Optimal control of a microbial growth model by means of substrate concentration and resource allocation

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Abstract: Resource allocation models have been proven to be a highly effective tool to study the growth of microorganisms. Here, we use one such growth model describing the metabolism of a given bacterium in an artificial (e.g. biotechnological) environment. This model involves two controls, one quantifying the protein precursors allocation (i.e. the cellular internal control) and the other representing the nutrient concentration in the culture. We seek to determine the controls that maximize the resulting growth rate of cells living in this controlled environment. We first carry out a theoretical analysis of this optimal control problem (OCP) by means of the Pontryagin's Maximum Principle (PMP). We show the bang-bang structure of the optimal resource allocation control. We find the environmental control to follow a bang-singular-bang structure, and give an expression for its singular arc depending on the state and costate given by the PMP. We solve the OCP in fixed final time using a direct optimization method, implemented on the BOCOP software. This resolution reveals an intrinsic period of the optimal control, corresponding to the solution of the periodic OCP in free final time. Moreover, we find a singular arc of the environment coinciding with the analytical expression given before. Our study highlights the optimality of periodic, non-constant environments in maximizing bacterial growth.

Keywords:

Microbial Growth, Optimal Control, Resource Allocation, Controlled Environment, Pontryagin's Maximum Principle.

1. INTRODUCTION

Microorganisms are widely used in many bio-industrial processes, such as food and drug production or waste-water treatment (Liao et al., 2016; Yegorov et al., 2019; Yabo et al., 2024). The optimization of these processes is thus an important question. Answering this question is non-trivial, partly due to the difficulty of studying the complex metabolism of microorganisms. A natural way to simplify this problem is by using appropriate mathematical models, which are often found to reproduce and explain many empirical observations on many microorganisms.

In this work, we use optimal control theory to approach this optimization problem, a tool which has been applied to optimize many bio-industrial processes (Park and Fred Ramirez, 1988; Harmand et al., 2019; Espinel-Ríos et al., 2024; Banga et al., 2005). Optimal control theory provides an ideal behavior which can be used as a benchmark for the comparison of other control strategies. See our previous work (Innerarity Imizcoz et al., 2024) for an example of such study of another control strategy.

Most articles investigating the mathematical modelization of the metabolism of microorganisms considered only steadystate conditions (Edwards et al., 2001; Ibarra et al., 2002; Lewis et al., 2010; Scott et al., 2014; Maitra and Dill, 2015). Other studies introduced dynamical modeling of the response of bacteria after a single shift in their environment (Giordano et al., 2016; Pavlov and Ehrenberg, 2013; van den Berg et al., 1998; Yabo et al., 2022; Ehrenberg et al., 2013; Yegorov et al., 2019; Waldherr et al., 2015). In our previous work (Innerarity Imizcoz et al., 2024), we studied the optimization of resource allocation in micro-organisms dwelling in a periodically-changing environment, using a simple model of the metabolism of a given cell. All of this work is based on the hypothesis that microorganisms optimize their growth as a result of evolution.

In this article we shall study the same resource allocation problem as in (Innerarity Imizcoz et al., 2024) but in a controlled environment, where nutrient concentration in the external medium of a cell can be set to any reasonable value at any time. This question addresses the general objective of maximizing biomass production in bioprocesses. In this situation, our control problem translates into an optimization of resources, not only within the cell itself but also by controlling the culture medium. The internal resource allocation of the cell can be externally manipulated by different means, of which the most promising one is probably optogenetics (Lindner and Diepold, 2022; Benisch et al., 2024).

The paper is organized as follows. We introduce the model of interest in Section 2, where the environment is another control variable. Then, we state the optimal control problem (OCP) in

Section 3. We address this OCP through Pontryagin's Maximum Principle (PMP) in Section 4. Finally, we numerically obtain the solution of the problem in Section 5.

