Optimization of a Micro-organisms Culture in a Fedbatch Bioreactor using an Intracellular Model

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***Abstract*— The production of biomass by micro-organisms is of great interest in more and more industrial fields. Their culture in fedbatch bioreactors is largely used and the optimization of the influent flow rate is a major challenge to set the micro-organisms in the highest performance conditions.**
Usually, sets of experiments are achieved to compute macroscopic models expressing the observed growth as a function of the concentrations of glucose, cell density, and any kind of external parameters (pH, temperature, etc.). In contrast, this paper relies on an intracellular model of a bacteria, which is used to determine the optimal profile of influent flow rate in a fedbatch bioreactor to produce as much biomass as possible. Compared to macroscopic models, the additional elements of this model better describe biological phenomena. The intracellular part of the model also provides a clear interpretation of the obtained optimal influent profile.

KEYWORDS: Bioprocess Optimization, Intracellular Process, Cellular Model, Fedbatch

I. Introduction

Bacterial cultures are a central interest in the production of biochemical products and a challenge is to make them grow as fast as possible. Usually, bacteria are placed in a medium containing all the needed nutrients for growth. The objective is then to maintain the highest culture performance, either for the bacterial growth or for other products of interest production.
Usually, bioreactors containing bacteria are analyzed and controlled using a growth rate model based on macroscopic observations as for example in [1], [2] or [3]. In this kind of model, the growth is linked to macroscopic variables such as biomass, substrate and excreted product concentrations in the bioreactor, density of cells or other physical variables. Such models can be as simple as linear relations, Monod kinetics including saturations, Haldane-like formulations with inhibitions taken into account, or combination of different kinetics. The fact remains that the growth rate always has a dominant role in the modelling of the process.
However, the growth rate strongly depends on biological factors and intracellular activity. Accurate and general modellings of cellular activity exist in steady-state. [4] and [5] present two of these models in steady state and [6] presents a dynamical model for cellular inner concentrations in metabolites and enzymes. These models are accurate and are able to predict the behaviour of the cells according to the external medium composition. Indeed, high numbers of internal variables and parameters (greater than 1000) are taken into account in these models, while considering some general assumptions.
In a previous work [7], we proposed an accurate tractable biological model for batch cultivation bioprocess, that combines these two modelling approaches (macroscopic and intracellular models). In the present paper, the model is extended to the case of a fedbatch cultivation bioprocess. In the latter operating mode, this model is further exploited to determine the optimal feeding rate in order to maximize the bacteria growth. The biological part of the model completes the usual macroscopic description and brings the optimal profile closer to biological considerations. This paper also shows that the biological constraints described in the model allow a clear interpretation of the limitations of the cells and a fair characterization of the optimality.
This paper is organized as follows: in Section II, the dynamics of the process is presented, further completed in Section III by the description of the related biological constraints in order to fully describe the cells growth. With this model, the optimal feeding strategy that maximizes the bacteria growth for a fedbatch bioreactor is derived, and presented in Section IV with some computational details. Then, in Section V, an analysis of the intracellular model is considered to interpret the determined optimal feeding strategy. Finally, some conclusions and perspectives are drawn in Section VI.

II. Dynamics of the bioprocess

A. Volume variations

Denoting F_{in} and F_{out} the influent and effluent flow rates respectively (both in $L.h^{-1}$), the variation of the bioreactor medium volume, V_{react}, is given by:

$$\frac{dV_{react}(t)}{dt} = F_{in}(t) - F_{out}(t) \tag{1}$$

The bioreactor is supposed to be perfectly stirred and namely the population of bacteria, N_{pop}, is supposed to be perfectly homogeneous in the medium. So, the number of bacteria leaving the reactor at each time instant equals the effluent flow rate multiplied by the relative concentration of bacteria in the bioreactor (N_{pop}/V_{react}). Hence, with $\mu(t)$ the specific growth rate, characterizing the natural growth of the bacterial culture, the evolution of bacteria population is given by (2).

$$\frac{dN_{pop}(t)}{dt} = \underbrace{\mu(t)\ N_{pop}(t)}_{Growth} - \underbrace{F_{out}(t)\frac{N_{pop}(t)}{V_{react}(t)}}_{Effluent} \tag{2}$$

