

# Optimal control of a fed-batch reactor with overflow metabolism <sup>★</sup>

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**Abstract:** Fast growing *E. coli* cells in glucose-aerobic conditions excrete fermentation by-products such as acetate. This phenomenon is known as overflow metabolism and can pose a major problem in industrial bio-processes. In this paper, we study optimal control strategies for feeding a fed-batch reactor subject to overflow metabolism. We consider that acetate has an inhibitor effect on the glucose uptake, and we also consider the cost associated to process duration. In our approach, using the Pontryagin Maximum Principle and numerical solutions we describe the optimal feeding policy that maximizes biomass productivity and minimizes the cost duration of the process. We show that a singular regime is possible, in which cells grow at a slow rate to prevent acetate formation. If the cost associated to the process is too high, only bang-bang solutions are allowed.

*Keywords:* Dynamics and control; Industrial biotechnology; Overflow metabolism; Fed-batch

## 1. INTRODUCTION

*Escherichia coli* (*E. coli*) is a bacterium that is naturally found in the intestine of humans and other mammals. This bacterium plays an important role in the biotechnology industry for large-scale production of proteins for therapeutic use (Baeshen et al. (2015)). Glucose is generally the preferred carbon source of *E. coli* (Bren et al. (2016)), and depending on growth conditions, *E. coli* combines two different metabolic strategies to harvest energy from glucose, aerobic respiration (oxygen required) and/or fermentation (not oxygen required) (Gerosa et al. (2015)). Respiration is more energy-efficient than fermentation. Nevertheless, in fast growing cells, some energy is obtained by fermentation, even in excess oxygen conditions. This phenomenon is referred as *overflow metabolism*. During fermentation (when overflow metabolism occurs), acetate is excreted to the medium as by-product. The accumulation of acetate has an inhibitory effect on cells growth (Luli and Strohl (1990)), which can pose a major problem in microbial bioprocesses. Note that overflow metabolism has been observed in many microorganisms (see the book of Vazquez (2017)). For example, fast growing yeast excrete ethanol which can inhibit their growth.

Several studies suggest the existence of a threshold glucose uptake rate, above which overflow metabolism happens (see the work of Basan et al. (2015) and the references therein). Thus, a straightforward strategy to increase biomass productivity, is to prevent acetate formation by forcing cells to uptake glucose from the medium below the threshold rate. This can be done in a fed-batch reactor restricting the feeding rate. Different authors have shown

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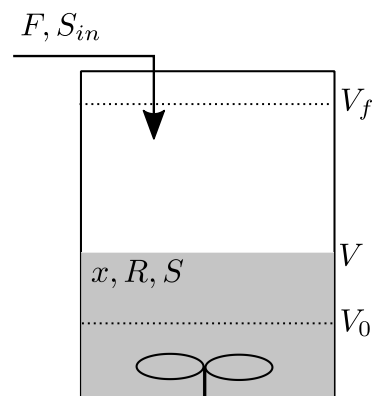


Fig. 1. A schematic diagram of a simplified fed-batch reactor. The initial volume of the bacterial culture is  $V_0$ . The volume increases as the fed-batch reactor is fed at a flow rate  $F$  with a concentration of glucose  $S_{in}$ . Feeding stops when the final volume  $V_f$  is reached. The evolution of the bulk concentrations of bacteria ( $X$ ), glucose ( $S$ ), and acetate ( $R$ ) depend on the feeding rate  $F$ .

experimentally that this strategy leads to high density cultures (see for example the works of Korz et al. (1995) and Babu et al. (2000)). In the context of yeast cultures, this strategy have been used to construct adaptive controls or extremum seeking algorithms for increasing biomass productivity (see the works of Valentinotti et al. (2003) and Dewasme et al. (2011)). One problem of this strategy, is that biomass is generated at a slower rate than the cells are capable of achieving. Therefore, the optimality of this strategy is not clear.