2. THE MODEL

We use the self-replicator model first stated in (Giordano et al., 2016), representing the reactions forming the metabolism of a micro-organism. The external medium (E_M) of this cell contains a substrate which can be absorbed and transformed into precursor metabolites (P) by the metabolic machinery (M) of the cell. The gene expression machinery (R) then converts these precursors into the macromolecules forming R and M. At each time t, the proportion of precursors used to fabricate R is denoted by the resource allocation parameter $\alpha(t)$. Figure 1 summarizes this process.

We can conveniently divide the variables by the total volume of the cell, which is assumed to be proportional to the total machinery mass, $V = \beta(M+R)$, with $\beta > 0$. We thus obtain the variables p, r and m, representing the respective concentration of their uppercase variables. We also suppose that reactions follow Michaelis-Menten dynamics. After normalization, these variables obey the following equations.



Fig. 1. Diagram of our model of bacterial metabolism. The control variable α represents the proportion of protein precursors allocated to producing *R*. In this article, the external medium E_M is the other control variable.

$$\begin{cases} \dot{p} = (1-r)E_M - (1+p)\frac{p}{K+p}r\\ \dot{r} = (\alpha-r)\frac{p}{K+p}r\\ \dot{V} = \frac{pr}{K+p}V, \end{cases}$$
(1)

where *m* does not need to appear in these equations as it can be defined by m = 1 - r. The resource allocation parameter $\alpha(t) \in [0, 1]$ is, as in (Giordano et al., 2016; Innerarity Imizcoz et al., 2024), a control exerted on the system. The parameter *K* is intrinsic to the metabolism of the cell, and will therefore be treated as a constant. In (Giordano et al., 2016) the parameter E_M , representing the richness of the external medium (summing up a Michaelis Menten function of the substrate), is considered to be constant. In our previous work (Innerarity Imizcoz et al., 2024), we considered the problem where E_M was a dynamic, periodic but fixed input. Here, we propose to set it as a control $0 \le E_M(t) \le E_M^{max} \forall t$, with $E_M^{max} > 0$. Thus, we have two controls on the system, α and E_M .

3. THE OPTIMAL CONTROL PROBLEM

We begin by considering the problem on a long, fixed time interval $t \in [0,T]$, with T > 0. We set reasonable initial conditions on the concentrations of metabolites in the cell



Fig. 2. The solution of the optimal control with fixed initial conditions. In red, the resource allocation control α and in green, the environment E_M . The bottom plot shows the phase plot of (p, r), where we can see a periodic pattern emerging.

 $(p, r)(0) = (p_0, r_0)$, as well as the constraint on the average substrate availability

$$\frac{1}{T}\int_0^T E_M dt = \overline{E}_M,$$

for a previously fixed $E_M \in [0, E_M^{\max}]$.

The admissible controls are measurable functions of the form $\alpha : [0,T] \rightarrow [0,1]$ and $E_M : [0,T] \rightarrow [0,E_M^{\max}]$ verifying the condition on the average \overline{E}_M . The objective is, as in (Giordano et al., 2016; Innerarity Imizcoz et al., 2024), the maximization of the final volume of micro-organisms. Since

$$V(t) = V(0) \exp \int_0^t \frac{pr}{K+p}(\tau) \ d\tau \ \forall t \ge 0,$$

we can rewrite our objective as the maximization of the integral

$$\int_0^T \mu(\tau) = \frac{pr}{K+p}(\tau) \ d\tau.$$

We will call the quantity $\mu(t)$ the specific growth rate of the cell.

The numerical solution (obtained with BOCOP software, see below) of the resulting problem when the initial conditions are $(p_0, r_0) = (0.024, 0.18)$ and the final time is T = 15 is shown in Figure 2. We can see a first transient arc, after which the controls follow a periodic pattern, corresponding to a periodic evolution of the state variables. Toward the end of the fixed time interval, the controls exit this periodic pattern to consume most of the protein precursors p on a second transient arc. In order to avoid these transient arcs, we will from now on set the periodic constraints (p, r)(0) = (p, r)(T).

