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Optimal allocation of bacterial resources in fed-batch reactors

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Abstract— **In bacteria, the mechanisms behind the allocation of resources to different cellular functions (i.e. growth or nutrient uptake) have been tuned to optimally adjust cell composition to changing environments. Simple dynamical models of bacterial growth have been key in predicting these mechanisms through a resource allocation perspective. This approach has also been applied to the production of added-value compounds, to determine how to externally adjust the cellular composition in order to maximize metabolite over biomass synthesis. This paper presents a resource allocation perspective of a fed-batch process, based on a multi-scale mechanistic model considering intracellular concentrations of the main cellular components, as well as the dynamics of the bioreactor. The model presented here is a simple coarse-grained self-replicator model of *E. coli*, accounting for empirical cellular trade-offs observed in previous experimental works. The problem of maximizing an added-value metabolite of interest is written as an Optimal Control problem, in terms of the internal resource allocation parameter and the feeding rate of the bioreactor. Numerical results are provided to better understand the role of cellular composition in fed-batch processing.**

I. Introduction

A common hypothesis when studying bacterial growth is that bacteria seek to maximize their growth rate [1]. From an evolutionary perspective, the latter is usually attributed to the pressure of natural selection: in environments with low nutrient levels, faster growth represents an evolutionary advantage allowing individuals to outgrow competitors. This has been the starting point of many studies focused on the adaptation of microorganisms to changing environments [2], [3], [4]. The premise is that the mechanisms behind the allocation of resources to different cellular functions (i.e. growth or nutrient uptake) have been tuned so as to optimally adjust cell composition to new environments. These studies commonly use simple coarse-grained self-replicator models of resource allocation in bacterial growth, capable of representing mechanistic trade-offs related to physical and biochemical limitations.

The study of resource allocation in bacteria has also been applied to the production of metabolites of interest [5], [6], [7], [8], [9]. While rather theoretical, the main contribution is a framework in which the control of bioprocesses occurs not only at a bioreactor level—which is standard in industrial practices—but also at a cellular scale. These approaches rely on the existence of cutting-edge biological techniques capable of externally modifying the cell composition of a bacterial population. For instance, *E. coli* can be re-engineered to control the transcription of RNA polymerase through the concentration of an inducible promoter, thus modifying the resource distribution in cells [10]. Another proven example is optogenetic control, also capable of regulating gene expression in bacteria in real time [11].

Resources in bacteria are limited due to physical constraints, and so controlling cellular composition is key for optimizing bioprocesses [12]. For instance, over-production of ribosomal proteins responsible for the production of macromolecules results in a low enzymatic composition, which slows down the uptake of nutrients, eventually affecting all cellular functions [2]. These simple principles can be represented in mathematical models by trade-offs resulting from optimization problems. The approach has been also applied in continuous bioreactors [13], [14] where the objective is to drive the system to a steady state with optimal cellular composition, thus maximizing the production of the heterologous protein of interest.

In this paper, fed-batch processing is studied from a resource allocation perspective. In fed-batch fermentation, a bioreactor with an initial culture is progressively filled through an inflow of rich medium. Thus, the volume of the culture increases until it reaches maximum volume. The nutrient feeding is usually controlled in terms of the nutrient concentration in the bioreactor, the biomass, the growth rate or the substrate uptake rate [15]. However, multi-scale models including bioreactor dynamics and intracellular composition of the bacterial culture have not been extensively studied. In this regard, resource allocation control represents a novel powerful tool to complement classic control schemes.

This paper starts with the introduction of the multi-scale model considering a simple coarse-grained bacterial model with basic cellular functions, and the bioreactor dynamics. The model is based on simplifying assumptions, but retains the main trade-offs and features of other bacterial growth models, such as a bounded ribosomal concentration in the cell. Then, the problem of maximizing the production of a metabolite of interest is posed, and later studied through a numerical optimal control analysis. The problem is tackled through a deterministic approach, and does not consider model and parametric uncertainties. An extension of this work is foreseen, with analytical results not included in this preliminary work.