In this work, we investigate if maintaining the uptake rate of glucose at a rate that prevents the acetate formation is an optimal strategy in fed-batch reactors for the production of *E. coli*. In our approach, we study an optimal

control problem with the criterion proposed by San and Stephanopoulos (1984). The criterion aims to maximize the quantity of bacteria at the end of the process, taking into account the cost associated to the process duration. To model *E. coli* growth, we consider a classical fed-batch reactor model and the recently proposed model by Mauri et al. (2020). Thus, we consider the existence of a threshold glucose uptake rate, above which overflow metabolism happens, and consequently the excretion of acetate.

In the context of fed-batch processes (without overflow metabolism), several optimal feeding strategies, with respect to different criteria, have been determined by the use of the Pontryagin Maximum Principle (PMP) (see for examples the works of Park and Ramirez (1988) and San and Stephanopoulos (1989)). These strategies are of “bang-bang” type, singular, or a combination of both. In general, characterizing the optimal solution of optimal control problems is quite challenging. Numerical solutions are of great help in this context. To study our problem, we apply the PMP to obtain some insights into the form of a singular arc. Then, using the software BOCOP (Bonnans et al. (2011)) (version 2.10), we obtain numerical simulations under different conditions. This approach allows us to characterize the optimal feeding strategies.

Our paper is organized as follows. In section 2, we describe the optimal control problem. In section 3, we apply the PMP, and we define a feedback control. In section 4, we solve numerically the optimal control problem and we describe the different structures of the optimal solutions. In section 5, we give some conclusions.

## 2. PROBLEM FORMULATION

We consider a fed-batch reactor (see Figure 1) with an *E. coli* population which density is denoted by  $x$ . This population grows at a specific growth rate  $\mu(\cdot)$ . The specific growth rate considers the carbon gain by glucose uptake and the carbon loss (in form of acetate) due to metabolic overflow *i.e.*

$$\mu(\cdot) = Y_S r_S(\cdot) - Y_R r_{of}(\cdot), \quad (1)$$

with  $r_S$  the glucose uptake rate,  $r_{of}$  the metabolic overflow rate (or acetate formation rate), and  $Y_S, Y_R$  yield coefficients. The glucose uptake rate depends on the glucose concentration ( $S$ ) and on the acetate concentration ( $R$ ):

$$r_S(S, R) = r_{S,max} \frac{S}{K_S + S} \frac{K_{i,R}}{K_{i,R} + R}, \quad (2)$$

where  $r_{S,max}$  is the maximal glucose uptake rate, and  $K_S, K_{i,R}$  are kinetic constants. Following Basan et al. (2015),  $r_{of}$  depends on  $r_S$  *i.e.*  $r_{of} = f(r_S)$ , with  $f$  defined as (see Figure 2):

$$f(r_S) := k \max\{0, r_S - r_{S0}\}, \quad (3)$$

with  $r_{S0} > 0$  the threshold glucose uptake rate above which acetate excretion occurs, and  $k > 0$ . We assume the following relation which is verified by the parameters estimated by Mauri et al. (2020):

$$Y_S - kY_R > 0. \quad (4)$$

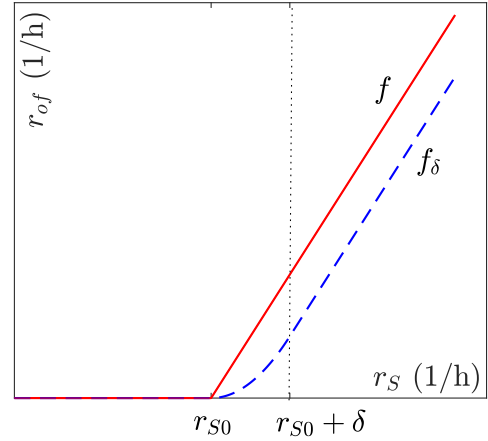


Fig. 2. Functions  $f$  (continuous line) and  $f_\delta$  (dash line). The function  $f$  (3) relates the acetate excretion rate ( $r_{of}$ ) with the glucose uptake rate ( $r_S$ ). The function  $f_\delta$  (defined in Section 3) is a smooth approximation of  $f$ .

