Optimal control of a bioreactor for biofuel production

R. Ghezzi∗, B. Piccoli†

February 7, 2014

Abstract

We propose a hybrid model for optimal control of a bioreactor which features a full coupling between a linear optimization problem and a dynamic one.

1 Introduction

Biofuels provide a concrete answer to the pressing need for renewable energy. The problem of increasing the efficiency and reducing the cost of biofuel production has been subject of intensive research [2, 9]. A feasible source to obtain biofuels consists in using ethanol produced by cyanobacteria and microalgae [12, 13]. This paper deals with the application of optimal control techniques to a general bioreactor for biofuel production.

Since the last decades, optimization of bioprocesses has been a line of research connecting optimal control to system biology, see [24] and references therein. Progress in plant genetic engineering has opened novel opportunities to use plants as bioreactors for safe and cost effective production of vaccine antigens. A review of methods and applications of plant, tissue and cell culture based expression strategies and their use as bioreactors for large scale production of pharmaceutically important proteins can be found in [23]. Exploiting bioreactors was also proved a fruitful method to deal with the problem of wastewater treatment in environmental engineering [19]. In this context a dynamic optimization problem arising frequently is the minimization of the time needed to reach a fixed target configuration for the bioreactor, see [22]. In this case, the trajectory evolves according to a system of nonlinear ODEs and may satisfy some boundary constraints as well. The right-hand side of the evolution equations involves not only control functions, i.e. parameters through which we can modify the dynamics, but also some unknown functions (e.g. biomass growth rate) of the evolving quantities themselves. Therefore, computing optimal controls through standard techniques (necessary conditions) is affected by the unknown functions’ behavior. An interesting approach to deal with this issue using observability techniques was proposed in [4], where an application to bioreactors is also provided (see also [11]).

Other applications of optimal control techniques have been studied in the field of biological systems [1]. In the context of medical treatment, chemotherapy was used [16] as a dynamic control to optimize treatment scheduling in early stage HIV-infected cases. In that paper the authors

∗Scuola Normale Superiore, Piazza dei Cavalieri 7, 56126 Pisa, Italy roberta.ghezzi@sns.it
†Rutgers University, Department of Mathematical Sciences and Center for Computational and Integrative Biology, Camden, NJ 08102, USA piccoli@camden.rutgers.edu
use the effect of chemotherapy on viral production to maximize benefits in terms of T cell count and minimize the systemic cost of the treatment. Time dependent control strategies were also exploited in models to contain the emergence of drug-resistant strains of tuberculosis [15]. Here controls are represented by efforts in finding patients in which virus is only latent and in completing treatment for patients in which virus is already active. The objective function balances the effect of minimizing the cases of latent and infectious drug-resistant tuberculosis and minimizing the cost of implementing the control treatments.

In this paper we are concerned with the optimization of a bioprocess for biofuel production. Namely, we consider a model for the metabolic activity of a microorganism (e.g. E. Coli or Saccharomyces Cerevisiae) in a fed-batch culture with different feeding substrates. The optimal control problem is to maximize the productivity of a certain side metabolite (e.g., ethanol) through different feeding rates.

A steady-state approach to model cellular metabolism is Flux Balance Analysis (FBA, see [20]). The main assumption of FBA is that metabolic activity of cells is performed in such a way that the growth rate of cells is maximized. Since genome-scale stoichiometric models for bacteria such as E. Coli are available, this translates in a linear program where the objective is cellular growth rate and constraints are given by metabolic reactions. Optimization is then performed by means of genetic manipulations on the bacteria (gene deletions and insertions). Other approaches based on flux balance analysis were proposed that take account of transcriptional and regulatory effects in [7], that couple the steady-state metabolic activity with a dynamic model [17, 18] and that integrate both aspects [8]. Optimizing ethanol productivity in fed-batch cultures modeled through Dynamic Flux Balance Analysis [14] consists in using outputs of FBA (cellular growth rate and metabolite fluxes) to update at each time step the dynamics for evolving extracellular quantities. Within this framework, control can be performed at two levels: intracellular controls (genetic modifications), which are implemented by acting on constraints of the FBA, and extracellular controls (of dynamic nature), which are implemented by changing feeding rates. Genetic strategies were deeply investigated in [14] for in silico evolution of a yeast strain in glucose and xylose media to maximize ethanol productivity with constant feeding rates.

In this paper, we analyze extracellular controls, i.e., feeding rates for glucose and xylose, which we allow to be time dependent. Namely, we cast the problem of optimizing ethanol for the bioreactor in the language of optimal control, where concentrations of feeding substrates are modified by an external agent in order to control ethanol production. Even though we do not consider genetic manipulations, our model can integrate intracellular controls. Our purpose is to develop up a general model which shares a full coupling between extracellular dynamics and intracellular metabolic activity: at each time instant solutions of ODEs feed fluxes to constraint the FBA, whereas FBA provides cellular growth rate and ethanol uptake ODEs see Figure 1. Since both the objective function and the constraints in FBA are linear (with respect to fluxes), outputs are piecewise linear. Dependence of fluxes on metabolite concentrations is modeled through Michaelis–Menten behavior, i.e., through rational functions. Therefore, FBA outputs appearing in dynamics are piecewise smooth functions of evolving quantities. The main idea is to implement the coupling between the intracellular and extracellular level through a hybrid control system where in each location the outputs of FBA are smooth. One of the advantages of a hybrid formulation is that it allows to take account of different timescales. One can either assume that information translates instantaneously from the micro level to the macro one (and viceversa) or one can implement delays, which are observed in experimental data. This is useful as there are configurations of the extra-
cellular environment (such as saturation of glucose or oxygen) which may cause a major change in the metabolic pathway involved inside the cell with a consequent delay in the variation of outputs of the FBA.

As a first step toward a unified model, we focus on the analysis of optimal trajectories contained in a connected component where FBA parameters are smooth. In other words, we study what happens in each location of the hybrid model. More precisely, the location is characterized by a control-affine system of the type

$$\dot{x} = F_0(x, y^x) + u_1 F_1(x) + u_2 F_2(x),$$

where \(x\) is a vector containing substrate concentrations (biomass, glucose, xylose, ethanol), \(u_1, u_2\) are feed rates of glucose and xylose, \(y^x\) are parameters coming from FBA, and \(F_0, F_1, F_2\) are smooth. We consider an optimal control problem in Mayer form where we optimize the total amount of ethanol at the final time. Since the system is control-affine, the maximization condition of the Pontryagin Maximum Principle allows to compute the control along an extremal trajectory as a feedback law, as long as the corresponding switching function (derivative of Hamiltonian with respect to control) has only isolated zeros. Otherwise, when the trajectory shows a singular arc, i.e., a time interval where a switching function vanishes identically, the main tool to find controls is exploiting conditions given by annihilation of higher order derivatives with respect to time. As our interest is driven by applications, one would rather avoid singular arcs, which represent an obstacle to efficient and reliable numerical simulations. The main result of the paper describes properties of extremal trajectories having singular arcs, under the assumption that parameters \(y^x\) are affine with respect to substrates’ fluxes and that growth is anaerobic. Notice that, due to specific properties of the optimal control problem considered here, one cannot expect that optimal singular arcs do not occur, even generically. Indeed, as the vector fields \(F_1, F_2\) are actually constant, we cannot use results on codimension of singular trajectories, such as [6], where the authors deal with control-affine systems satisfying generic assumptions. We also consider the role of oxygen as a control. In the case where feeding rates of glucose and xylose are piecewise constant, we characterize the solution of the adjoint system along singular arcs.

