5\} & \Delta x_1 &= 0.05, \\ \chi_{x_2} &= \{0, 0.05, \dots, 8.0\} & \Delta x_2 &= 0.05, \\ \chi_u &= \{0, 0.01, \dots, 1.0\} & \Delta u &= 0.01, \end{aligned}$$

that is to say the number of simulation steps was  $T = 1,400$ , according to the fixed final time  $t_f = 14$  days. In turn, the value function  $(V(t, x) : \mathbb{R} \times \mathbb{R}^2 \rightarrow \mathbb{R})$  was solved numerically, the surface of the value function for a fixed time  $t_1 = 3.5$  days is shown in Figure 1 and the corresponding control surface is shown in Figure 2.

In order to validate the numerical solution, let us define the error function of the optimal policy given in eq. (12) as follows

$$\text{Error} \equiv E(t, x) := \frac{\partial}{\partial t} V(t, x) + \inf \left\{ L(x, u_D) + \left\langle \frac{\partial}{\partial x} V(t, x), F(x, u_D) \right\rangle \right\},$$

which, in principle, must be close enough to zero. i. e.

$$E(t, x) \approx 0.$$

In Figure 3 is shown the numeric error at the fixed time  $t_1 = 3.5$  days. Note the magnitude order for the error is around  $10^{-1}$ , which depends on the chosen time  $t_1$  as well as the discretization of states and control. Clearly, smaller steps will produce a better approximation of the value function surface, but will increase significantly the computation time.

At this stage, one may say that a good approximation of the value function and the optimal control was obtained, since trying smaller discretization steps will not improved significantly the presented result.

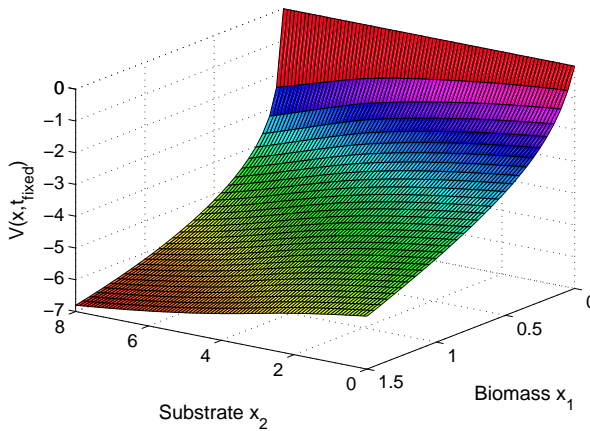
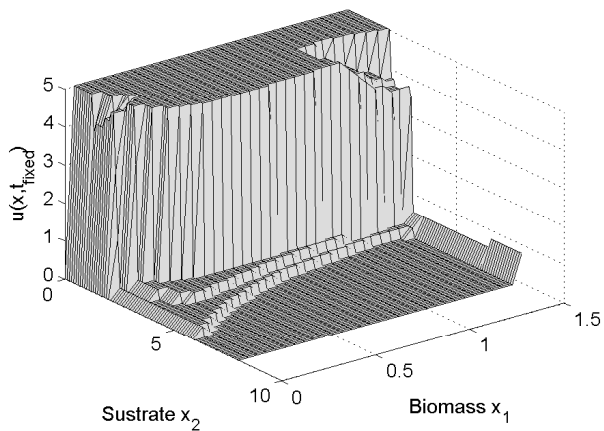


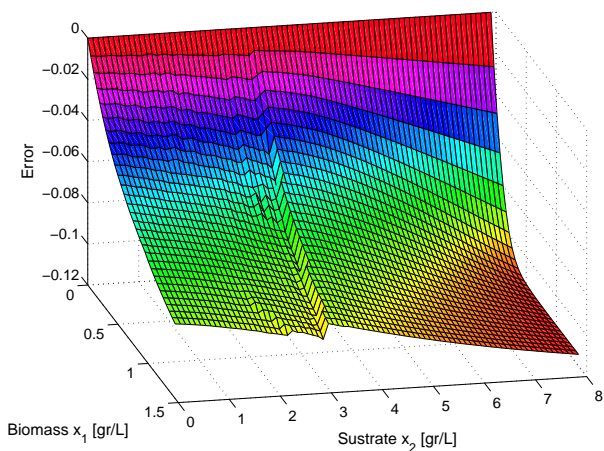
Fig. 1. Surface of the value function for  $t_{fixed} = 3.5$  days.