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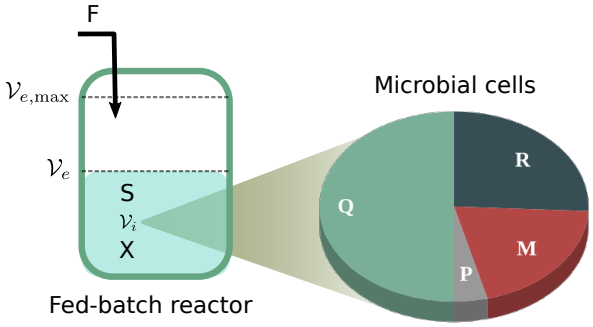

Fig. 1. A schematic diagram of a simplified fed-batch bioreactor containing the substrate S, the bacterial population $\mathcal{V}_i$ and the metabolite of interest X. The inflow of rich medium F produces an increase of the volume of the culture $\mathcal{V}_e$, that is constrained to a maximum volume $\mathcal{V}_{e,\max}$.

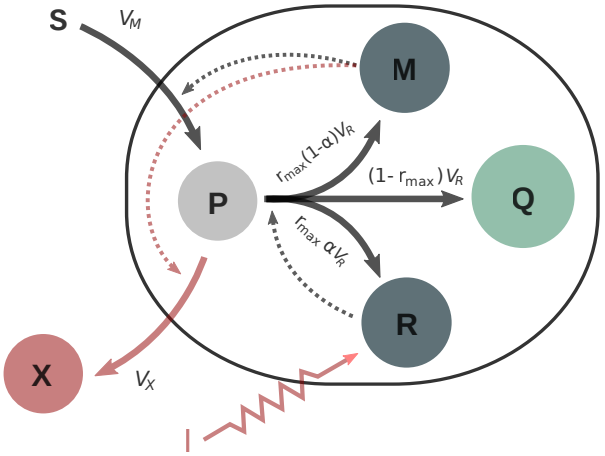

Fig. 2. Scheme of the coarse-grained self-replicator model. The substrate S is consumed by bacteria, forming precursor metabolites P through the action of the metabolic machinery M. Then, the precursors are used to produce macromolecules of the gene expression machinery R, the metabolic machinery M, the housekeeping machinery Q, and metabolites of interest X. The external control I externally affects the natural allocation parameter α, so as to channel resources into the production of X.

II. Self-replicator model

A. Model definition

The dynamical model at the extracellular level consists of a fed-batch bioreactor with culture volume $\mathcal{V}_e$, subject to an input of rich medium F measured in liters per hour (Figure 1). The culture in the bioreactor is composed of the substrate S, the bacterial population $\mathcal{V}_i$ and the metabolites of interest X which is excreted from the bacterial cells. Based on [2], [5], microbial cells are represented through a self-replicator model composed of the precursor metabolites P, the mass of the metabolic machinery M, the mass of the gene expression machinery R and the mass of the housekeeping machinery Q. As shown in Figure 2, the substrate S in the bioreactor is consumed by bacteria and transformed into P at rate V_M through a reaction catalyzed by M. The precursors P are transformed into M, Q, R and X at rates $r_{\max}(1-\alpha)V_R$, $(1-r_{\max})V_R$, $r_{\max}\alpha V_R$, and V_X, respectively. The reactions producing M, Q and R are catalyzed by R, and the reactions synthesizing P and X are catalyzed by M. In short, the ribosomal proteins R produce new proteins, and the metabolic proteins M are responsible for the uptake of nutrients into the cell, and the production of X. The latter is a well-known trade-off in biology, and is represented through the parameter α, defined as a time function $\alpha(t) \in [0,1]$. As described in the introduction, we suppose the allocation variable α can be controlled through an external signal I. The action of the external control is introduced through a multiplicative term, such that the final control $u(t)$ is given by

$$u(t) = I(t) \times \alpha(t),$$

with $I \in [0,1]$. This implies that the external control can either preserve the natural bacterial allocation ($I = 1$) or fully arrest the production of ribosomal proteins ($I = 0$). In this first approach, we suppose u can be directly controlled, without considering the interplay between I and α. Thus, the dynamics of the system are described by

$$\begin{cases} \dot{S} = V_{S_{in}} - V_M, \\ \dot{P} = V_M - V_R - V_X, \\ \dot{M} = r_{\max}(1-u)V_R, \\ \dot{R} = r_{\max} u V_R, \\ \dot{Q} = (1-r_{\max})V_R, \\ \dot{X} = V_X, \\ \dot{\mathcal{V}}_e = F, \end{cases} \tag{1}$$