It is straightforward to verify that (4) implies  $\frac{\partial \mu}{\partial S} > 0$  and  $\frac{\partial \mu}{\partial R} < 0$ . The growth of bacteria in the fed-batch reactor is modeled by :

$$\begin{aligned} \frac{dx}{dt} &= \left( \mu(S, R) - \frac{F}{V} \right) x \\ \frac{dS}{dt} &= \frac{F}{V} (S_{in} - S) - r_S(S, R)x \\ \frac{dR}{dt} &= -\frac{F}{V} R + r_{of}(S, R)x \\ \frac{dV}{dt} &= F \\ x(0) &= x_0, S(0) = S_0, R(0) = R_0, \\ V(0) &= V_0, V(t_f) = V_f \end{aligned} \quad (5)$$

Feed rate  $F$  is the control variable, and  $V$  is the volume. The initial values (at  $t = 0$ ) of  $x, S, R$  and  $V$  are specified, as well as the final value of  $V$  ( $V_f$ ). We want to maximize the total biomass production in the reactor together while minimizing the process duration. We consider the criterion proposed by San and Stephanopoulos (1984):

$$\begin{aligned} \max_F & x(t_f)V(t_f) - c_1 \int_{t_0}^{t_f} dt, \\ & 0 \leq F \leq F_{max}, \end{aligned} \quad (6)$$

where  $c_1$  is a composite overall time cost in units of cell biomass per unit of time, and  $F_{max}$  is the maximal flow rate allowed in the system. The terminal time  $t_f$  is not fixed in this formulation.

## 3. NECESSARY OPTIMALITY CONDITIONS

The classical PMP requires the continuous differentiability of the dynamics with respect to the state variables. In our

model, overflow metabolism is described by the maximum function, which is not differentiable. Thus, to apply the PMP to (5)-(6), we consider a smooth approximation of  $f$ . Let  $\delta$  be a positive real number, we define the function  $f_\delta$  through the following properties (see Figure 2):

- $f_\delta(r_S) = 0$ , for all  $r_S \leq r_{S0}$ ,
- $f_\delta(r_S) > 0$ ,  $f'_\delta(r_S) > 0$  for all  $r_S \in (r_{S0}, r_{S0} + \delta)$ ,
- $f'_\delta(r_S) = k$  for all  $r_S \geq r_{S0} + \delta$ .

It is clear that  $f_\delta \rightarrow f$  as  $\delta \rightarrow 0^+$  uniformly. The necessary conditions for optimization of the problem (5)-(6), with  $f$  replaced by  $f_\delta$ , are determined by the PMP. The associated Hamiltonian is given by:

$$\begin{aligned} H = & -c_1 + \lambda_x \left( Y_S r_S - Y_R f_\delta(r_S) - \frac{F}{V} \right) x \\ & + \lambda_S \left( \frac{F}{V} (S_{in} - S) - r_S x \right) \\ & + \lambda_R \left( -\frac{F}{V} R + f_\delta(r_S) x \right) + \lambda_V F. \end{aligned} \quad (7)$$

For the adjoint variables, let us define  $\Lambda_S = \lambda_S - Y_S \lambda_x$  and  $\Lambda_R = \lambda_R - Y_R \lambda_x$ . Then, for  $\lambda_x$ ,  $\lambda_S$ ,  $\lambda_R$ , and  $\lambda_V$ , the dynamics are given by:

$$\begin{aligned} \frac{d\lambda_x}{dt} &= r_S \Lambda_S - f_\delta(r_S) \Lambda_R + \frac{F}{V} \lambda_x, \\ \frac{d\lambda_S}{dt} &= x \frac{\partial r_S}{\partial S} (\Lambda_S - f'_\delta(r_S) \Lambda_R) + \frac{F}{V} \lambda_S, \\ \frac{d\lambda_R}{dt} &= x \frac{\partial r_S}{\partial R} (\Lambda_S - f'_\delta(r_S) \Lambda_R) + \frac{F}{V} \lambda_R, \\ \frac{d\lambda_V}{dt} &= \frac{F}{V^2} (-\lambda_x x + \lambda_S (s_{in} - s) - \lambda_R R). \end{aligned} \quad (8)$$