### 5.2. Pontryagin’s Maximum Principle

Recall that when implementing the bang-singular-bang optimal control, issued from Pontryagin’s maximum principle, it is necessary to determine the switching function (9)



**Fig. 2.** Control surface for  $t_{fixed} = 3.5$  days.



**Fig. 3.** Hamilton-Jacobi-Bellman (HJB) equation error at  $t_{fixed} = 3.5$  days.

by solving first the Lagrangian eq. (7). Now, when necessary and sufficient conditions match in an optimization problem [16], one has the relation

$$-\frac{\partial}{\partial x}V(t, x) = \lambda(t). \tag{15}$$

Therefore, one may try to infer the initial conditions of the co-state vector  $\lambda$  from the numerical results of the HJB equation. According to this way of thinking, the evolution

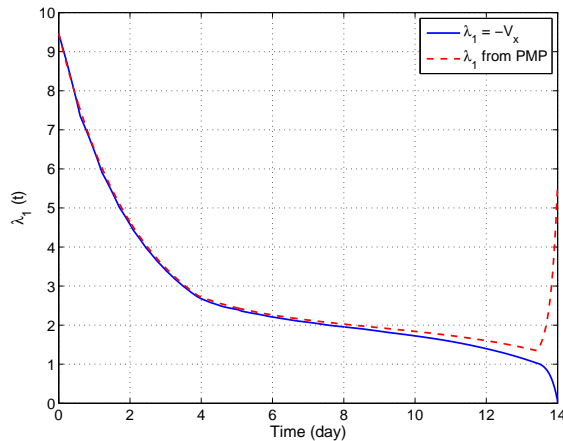
of the adjoint systems (7), taking as initial conditions  $\lambda(t_0) = -\frac{\partial}{\partial x}V(t_0, x_0)$ , is shown in Figure 4 and 5, where  $x_0$  are the initial conditions ( $x_0 = x(t_0)$ ). Notice that, the curves are pretty similar to the ones determined from the expression (15) which are reported in the same graphics for comparison purposes.

On the basis of the previous results, one may assume that the initial conditions for the function  $\lambda$  have been found, so that the optimization problem is completely solved.

**Remark 5.2.** Recall that in order to obtain the singular arc of Lemma 3.1, equation (11) must be satisfied. However, from a numerical view point it is impossible to satisfy such exact equalities. So, given an arbitrarily small  $\varepsilon > 0$ , it is convenient to say that one reaches the singular control region whenever  $|h(x, \lambda)| < \varepsilon$ . This consideration introduces another important difficulty of choosing a suitable  $\varepsilon$  when trying to apply PMP.

**Remark 5.3.** Notice that, the optimization problem within the PMP approach allows to obtain important information, namely the existence of a singular control and, even more, the analytic deduction of the optimal control. It can be verified numerically that in steady state condition the proposed singular control policy will coincide with the optimal  $u_{ss}^{opt}$  (see eq. (4) which is often referenced in the biotechnological literature.

**Remark 5.4.** Concerning the complementarity of the Bellman’s Optimality Principle and the Pontryagin’s Maximum Principle, it is clear that both approaches can be used simultaneously in practical experiments. The former one is more adequate to establish the time intervals for the bang-singular-bang control, while the latest one leads to the analytical solution (10) consisting of a smooth state dependent control profile.