where $V_{S_{in}}$ is the constant inflow mass of substrate per unit of time, and V_M, V_R and V_X the reaction rates. The constant $r_{\max}$ corresponds to the maximal ribosomal concentration found in microbial cells, which is around 0.5 for *E. coli*, according to experimental results [16]. We define the structural volume of the bacterial population $\mathcal{V}_i$ measured in liters as

$$\mathcal{V}_i \doteq \beta(M+R+Q), \tag{2}$$

where β is a constant relating biomass and bacterial volume. This definition considers the precursor mass P to be negligible with respect to the mass of macromolecules M, R and Q, as it is commonly observed in bacterial composition [2]. We define the intracellular concentrations

$$p \doteq \frac{P}{\mathcal{V}_i}, \qquad m \doteq \frac{M}{\mathcal{V}_i}, \qquad r \doteq \frac{R}{\mathcal{V}_i}, \qquad q \doteq \frac{Q}{\mathcal{V}_i}, \tag{3}$$

and define relative rates involved in the processes as

$$v_M(s,m) \doteq \frac{V_M}{\mathcal{V}_i}, \quad v_R(p,r) \doteq \frac{V_R}{\mathcal{V}_i}, \quad v_X(p,m) \doteq \frac{V_X}{\mathcal{V}_i}.$$

Likewise, we define the concentration of substrate in the culture

$$s = \frac{S}{\mathcal{V}_e}.$$

From (2) and (3), we have that

$$\beta(m + r + q) = 1.$$

By hypothesis, the concentration of housekeeping proteins in the cell is internally autoregulated [17], and thus we set

$$\beta(r + m) = r_{\max}, \qquad \beta q = q_{\max} = 1 - r_{\max}.$$

The inflow rate is defined as $V_{S_{in}} = F s_{in}$ where s_{in} is the constant concentration of the nutrient input measured in grams per litres. Moreover, the time evolution of the volume of the bacterial population $\dot{\mathcal{V}}_i$ is calculated using (1) and (2):

$$\dot{\mathcal{V}}_i = \beta(\dot{M} + \dot{R} + \dot{Q}) = \beta V_R.$$

From this expression, we define the bacterial growth rate μ as

$$\mu \doteq \frac{\dot{\mathcal{V}}_i}{\mathcal{V}_i} = \beta v_R(p, r).$$

The bacterial model also accounts for empirical observations in growing bacterial cultures [18]:

- a minimal concentration of ribosomes $r_{\min}$ is required to have growth,
- all synthesis rates are linear in m and r.

Taking into account the latter, we define the kinetics of the problem as

$$\begin{aligned} v_M(s, m) &\doteq w_M(s)(r_{\max} - r), \\ v_R(p, r) &\doteq w_R(p)(r - r_{\min}), \\ v_X(p, m) &\doteq w_X(p)(r_{\max} - r), \end{aligned}$$

with functions w given by Michaelis-Menten kinetics:

$$\begin{aligned} w_M(s) &\doteq k_M \frac{s}{K_M + s}, \\ w_R(p) &\doteq k_R \frac{p}{K_R + p}, \\ w_X(p) &\doteq k_X \frac{p}{K_X + p}, \end{aligned}$$

where the parameters k_M, k_R, k_X, K_M, K_R and K_X are selected from the literature [2], [5]. We define the non-dimensional timescale $\hat{t} = k_R t$, as well as the mass fractions of the bacterial volume $\mathcal{V}_i$: $\hat{p} \doteq \beta p$, $\hat{r} \doteq \beta r$, $\hat{r}_{\min} \doteq \beta r_{\min}$, $\hat{m} \doteq \beta m = r_{\max} - r$, $\hat{q} \doteq \beta q = 1 - r_{\max}$, $\hat{X} \doteq \beta X$. Additionally, we define non-dimensional synthesis rates $\hat{w}_R(p) = w_R(p)/k_R$, $\hat{w}_M(s) = w_M(s)/k_R$ and $\hat{w}_X(p) = w_X(p)/k_R$. Then, dropping all hats, the model becomes

$$\begin{cases} \dot{s} = \dfrac{F}{\mathcal{V}_e}(s_{in} - s) - v_M(s)(r_{\max} - r)\dfrac{\mathcal{V}_i}{\mathcal{V}_e}, \\ \dot{p} = v_M(s)(r_{\max} - r) - v_X(p)(r_{\max} - r) \\ \qquad -\mu(p)(r - r_{\min})(p + 1), \\ \dot{r} = (r_{\max} u - r)\mu(p)(r - r_{\min}), \\ \dot{X} = v_X(p)(r_{\max} - r)\mathcal{V}_i, \\ \dot{\mathcal{V}}_e = F, \\ \dot{\mathcal{V}}_i = \mu(p)(r - r_{\min})\mathcal{V}_i. \end{cases} \tag{S}$$