The structure of the paper is the following. In Section 2 we recall the general model of a bioreactor and formulate the optimal control problem where the cost to be maximized is ethanol productivity of the bioreactor (i.e., a certain function of the total amount of ethanol produced
at the final time.) In Section 3 we state and prove the main result concerning optimal singular trajectories. In Section 4 we discuss the role of oxygen as a control.

2 Problem formulation

Bioreactors are processes where a living microorganism metabolizes some substrates and consequently grows and produces other metabolites. In [14] the authors consider in silico evolution of a yeast strain, *Saccharomyces cerevisiae*, which grows in a fed-batch culture with glucose and xylose and produces ethanol. The dynamic model can be applied to a general bioreactor.

We denote by $V$ the total culture volume, which is assumed to grow linearly with respect to time with constant rate $F$. Biomass concentration is denoted by $X$. The total biomass in the culture evolves linearly with a growth rate $\mu$. Concentrations of feeding substrates (glucose $G$ and xylose $Z$) are characterized by an evolution which takes account of a feeding rate and a compensation term due to metabolism of the microorganism. Feeding rates for substrates represent the control we perform on the system and are denoted by $u_1, u_2$. The organism metabolizes glucose and xylose with specific rates (or fluxes) $v_g, v_z$. Oxygen concentration $O$ is also involved in the bioprocess and represent a further control whose role we analyze in Section 4. The produced metabolite we study is ethanol. Denoting ethanol concentration by $E$, we assume that the organism produces ethanol proportionally to the total biomass through a specific rate $v_e$. Therefore, the control system we analyze is

$$
\begin{align*}
\dot{V} &= F \\
V \dot{X} &= \mu(G,Z,E,O)V X \\
V \dot{G} &= F u_1 - v_g(G,E)V X \\
V \dot{Z} &= F u_2 - v_z(G,Z,E)V X \\
V \dot{E} &= v_e(G,Z,E,O)V X.
\end{align*}
$$

(1)

It is evident from system (1) the parameters $\mu, v_g, v_z, v_e$ depend on evolving variables. First of all, oxygen uptake kinetics follows Michaelis-Menten kinetics

$$
v_o(O) = v_{o,\text{max}} \frac{O}{k_o + O},
$$

whereas glucose uptake kinetics has an additional regulatory term to capture growth rate suppression due to high ethanol concentration, i.e.,

$$
v_g(G,E) = v_{g,\text{max}} \frac{G}{k_g + G \left(\frac{1}{1 + E/k_{ie}^{g}}\right)}.
$$

Xylose uptake kinetics has a similar form with another regulatory term to account for inhibited xylose metabolism in presence of the preferred substrate (glucose),

$$
v_z(G, Z, E) = v_{z,\text{max}} \frac{Z}{k_z + Z \left(\frac{1}{1 + E/k_{ie}^{z}}\right)} \frac{1}{1 + G/k_{ig}}.
$$

Parameters $v_{o,\text{max}}, v_{g,\text{max}}, v_{z,\text{max}}, k_g, k_z, k_{ie}^{g}, k_{ie}^{z}, k_{ig}$ are positive and constant. As for $\mu, v_e$, the model is based on the principle that the metabolic activity of the microorganism is performed so that
biomass growth rate is maximized. This is equivalent to say that \( \mu \) and \( v_e \) are outputs of an optimization problem stated as

\[
\begin{align*}
\mu(G, Z, E, O) &= \max_{\bar{v} \in \mathbb{R}^n} \sum_{j=1}^{n} w_j \bar{v}_j \\
\text{s.t.} \quad S\bar{v} &= 0 \\
\bar{v}_g &= v_g(G, E) \\
\bar{v}_z &= v_z(G, Z, E) \\
\bar{v}_o &= v_o(O) \\
0 &\leq \bar{v}_j \leq \bar{v}_j, \quad j = 1, \ldots, n
\end{align*}
\]  

\( v_e(G, Z, E, O) = \arg\max \mu(G, Z, E, O). \)  

In (2), \( \bar{v} \in \mathbb{R}^n \) is the vector of fluxes (among which glucose, xylose, oxygen and ethanol fluxes) considered in the model; \( w \in [0, 1]^n \) is the vector of weights which determines fluxes producing biomass; \( S \in M^{r \times n}(\mathbb{R}) \) is the stoichiometric matrix which encode the metabolic network inside the cell (involving \( r \) reactions and \( n \) metabolites); \( \bar{v} \) is an upper bound associated with the microorganism, (see [20] for an exhaustive treatment of metabolic networks in systems biology.) For our purposes, \( w, S, \bar{v} \) are considered as given parameters. They depend on the specific strain of microorganism used in the bioreactor and are usually determined through experimental data see for instance supplementary data of [8] for E. Coli or [13] for Saccharomyces Cerevisiae.

Equation (3) is to be read as follows. Let \( V^{\max} \) denote the set of vectors \( \bar{v} \) satisfying the constraints above and realizing the maximum, i.e., \( \mu(G, Z, E, O) = \sum_j w_j \bar{v}_j \). Then \( v_e(G, Z, E, O) \) is the maximum of \( \bar{v}_e \) as \( \bar{v} \) varies in \( V^{\max} \). We fix the final time \( t_f > 0 \) and an initial condition \((V_0, X_0, G_0, Z_0, O_0)\) and we seek to optimize ethanol productivity along a trajectory of (1), i.e.,

\[
\max_{(u_1, u_2) \in \mathcal{U}} \frac{V(t_f)E(t_f)}{\int_0^{t_f} (u_1(s) + u_2(s)) \, ds}
\]

(4)

where the class of admissible controls is

\[
\mathcal{U} = \{(u_1, u_2) \in L^\infty([0, t_f])^2 \mid u_i(t) \in [0, 1] \text{ for almost every } t\}.
\]

We may assume that the functional (4) does not diverge, as the trivial control strategy where there is no feed, i.e., \((u_1(t), u_2(t)) \equiv (0, 0)\), is not optimal.

The problem shares a full coupling between a classical optimal control problem (1), (4), which describes the dynamics outside the cell, and a linear optimization problem (2), which models the metabolic activity inside the cell. In other words, at each time instant \( t \), on the one hand, one needs to solve the LP (2) to obtain \( \mu, v_e \) at time \( t \) to plug into (1); on the other hand, dynamics in (1) must be integrated to in order to get the triple \((G(t), Z(t), E(t))\) that allows to determine constraints in (2).

Since in (2) both the objective function and the constraints are linear with respect to \( \bar{v} \), the outputs \( \mu \) and \( v_e \) are piecewise linear with respect to \( \bar{v} \). Therefore \( \mu, v_e \) are piecewise smooth as functions of \((Z, G, E, O)\). In the sequel we assume that the outputs of (2) are smooth. This amounts
to say that the system evolves in a domain where \( \mu, v \) are smooth as functions of \( (Z, G, E, O) \).