**Fig. 4.** Numerical construction for  $\lambda_1$ .

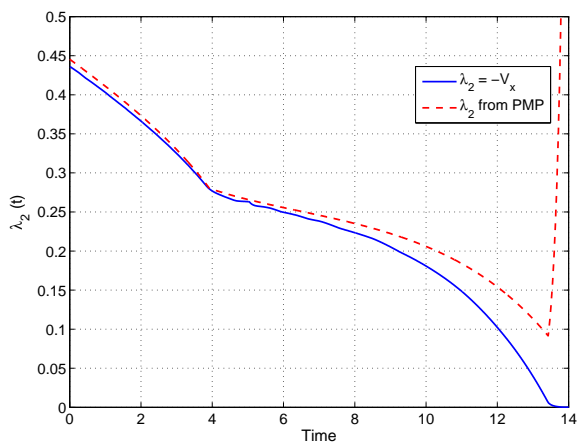


Fig. 5. Numerical construction for  $\lambda_2$ .

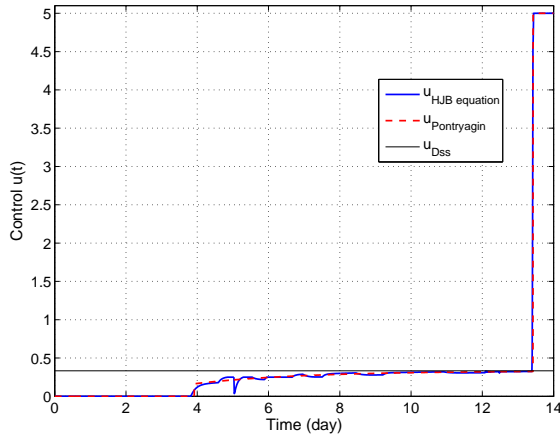
### 5.3. Control implementation

With respect to the optimal control, it turns out that the solution based on the two optimization methods, above discussed, have given practically the same control policy, see Figure 6. Also, according to the Pontryagin's maximum principle, one may appreciate the bang-singular-bang characteristic of the control profile. Notice that here, the optimal dilution strategy takes into account the inoculation process and for a large enough time ( $t = 10$  days) it reaches, in a smooth way, the optimal stationary solution  $u_{ss}^{opt}$  (see eq. (4)). Finally, at  $t = 13$  days, a washing condition is observed in the culture, because one is dealing with an optimal problem of fixed final time and therefore biomass production is faced to an extremely high dilution rate.

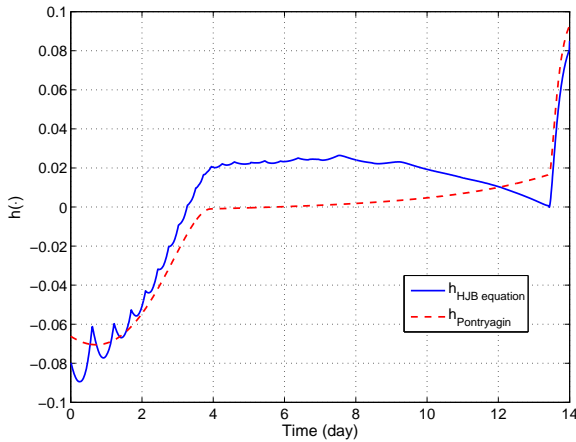
In order to complement the study, the switching functions  $h(\cdot)$  deduced from PMP and HJB equation are presented in Figure 7, respectively. Due to numerical considerations, the functions do not completely vanish and differ in magnitude order of  $10^{-2}$ , see Remark 5.2. Nevertheless, such approximation seems to be reasonable in view of the singular-bang-singular control that is depicted in Figure 6.

### 5.4. Experimental results

In this section, to complement the discussion, the theoretical results and the experimental ones are reported together when convenient. The biomass growth is shown in Figure 8, continuous lines represent simulation results while piecewise continuous lines represent experimental results, respectively. It is clear that optimal control from the PMP or HJB approaches gave higher biomass concentrations reaching 1.2 g/L compared with 0.6 g/L, this last using the stationary optimal dilution rate (see eq. (4)), at  $t = 10$  days, for instance. One can also observe the proximity of the simulation results with respect to the experimental ones, which certainly confirms the mathematical



**Fig. 6.** Optimal control for a 14 days experiment.



**Fig. 7.** Switching Function.

speculations from the optimal control analysis.