4. PONTRYAGIN'S MAXIMUM PRINCIPLE

To integrate the constraint on the average nutrient availability into our model, we define the variable e following the differ-

ential equation

$$\dot{e} = \frac{1}{T} E_M,$$

such that $\frac{1}{T} \int_0^T E_M dt = e(T) - e(0)$. The expanded system thus reads

 $\begin{cases} \dot{p} = (1-r)E_M - (1+p)\frac{p}{K+p}r\\ \dot{r} = (\alpha - r)\frac{p}{K+p}r\\ \dot{e} = \frac{E_M}{T}. \end{cases}$

The Hamiltonian of this modified system is given by

$$\begin{split} H(\cdot) &= \lambda_p \dot{p} + \lambda_r \dot{r} + \lambda_e \dot{e} + \lambda_0 (\frac{pr}{K+p}) \\ &= \lambda_p \left[(1-r)E_M - (1+p)\frac{pr}{K+p} \right] + \lambda_r (\alpha - r)\frac{pr}{K+p} \\ &+ \lambda_e \frac{E_M}{T} + \lambda_0 \frac{pr}{K+p}. \end{split}$$

With $\lambda_0 \ge 0$ and the adjoint variables $\lambda = (\lambda_p, \lambda_r, \lambda_e)$ fulfilling the equations

$$\begin{aligned} \dot{\lambda_p} &= -\frac{\partial H}{\partial p} = \lambda_p \frac{r(2Kp+K+p^2)}{(K+p)^2} + \lambda_r (r-\alpha) \frac{Kr}{(K+p)^2} \\ &-\lambda_0 \frac{Kr}{(K+p)^2} \end{aligned} \\ \dot{\lambda_r} &= -\frac{\partial H}{\partial r} = \lambda_p \left[E_M + \frac{(1+p)p}{K+p} \right] + \lambda_r \frac{p(2r-\alpha)}{K+p} - \lambda_0 \frac{p}{K+p} \\ \dot{\lambda_e} &= -\frac{\partial H}{\partial e} = 0. \end{aligned}$$

Therefore, λ_e is a constant.

By the periodicity of the state, the adjoint variables must also fulfill $(\lambda_p, \lambda_r)(0) = (\lambda_p, \lambda_r)(T)$.

We have the maximization condition

$$\begin{split} H(t,p,r,\lambda,\lambda_0,\alpha(t),E_M(t)) &= \\ \max_{u,v\in[0,1]\times[0,E_M^{\max}]} H(t,p,r,\lambda,\lambda_0,u,v) \text{ a.e. on } [0,+\infty). \end{split}$$

Finally, the Hamiltonian follows the equation

$$\frac{dH}{dt} = \frac{\partial H}{\partial t} = 0,$$

and so is constant. Since the Hamiltonian is affine in both controls, this gives the following proposition.

Proposition 4.1. The optimal control $(\alpha_{opt}, E_M^{opt})$, is given by

and

$$\begin{split} \alpha_{opt} &= \begin{cases} 1 & if \, \Phi_{\alpha} > 0 \\ 0 & if \, \Phi_{\alpha} < 0 \\ ? & if \, \Phi_{\alpha} = 0 \end{cases} \\ E_{M}^{opt} &= \begin{cases} E_{M}^{\max} & if \, \Phi_{E_{M}} > 0 \\ 0 & if \, \Phi_{E_{M}} < 0 \\ ? & if \, \Phi_{E_{M}} = 0, \end{cases} \end{split}$$

$$\Phi_{\alpha} = \lambda_r,$$

$$\Phi_{E_M} = \lambda_p (1 - r) + \frac{\lambda_e}{T}$$

are the switching function of α and E_M , respectively. Additionally, if $\alpha = 0$ on the singular arc for E_M (a time interval $t \in [t_1, t_2]$, with $t_1 < t_2$, over which $\Phi_{E_M} = 0$), the expression for this control on this arc is given by

 $E_M = A/B,$

where

$$A = r((-(2p(K - r + 1)) + K(r - 1) + p^{2}(r - 3))(\lambda_{0} - \lambda_{r}r) + \lambda_{p}(p + 1)(p(K - 2r + 2) - Kr + K - p^{2}(r - 2))),$$

$$B = 2(r-1)(K+p)(\lambda_p(K+(p+1)r-1) + (r-1)(\lambda_r r - \lambda_0)).$$

Proof. These expressions come from the maximization condition on the Hamiltonian H and from the fact that it is affine in both controls. The expression on the singular arc E_M^s , which depends on the state and adjoint state variables, can be obtained by taking the second-order time derivative $\Phi_{E_M}^{\prime\prime}$ of the switching function for this control. Then it suffices to remark that, if E_M has a singular arc, then $\Phi_{E_M}^{\prime\prime}$ is null on that arc, and to solve for the value of E_M .