III. Maximization of metabolite production

The objective in this work is to maximize the production of the metabolite at final time t_f, which is given by

$$X(t_f) = \int_0^{t_f} v_X(p)(r - r_{\max})\mathcal{V}_i \, dt,$$

under the state constraint

$$\mathcal{V}_e(t) \leq \mathcal{V}_{e,\max}, \tag{PC}$$

for the whole interval $[0, t_f]$. The constraint (PC) implies that the bioreactor can be filled up with rich medium to a certain extent, which is part of the classical operation mode of fed-batch processes. We first see that, for all controls u and F, the trajectories lie in the same invariant set.

Lemma 1: The set

$$\begin{aligned} \Gamma = \big\{(s, p, r, X, \mathcal{V}_e, \mathcal{V}_i) \in \mathbb{R}^6 \,:\, & s_{in} \geq s \geq 0,\ p \geq 0,\ X \geq 0, \\ & r_{\max} \geq r \geq r_{\min},\ \mathcal{V}_e \geq 0,\ \mathcal{V}_i \geq 0\big\} \end{aligned}$$

is positively invariant for the initial value problem.

Thus, we define the initial conditions

$$\begin{aligned} s(0) = s_0 \in (0, s_{in}), \quad p(0) = p_0 > 0, \\ r(0) = r_0 \in (r_{\min}, r_{\max}), \quad X(0) = 0, \\ \mathcal{V}_e(0) = \mathcal{V}_{e,0} \in (0, \mathcal{V}_{e,\max}), \quad \mathcal{V}_i(0) = \mathcal{V}_{i,0} > 0. \end{aligned} \tag{IC}$$

The optimal control problem can be written as

$$\begin{cases} \textit{maximize} & X(t_f) \\ \textit{subject to} & \textit{dynamics of} \text{ (S)}, \\ & \textit{initial conditions} \text{ (IC)}, \\ & \textit{path constraint} \text{ (PC)}, \\ & u(\cdot) \in \mathcal{U},\ F(\cdot) \in \mathcal{F}, \end{cases} \tag{OCP}$$

where $\mathcal{U}$ and $\mathcal{F}$ are the set of admissible controllers, which are Lebesgue measurable real-valued functions defined on the time interval $[0, t_f]$ and satisfying $u(t) \in [0, 1]$ and $F(t) \in [0, F_{\max}]$, respectively. We define the total mass in

grams in the bioreactor

$$\gamma = s\mathcal{V}_e + (p+1)\mathcal{V}_i + X$$

which obeys the dynamics

$$\dot{\gamma} = F s_{in}.$$

Given that there is no protein degradation, the total mass γ is an increasing function of time for all t. Additionally, the quantity $z = \gamma - s_{in}\mathcal{V}_e$ is constant, which means that the total mass variation in the bioreactor $\Delta\gamma = s_{in}\Delta\mathcal{V}_e$, and thus

$$\Delta\mathcal{V}_i + X(t_f) = s_{in}\Delta\mathcal{V}_e - \Delta P - \Delta S. \tag{4}$$

In particular, when all the substrate in the bioreactor has been consumed, and all the available precursors have been depleted (which cannot occur in finite time), (4) becomes

$$\mathcal{V}_i^* + X^* = s_{in}\Delta\mathcal{V}_e + S(0) + P(0) + \mathcal{V}_i(0),$$