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Also, assuming the volume of each bacteria, v_b, constant with time, the total volume of bacteria, $V_{pop}(t) = v_b\ N_{pop}(t)$, follows the same dynamics:

$$\frac{dV_{pop}(t)}{dt} = \left(\mu(t) - \frac{F_{out}(t)}{V_{react}(t)}\right)\ V_{pop}(t) \tag{3}$$

B. Concentration variations in terms of fluxes

The mass balance of the compounds in presence is derived from the exchange of material between species. Like in [8], a part of the exchanges is material fluxes per unit of bacterial volume. In addition, the influent and effluent flow rates are responsible of material variation. Hence, the variation of the quantities of each intracellular and extracellular species ξ in presence, n_ξ, can be expressed in terms of total bacterial volume, reactions fluxes per unit of bacterial volume, $\nu^\xi_{produced}$ and $\nu^\xi_{consumed}$, and influent and effluent flow rates as in (4).

$$\begin{array}{rcl} \frac{dn_\xi(t)}{dt} = & (\nu^\xi_{produced}(t) - \nu^\xi_{consumed}(t))\ V_{pop}(t) \\ & +\xi^0\ F_{in}(t) - \frac{n_\xi(t)}{V_{react}(t)}\ F_{out}(t) \end{array} \tag{4}$$

where ξ^0 is the concentration of the species ξ in the influent (null in case of intracellular specie).
Expressing the concentration of an internal compound ξ_{int}, denoted $[\xi_{int}]$, with respect to the volume of bacteria V_{pop}, and the concentration of an external compound ξ_{ext}, denoted $[\xi_{ext}]$, with respect to the bioreactor volume V_{react}, it comes:

$$\frac{d[\xi_{int}]}{dt} = \frac{d\frac{n_{\xi_{int}}}{V_{pop}}}{dt} = \frac{1}{V_{pop}}\ \underbrace{\frac{dn_{\xi_{int}}}{dt}}_{eq.(4)} - \frac{[\xi_{int}]}{V_{pop}}\ \underbrace{\frac{dV_{pop}}{dt}}_{eq.(3)} \tag{5}$$

and for external concentrations:

$$\frac{d[\xi_{ext}]}{dt} = \frac{d\frac{n_{\xi_{ext}}}{V_{react}}}{dt} = \frac{1}{V_{react}}\ \underbrace{\frac{dn_{\xi_{ext}}}{dt}}_{eq.(4)} - \frac{[\xi_{ext}]}{V_{react}}\ \underbrace{\frac{dV_{react}}{dt}}_{eq.(1)} \tag{6}$$

Merging the equation (5) with (4) and (3), and the equation (6) with (4) and (1), leads to:

$$\left\{\begin{array}{rcl} \frac{d[\xi_{int}]}{dt} = & \pm\nu^{\xi_{int}} - \mu[\xi_{int}] \\ \frac{d[\xi_{ext}]}{dt} = & \pm\nu^{\xi_{ext}}\ \frac{V_{pop}}{V_{react}} + (\xi^0 - [\xi_{ext}])\ \frac{F_{in}}{V_{react}} \end{array}\right. \tag{7}$$

where $\pm\nu^\xi = \nu^\xi_{produced} - \nu^\xi_{consumed}$.

Remark: There is a strong analogy with the dynamics described in [1] for the growth of one population of micro-organisms on a single limiting substrate, recalled:

$$\left\{\begin{array}{rcl} \frac{dV_{react}}{dt} & = & F_{in} - F_{out} \\ \frac{dV_{pop}}{dt} & = & \mu V_{pop} - F_{out}\frac{V_{pop}}{V_{react}} \\ \frac{d[S]}{dt} & = & -Y_S\mu\frac{V_{pop}}{V_{react}} + (S^0 - [S])\ \frac{F_{in}}{V_{react}} \end{array}\right. \tag{8}$$

with the same notations as previously and with Y_S the "yield coefficient in substrate consumption by the biomass", and S the single external substrate. Hence, in this framework, the following analogy can be made between the two models:

$$\pm\nu^S = -Y_S\ \mu \tag{9}$$

Assuming there is no production of S and only its consumption, $Y_S = \nu^S_{consumed}/\mu$ is a rational definition for the notion of yield: the ratio of the consumption over the growth.