Under this assumption, we study optimal trajectories for problem (1), (4). Let us give a more compact formulation of the optimization problem above. We will deal first with the case where growth is anaerobic, that is, oxygen concentration \( O \) is zero (see Section 4 for the analysis where oxygen is taken as a control.) Let us rename

\[
x_3 = VG, \ x_4 = VZ, \ x_5 = VE, \ x_6 = VX, \ x_7 = V.
\]

It is natural to add two new variables to (1) by setting

\[
x_i(t) = \int_0^t u_i(s)ds, \ i = 1, 2,
\]

so that the control system (1) reads

\[
\begin{aligned}
\dot{x}_1 &= u_1 \\
\dot{x}_2 &= u_2 \\
\dot{x}_3 &= Fu_1 - v_g(x_3, x_5, x_7)x_6 \\
\dot{x}_4 &= Fu_2 - v_z(x_3, x_4, x_5, x_7)x_6 \\
\dot{x}_5 &= v_e(x_3, x_4, x_5, x_7)x_6 \\
\dot{x}_6 &= \mu(x_3, x_4, x_5, x_7)x_6 \\
\dot{x}_7 &= F
\end{aligned}
\]

where

\[
v_g(x_3, x_5, x_7) = \frac{x_3x_7}{v_{g\max}(k_g x_7 + x_3)(x_7 + x_5/k_{ie})} (7)
\]

\[
v_z(x_3, x_4, x_5, x_7) = \frac{x_4x_7^2}{v_{z\max}(k_z x_7 + x_4)(x_7 + x_3/k_{ig})(x_7 + x_5/k_{ie})}, (8)
\]

and \( v_e(x_3, x_4, x_5, x_7), \mu(x_3, x_4, x_5, x_7) \) are defined in the obvious way by (2), (3) using (5).

The equation for \( x_7 \) can be trivially integrated. Indeed, the total volume of the culture plays the same role as time along the experiment. Here we prefer to keep the state variable \( x_7 \) in order the control system to be autonomous.

Setting \( x = (x_1, x_2, x_3, x_4, x_5, x_6, x_7) \), the optimization in (4) becomes

\[
\max_{(u_1, u_2) \in \mathcal{U}} \psi(x(t_f)), (9)
\]

where \( \psi(x) = \frac{x_3}{x_1 + x_2} \). The latter is smooth on the subset \( \mathcal{R} = \{ x \in \mathbb{R}^7 \mid x_1 + x_2 > 0 \} \). Every trajectory starting at a point with \( x_1(0) + x_2(0) > 0 \) satisfies the state constraint \( x_1(t) + x_2(t) > 0 \), i.e., it belongs to the region \( \mathcal{R} \) for every \( t > 0 \).

The control system (6) is affine with respect to controls and can be written in the form

\[
\dot{x} = f(x, u), \ x \in \mathbb{R}^7
\]
where \( u = (u_1, u_2), f(x, u) = F_0(x) + u_1 F_1(x) + u_2 F_2(x) \) and

\[
F_0(x) = \begin{pmatrix}
0 \\
0 \\
-v_g(x_3, x_5, x_7)x_6 \\
v_e(x_3, x_4, x_5, x_7)x_6 \\
\mu(x_3, x_4, x_5, x_7)x_6 \\
F
\end{pmatrix}, \quad F_1(x) = \begin{pmatrix}
1 \\
0 \\
F \\
0 \\
0 \\
0
\end{pmatrix}, \quad F_2(x) = \begin{pmatrix}
0 \\
0 \\
0 \\
0 \\
0 \\
0
\end{pmatrix}.
\]

We end this section by stating a result which ensures the existence of optimal solutions to (1), (2) with suitable initial conditions.

**Proposition 1** Assume \( v_e, \mu \in C^\infty(\mathbb{R}^4, \mathbb{R}^+) \) and let \( x^0 \) belong to

\[
D = \{ x \in \mathbb{R}^7 \mid x_i \geq 0, i = 1 \ldots 6, x_1 + x_2 > 0, x_7 > 0 \}.
\]

Then there exist an optimal solution to (6), (9) satisfying the initial constraint \( x(0) = x^0 \).

**Sketch of proof.** Under the assumption that \( \mu, v_e \) are smooth, every trajectory of the control system starting at a point \( x^0 \) in the set (11), is well-defined for all \( t > 0 \) and satisfies \( x_i(t) \geq 0 \) for every \( t > 0 \). Indeed, let \( D_0 = \{ x \in \mathbb{R}^7 \mid x_i \geq 0, i = 1 \ldots 6, x_7 \geq x_7^0 \} \). Then the dynamics is smooth and satisfies the sublinear bound

\[
|f(x, u)| \leq C(1 + |x|), \quad \forall x \in D_0, \; (u_1, u_2) \in [0, 1]^2,
\]

for a certain constant \( C > 0 \). Let \( T \) be such that a trajectory starting at \( x^0 \) is well-defined on \([0, T]\). Then \( x(t) \in D_0 \) for every \( t \in [0, T] \). Indeed, inequalities \( x_i(t) \geq 0 \) for \( i = 1, 2, \) and \( x_7(t) \geq x_7^0 \) are trivially satisfied. Similarly, since the equation for \( x_6 \) is linear with respect to \( x_6 \), we have \( x_6(t) \geq 0 \). By assumption (and thanks to the constraints \( 0 \leq \bar{v}_j \leq \tilde{v}_j \) in (2)), ethanol flux \( v_e(x_3, x_4, x_5, x_7) \) is non negative for every \( t \), whence \( x_5(t) \geq 0 \). Finally, let \( \bar{t} \) be such that \( x_3(\bar{t}) = 0 \). Then \( \dot{x}_3(\bar{t}) = Fu_2(\bar{t}) \geq 0, \) hence \( x_3(t) \geq 0 \) for \( t > \bar{t} \). In the same way one infers that \( x_4(t) \geq 0 \) for every \( t \). Hence trajectories starting at a point of the set (10) are well-defined for every \( t > 0 \) and \( x(t) \in D_0 \) for all \( t > 0 \). Moreover, because \( x_1^0 + x_2^0 > 0 \), we have \( x_1(t_f) + x_2(t_f) > 0 \), that is, the final point of any trajectory belongs to the \( \mathcal{R} \) on which the cost functional (2) is smooth.

Since the dynamics (6) is control affine, the set of velocities \( \{ f(x, u) \mid u \in [0, 1]^2 \} \) is convex for every \( x \). Therefore, the existence of an optimal solution is a consequence of classical results (see [3, Theorem 5.1.1]).

### 3 Analysis of extremal trajectories

In this section we study optimal trajectories of (6), (7) starting from a given initial condition \( x^0 \in D \). The Hamiltonian associated with the optimal control problem is

\[
H(x, \lambda, u) = \langle \lambda, F_0(x) \rangle + u_1 \langle \lambda, F_1(x) \rangle + u_2 \langle \lambda, F_2(x) \rangle,
\]

where \( \lambda = (\lambda_1, \lambda_2, \lambda_3, \lambda_4, \lambda_5, \lambda_6, \lambda_7) \in \mathbb{R}^7 \) denotes the covector.
Let \( u^*(t) = (u_1^*(t), u_2^*(t)) \) be an admissible control such that the corresponding trajectory of \( \mathbf{G} \) starting at \( x^0 \), denoted by \( x(t) \), is optimal. Applying the Pontryagin Maximum Principle \([21]\), there exists a solution \( \lambda(t) \neq 0 \) of the adjoint system

\[
\dot{\lambda}(t) = -\frac{\partial H}{\partial x}(x(t), \lambda(t), u^*(t)), \quad \lambda(t_f) = \nabla \psi(x(t_f)),
\]