showing that the final mass in the bioreactor does not depend on the choice of (u, F) (except in trivial cases such as $F = 0$ for all t). Indeed, for every control (u, F) that takes $\mathcal{V}_e$ to $\mathcal{V}_{e,\max}$, the quantity $\mathcal{V}_i^* + X^*$ is fixed. Thus, the control (u, F) would rather play a role in the ratio $X^*/\mathcal{V}_i^*$. Based on Pontryagin's Maximum Principle [19], we denote the adjoint state $(\lambda_s, \lambda_p, \lambda_r, \lambda_X, \lambda_{\mathcal{V}_e}, \lambda_{\mathcal{V}_i})$, define η as the multiplier associated with the path constraint (PC), and we write the Hamiltonian

$$\begin{aligned} H = &\left(\frac{F}{\mathcal{V}_e}(s_{in} - s) - v_M(s)(r_{\max} - r)\frac{\mathcal{V}_i}{\mathcal{V}_e}\right)\lambda_s \\ &+ \left(v_M(s)(r_{\max} - r) - v_X(p)(r_{\max} - r)\right)\lambda_p \\ &- \mu(p)(r - r_{\min})(p+1)\lambda_p + F\lambda_{\mathcal{V}_e} \\ &+ (r_{\max}u - r)\mu(p)(r - r_{\min})\lambda_r \\ &+ \mu(p)(r - r_{\min})\mathcal{V}_i\lambda_{\mathcal{V}_i} + v_X(p)(r_{\max} - r)\mathcal{V}_i\lambda_X \\ &+ \eta(\mathcal{V}_e - \mathcal{V}_{e,\max}) \end{aligned}$$

which is affine in both controls as

$$H = H_0 + uH_1 + FH_2 + \eta(\mathcal{V}_e - \mathcal{V}_{e,\max}),$$

with

$$\begin{aligned} H_1 &= r_{\max}\mu(p)(r - r_{\min})\lambda_r, \\ H_2 &= \frac{\lambda_s}{\mathcal{V}_e}(s_{in} - s) + \lambda_{\mathcal{V}_e}. \end{aligned}$$

A solution of (OCP) (one can prove existence of solution thanks to Filippov's theorem [20], which we do not discuss here) may combine bang, singular and boundary controls, both for controls u and F. In particular, as the culture volume $\mathcal{V}_e$ is increasing, while $\mathcal{V}_e < \mathcal{V}_{e,\max}$ the path constraint is inactive and the multiplier η must be zero by complementarity. In this case, possible values of the two controls given by the maximum principle are

$$u(t) = \begin{cases} 0 & \text{if } H_1 < 0, \\ 1 & \text{if } H_1 > 0, \\ u_{\text{sing}}(t) & \text{if } H_1 = 0. \end{cases}$$

$$F(t) = \begin{cases} 0 & \text{if } H_2 < 0, \\ F_{\max} & \text{if } H_2 > 0, \\ F_{\text{sing}}(t) & \text{if } H_2 = 0. \end{cases}$$

The singular arcs u_{sing} and F_{sing} occur when the functions H_1 and H_2 vanish over a subinterval of time. An explicit expression of the singular arcs in terms of the state and the adjoint state can be obtained by differentiating the functions H_1 and H_2 until the controls appear explicitly. Now, if $\mathcal{V}_e$ reaches $\mathcal{V}_{e,\max}$ at some time $\tau < t_f$, it is clear that the constraint remains active on $[\tau, t_f]$. Then, as $\mathcal{V}_e$ is constant, one has $F = 0$ along this boundary arc. We do not discuss further the structure of the solution here, and present preliminary numerical computations in the following section.

IV. Numerical simulations

The computations of the optimal solutions were performed with Bocop [21], which uses a direct method. The time discretization algorithm used is Lobato IIIC (implicit, 4-stage, order 6) with 2000 time steps. Given the increasing nature of γ, the maximum amount of mass is obtained at final time $t = t_f$, which defines $\gamma_{\max} = \gamma(t_f)$. We first analyze the impact of the initial cellular composition in the optimal trajectories by modifying the balance between ribosomal proteins R and enzymatic proteins M. Then, we study the solutions for different maximal metabolite synthesis rates k_X. All simulations are performed with a fixed final time $t_f = 70$, and with parameters and initial conditions based on literature values [2], [22], [23], [24]: $s_0 = 0.015$, $p_0 = 0.05$, $V_{e,0} = 0.5$ L, $V_{e,\max} = 5$ L, $V_{i,0} = 0.003$ L and $F_{\max} = 0.08$ L/h. All figures (from 3 to 8) show the optimal controls u and F, as well as the time evolution of all the masses in the bioreactor X, S, P, M, R and Q. According to the model definition, the mass of precursors P remains marginal with respect to the others, and thus not visible in the plots.