C. Pool aggregation and fluxes

1) Pool definition: In the bioreactor medium, there are

- bacteria with a volume V_{pop}
- a single substrate S_{ext}

As in [7], this work relies on the aggregation of the different intracellular species into pools in order to deal with the trade-off between simplicity and tractability. Almost all cellular components can be categorized in one of these pools:

- S_{in} pool gathers all the intracellular metabolic precursors including intracellular forms of substrate,
- e_B are all the proteins involved in metabolic network and the proteins complexes engaged in molecular machineries,
- e_T are the membranous proteins, part of which is involved in the substrate uptake,
- e_R are the ribosomes,
- X_B pool consists in all the metabolites and macro-components of the cell.

2) Flux expression: The considered reactions in this model are the same as in [7], that is to say:

- $S_{ext} \xrightarrow{\nu_{import}} S_{in}$, importing the substrate from the external medium to the cells thanks to membranous proteins e_T,
- $S_{in} \xrightarrow{\nu_{X_B}} \alpha_B.X_B$, producing metabolites and macro-components from S_{in} under the action of enzymes and molecular machineries e_B,
- $S_{in} \xrightarrow{\nu_{e_B}} \alpha_{e_B}.e_B$, creating the metabolic enzymes by ribosomes e_R,
- $S_{in} \xrightarrow{\nu_{e_T}} \alpha_{e_T}.e_T$, creating membranous proteins by ribosomes e_R,
- $S_{in} \xrightarrow{\nu_{e_R}} \alpha_{e_R}.e_R$, producing ribosomes by themselves.

A reaction catalysed by an enzyme has a flux equal to:

$$\nu = \text{efficiency } [\text{enzyme concentration}] \tag{10}$$

The efficiencies are determined as in [7]:

$$\begin{array}{rcll} \nu_{import} & = & \frac{r_{imp}v_T[S_{ext}]}{[S_{ext}] + K_T(1 + [S_{in}]/K_S)} & [e_T] \\ \nu_{X_B} & = & k_B[S_{in}] & [e_B] \\ \nu_{e_B} & = & u_{e_B}[S_{in}] & [e_R] \\ \nu_{e_T} & = & u_{e_T}[S_{in}] & [e_R] \\ \nu_{e_R} & = & u_{e_R}[S_{in}] & [e_R] \end{array} \tag{11}$$

where v_T is the maximal import efficiency of transporters, r_{imp} is the proportion of active transporters in the membrane, K_T is a constant representing the affinity between e_T and the substrate S_{ext}, K_S is a constant representing the inhibition of the import flux relative to $[S_{in}]$, k_B is the relative efficiency of metabolism with respect to $[S_{in}]$, u_{e_B} (resp. u_{e_T}, u_{e_R}) is level of expression of e_B (resp. e_T, e_R).
Concerning the import flux ν_{import}, the efficiency is supposed to be saturated by the concentrations in external substrate as it is observed on experiments. The transfer is

also supposed to be inhibited by the internal concentration in substrate in order to avoid too high intracellular concentrations. The other efficiencies are linear with respect to internal substrate concentration meaning that the absence of substrate turns these fluxes off.

D. Concentration dynamics

Considering the fluxes expressions given in (11) and the general formulation of the concentration dynamics (7) and with (1) and (3), the description of the bioprocess dynamics is derived in (12).

$$\begin{cases} \dot{[S_{in}]} = \dfrac{r_{imp} v_T [S_{ext}][e_T]}{[S_{ext}] + K_T(1+[S_{in}]/K_S)} \\ \qquad - k_B[e_B][S_{in}] - \displaystyle\sum_{i\in\{B,T,R\}} u_{e_i}[e_R][S_{in}] - \mu[S_{in}] \\ \dot{[e_B]} = \alpha_{e_B} u_{e_B}[e_R][S_{in}] - \mu[e_B] \\ \dot{[e_T]} = \alpha_{e_T} u_{e_T}[e_R][S_{in}] - \mu[e_T] \\ \dot{[e_R]} = \alpha_{e_R} u_{e_R}[e_R][S_{in}] - \mu[e_R] \\ \dot{[X_B]} = \alpha_B k_B[e_B][S_{in}] - \mu[X_B] \\ \dot{[S_{ext}]} = -\dfrac{r_{imp} v_T [S_{ext}][e_T]}{[S_{ext}] + K_T(1+[S_{in}]/K_S)} \dfrac{V_{pop}}{V_{react}} \\ \qquad + (S^0_{ext} - [S_{ext}]) \dfrac{F_{in}}{V_{react}} \\ \dot{V}_{pop} = \mu\ V_{pop} - F_{out}\ \dfrac{V_{pop}}{V_{react}} \\ \dot{V}_{react} = F_{in} - F_{out} \end{cases} \tag{12}$$

The expression of the specific growth rate is detailed hereafter. The values of the parameters can be found in literature ([9]) and are gathered in table I.