which reads

\[
\begin{cases}
\dot{\lambda}_1 = 0 \\
\dot{\lambda}_2 = 0 \\
\dot{\lambda}_3 = x_6 \left( \frac{\partial v}{\partial x_3} \lambda_3 + \frac{\partial v}{\partial x_4} \lambda_4 - \frac{\partial v}{\partial x_5} \lambda_5 - \frac{\partial v}{\partial x_6} \lambda_6 \right) \\
\dot{\lambda}_4 = x_6 \left( \frac{\partial v}{\partial x_3} \lambda_4 - \frac{\partial v}{\partial x_4} \lambda_5 - \frac{\partial v}{\partial x_5} \lambda_6 \right) \\
\dot{\lambda}_5 = x_6 \left( \frac{\partial v}{\partial x_3} \lambda_5 + \frac{\partial v}{\partial x_4} \lambda_4 - \frac{\partial v}{\partial x_5} \lambda_5 - \frac{\partial v}{\partial x_6} \lambda_6 \right) \\
\dot{\lambda}_6 = v_y \lambda_3 + v_z \lambda_4 - v_e \lambda_5 - \mu \lambda_6 \\
\dot{\lambda}_7 = x_6 \left( \frac{\partial v}{\partial x_7} \lambda_3 + \frac{\partial v}{\partial x_4} \lambda_4 - \frac{\partial v}{\partial x_5} \lambda_5 - \frac{\partial v}{\partial x_6} \lambda_6 \right),
\end{cases}
\]

such that, for almost every \( t \in [0, t_f] \),

\[
H(x(t), \lambda(t), u^*(t)) = \max_{0 \leq \omega_1 \leq 1, 0 \leq \omega_2 \leq 1} \left( \langle \lambda(t), F_0(x(t)) \rangle + \omega_1 \langle \lambda(t), F_1(x(t)) \rangle + \omega_2 \langle \lambda(t), F_2(x(t)) \rangle \right),
\]

(see also \([3]\) Theorem 6.1.1]). The transversality condition for the covector at final time is

\[
\lambda(t_f) = \left( -\frac{x_5(t_f)}{(x_1(t_f) + x_2(t_f))^2}, -\frac{x_5(t_f)}{(x_1(t_f) + x_2(t_f))^2}, 0, 0, \frac{1}{x_1(t_f) + x_2(t_f)}, 0, 0 \right),
\]

and, since the Hamiltonian does not depend on \( x_1, x_2 \), we obtain

\[
\lambda_1(t) \equiv \lambda_2(t) \equiv -\frac{x_5(t_f)}{(x_1(t_f) + x_2(t_f))^2}.
\]

Define the switching functions

\[
\varphi_i(t) = \langle \lambda(t), F_i(x(t)) \rangle, \quad i = 1, 2.
\]

Since the Hamiltonian is affine with respect to controls, for every \( t \) such that \( \varphi_1(t) \varphi_2(t) \neq 0 \) the control \( u^*(t) \) is uniquely determined by \( u_i^*(t) = \frac{1 + \text{sign} \varphi_i(t)}{2}, \quad i = 1, 2 \).

We say that an extremal \( x(\cdot) \) has a singular arc on \( [a, b] \subset [0, t_f] \), with \( a < b \), if \( \varphi_1|_{[a,b]} \equiv 0 \) or \( \varphi_2|_{[a,b]} \equiv 0 \). In general more degenerate singular arcs can occur: for instance, arcs where one among the switching functions vanishes identically and the other one has non-isolated zeros and does not vanish identically. In this case, the corresponding component of the control features an accumulation of switchings. Accumulations of zeros of the switching functions can indeed happen along an optimal trajectory and they are known as chattering or Fuller’s phenomena (see \([10]\)). In this paper we assume that chattering phenomena do not occur. Even though such pathological phenomenon can happen, novel techniques have been recently developed to overcome it, see \([5]\).

When seeking an optimal synthesis, a fundamental step is to reduce the problem to a finite-dimensional one. For instance, this can be done if, by means of necessary conditions, one deduces
that every extremal trajectory is a finite concatenation of bang arcs, where the control has a simple form. In general this is hard to prove, still one can expect that only “a few” extremal trajectories are “bad”, i.e., they do not satisfy this property. To do so, a first step is the result below, which describes the covector along badly behaved extremal trajectories for the optimal control problem (6), (9).

Concerning the presence of optimal singular trajectories for control-affine systems, a genericity result has been shown in [6]. In that paper, the authors prove that, given a quadratic cost, generically with respect to the control system there do not exist nontrivial optimal singular trajectories [6, Corollary 2.9]. Notice that this result does not apply here because system (6) is not generic, $F_1, F_2$ being constant vector fields.

**Theorem 1** Assume that $\mu, v_e$ are affine with respect to $(v_g, v_z)$, i.e.,

$$
\begin{align*}
\mu &= a_1 v_g + a_2 v_z + \bar{\mu} \\
v_e &= b_1 v_g + b_2 v_z + \bar{v},
\end{align*}
$$

with $a_1, a_2, b_1, b_2, \bar{\mu}, \bar{v}$, positive constant such that

$$
\begin{align*}
a_1 b_2 - a_2 b_1 &\neq 0 \\
(b_1 - b_2) \bar{\mu} + (a_2 - a_1) \bar{v} &\neq 0 \\
b_1 \bar{\mu} - a_1 \bar{v} &\neq 0.
\end{align*}
$$

Let $x(\cdot)$ be an optimal trajectory having a singular arc on $[a, b] \subset [0, t_f]$ such that the sets of zeros of $\varphi_1$ and $\varphi_2$ are finite unions of intervals. Then one among $\varphi_1, \varphi_2$ does not vanish identically on $[a, b]$.

If a singular arc of type $\varphi_1 \equiv 0, \varphi_2 \not\equiv 0$ is optimal, then $u_2^* = \frac{1 + \text{sign} \varphi_2(t)}{2}$ and

- if $\langle \lambda, [F_1, [F_0, F_1]] \rangle \equiv 0$ then $\lambda_4(t)$ is uniquely determined by $x(\cdot)$ and $x(t_f)$ and $\lambda_j$ is constant for every $j \neq 4$.
- if $\langle \lambda, [F_1, [F_0, F_1]] \rangle$ has only isolated zeros then $u_1^*(t)$ is uniquely determined as a feedback.

**Proof.** Since there is no chattering, on $[a, b]$ either both switching functions vanish identically or one is identically zero and the other one has only isolated zeros.

Assume first that $\varphi_1|_{[a, b]} \equiv 0$ and $\varphi_2|_{[a, b]} \equiv 0$. Then

$$
\begin{align*}
\lambda_1(t) + F \lambda_3(t) &\equiv 0 \\
\lambda_2(t) + F \lambda_4(t) &\equiv 0.
\end{align*}
$$

Jointly with (12), we deduce that

$$
\lambda_3(t) \equiv \lambda_4(t) \equiv -\frac{\lambda_1(t_f)}{F}.
$$

Therefore, $\lambda_1(t), \lambda_2(t), \lambda_3(t), \lambda_4(t)$ are uniquely determined as functions of $x(t_f)$. In the following we set $\bar{\lambda}_1(t) = \lambda_1(t_f), \bar{\lambda}_2(t) = \lambda_1(t_f), \bar{\lambda}_3(t) = -\lambda_1(t_f)/F, \bar{\lambda}_4(t) = -\lambda_1(t_f)/F$. 