A. The role of the cellular composition

In this section, optimal trajectories with different initial cellular compositions are shown.:

- $r_0 = r_{\min}$ and $m_0 = r_{\max} - r_{\min}$ in Figure 3,
- $r_0 = m_0 = (r_{\min} - r_{\max})/2$ in Figure 4,
- $r_0 = r_{\max}$ and $m_0 = 0$ in Figure 5,

where m_0 corresponds to the initial enzymatic concentration $m(0) = 1 - r_0$. The exponential regime of the feeding rate F is not present in the results, as control F follows a bang-boundary control structure, resulting in a linear increase of the culture $\mathcal{V}_e$ until it reaches $\mathcal{V}_{e,\max}$, point at which the total mass in the bioreactor γ remains constant until the end of the bioprocess. The allocation control u has a bang-singular-bang structure, and in all cases the singular arc is a decreasing curve around an intermediate value $u = 0.5$,

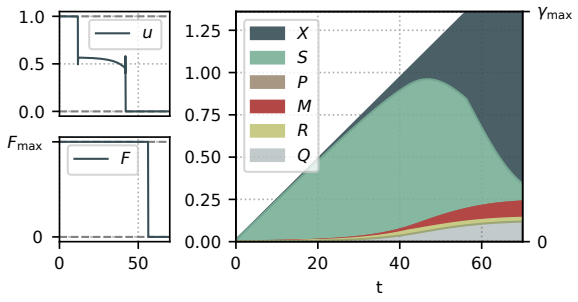

Fig. 3. Optimal process evolution. The right plot describes the time evolution of the masses of each component. The initial ribosomal mass fraction is $r_0 = r_{\min}$. The r

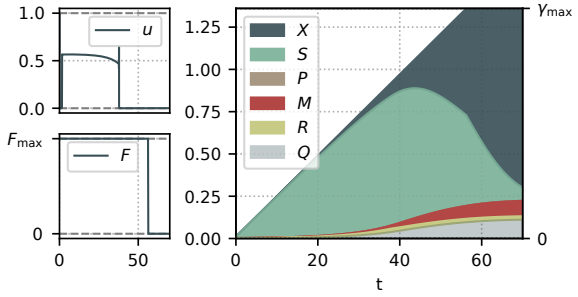

Fig. 4. Optimal process evolution. The right plot describes the time evolution of the masses of each component. The initial ribosomal mass fraction is $r_0 = 0.3$.

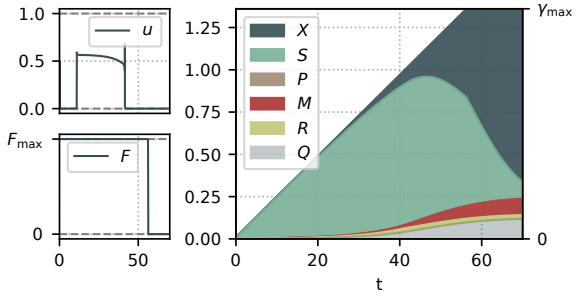

Fig. 5. Optimal process evolution. The right plot describes the time evolution of the masses of each component. The initial ribosomal mass fraction is $r_0 = r_{\max}$.

followed by a bang arc $u = 0$ at the end of the process, which matches previous results in the literature for other classes of bioreactors [5]. However, the first bang is clearly linked to the initial ribosomal concentration r_0, as it is 0 for $r_0 = r_{\max}$, 1 for $r_0 = r_{\min}$ and almost non-existent for the intermediate $r_0 = (r_{\max} - r_{\min})/2$). These strategies match classical inducer-based control strategies, where growth is externally halted after achieving a certain necessary bacterial volume $\mathcal{V}_i$. The latter is represented through the last bang arc $u = 0$, which maximizes the production of enzymatic proteins M catalyzing the production of metabolite X, while purposely stopping the production of ribosomal proteins R. In all cases, the metabolite represents almost 80% of the total mass.

B. The role of the maximum metabolite synthesis rate

In this section, different values of the maximum metabolite production rate k_X are explored:

- $k_X = 0.5k_R$ in Figure 6,
- $k_X = k_R$ in Figure 7,
- $k_X = 1.5k_R$ in Figure 8.