It is worth noticing that, for a time long enough, the optimal control considered here consist of a three steps control. First, it seems that biomass production is penalized in order to achieve certain amount of microalgae cells ( $u = 0$ ). Secondly, optimal control moves smoothly to the optimal control in steady state to ensure high productivity. Finally, the optimal control indicates very high dilution rates to recover all microalgae cells that remain in the bioreactor, see Figure 6.

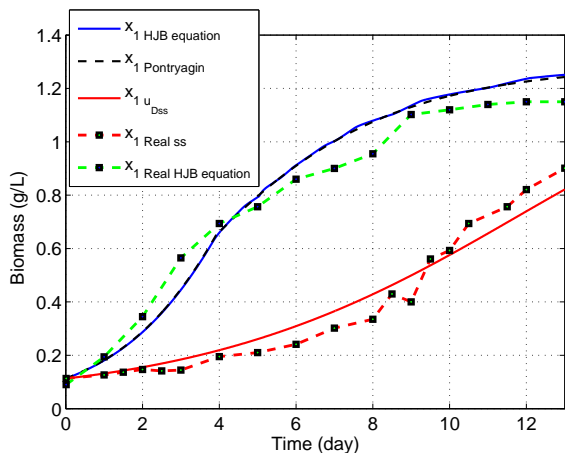


Fig. 8. Biomass growth for a 14 days experiment.

According to the three steps optimal control policy, a relatively low productivity at the beginning of the experiments was obtained ( $u = 0$ ). However, after  $t = 7$  days, biomass productivity started to increase significantly so that, at the final time was considerably higher than productivity with any other dilution rate strategy, see Figure 9.

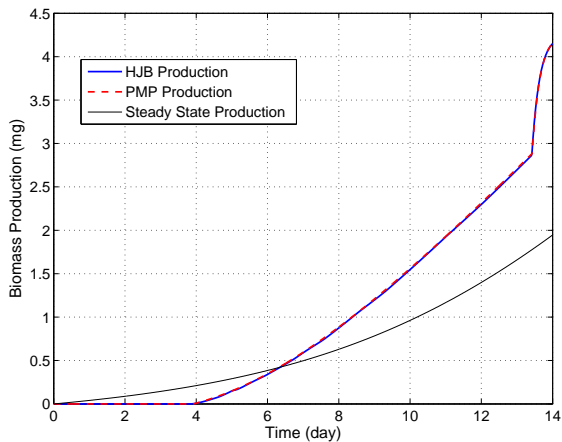


Fig. 9. Production for a 14 days experiment.

It is worth noticing the above mentioned three stages of the optimal control. Within this respect, increasing the experiment time will only increase the singular control dwell time. In turn, when reducing the experiment time one might be faced to only a two

stage control scheme (bang-bang control).

## 6. CONCLUSIONS

The analysis of the optimal dilution rate from inoculation time was presented. It is shown that the time from inoculation to steady state operation conditions can be reduced, and, even more, there exists an optimal strategy to improve productivity. This last has the benefice of a better usage of substrate.

Pontryagin's Maximum Principle allowed to determine the existence of a bang – singular – bang optimal control policy. In a complementary manner, the Hamilton–Jacobi–Bellman approach allowed to construct the optimal control solution in backward time. Hence, as it is known, there is a strict relation between both approaches helping to infer the initial conditions for the solution of the co-state Lagrangian system. From a numerical point of view, the complementarity of both approaches is reinforced by the fact they have produced practically the same optimal solution. Finally, such sufficient and necessary conditions were tested in real time experiments.

As future work, in the field of optimal control oriented to bio-process, one might consider different models to include secondary metabolites, which may exhibit more complex dynamics and some of them are of important commercial value.

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