TABLE I

PARAMETERS VALUES. ALL FROM [9], EXCEPT FOR THOSE MARKED WITH [*] FROM [7]. "aa" STANDS FOR AMINO ACIDS

Param.	Value	$(Unit)$
v_T	$1.33\,10^6$	$(L\,h^{-1}\,mmol^{-1})$
r_{imp}	0.01	
K_T	0.8	$(mmol\,L^{-1})$
K_S	1	$(mmol\,L^{-1})$
k_B	$4.32\,10^4$	$(L\,h^{-1}\,mmol^{-1})$
aa_{e_B}	360	(aa)
aa_{e_T}	360	(aa)
aa_{e_R}	10100	(aa)
$aa_{S_{in}}$	0.8	(aa)
aa_{X_B}	5.97	(aa)
$\overline{D}$	16.5	$(mol\,aa\,L^{-1})$
v_{k_T}	$9.72\,10^4$	(aa)
K_{k_T}	0.5	(h^{-1})
u_{e_B}	$1.86\,10^5$ [*]	$(L\,h^{-1}\,mmol^{-1})$
u_{e_T}	$3.39\,10^4$ [*]	$(L\,h^{-1}\,mmol^{-1})$
u_{e_R}	$9.94\,10^4$[*]	$(L\,h^{-1}\,mmol^{-1})$
α_{e_B}	$2.2\,10^{-3}$	
α_{e_T}	$2.2\,10^{-3}$	
α_{e_R}	$9.5\,10^{-5}$	
α_B	$1.67\,10^{-4}$	

III. BIOLOGICAL LIMITATIONS

The above state variables and dynamics have to respect some biological constraints to reproduce reliably the biological behaviour of the cells. Namely, the growth rate and the limiting ribosome capacity have to be taken into account.

A. Intracellular density regulation

In this model, the specific growth rate μ is assumed to be linked to the mass accumulation in the form of proteins and ribosomes. The intracellular density in joined amino acids, denoted $\overline{D}$, is known to be constant over time [10].
Typically, it means that the pools S_{in} and X_B do not account in the intracellular density, as in [11].
The density is expressed as:

$$\overline{D} = \sum_{i\in\{B,T,R\}} aa_{e_i}[e_i] \tag{13}$$

and thus, its derivative is null, leading to:

$$\begin{aligned} \dot{\overline{D}} = 0 \quad &= \sum_{B,T,R} aa_{e_i}\dot{[e_i]} \\ 0 \quad &= \sum_{B,T,R} aa_{e_i}\Big(\alpha_{e_i} u_{e_i}[e_R][S_{in}] - \mu[e_i]\Big) \\ 0 \quad &= \sum_{B,T,R} \Big(aa_{e_i}\alpha_{e_i} u_{e_i}[e_R][S_{in}]\Big) - \mu \sum_{B,T,R} aa_{e_i}[e_i] \\ 0 \quad &= \sum_{B,T,R} \Big(aa_{e_i}\alpha_{e_i} u_{e_i}[e_R][S_{in}]\Big) - \mu\overline{D} \end{aligned} \tag{14}$$

Consequently,

$$\mu = \frac{1}{\overline{D}} \sum_{B,T,R} \Big(aa_{e_i}\alpha_{e_i} u_{e_i}\Big)[e_R][S_{in}] \tag{15}$$

The specific growth rate is thus determined from the concentrations in ribosomes and in internal substrate.

Computational remark: In order to avoid numerical chattering, a first-order filter with very fast time constant compared to $1/\mu$ can be added either to μ or to $\overline{D}$ defined as (15) and (13), respectively.
Biological remark: the density regulation mechanisms statement notably stands for rod-shaped bacteria like *Escherichia coli* and *Bacillus subtilis*.