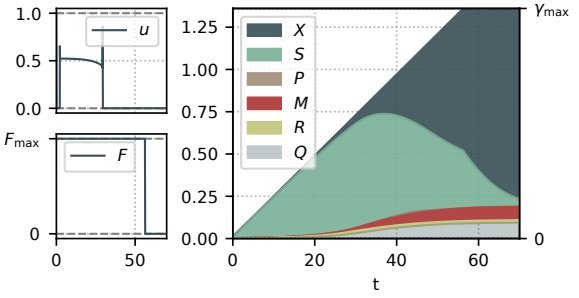

Fig. 6. Optimal process evolution. The right plot describes the time evolution of the masses of each component. The maximum metabolite synthesis rate is $k_X = 0.5k_R$.

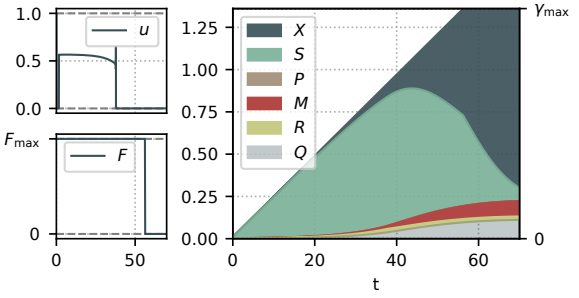

Fig. 7. Optimal process evolution. The right plot describes the time evolution of the masses of each component. The maximum metabolite synthesis rate is $k_X = k_R$.

Results are obtained for an initial ribosomal concentration $r_0 = (r_{\max} - r_{\min})/2$, which produces an initial bang arc almost imperceptible. The main difference between allocation controls u is the length of the singular arc with respect to the final bang arc, which appears to be proportional to k_X. We see that, for $k_X = 1.5k_R$, at $t = t_f$ there remains an important mass of substrate in the bioreactor, while for $k_X = 0.5k_R$, almost all the substrate is depleted by the end of the process. One would expect that, for low values of k_X, most of the substrate would be used to produce biomass due to the difference in maximum rates. In this sense, the role of the

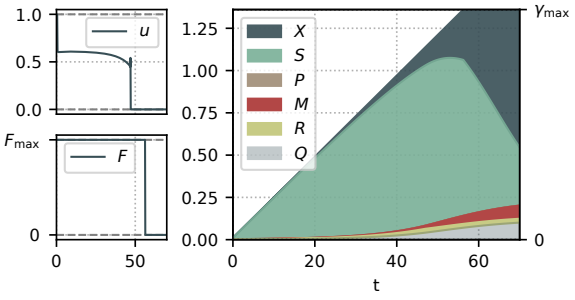

Fig. 8. Optimal process evolution. The right plot describes the time evolution of the masses of each component. The maximum metabolite synthesis rate is $k_X = 1.5k_R$.

allocation control is instrumental, as it prevents bacteria from allocating all resources to the production of biomass, at the expense of slowing down metabolite synthesis. Additionally, results show a link between the metabolite production rate and the switch time at which the production of ribosomal proteins should be externally turned off.

V. Discussion

Thanks to recent ground-breaking advancements in biotechnology, we are now able to manipulate bioprocesses at the intracellular level. However, there is a lack of understanding of how to optimally interfere with the natural cellular processes in order to achieve synthetic objectives. This paper tackled this problem in the context of fed-batch processing. The phenomenon is studied through a multi-scale dynamical model accounting for the bioreactor-level processes (such as the nutrient and the biomass) and the cellular-level dynamics (such as gene expression, metabolism and nutrient uptake). The bacterial model is calibrated so as to account for *E. coli* bacterium. We propose the objective of maximizing the production of a metabolite of interest through an Optimal Control problem, by regulating the allocation of cellular resources, as well as the feeding rate of the bioreactor. Numerical results show how the intracellular concentration and the rate of production of the metabolite of interest can affect the dynamical resource allocation strategy required to maximize such metabolite. An extension of this work will include a dynamical analysis of the system, as well a more detailed study of the optimal control problem. For instance, and as it occurred in previous results, the chosen final time could play an important role in the solution. Ultimately, the open-loop strategies could serve as a baseline to develop closed-loop control techniques.

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