9
Conditions $\varphi_1 \equiv 0, \varphi_2 \equiv 0$ imply jointly with the third and fourth equation in (11) that the pair $(\lambda_5, \lambda_6)$ must satisfy

$$\begin{pmatrix} \frac{\partial u_v}{\partial x_3} & \frac{\partial u_v}{\partial x_4} \\ \frac{\partial u_w}{\partial x_3} & \frac{\partial u_w}{\partial x_4} \end{pmatrix} \begin{pmatrix} \lambda_5 \\ \lambda_6 \end{pmatrix} = - \begin{pmatrix} \lambda_1(t_f) \frac{\partial u_v}{\partial x_3} + \lambda_2(t_f) \frac{\partial u_w}{\partial x_3} \\ \lambda_1(t_f) \frac{\partial u_v}{\partial x_4} + \lambda_2(t_f) \frac{\partial u_w}{\partial x_4} \end{pmatrix}$$  \quad (14)

System (14) can be seen as a linear system in $(\lambda_5(t), \lambda_6(t))$ whose coefficient depend only on $x(t)$ and on $x(t_f)$. When $\mu, v_\epsilon$ are given as in (13), the determinant of (14) is a positive multiple of $a_1b_2 - a_2b_1$. Hence, by assumption, it never vanishes and (14) admits a unique solution. In other words, we can express $(\lambda_5(t), \lambda_6(t))$ as a functions of $x(t)$ and $x(t_f)$. In what follows, we denote by $\lambda_5(t), \lambda_6(t)$ the solution of (14). Consider now the system

$$\begin{align*}
\bar{\varphi}_1 &= \langle \lambda, [F_0, [F_0, F_1]] \rangle + u_1 \langle \lambda, [F_1, [F_0, F_1]] \rangle + u_2 \langle \lambda, [F_2, [F_0, F_1]] \rangle \equiv 0, \\
\bar{\varphi}_2 &= \langle \lambda, [F_0, [F_0, F_2]] \rangle + u_1 \langle \lambda, [F_1, [F_0, F_2]] \rangle + u_2 \langle \lambda, [F_2, [F_0, F_2]] \rangle \equiv 0.
\end{align*}$$  \quad (15)

Taking $\lambda = (\bar{\lambda}_1, \bar{\lambda}_2, \bar{\lambda}_3, \bar{\lambda}_4, \bar{\lambda}_5, \bar{\lambda}_6, \bar{\lambda}_7)$, due to the form of $\mu, v_\epsilon$ a computation shows that

$$\langle \lambda, [F_1, [F_0, F_1]] \rangle \equiv 0, \quad \langle \lambda, [F_2, [F_0, F_1]] \rangle \equiv 0, \quad \langle \lambda, [F_1, [F_0, F_2]] \rangle \equiv 0, \quad \langle \lambda, [F_2, [F_0, F_2]] \rangle \equiv 0.$$  

Hence, in order system (15) to be compatible, one needs

$$\langle \lambda, [F_0, [F_0, F_1]] \rangle \equiv 0, \quad \langle \lambda, [F_0, [F_0, F_2]] \rangle \equiv 0.$$  

Nevertheless, we have

$$\langle \lambda, [F_0, [F_0, F_2]] \rangle = \frac{a_2Fk_\kappa^z k_i g v_\max x_6 x_7^2((b_1 \bar{\lambda}_4 - b_2 \bar{\lambda}_3)\bar{\mu} + (a_2 \bar{\lambda}_3 - a_1 \bar{\lambda}_4)\bar{v})}{(a_2 b_1 - a_1 b_2)(x_5 + k_\kappa^z x_7)(x_3 + k_i g x_7)(x_4 + k_\zeta x_7)^2}.$$  

Hence, since $\bar{\lambda}_3 = \bar{\lambda}_4 \neq 0$ and $a_1 b_2 - a_2 b_1 \neq 0$, $(b_1 - b_2)\bar{\mu} + (a_2 - a_1)\bar{v} \neq 0$, we get a contradiction. Therefore, there cannot exist optimal singular arcs where both $\varphi_1$ and $\varphi_2$ vanish identically.

Assume now that $\varphi_1|_{[a, b]} \equiv 0$ and $\varphi_2(t)$ has only isolated zeros in $[a, b]$. In particular,

$$\lambda_3(t) \equiv -\frac{\lambda_1(t_f)}{F} > 0,$$

and $u_2^*(t) = \frac{1 + \text{sign}(\varphi_2(t))}{2}$, that is, $u_2^*$ is given as a feedback law. Let $\bar{\lambda}_1(t) \equiv \lambda_1(t_f), \bar{\lambda}_3(t) \equiv -\lambda_1(t_f)/F$. From condition $\varphi_1 \equiv 0$, which is equivalent to $\bar{\lambda}_3 \equiv 0$, we obtain that

$$\begin{align*}
k_\kappa^g k_i g v_\max \bar{\lambda}_3 - b_1 \lambda_5 - a_1 \lambda_6 + k_\kappa^z k_i g v_\max x_4 \frac{-\lambda_4 + b_2 \lambda_5 + a_2 \lambda_6}{(x_5 + k_\kappa^z x_7)(x_3 + k_i g x_7)^2(x_4 + k_\zeta x_7)} \equiv 0 \quad (17)
\end{align*}$$

Setting

$$A = k_\kappa^g k_i g v_\max \bar{\lambda}_3 - b_1 \lambda_5 - a_1 \lambda_6, \quad B = k_\kappa^z k_i g v_\max \frac{-\lambda_4 + b_2 \lambda_5 + a_2 \lambda_6}{(x_5 + k_\kappa^z x_7)(x_3 + k_i g x_7)^2(x_4 + k_\zeta x_7)},$$

equation (17) becomes

$$A + x_4 B \equiv 0.$$  

10
Let \( c(t) = \langle \lambda, [F_1, [F_0, F_1]] \rangle \) be the coefficient of \( u_1 \) in condition \( \bar{\varphi}_1 \equiv 0 \), see (15). A computation shows that
\[
c(t) = -2F^2 x_6 x_7^2 \left( \frac{A}{x_3 + k_g x_7} + x_4 \frac{B}{x_3 + k_{ig} x_7} \right).
\] (18)

Since there is no chattering, zeros of \( c(\cdot) \) are finite union of intervals. Hence, we distinguish two cases: first \( c|_{[a,b]} \equiv 0 \), second \( c(\cdot) \) has only isolated zeros in \( [a,b] \). In the second case condition \( \bar{\varphi}_1 \equiv 0 \) allows to determine \( u_1^*(t) \), for all \( t \) such that \( c(t) \neq 0 \), by
\[
u_1^*(t) = - \nu_2^* \frac{\langle \lambda, [F_2, [F_0, F_1]] \rangle + \langle \lambda, [F_0, [F_0, F_1]] \rangle}{\langle \lambda, [F_1, [F_0, F_1]] \rangle}.
\]

Let now \( c(t) \equiv 0 \). In this case, from (17) and (15) and since \( k_g \neq k_{ig} \), we deduce that \( A \equiv 0 \) and \( x_4 B \equiv 0 \), which are equivalent to
\[
\begin{cases}
\lambda_3 - b_1 \lambda_5(t) - a_1 \lambda_6(t) = 0 \\
x_4(t) (-\lambda_4(t) + b_2 \lambda_5(t) + a_2 \lambda_6(t)) = 0
\end{cases}
\]

Using these relation and the behavior of \( \mu, v_\varepsilon \), the dynamics for \( \lambda_5 \) in (11) gives \( \dot{\lambda}_5 \equiv 0 \). Hence, differentiating the first equation with respect to time we obtain that \( \dot{\lambda}_6 \equiv 0 \). Therefore, imposing that the right hand side in the dynamics for \( \lambda_6 \) vanishes identically we obtain that the pair \( (\lambda_5, \lambda_6) \) is constant. Indeed, it is the unique solution to the linear system
\[
\begin{cases}
A \equiv 0, \\
\frac{d}{dt} A \equiv 0,
\end{cases}
\]
whose coefficients depending only on \( x, \bar{\lambda}_3 \) and which has determinant \( a_1 (b_1 \bar{\mu} - a_1 \bar{v}) \). By assumption, this determinant is nonzero and the unique solution of the system is
\[
\bar{\lambda}_5(t) \equiv \bar{\lambda}_3 \frac{\mu}{b_1 \bar{\mu} - a_1 \bar{v}}, \quad \bar{\lambda}_6(t) \equiv - \bar{\lambda}_3 \frac{\bar{v}}{b_1 \bar{\mu} - a_1 \bar{v}}.
\]

Assuming that the zeros of \( x_4 \) and \( B \) are finite union of intervals, then \( [a,b] = \cup_{i=1}^{N} [a_i, b_i] \), where \( a_{i+1} = b_i \) and on each \([a_i, b_i], x_4 \equiv 0 \) or \( B \equiv 0 \).