B. Translation limitation

The ribosomes capacity represents the number of amino acids one ribosome can join together to form peptide chains. As observed in [12], this capacity, k_T, is limited by the growth rate as follows:

$$k_T = \frac{v_{k_T}\mu}{K_{k_T} + \mu} \tag{16}$$

where v_{k_T} is the maximal translation capacity and K_{k_T} is a constant representing the growth rate for which the capacity is half its maximal value. This leads to a constraint on the enzyme and ribosome creation fluxes:

$$\underbrace{\sum_{i\in\{B,T,R\}} \nu_{e_i}}_{\text{Total enzymes production}} \quad \leq \quad \underbrace{\frac{v_{k_T}\mu}{K_{k_T}+\mu}}_{\text{Ribosomes capacity}} \quad [e_R] \tag{17}$$

Hence, from (11):

$$[S_{in}] \sum_{i \in \{B,T,R\}} u_{e_i} \leq \frac{v_{k_T} \mu}{K_{k_T} + \mu} \tag{18}$$

This constraint must be satisfied at any time.

IV. Optimal feeding strategy for a fedbatch bioreactor

In the following, the study will focus on a fedbatch bioreactor, i.e. a culture without any effluent flow:

$$F_{out} = 0 \tag{19}$$

The problem considered below consists in maximizing the bacteria growth within a fixed cultivation duration, using the modelling and the constraints developed in Section II and III, and parameters from table I, acting only on the influent flow rate, F_{in}. The derived optimization problem is then expressed as:

$$\max_{F_{in}(t)} V_{pop}(t = t_f)$$
$$\text{subject to,} \begin{cases} \text{concentrations dynamics (12)} \\ \text{growth rate (15)} \\ \text{translation constraint (18)} \\ \text{fixed initial conditions} \\ 0 \leq F_{in}(t) \leq F_{MAX} \\ 0 \leq V_{react}(t) \leq V_{MAX} \\ \text{fixed } t_f \end{cases} \tag{20}$$

F_{MAX} (resp. V_{MAX}) is the upper bound on F_{in} (resp. V_{react}). Considered initial values, bounds, final time and influent flow characteristics are gathered in table II.

TABLE II
Simulation conditions.

Variable	Value ($Unit$)	Var.	Value ($Unit$)
$[e_B]_0$	26 ($mmol\,L^{-1}$)	V_{pop_0}	0.015 (L)
$[e_T]_0$	14 ($mmol\,L^{-1}$)	V_{react_0}	3 (L)
$[e_R]_0$	0.21 ($mmol\,L^{-1}$)	t_f	10 (h)
$[S_{in}]_0$	0.43 ($mmol\,L^{-1}$)	F_{MAX}	0.7 ($L\,h^{-1}$)
$[X_B]_0$	0.06 ($mmol\,L^{-1}$)	V_{MAX}	10 (L)
$[S_{ext}]_0$	5 ($mmol\,L^{-1}$)	S^0_{ext}	60 ($mmol\,L^{-1}$)

The solution of problem (20) can be determined by a sequential approach as in [13] and [3]. It means that only the control variable, F_{in}, is described by piecewise polynomials and the optimization is made with respect to these polynomial coefficients. The problem is then formulated as a nonlinear programming problem.
Here, the command F_{in} is discretized with a zero-order hold with a 7min time step, and equations (12), (15), (18) are also discretized with an explicit Euler method of sampling time 0.06min. The optimization problem is solved using a Sequential Quadratic Programming algorithm presented in [14].

The obtained optimal influent flow rate profile is depicted in Fig. 1.

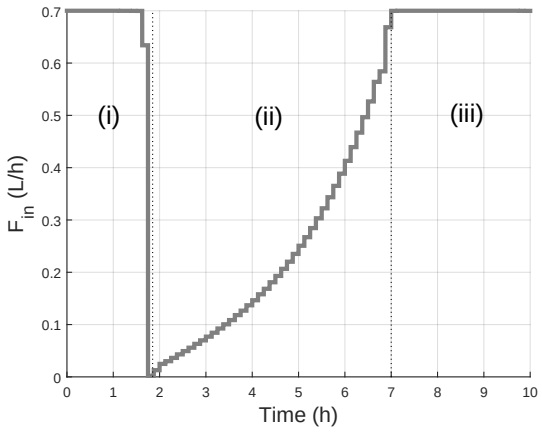


Fig. 1. Time evolution of the optimal influent flow rate

The strategy appears to be split in 3 stages:

(i) $F_{in}(t) = F_{MAX}$,

(ii) a sort of exponential curve,

(iii) the "exponential" seems to saturate at $F_{in} = F_{MAX}$.