with  $\lambda_x(t_f) = V_f$  and  $\lambda_S(t_f) = \lambda_R(t_f) = 0$ . Since the Hamiltonian is linear in the control variable ( $F$ ), the structure of an optimal control  $\hat{F}$  is determined by the sign of the *switching function*  $H_F := \frac{\partial H}{\partial F}$ . Indeed,

$$\hat{F} = \begin{cases} F_{max} & \text{if } H_F > 0, \\ 0 & \text{if } H_F < 0, \end{cases}$$

with

$$H_F = \frac{1}{V} (-\lambda_x x + \lambda_S (S_{in} - S) - \lambda_R R) + \lambda_V. \quad (9)$$

If  $H_F$  vanishes over an interval of time  $I$ , a singular regime takes place. The following result gives necessary conditions for the existence of a singular regime.

*Proposition 1.* Consider the problem (5)-(6) with  $f$  replaced by  $f_\delta$  and assume that  $S(0) \leq S_{in}$ . If  $H_F$  (defined in (9)) vanishes in a sub-interval of time  $I$ , then:

$$0 \leq r_S(t) - r_{S0} \leq \delta, \quad (10)$$

for all  $t \in I$ .

**Proof.** The proof is given in the Appendix.  $\square$

Proposition 1 suggests that if an optimal trajectory of (5)-(6) presents a singular arc during a subinterval of time  $I$ , then  $r_S(t) = r_{S0}$  for all  $t \in I$  (take  $\delta$  small enough in (10)).

In such a case, the singular arc, denoted  $F_{sing}$ , satisfies  $\frac{dr_S}{dt} \Big|_{r_S=r_{S0}} = 0$  i.e.

$$F_{sing} = \frac{r_{S0} x V}{S_{in} - S + \frac{SR(K_S+S)}{K_S(K_{iR}+R)}}. \quad (11)$$

As we will show in the next section,  $F_{sing}$  can be a singular arc of the optimal solution of (5)-(6). To end this section, based on  $F_{sing}$ , we define a feedback control  $\tilde{F}$  that will be useful for describing the structure of optimal controls in the next section:

$$\tilde{F} = \begin{cases} 0 & \text{if } r_S > r_{S0} \text{ or } V \geq V_f, \\ \min\{F_{max}, F_{sing}\} & \text{if } r_S \leq r_{S0} \text{ and } V < V_f. \end{cases} \quad (12)$$

If the feedback control  $\tilde{F}$  is applied when  $r_S > r_{S0}$  and  $V < V_f$ , then the reactor will be operated in batch mode ( $F = 0$ ), which results in a decrease of  $r_S$ . The batch mode stops when  $r_S$  equals  $r_{S0}$ . After that,  $\tilde{F} = F_{sing}$  and  $r_S$  remains equal to  $r_{S0}$  provided  $F_{sing} \leq F_{max}$ . If  $\tilde{F}$  switches from  $F_{sing}$  to  $F_{max}$  ( $F_{sing} > F_{max}$  and  $V < V_f$ ), then  $r_S$  decreases. Thus,  $r_S$  remains equal than or lower than  $r_{S0}$  until the final volume ( $V_f$ ) is achieved. Then, the reactor is operated again in batch mode. This feeding strategy ( $\tilde{F}$ ) is comparable to that proposed by Korz et al. (1995). As we will see in the next section, in some cases  $\tilde{F}$  corresponds to an optimal control.

#### 4. STRUCTURE OF THE OPTIMAL CONTROL

We solve numerically the problem (5)-(6) for different values of  $S_0$ ,  $x_0$ , and  $c_1$ , with parameters from Table 1. We use a direct method implemented in the software BOCOP (Bonnans et al. (2011)) (version 2.10). The problem is discretized by a two-stage Gauss-Legendre method of order 4 with 300 time steps. We consider a constant initialization, and the tolerance for IPOPT NLP solver is set at  $10^{-12}$ .