Let \( x_4 \equiv 0 \). Then plugging \( \lambda_5, \lambda_6 \) into (11), the equation for \( \lambda_4 \) becomes
\[
\dot{\lambda}_4 = \frac{k_{iez} k_{ig} v_\varepsilon x_6 x_7}{k_z (x_5 + k_{ie} x_7) (x_3 + k_{ig} x_7)} (\dot{\lambda}_4 - b_2 \bar{\lambda}_5 - a_2 \bar{\lambda}_6).
\] (19)

Hence \( \lambda_4(t) \) is uniquely determined as a function of \( x(\cdot) \) and \( x(t_f) \), for the right hand side of (19) depends only on \( x(\cdot), x(t_f) \) and not on other components of \( \lambda \). Moreover, that \( x_4 \equiv 0 \) implies jointly with the evolution equation for \( x_4 \) that \( u_2^* \equiv 0 \). Hence, from the condition \( H = const \) we deduce that \( \lambda_7 \) is constant.

Let now \( B \equiv 0 \). Then from
\[
\dot{\lambda}_4 = - k_z x_6 x_7^3 \frac{x_3 + k_{ig} x_7}{x_4 + k_z x_7} B.
\]
one deduces that $\lambda_4$ is constant, and it is given by

$$\dot{\lambda}_4(t) \equiv \frac{b_2\bar{\mu} - a_2\bar{v}}{b_1\bar{\mu} - a_1\bar{v}}.$$ 

Again, the condition $H = \text{const}$ implies that $\lambda_7$ is constant, whence the whole covector is constant.

4 Oxigen as a control

In this section we assume $(x_3^0, x_4^0, x_5^0, x_6^0, x_7^0)$ is given such that there exists $(\bar{x}_1, \bar{x}_2, x_3^0, x_4^0, x_5^0, x_6^0, x_7^0) \in \mathcal{D}$ for which a solution of the optimal control problem in the previous section is a trajectory corresponding to a piecewise constant control $(u_1^*(t), u_2^*(t))$.

We assume that that the oxygen affects the system and that we control oxygen concentration. Let $u_3 = O$. We model $\mu, v_e$ as

$$\mu = \tilde{\mu}(x_3, x_4, x_5, x_7)\sigma(u_3), \quad v_e = \tilde{v}_e(x_3, x_4, x_5, x_7)\frac{1}{\sigma(u_3)},$$

where $\sigma(u_3)$ is an affine function of $v_o = \frac{u_3}{u_3 + u_4}$ and $\tilde{\mu}, \tilde{v}_e$ are positive functions. We assume that $\sigma : \mathbb{R}^+ \to \mathbb{R}$ satisfies

$$\sigma(0) = l > 0, \quad \lim_{z \to +\infty} \sigma(z) = L > l,$$  

and $\sigma'(z) > 0$ for every $z \geq 0$. We study a new optimal control problem

$$\max_{0 \leq u_3 \leq 1} x_5(t_f)$$

subject to

$$\begin{cases}
\dot{x}_3 = F u_1^* - v(x_3, x_5, x_7)x_6 \\
\dot{x}_4 = F u_2^* - v(x_3, x_4, x_5, x_7)x_6 \\
\dot{x}_5 = \tilde{v}_e(x_3, x_4, x_5, x_7)x_6\frac{1}{\sigma(u_3)} \\
\dot{x}_6 = \tilde{\mu}(x_3, x_4, x_5, x_7)x_6\sigma(u_3) \\
\dot{x}_7 = F,
\end{cases}$$

and

$$(x_3(0), x_4(0), x_5(0), x_6(0), x_7(0)) = (x_3^0, x_4^0, x_5^0, x_6^0, x_7^0).$$

For simplicity, let us denote by $x$ the tuple $(x_3, x_4, x_5, x_6, x_7)$ and by $\lambda$ the corresponding covector $(\lambda_3, \lambda_4, \lambda_5, \lambda_6, \lambda_7)$. The Hamiltonian becomes

$$H(x, \lambda, u_3) = \lambda_3(F u_1^* - v x_6) + \lambda_4(F u_2^* - v x_6) + x_6 \left( \frac{\tilde{v}_e}{\sigma(u_3)} + \lambda_6\tilde{\mu}\sigma(u_3) \right) + \lambda_7 F,$$
and the covector satisfies

\[
\begin{align*}
\lambda_3 &= x_6 \left( \frac{\partial v}{\partial x_3} \lambda_3 + \frac{\partial v_e}{\partial x_3} \lambda_4 - \frac{\partial \nu}{\partial x_3} \lambda_5 - \frac{\partial \mu}{\partial x_3} \lambda_6 \right) \\
\lambda_4 &= x_6 \left( \frac{\partial v}{\partial x_3} \lambda_3 + \frac{\partial v_e}{\partial x_3} \lambda_4 - \frac{\partial \mu}{\partial x_3} \lambda_6 \right) \\
\lambda_5 &= x_6 \left( \frac{\partial v}{\partial x_3} \lambda_3 + \frac{\partial v_e}{\partial x_3} \lambda_4 - \frac{\partial \mu}{\partial x_3} \lambda_6 \right) \\
\lambda_6 &= v_g \lambda_3 + v_z \lambda_4 - v_e \lambda_5 - \mu \lambda_6 \\
\lambda_7 &= x_6 \left( \frac{\partial v}{\partial x_3} \lambda_3 + \frac{\partial v_e}{\partial x_3} \lambda_4 - \frac{\partial \nu}{\partial x_3} \lambda_5 - \frac{\partial \mu}{\partial x_3} \lambda_6 \right)
\end{align*}
\]

(24)

The transversality conditions read \( \lambda(t_f) = \nabla \psi(x(t_f)) = (0, 0, 1, 0, 0) \) hence \( \lambda_5(t_f) = 1, \lambda_6(t_f) = 0 \).

To maximize \( H(x, \lambda, u_3) \) with respect to \( u_3 \), we compute

\[
\frac{\partial H}{\partial u_3} = x_6 \sigma'(u_3) \left( -\lambda_5 \frac{\hat{v}_e}{\sigma(u_3)^2} + \lambda_6 \hat{\mu} \right).
\]

Clearly, \( x_6(t) > 0 \) for every \( t \). Notice that at the final time the maximization condition implies that \( u_3(t_f) \) maximizes \( x_6(t_f)^2 \hat{v}_e(t_f)/\sigma(u_3) \). If \( \hat{v}_e(t_f) > 0 \) then \( u_3(t_f) = 0 \), otherwise, \( u_3(t_f) = 1 \).

We say that \( x(\cdot) \) has a singular arc on \([a, b] \subset [0, t_f]\), with \( a < b \), if \( \frac{\partial H}{\partial u_3} \equiv 0 \). As noted in the previous section, more degenerate situations can happen: for instance, the set of zeros of \( \frac{\partial H}{\partial u_3} \equiv 0 \) can have non-isolated points without containing a whole interval. In the following theorem we assume that the set of zeros of \( \frac{\partial H}{\partial u_3} \equiv 0 \) is given by a whole interval and we exclude chattering phenomena.