By the periodicity of all the variables of the extended system, we have that the switching functions of the controls fulfill $\Phi_{\alpha}(0) = \Phi_{\alpha}(T)$ and $\Phi_{E_M}(0) = \Phi_{E_M}(T)$. If either of this function, which we will denote Φ , is non null at the initial time, we can formulate the following property. If $\Phi(0) > 0$, then the associated control begins and ends by a bang arc where it takes its maximal value. Symmetrically, if $\Phi(0) < 0$, then the control which depends on its sign will both begin and end by an arc where it is equal to zero.

4.1 Singular arcs

We can have that α has a singular arc, that E_M is singular over an interval, or both at the same time.

An arc where only α is singular must be contained in a single bang arc for E_M . Indeed, if α is singular over an interval then

$$\lambda_r = 0 \implies 0 = \dot{\lambda_r} = \lambda_p \left[E_M + \frac{(1+p)p}{K+p} \right] - \lambda_0 \frac{p}{K+p}$$

In particular, this last expression is continuous. If E_M presents some discontinuity, since all the other variables that appear are continuous, it must be that $\lambda_p = 0$. Combining this with the equation above, one finds that $\lambda_0 \frac{p}{K+p} = 0$, and therefore, by the positivity of p, we must have that $\lambda_0 = 0$, which contradicts optimality conditions. So E_M is constant over any singular arc for α unless E_M is also singular.

Moreover, combining this equation with the fact that the Hamiltonian is constant,

$$\lambda_p \left[(1-r)E_M - (1+p)\frac{pr}{K+p} \right] + \lambda_e \frac{E_M}{T} + \lambda_0 \frac{pr}{K+p} = \text{constant},$$

we find that $(\lambda_p + \frac{\lambda_e}{T}) E_M$ is also constant over this arc. In this case, since E_M is constant, we have that $\dot{\lambda_p} = 0$. We can combine the equations $\dot{\lambda_r} = 0$ and $\dot{\lambda_p} = 0$ as in (Innerarity Imizcoz et al., 2024) to find that in a singular arc α , p and rmust take specific values depending on the value of E_M , which are given in (Innerarity Imizcoz et al., 2024) under the names $\alpha = \alpha_{opt}^*(E_M)$ and $(p, r) = (p_{opt}^*, r_{opt}^*)(E_M)$. Since for $E_M = 0$ we have $p_{opt}^* = r_{opt}^* = 0$, which is impossible by the positivity of each of these variables, it must hold that over a singular arc for α but not for E_M , this last control must take its maximal value. Likewise, if E_M is in a singular arc and α is in a bang-bang arc, then α cannot switch. As we have just done, we can prove this by noticing that the switching function for E_M is

$$\Phi_{E_M} = \lambda_p (1-r) + \frac{\lambda_e}{T} = 0$$

over this whole interval. Therefore,

$$\begin{split} \hat{\Phi} &= \dot{\Phi}_{E_M} = \dot{\lambda_p} (1-r) - \lambda_p \dot{r} \\ &= (1-r) \cdot \\ & \left[\lambda_p \frac{r(2Kp+K+p^2)}{(K+p)^2} + \lambda_r (r-\alpha) \frac{Kr}{(K+p)^2} - \lambda_0 \frac{Kr}{(K+p)^2} \right] \\ &- \lambda_p (\alpha - r) \frac{pr}{K+p}. \end{split}$$

In particular, this expression is continuous, and thus if α is discontinuous at some time *t*, we must have that the coefficient of this control is null, i.e.