Figure 3 shows the optimal control strategy for different initial conditions and values of  $c_1$ . For brevity, we only show some plots representing the different structures that were observed. To describe the different solutions we recall the feedback control  $\tilde{F}$  defined in (12). If  $x_0 = 0.1$  g/L,  $S_0 = 20$  g/L, and  $c_1 = 0.1$  g/h (Figure 3A), the optimal control coincides with the feedback control  $\tilde{F}$  during all the process duration. If  $x_0 = 0.1$  g/L,  $S_0 = 0$  g/L, and  $c_1 = 0.1$  g/h (Figure 3B), the feeding rate is maximum during the first 30 minutes, and then the feedback control  $\tilde{F}$  is applied until the final time. If  $x_0 = 5$  g/L,  $S_0 = 0$  g/L, and  $c_1 = 0.1$  g/h (Figure 3C), then during a very short period of time the flow rate is maximum. During this time, the value of  $r_S$  increases from 0 to  $r_{S0}$ . Then, the feedback control  $\tilde{F}$  is applied until the end, keeping almost all the time the glucose uptake rate set to  $r_{S0}$ . If  $x_0 = 0.1$  g/L,  $S_0 = 20$  g/L, and  $c_1 = 0.5$  g/h (Figure 3D), a bang-bang control, switching from 0 to  $F_{max}$ , is observed during the startup. Then, the control switches from  $F_{max}$  to  $\tilde{F}$ , and  $\tilde{F}$  is applied until the final time.

Figures 4 and 5 show the optimal profile of the optimal control for different values of  $S_0$  and  $c_1$ . Figure 4 shows that as  $c_1$  increases, a singular regime occurs during a