Next Section will analyse and interpret this obtained profile.

V. Interpretation

The intra-cellular concentrations have a fast dynamics compared to the extracellular variables ($[S_{ext}]$, V_{pop} and V_{react}) which have slower dynamics. Therefore, a model reduction can be applied, based on the assumption that $\dot{[\xi_{int}]} = 0$. Thus, the objective is to determine $[\xi_{int}]$ as a function of the slow external variables.

A. Model reduction

In the following, the equilibrium of the internal concentration is assumed to be reached and $\overline{[\xi_{int}]}$ denotes the concentration of internal compound ξ_{int} at equilibrium.

From the ribosomes equilibrium in (12):

$$\overline{\mu} = \alpha_{e_R} u_{e_R} \overline{[S_{in}]} \tag{21}$$

From the enzymes e_B and e_T equilibrium in (12) and from (21), it can be shown that:

$$\begin{cases} \overline{[e_B]} = \frac{\alpha_{e_B} u_{e_B} \overline{[e_R]}\ \overline{[S_{in}]}}{\overline{\mu}} = \frac{\alpha_{e_B} u_{e_B}}{\alpha_{e_R} u_{e_R}} \overline{[e_R]} \\ \overline{[e_T]} = \frac{\alpha_{e_T} u_{e_T} \overline{[e_R]}\ \overline{[S_{in}]}}{\overline{\mu}} = \frac{\alpha_{e_T} u_{e_T}}{\alpha_{e_R} u_{e_R}} \overline{[e_R]} \end{cases} \tag{22}$$

The constant density in proteins and ribosomes and (22) gives the relation:

$$\overline{[e_R]} = \frac{\alpha_{e_R} u_{e_R}}{\sum_{i \in \{B,T,R\}} a a_{e_i} \alpha_{e_i} u_{e_i}} \overline{D} \tag{23}$$

And once again (22):

$$\overline{[e_j]} = \frac{\alpha_{e_j} u_{e_j}}{\sum_{i \in \{B,T,R\}} a a_{e_i} \alpha_{e_i} u_{e_i}} \overline{D}, j \in \{B,T,R\} \tag{24}$$

This means that the enzyme and ribosome concentrations in steady-state depend on the density $\overline{D}$.

Besides, the concentration of intracellular metabolites and macrocomponents is deduced from its equilibrium:

$$\overline{[X_B]} = \frac{\alpha_B k_B}{\alpha_{e_R} u_{e_R}} \overline{[e_B]} = \frac{\alpha_B k_B}{\alpha_{e_R} u_{e_R}} \frac{\alpha_{e_B} u_{e_B}}{\sum\limits_{i \in \{B,T,R\}} a a_{e_i} \alpha_{e_i} u_{e_i}} \overline{D} \tag{25}$$

Moreover, (21) can be included in the equation of the equilibrium of S_{in} as in (26).

$$\begin{aligned} 0 = & - \overline{[e_T]} v_T r_{imp} [S_{ext}] \\ & + (k_B \overline{[e_B]} + \sum_{B,T,R} u_{e_i} \overline{[e_R]}) \ ([S_{ext}] + K_T) \ \overline{[S_{in}]} \\ & + \Big((k_B \overline{[e_B]} + \sum_{B,T,R} u_{e_i} \overline{[e_R]}) \ K_T / K_S \\ & \quad + \alpha_{e_R} u_{e_R} \ ([S_{ext}] + K_T) \Big) \ \overline{[S_{in}]}^2 \\ & + \alpha_{e_R} u_{e_R} K_T / K_S \overline{[S_{in}]}^3 \end{aligned} \tag{26}$$

Assuming $\overline{D}$ independent from $\overline{[S_{in}]}$, (26) can be seen as a third degree polynomial in $\overline{[S_{in}]}$ with coefficients depending on $[S_{ext}]$. The roots of this polynomial in $\overline{[S_{in}]}$ can be computed with the numerical values of table I and several values of $[S_{ext}]$. For every value of $[S_{ext}]$, the polynomial has two conjugate complex roots and a real positive one. Plotting the latter root as a function of $[S_{ext}]$ is similar to a Michaelis-Menten shape: $\overline{[S_{in}]} \approx \frac{\alpha [S_{ext}]}{\beta + [S_{ext}]}$.