If the components \( \lambda_5, \lambda_6 \) of a solution of (23) have only isolated zeros on \([a, b]\) then the only possibility for a singular arc, i.e., for \( \frac{\partial H}{\partial u_3} \) to vanish identically, is that \( \lambda_5 \lambda_6 > 0 \) and \( \sqrt{\frac{\lambda_5 \hat{v}_e}{\lambda_6 \hat{\mu}}} \in [t, \sigma(1)] \) almost everywhere on \([a, b]\). In that case, \( u_3 = \sigma^{-1} \left( \sqrt{\frac{\lambda_5 \hat{v}_e}{\lambda_6 \hat{\mu}}} \right) \). Such singular arc can indeed happen.

In other words, \( \frac{\partial H}{\partial u_3} \equiv 0 \) does not imply \( \lambda_5 \equiv 0, \lambda_6 \equiv 0 \). To see this, note that if there exists \( t_0 \) such that \( \lambda_5(t_0) \neq 0 \) and \( \lambda_6(t_0) = 0 \) then \( \frac{\partial H}{\partial u_3}(t_0) \neq 0 \). Similarly, if there exists \( t_0 \) such that \( \lambda_5(t_0) = 0 \) and \( \lambda_6(t_0) \neq 0 \) then \( \frac{\partial H}{\partial u_3}(t_0) \neq 0 \). If \( \lambda_5(t_0) \lambda_6(t_0) < 0 \) then \( \frac{\partial H}{\partial u_3}(t_0) \neq 0 \). On the contrary, if \( \lambda_5(t_0) \lambda_6(t_0) > 0 \), then the equation \( \frac{\partial H}{\partial u_3}(t) = 0 \) has a solution \( u_3 \in [0, 1] \) if and only if \( \sqrt{\frac{\lambda_5(t_0) \hat{v}_e(t_0)}{\lambda_6(t_0) \hat{\mu}(t_0)}} \in [t, \sigma(1)] \).

If we assume that there are no chattering phenomena, then a singular arc is either of the type above or it satisfies \( \lambda_5 \equiv 0, \lambda_6 \equiv 0 \). In the latter case, under assumptions (20) on the behavior of \( \sigma \) and some additional conditions on \( x_3, x_4 \), we show the following assertion.

**Proposition 2** Assume that the inhibition constants for glucose and xylose are different, i.e., \( k_{ie}^g \neq k_{ie}^x \). Let \( x : [0, t_f] \rightarrow \mathbb{R}^5 \) be an optimal trajectory such that, for every \( t \in (0, t_f), x_3(t) + x_4(t) > 0 \) and both sets \( \{ t \mid x_3(t) = 0 \} \) and \( \{ t \mid x_4(t) = 0 \} \) are finite unions of intervals. If \( x(\cdot) \) has a singular arc on \([a, b]\) such that \( \lambda_5 \equiv 0 \) and \( \lambda_6 \equiv 0 \), then \( \lambda_7 \) is constant and there are two possibilities:

- \( x_3 \big|_{[a, b]} \equiv 0, \lambda_3(t) = \lambda_3(a) \int_0^t (x_6(s) \frac{\partial v}{\partial x_3}(s)) \, ds, \lambda_4 \) vanishes identically on \([a, b] \);
- \( x_4 \big|_{[a, b]} \equiv 0, \lambda_4(t) = \lambda_4(a) \int_0^t (x_6(s) \frac{\partial v}{\partial x_3}(s)) \, ds, \lambda_3 \) vanishes identically on \([a, b] \).
Note that we cannot prove the absence of optimal singular trajectories, not even for generic initial conditions. Proposition 2 only provides properties of optimal singular trajectories where \( \lambda_5 \equiv 0 \) and \( \lambda_6 \equiv 0 \).

Since \( v_g, v_z \) are given by equations (7), (8), one easily deduce that at a certain time \( t \), \( v_g(t) \), respectively \( v_z(t) \), is positive if and only if \( x_3(t) > 0 \), respectively \( x_4(t) > 0 \). Moreover, ethanol flux \( v_e \) is positive\(^1\) if at least one among glucose and xylose concentrations is positive. Therefore, since in Section 3 we optimize the final amount of ethanol through control of feeding rates of xylose and glucose, it is reasonable to assume that the open loop controls \( u^*_1(t), u^*_2(t) \) are such that \( v_g(t) + v_z(t) > 0 \) for every \( t \).

**Proof of Proposition 2.** Assume \( x(\cdot) \) to be an optimal trajectory having a singular arc on \([a, b]\) such that \( \lambda_5 \equiv 0 \) and \( \lambda_6 \equiv 0 \). Since the functional to maximize (21) is \( \psi(x) = x_5 \), we have \( \lambda_5(t_f) = 1 \), which implies \( b < t_f \). Imposing conditions \( \dot{\lambda}_5 \equiv 0 \) and \( \dot{\lambda}_6 \equiv 0 \), we obtain a linear homogeneous system in \((\lambda_3, \lambda_4)\),

\[
\begin{align*}
\frac{\partial v_{g}}{\partial x_5} \lambda_3 + \frac{\partial v_{g}}{\partial x_5} \lambda_4 &= 0 \\
v_g \lambda_3 + v_z \lambda_4 &= 0.
\end{align*}
\]

An easy computation shows that the determinant of system (25) is proportional to

\[x_3 x_4 (k^g_{ie} - k^z_{ie}),\]

through a nonvanishing factor. By assumption, \( k^g_{ie} - k^z_{ie} \neq 0 \).

**Case 1.** Assume there exists \( t_0 \in [a, b] \) such that \( x_3(t_0)x_4(t_0) > 0 \) then \( (\lambda_3, \lambda_4)(t_0) = 0 \). Hence \( (\lambda_3, \lambda_4)|_{[a, b]} \equiv 0 \). Imposing that \( \lambda \) satisfies the Hamiltonian system on \([a, b]\), since the equations for \( (\lambda_3, \lambda_4, \lambda_5, \lambda_6) \) do not depend on \( \lambda_7 \) and are homogeneous in \((\lambda_3, \lambda_4, \lambda_5, \lambda_6)\) we deduce that \( \lambda_3, \lambda_4, \lambda_5, \lambda_6 \) vanish identically on the whole interval \([0, t_f]\), which contradicts \( \lambda_5(t_f) = 1 \).

**Case 2.** Assume now \( x_3 x_4 \equiv 0 \) on \([a, b]\). Then, since zeros of \( x_3, x_4 \) are finite union of intervals and \( x_3 + x_4 > 0 \), there exists a finite decomposition \([a, b] = \bigcup_{i=1}^{n} [a_i, b_i]\), \( b_i = a_{i+1} \) where on each subinterval exactly one among \( x_3, x_4 \) vanishes identically and the other one is strictly positive. We are going to show that \( m \) is equal to 1.