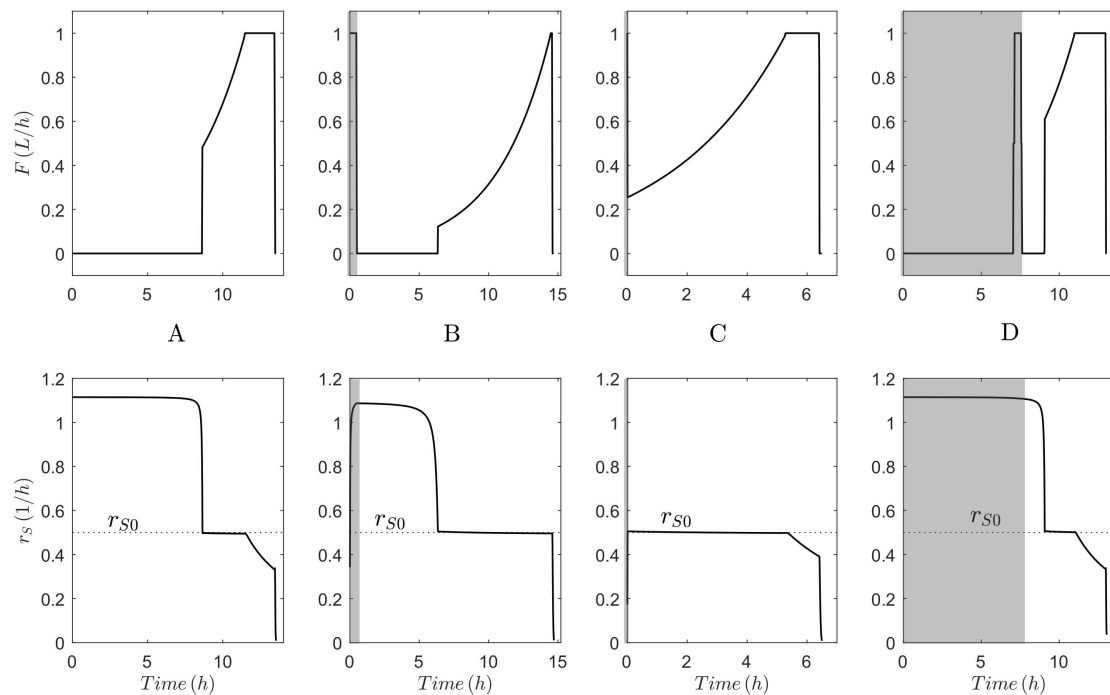


Fig. 3. Optimal solution of the problem (5)-(6) for different values of  $S_0$ ,  $x_0$ , and  $c_1$ . The not-shaded area represents the interval of time while the control  $\tilde{F}$  (defined in (12)) is applied. Parameters are taken from Table 1 **A.**  $S_0 = 20 \text{ g/L}$ ,  $x_0 = 0.1 \text{ g/L}$ , and  $c_1 = 0.1 \text{ g/h}$ . **B.**  $S_0 = 0 \text{ g/L}$ ,  $x_0 = 0.1 \text{ g/L}$ , and  $c_1 = 0.1 \text{ g/h}$ . **C.**  $S_0 = 0 \text{ g/L}$ ,  $x_0 = 5 \text{ g/L}$ , and  $c_1 = 0.1 \text{ g/h}$ . **D.**  $S_0 = 20 \text{ g/L}$ ,  $x_0 = 0.1 \text{ g/L}$ , and  $c_1 = 0.5 \text{ g/h}$ .

shorter interval of time, and a bang-bang solution (during the startup, shaded area), switching from  $F_{max}$  to 0, occurs during a longer time. For values of  $c_1$  equal than or higher than  $0.9 \text{ g/L}$ , there is not singular arc. Similarly to Figure 4, Figure 5 shows the same effect when increasing  $c_1$ . However, the bang-bang solution associated to  $c_1$  during the startup, switches from 0 to  $F_{max}$ .

Numerical simulations suggest the existence of a time  $t^* \in [0, t_f)$  (the end of the shaded areas in Figures 3, 4, and 5), such that the feedback control  $\tilde{F}$  is applied from the time  $t^*$  until the final time (not-shaded areas in Figures 3, 4, and 5). If  $t^* > 0$ , during the interval of time  $[0, t^*]$ , the optimal control is equal to  $F_{max}$  (Figures 3B and 3C) or is bang-bang, switching from 0 to  $F_{max}$  (Figure 3D). If  $t^* = 0$ , the optimal control coincides with  $\tilde{F}$  (Figure 3A). As shown in Figures 4 and 5, the value of  $t^*$  is related to the value of  $c_1$ . Indeed, if  $c_1$  is too high,  $\tilde{F}$  is only applied when the final volume is reached (Figures 4 and 5).

Table 2 shows the biomass productivity and the cost duration associated to Figures 4 and 5. Biomass productivity does not change very much with changes on  $c_1$ . This is probably due to the initial and final volumes. Unfortunately, we did not obtain convergence of the numerical method for large volumes to test this hypothesis.

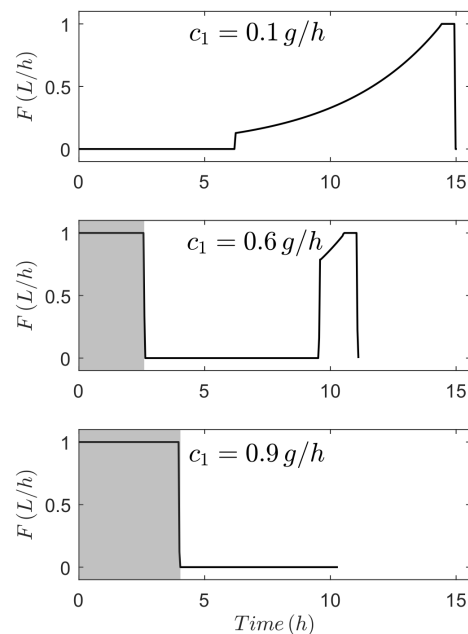


Fig. 4. Optimal feeding profile for different values of  $c_1$ . The not-shaded area represents the interval of time while the control  $\tilde{F}$  (defined in (12)) is applied. Parameters are taken from Table 1 ( $S_0 = 5 \text{ g/L}$  and  $x_0 = 0.1 \text{ g/L}$ )

Table 1. Parameters and initial conditions.