Consequently, from (21), this model reduction shows that the behaviour is similar to the one obtained through a macroscopic approach considering a Monod kinetics law.

B. Focus on the growth rate

As the internal steady state growth rate follows a Monod-like kinetics, the optimal strategy to maximize the growth appears to be to maximize the external substrate concentration. This is the classical strategy when not considering inhibition of the substrate, when the growth strictly increases with the substrate concentration.
The asymptotic specific growth rate, μ_{sup}, is given by (27) (from (26) when $[S_{ext}]$ tends to infinity).

$$\begin{aligned} 0 = & \ -\overline{[e_T]} v_T r_{imp} + \overline{E} \frac{\mu_{sup}}{\alpha_{e_R} u_{e_R}} + \alpha_{e_R} u_{e_R} \frac{\mu_{sup}^2}{\alpha_{e_R}^2 u_{e_R}^2} \\ \mu_{sup} = & \ \frac{-\overline{E} + \sqrt{\overline{E}^2 + 4 \alpha_{e_R} u_{e_R} v_T r_{imp} \overline{[e_T]}}}{2} \end{aligned} \tag{27}$$

with $\overline{E} = k_B \overline{[e_B]} + \sum\limits_{B,T,R} u_{e_i} \overline{[e_R]}$.

Nonetheless, in this context, the translation constraint (18) becomes a limitation on μ as in (28).

$$\begin{aligned} \overline{[S_{in}]} \sum_{i \in \{B,T,R\}} u_{e_i} & \leq \frac{v_{k_T} \overline{\mu}}{K_{k_T} + \overline{\mu}} \\ \frac{\overline{\mu}}{\alpha_{e_R} u_{e_R}} \sum_{i \in \{B,T,R\}} u_{e_i} & \leq \frac{v_{k_T} \overline{\mu}}{K_{k_T} + \overline{\mu}} \\ \overline{\mu} & \leq \frac{v_{k_T} \alpha_{e_R} u_{e_R}}{\sum\limits_{i \in \{B,T,R\}} u_{e_i}} - K_{k_T} \triangleq \mu_{trans} \end{aligned} \tag{28}$$

This threshold μ_{trans} has to be compared to μ_{sup}. With the numerical values of table I, it comes:

$$\begin{cases} \mu_{sup} = & 0.50 h^{-1} \\ \mu_{trans} = & 0.48 h^{-1} \end{cases} \tag{29}$$

Thus, the translation constraint can limit the growth. In fact, under the assumption of intracellular steady state, a too important concentration of external substrate burdens the cells and the translation constraint is saturated.
The translation constraint and the steady-state growth rate are both represented in terms of $[S_{ext}]$ on Fig. 2.

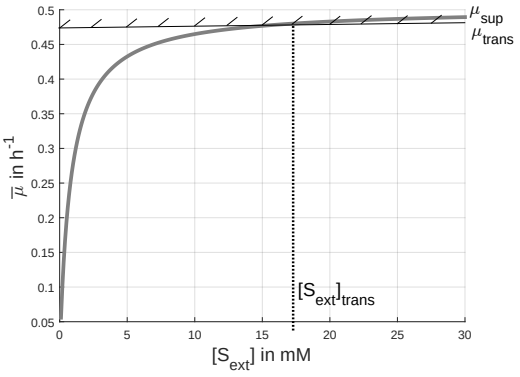


Fig. 2. Growth rate as a function of external substrate concentration, assuming internal steady-state is reached. In grey, the evolution of $\overline{\mu}$ in steady state, without translation constraint. In black, the equivalent translation constraint in terms of saturation on growth rate, implicitly limiting $[S_{ext}]$ (dotted black line). mM stands for $mmol\,L^{-1}$.