Consider a subinterval \([a_i, b_i]\) where \( x_3 \equiv 0 \). Then system (25) implies \( \lambda_4 \equiv 0 \) on \([a_i, b_i]\). Moreover, since the evolution equation for \( \lambda_4 \) does not involve \( \lambda_3 \) and \( \lambda_5 \equiv \lambda_6 \equiv 0 \) on \([a, b]\), we deduce that \( \lambda_4 \) vanishes identically on the whole interval \([a, b]\). Using the evolution equation for \( \lambda_3, \lambda_7 \) we obtain

\[
\begin{align*}
\dot{\lambda}_3 &= x_6 \left( \frac{\partial v_{g}}{\partial x_3} \lambda_3 + \frac{\partial v_z}{\partial x_3} \lambda_4 \right) = x_6 \frac{\partial v_{g}}{\partial x_3} \lambda_3 \\
\dot{\lambda}_7 &= x_6 \left( \frac{\partial v_{g}}{\partial x_7} \lambda_3 + \frac{\partial v_z}{\partial x_7} \lambda_4 \right) = x_6 \frac{\partial v_{g}}{\partial x_7} \lambda_3 \equiv 0,
\end{align*}
\]

where the last identity is a consequence of \( v_g \) being proportional to \( x_3 \). Hence \( \lambda_3|_{[a_i, b_i]} \) is completely determined as functions of \( x \) and \( \lambda_7 \) is constant on \([a_i, b_i]\).

\(^1\)This is a consequence of the fact that growth of bacteria in the bioreactor happens if substrates are given. Also, ethanol is a byproduct of the metabolic activity of the growing bacteria. In other words, biomass growth rate \( \mu \), and consequently ethanol flux \( v_e \), is positive only if sugars are given.
Consider now a subinterval \([a_j, b_j]\) where \(x_4 \equiv 0\). Then system (25) implies \(\lambda_3 \equiv 0\) on \([a_j, b_j]\).

Using the evolution equation for \(\lambda_4, \lambda_7\) we obtain
\[
\dot{\lambda}_4 = \frac{\partial v_z}{\partial x_4} x_6 \lambda_4 \\
\dot{\lambda}_7 = x_6 \left( \frac{\partial v_9}{\partial x_7} \lambda_3 + \frac{\partial v_z}{\partial x_7} \lambda_4 \right) \equiv 0,
\]
where the last identity is a consequence of \(v_z\) being proportional to \(x_4\). Hence, on \([a_j, b_j]\) \(\lambda_4\) is determined as a function of \(x\) and \(\lambda_3\) vanishes identically.

Let now \(m \geq 2\), i.e., assume that there exists \([a_1, b_1] \subset [a, b]\) on which \(x_3 \equiv 0\) and \([a_2, b_2] \subset [a, b]\) on which \(x_4 \equiv 0\) and \(b_1 \leq a_1\). Then \(\lambda_4\) vanishes on the whole \([a, b]\) and \(\lambda_3\) vanishes on \([a_2, b_2]\). Hence on \([a_2, b_2]\) we have \(\lambda_i \equiv 0\) for every \(i = 3, 4, 5, 6\). Since the dynamics for \(\lambda_3, \lambda_4, \lambda_5, \lambda_6\) is linear and homogeneous (and it does not depend on \(\lambda_7\)), this implies \(\lambda_i \equiv 0\) on the whole interval \([0, t_f]\). This contradicts \(\lambda_5(t_f) = 1\). Therefore \(m = 1\) and the statement is proved.

\[\blacksquare\]

References

[1] J. Alford. Bioprocess control: Advances and challenges. *Computers & Chemical Engineering*, 30(10-12):1464–1475, 2006.

[2] P. T. Benavides and U. Diwekar. Optimal control of biodiesel production in a batch reactor: Part i: Deterministic control. *Fuel*, 94(0):211 – 217, 2012.

[3] A. Bressan and B. Piccoli. *Introduction to the mathematical theory of control*, volume 2 of *AIMS Series on Applied Mathematics*. American Institute of Mathematical Sciences (AIMS), Springfield, MO, 2007.

[4] É. Busvelle and J.-P. Gauthier. On determining unknown functions in differential systems, with an application to biological reactors. *ESAIM Control Optim. Calc. Var.*, 9:509–551, 2003.

[5] M. Caponigro, R. Ghezzi, B. Piccoli, and E. Trélat. Regularization of chattering phenomena via bounded variation control. preprint 2013, arXiv:1303.5796.

[6] Y. Chitour, F. Jean, and E. Trélat. Singular trajectories of control-affine systems. *SIAM J. Control Optim.*, 47(2):1078–1095, 2008.

[7] M. W. Covert, C. Schilling, and B. Palsson. Regulation of gene expression in flux balance models of metabolism. *Biophys J.*, 83(3):1331–1340, 2002.

[8] M. W. Covert, N. Xiao, T. J. Chen, and J. R. Karr. Integrating metabolic, transcriptional regulatory and signal transduction models in *escherichia coli*. *Bioinformatics*, 24(18):2044–2050, 2008.

[9] T. Eevera, K. Rajendran, and S. Saradha. Biodiesel production process optimization and characterization to assess the suitability of the product for varied environmental conditions. *Renewable Energy*, 34(3):762 – 765, 2009.

[10] A. T. Fuller. Study of an optimum non-linear control system. *J. Electronics Control (1)*, 15:63–71, 1963.

[11] J.-P. Gauthier, H. Hammouri, and S. Othman. A simple observer for nonlinear systems applications to bioreactors. *IEEE Trans. Automat. Control*, 37(6):875–880, 1992.

[12] J. L. Hjersted and M. A. Henson. Optimization of fed-batch *saccharomyces cerevisiae* fermentation using dynamic flux balance models. *Biotechnol. Prog.*, 22:1239–1248, 2006.
[13] J. L. Hjersted and M. A. Henson. Steady-state and dynamic flux balance analysis of ethanol production by saccharomyces cerevisiae. *IET Systems Biology*, 3:167–179, 2009.

[14] J. L. Hjersted, M. A. Henson, and R. Mahadevan. Genome-Scale Analysis of *Saccharomyces Cerevisiae* Metabolism and Ethanol Production in Fed-Batch Culture. *Biotechnology and Bioengineering*, 97(5):1190–1204, 2007.

[15] E. Jung, S. Lenhart, and Z. Feng. Optimal control of treatments in a two-strain tuberculosis model. *Discrete and Continuous Dynamical Systems–Series B*, 2(4):473–482, November 2002.

[16] D. Kirschner, S. Lenhart, and S. Serbin. Optimal control of the chemotherapy of HIV. *J. Math. Biol.*, 35:775–792, 1997.

[17] A. Kremling, K. Bettenbrock, and E. Gilles. Analysis of global control of Escherichia coli carbohydrate uptake. *BMC Systems Biology*, 1(42), 2007.

[18] R. Mahadevan, J. Edwards, and F. r. Doyle. Dynamic flux balance analysis of diauxic growth in Escherichia coli. *J Theor Biol.*, 213(1):73–88, 2001.

[19] J. Moreno. Optimal time control of bioreactors for the wastewater treatment. *Optimal Control Applications Methods*, 20(3):145–164, 1999.

[20] B. O. Palsson. *Systems Biology - Property of Reconstructed Networks*. Cambridge University Press, 2006.

[21] L. S. Pontryagin, V. G. Boltyanski˘ı, R. V. Gamkrelidze, and E. F. Mishchenko. *The Mathematical Theory of Optimal Processes*. “Nauka”, Moscow, fourth edition, 1983.

[22] A. Rapaport and D. Dochain. Minimal time control of fed-batch processes with growth functions having several maxima. *IEEE Trans. Automat. Contr.*, 56(11):2671–2676, 2011.

[23] S. Tiwari, P. Verma, P. Singh, and R. Tuli. Plants as bioreactors for the production of vaccine antigens. *Biotechnology Advances*, 27(4):449–467, 2009.

[24] K. Yamuna Rani and V. S. Ramachandra Rao. Control of fermenters - a review. *Bioprocess and Biosystems Engineering*, 21:77–88, 1999. 10.1007/PL00009066.