Parameter	Value	Unit	Remark
$r_{S,max}$	1.12	$h^{-1}$	Guardia and Calvo (2001)
$K_S$	0.1	$g/L$	Guardia and Calvo (2001)
$K_{i,R}$	4	$g/L$	Guardia and Calvo (2001)
$k$	0.25	—	
$r_{S0}$	0.5	$h^{-1}$	
$Y_S$	0.52	$gX/gS$	Xu et al. (1999)
$Y_R$	0.4	$gX/gR$	Guardia and Calvo (2001)
$c_1$	0.1 – 1.2	$g/h$	
$S_{in}$	10	$g/L$	
$F_{max}$	1	$L/h$	
$V_0$	1	$L$	
$V_f$	5	$L$	
$x_0$	0.1 – 5	$g/L$	
$S_0$	0 – 20	$g/L$	
$R_0$	1	$g/L$	

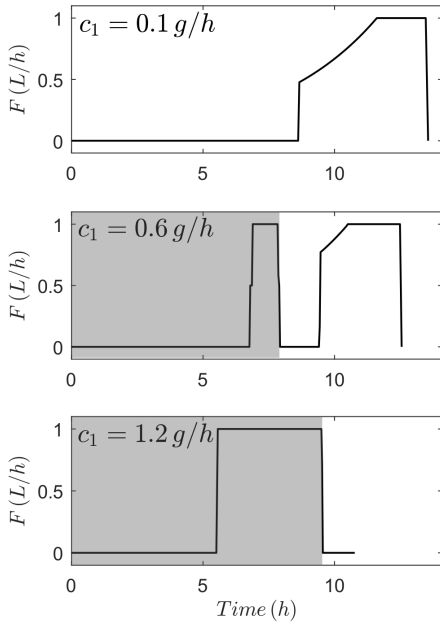


Fig. 5. Optimal feeding profile for different values of  $c_1$ . The not-shaded area represents the interval of time while the control  $\tilde{F}$  (defined in (12)) is applied. Kinetic parameters are taken from Table 1 ( $S_0 = 20 g/L$  and  $x_0 = 0.1 g/L$ ).

Table 2. Biomass production ( $x(t_f)V_f$ ) and cost associated to process duration ( $c_1 \int_{t_0}^{t_f} dt$ ) for the different conditions represented in Figures 4 and 5.

	$c_1$ (g/h)	$x(t_f)V_f$ (g)	$c_1 \int_{t_0}^{t_f} dt$ (g)
Figure 4	0.1	23.3	1.6
	0.6	22.2	7.3
	0.9	21.25	10
Figure 5	0.1	30.7	1.6
	0.6	29.7	8.3
	1.2	28.7	14.8

## 5. CONCLUSIONS AND FUTURE WORK

The optimal feeding rate changes with the initial conditions,  $x_0$  and  $S_0$ , and the process duration cost,  $c_1$ . In some cases, the optimal feeding rate is given by the feedback optimal control ( $\tilde{F}$ ). This control prevents acetate formation by forcing cells to uptake glucose from the medium below the threshold uptake rate ( $r_{S0}$ ), even if biomass is generated at a slower rate than the cells are capable of achieving. This confirms that the simple strategy proposed by Korz et al. (1995) may be optimal. However, as the cost associated to the process duration increases, the optimal feeding strategy combines an initial bang-bang control with the feedback control. This is explained by the fact that for a high operational cost, it is convenient to accelerate the process by feeding at maximal rate. Indeed, if the operational cost is heavily weighted, the optimal solutions are of bang-bang type, which is consistent with the results of Cazzador (1988). Feeding at maximal rate during the startup is not necessarily associated to high operational cost, but to a small initial glucose uptake rate. Feeding at maximal rate during the startup may be necessary to increase the glucose uptake rate to a level equal than or higher than the critical uptake rate ( $r_{S0}$ ), so that the feedback control  $\tilde{F}$  is optimal for the rest of the process.