$$\left(-\lambda_r \frac{Kr(1-r)}{(K+p)^2} - \lambda_p \frac{pr}{K+p}\right)(t) = 0$$

Since α can only be discontinuous if it switches, or over a singular arc, which in any case implies that $\lambda_r = 0$, this implies that $\lambda_r(t) = \lambda_p(t) = 0$. Since the switching function of E_M is zero, we also have that $\lambda_e(t) = 0$. Finally, by the expression of E_M , we find that $\lambda_0 = 0$ too. Therefore, $(\lambda, \lambda_0) = 0$, which contradicts the conditions of Pontryagin's Maximum Principle. We have just proved that over any singular arc for E_M , the resource allocation control α must be continuous. In particular, if α is not singular, then the singular arc for E_M must be contained in a single bang arc for α . This is in accordance with what we will observe in the following section.

5. NUMERICAL RESULTS

We add e(0) = 0 and $e(T) \leq \overline{E}_M$ to the previously defined periodic boundary conditions (p, r)(0) = (p, r)(T), so that $\frac{1}{T} \int_0^T E_M dt = e(T) - e(0) \leq \overline{E}_M$, i.e. one does not consume more resources than available. We shall see that this is, in practice, equivalent to simply setting $e(T) = \overline{E}_M$, as the optimal strategy is logically to use all available resources.

We have solved this problem using the numerical resolution software BOCOP (Team Commands, 2017), with a final time of T = 5 and the constant K = 0.003 given by (Giordano et al., 2016), together with the constants $\overline{E}_M = 0.6$ and $E_M^{\text{max}} = 1$. The numerical parameters used are N = 4000 time steps and a relative tolerance of 10^{-14} .

5.1 Resolution of the OCP in fixed final time

We observe in Figure 3 that the controls follow a bang-bang structure, taking alternatively their maximal and minimal values. Additionally, the environment E_M presents a singular arc, which is contained in a single bang arc for α , in agreement with our theoretical analysis. We can also appreciate an apparent periodic pattern, with a subperiod about $T \approx 0.6$ emerging. The optimality of a periodic environment with respect to an equivalent constant one (here $E_M = \overline{E}_M$) is a property that has been observed in a number of biological optimal control problems, such as (Ali Al-Radhawi et al., 2021). We will investigate this subperiod in the following subsection.



Fig. 3. The two controls α (red) and E_M (green) for a fixed long period and periodic boundary conditions. We observe a periodic pattern appearing.

5.2 Free final time OCP

We define the problem where the final time T is an optimization parameter with the objective of finding the origin of the subperiod we observed in the previous subsection. We seek to find

$$(\alpha_{\text{opt}}, E_{\text{opt}}^M, T_{\text{opt}})$$
 maximizing $\overline{\mu} = \frac{1}{T_{\text{opt}}} \int_0^{T_{\text{opt}}} \mu(\tau) d\tau$,

where $T_{opt} > 0$. We call $\overline{\mu}$ the average growth rate of the cell.

In order to integrate T into the equations describing the system, we normalize it by dividing the time variable by T, so that the equivalent system reads

$$\begin{cases} \frac{dp}{d\hat{t}}(\hat{t} = t/T) = T \left[(1-r)E_M - (1+p)\frac{p}{K+p}r \right] \\ \frac{dr}{d\hat{t}}(\hat{t} = t/T) = T \left[(\alpha - r)\frac{p}{K+p}r \right] \\ \frac{dV}{d\hat{t}}(\hat{t} = t/T) = T\frac{pr}{K+p}V \\ \frac{de}{d\hat{t}}(\hat{t} = t/T) = E_M, \end{cases}$$
(2)

where the boundary conditions are now (p,r)(0) = (p,r)(1), e(0) = 0 and $e(1) \leq \overline{E}_M$. The maximization objective can be rewritten as

$$\overline{\mu} = \frac{1}{T_{\text{opt}}} \int_0^{T_{\text{opt}}} \mu(\tau = t) \ d\tau = \int_0^1 \mu(\tau = T\hat{t}) \ d\hat{t}.$$