It can be seen that the constraint indirectly prevents the substrate from being too concentrated. Hence, the optimal feeding strategy is not to fill the bioreactor at a maximum to increase the substrate concentration as in a Monod-like macroscopic model (see [15]) but to keep the system around the point $\mu = \mu_{trans}$ and so, $[S_{ext}] = [S_{ext}]_{trans}$.

C. Time evolutions

Simulation is run with computational details from Section IV and the influent profile $F_{in}(t)$ depicted in Fig. 1.
Fig. 3 gathers the time evolutions of the growth rate and the external substrate concentration, and the representation of the real μ versus $[S_{ext}]$ to complete Fig. 2.
The same three stages as in Fig. 1 can be observed:

(i) both of the substrate concentration and the growth rate increase,
(ii) the substrate concentration and the growth rate are maintained constant. The specific growth rate value equals μ_{trans},
(iii) both of the substrate concentration and the growth rate decrease, corresponding to $F_{in} = F_{MAX}$.

As depicted in Fig. 3.c, obtained growth rate μ is very close to the theoretical one, $\overline{\mu}$. The difference comes from: (1) the control law discretization, (2) optimization algorithm convergence, and (3) numerical approximation while performing model reduction. Finally, the optimal operating point of the system corresponds to $\mu \approx \mu_{trans}$ and $[S_{ext}] \approx [S_{ext}]_{trans}$. The optimal feeding strategy thus consists in driving the system to this point and maintaining it there until F_{in} reaches its upper bound. The optimal strategy can be split into three stages:

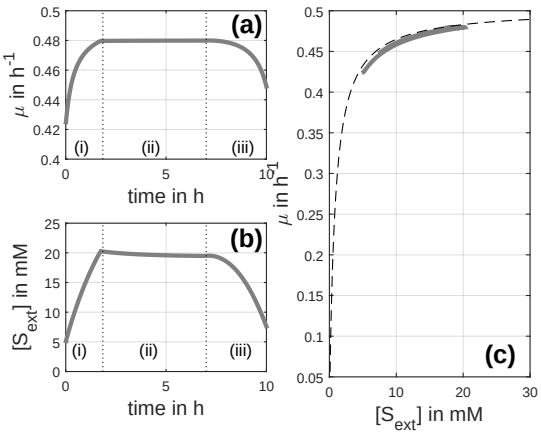


Fig. 3. (a) the growth rate μ. (b) $[S_{ext}]$ as a function of time. (c) in grey, μ evolution versus $[S_{ext}]$ and in dashed black, recalling $\overline{\mu}$ from Fig.2. mM stands for $mmol\, L^{-1}$.

(i) as long as $[S_{ext}] < [S_{ext}]^*$, increase $[S_{ext}]$: $F_{in}(t) = F_{MAX}$,
(ii) as long as $F_{opt}(t) < F_{MAX}$, keep $\mu = \mu_{trans}$, i.e. the translation constraint saturated: $F_{in}(t) = F_{opt}(t)$,
(iii) as long as $V_{react} < V_{MAX}$, $F_{in}(t) = F_{MAX}$.

VI. Conclusions and perspectives

The biological constraints and the information they provide on the cell behaviour are complementary to the evolution of every compound in the cells and the reactor. They have to be exploited in the design of control laws as the dynamics of extracellular compounds already are. It is essential to understand what is important for the cells and so how bacteria can be at best in a bioreactor to grow.

In this paper, we have shown that an optimal control law profile is perfectly described if we consider the internal limitation in terms of proteins translation limitation. The optimal feeding flow is the one which maintains the internal state of the cells in such a way that resources are present in the maximal quantity with respect to this translation constraint.

This optimal feed-rate was determined by means of an intracellular model. A current drawback of this kind of intracellular models is that they have to be assessed by experiments to be fully trustworthy. For instance, some model parameters must be determined. The uncertainty on their values and its impact on the model accuracy and control law optimality will be further investigated in a future work.

The optimal flow rate can be moved into a control problem: the optimal operating of the bioreactor can then be expressed as the regulation of the specific growth rate to a given value that corresponds to the maximal capacity of the ribosomes, or equivalently, to regulate the external substrate concentration to the corresponding objective value. Moreover, the control of inner parameters as gene level expressions should be also optimized to improve further the performance of the bioprocess operation. This is investigated in an ongoing work.

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