As a future work, we will consider the acetate consumption. According to Wolfe (2005), *E. coli* can consume acetate, but only after the glucose is totally consumed. Another future work, follows the works of Harvey et al. (2014) and Bernstein et al. (2012). It considers a consortium with another *E. coli* strain that grows on consuming acetate.

## APPENDIX

Here we prove Proposition 1. We recall the notations of Section 3. If  $H_F$  vanishes during a sub-interval of time, then  $\frac{dH_F}{dt} = 0$ . Let us define  $W = \Lambda_S - f'_\delta(r_S)\Lambda_R$ . It can be shown that:

$$\frac{dH_F}{dt} = \frac{x}{V}W \left( (S_{in} - S)\frac{\partial r_S}{\partial S} - R\frac{\partial r_S}{\partial R} \right).$$

Assume that  $S < S_{in}$ . Since  $\frac{\partial r_S}{\partial S} > 0$  and  $\frac{\partial r_S}{\partial R} < 0$ , the sign of  $W$  determines the monotonicity of  $H_F$ . The derivative of  $W$  with respect to the time gives:

$$\begin{aligned} \frac{dW}{dt} &= -\Lambda f''_\delta(r_S)\frac{dr_S}{dt} \\ &+ W \left( x\frac{\partial r_S}{\partial S} + \frac{F}{V} - x f'_\delta(r_S)\frac{\partial r_S}{\partial R} \right) \\ &+ (r_S\Lambda_S - f_\delta(r_S)\Lambda_R)(Y_R f'_\delta(r_S) - Y_S). \end{aligned} \quad (13)$$

*Lemma 2.* Let us define  $\alpha = x[f_\delta(r_S) - r_S f'_\delta(r_S)]$  and  $S(0) \leq S_{in}$ . If  $H_F = 0$  in a sub-interval of time  $I$ , then for all  $t \in I$ :

- $\alpha(t) \neq 0$ ,
- $\Lambda_R(t) = \frac{c_1}{\alpha(t)x(t)}$ .

**Proof.** If  $H_F = 0$  in a sub-interval of time, then  $\frac{dH_F}{dt} = W = 0$ . Then,  $A\lambda = b$ , with  $A$  given by

$$\begin{bmatrix} \left(\mu - \frac{F}{V}\right)x & \frac{F}{V}(S_{in} - S) - r_S x - \frac{F}{V}R + f_\delta(r_S)x & F \\ -x & S_{in} - S & -R & V \\ Y_R f'_\delta(r_S) - Y_S & 1 & -f'_\delta(r_S) & 0 \end{bmatrix},$$

$\lambda = [\lambda_x, \lambda_S, \lambda_R, \lambda_V]^T$ , and  $b = [c_1, 0, 0]^T$ . If  $\alpha = 0$ , the equality  $A\lambda = b$  leads to  $c_1 = 0$ , which is a contradiction. Thus, a) is proved. Now, for  $\alpha \neq 0$ , any solution of  $A\lambda = b$  satisfies  $\lambda_R = \frac{c_1}{x[f_\delta(r_S) - r_S f'_\delta(r_S)]} + Y_R \lambda_x$ . From where the proof b) follows.  $\square$

**Lemma 3.** If  $S(0) \leq S_{in}$  and  $H_F = 0$  during a sub-interval of time, then  $f''_\delta(r_S) > 0$ .

**Proof.** By contradiction, let us assume that  $H_F = 0$  and  $f''_\delta(r_S) = 0$ . In view of Lemma 2, necessarily  $f'_\delta(r_S) = k$ . Since  $W = 0$ , we obtain that  $\Lambda_S = k\Lambda_R$ . Recalling (13), we have:

$$\frac{dW}{dt} = \Lambda_R(kr_S - f_\delta(r_S))(Y_R k - Y_S).$$

From Lemma 2 and (4), we obtain that:

$$\frac{dW}{dt} = -\frac{c_1}{x}(Y_R f'_\delta(r_S) - Y_S) > 0.$$

This contradicts the fact that  $W = 0$ .  $\square$

**Proof.** (Proposition 1) It follows from Lemmas 2 and 3.  $\square$

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