We notice that, if the tuple $(\alpha_{opt}, E_{opt}^M, T_{opt})$ is a solution of the problem, then $((\alpha_{opt}, E_{opt}^M)(t \pmod{T}), n \cdot T_{opt})$ is also a solution $\forall n \in N$. In order to obtain a unique solution for the problem, we define T_{opt} as the *smallest* T that maximizes the average growth rate. As we observed a subperiod of about $T_{obs} \approx 0.6$ in Figure 3, we set the bound $T \in [0.3, 0.9] = [T_{obs}/2, 3T_{obs}/2]$. The numerical resolution of this problem gives the controls shown in Figure 4, with $T_{opt} \approx 0.656$.

If instead we let $T \in [0.9, 1.5] = [3T_{obs}/2, 5T_{obs}/2]$ we obtain the controls shown in Figure 5, with $T \approx 1.313 \approx 2T_{opt}$. We see how the pattern from Figure 4 is repeated twice in this case.

The difference between the resulting average growth rates of both problems, the one with a fixed final time and the one with a free final time is of the order of 10^{-6} , which means that repeating the solution to this free final time problem is indeed the optimal strategy in long time. We also observe that the subperiod appearing in the solution of our original fixed-time problem corresponds to the solution of the free-time one.



Fig. 4. The two controls α and E_M of the solution of the freetime problem. We observe the same periodic pattern as in Figure 3.



Fig. 5. The two controls α and E_M for a free longer period. We observe the same periodic pattern repeating twice.



Fig. 6. Plot of the control E_M (green) and of the analytical value for its singular arc (gray). Both plots coincide on the singular arc of E_M . On the bottom plot, Φ_{E_M} , the switching function of E_M . The interval over which Φ_{E_M} is zero corresponds to the singular arc of E_M .

5.3 The singular arc of E_M

The plot of the environmental control E_M presents a singular arc, both in Figure 3 and 4, which is contained in a bang arc $\alpha = 0$ for the other control. By Theorem 4.1, we have an expression depending on the state and adjoint state variables for the value of E_M in this singular arc. We have plotted the value of this expression given by the numerical resolution of the problem with free final time in Figure 6, together with the computed value of the environmental control. Both plots overlap on the singular arc of E_M .

6. CONCLUSION

In this work, we studied the optimization of cellular growth of micro-organisms living in a controlled environment. We modified the environmental input of the model proposed in (Giordano et al., 2016) to set it as a control variable on the system, in order to better represent human-controlled environments, such as bio-reactors. This control was considered in addition to the cellular internal resource allocation control which was introduced in (Giordano et al., 2016).

We defined an optimal control problem, which we theoretically studied using Pontryagin's Maximum Principle (PMP). Using this theorem, we proved that the optimal resource allocation control follows a bang-bang structure. We also showed that the environmental control must be bang-singular-bang, and found an analytical expression for the value of its singular arc, which depends on the state and costate variables given by the PMP.

We computed the solution of the OCP, and observed the emergence of the same subperiod in both controls. We solved the related periodic OCP in free time, whose solution coincides with the subperiod we had previously observed.

We numerically observed a singular arc for the environmental control, which was included in a bang arc for the resource allocation control. We compared the analytical expression for the value of this singular arc found previously with the value of the control given by the numerical resolution of the OCP, and found that they coincide.

This article highlights the surprising fact that a periodic environment can emerge from a controlled one and result in higher growth rates than an equivalent constant one. Moreover, the optimal controls present an intrinsic optimal period which is independent of the final time of the OCP. In a future work, we intend to deepen our study of this optimal period, and find biological reasons to explain its emergence.

ACKNOWLEDGMENTS

This work has been supported by the French government, through the EUR DS4H Investments in the Future Project managed by the National Research Agency (ANR) with the reference number ANR-17-EURE-0004. It was also supported by the French government through the France 2030 investment plan managed by the National Research Agency (ANR), as part of the Initiative of Excellence Université Côte d'Azur under reference number ANR- 15-IDEX-01.

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