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A Closed-loop Exponential Feeding Law for Multi-substrate Fermentation Processes

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Abstract: This article addresses the computation of invariant and stabilizing control laws for dual-substrate fed-batch fermentors. The design is based on two commonly used model structures. It will be shown how to derive partial state feedbacks, using only biomass and volume as measures, that keep the substrates at a desired concentration provided the model is good enough and does not change with time. In the paper an analysis of invariance and a study of global stability within the framework of partial stability is provided.

Keywords: Fermentation processes; nonlinear control; invariance; stabilizing feedback; stability analysis.

1. INTRODUCTION

A bio-reactor is a tank in which several microbial growth and enzyme-catalyzed reactions occur simultaneously in a liquid medium. In fed-batch bio-reactors there is one or more feeding flows F_i entering the tank and supplying it with nutrients until its limit capacity is reached.

The models commonly used for control purposes are **non-structured**, i.e. the cell is regarded as a black box without delving into the intracellular mechanisms, and **non-segregated**, i.e. assuming an average cell. Also, the conditions and concentrations in the tank are supposed to be homogeneous. Finally, in a dual-substrate fed-batch two nutrients or substrates are separately supplied in a short or limiting concentration along with many other components composing the medium. These substrates may play different roles or functions, e.g. one being a carbon source and the other a nitrogen source.

As stated in the abstract, the control design is based on two commonly used models for multi-substrate systems. It is also assumed, in this first approach, there is no product inhibiting or somehow significantly affecting growth and/or substrate consumption. Hence, it is not necessary to include it in the model. This is described in section 2, in which a set of typical control specifications is also described.

In section 3 a presentation is given of a closed-loop control law using only on-line measurements of biomass and volume. It is analyzed both from an intuitive point of view and analytically, proving that it constitutes an invariant control law. That is, it keeps the system within a given subset of the state space provided the initial conditions are adequate.

In section 4 the concept of partial stability is introduced and then applied for proving that the control law is also stabilizing. This is always true for systems with monotonously increasing or Monod-like kinetic functions

and under some additional conditions for systems with non-monotonous or Haldane-like kinetic functions.

In section 5 some simulations are provided illustrating the controller performance. Finally, in section 6 conclusions and future lines are given.

2. CONTROL PROBLEM

Two model structures are commonly used to describe dual-substrate fed-batch fermentors. The main differences lying with the specific kinetic functions to be used. See Anjou [2001], Chanprateep [2002], Chan [2003], Bielefeldt [2005], Bailey [1986], Bae [1996], Sonmezisik [1998] and Beyenal [2003]. Both can be summarized in the following expressions:

$$
\begin{aligned}
\dot{x} &= f(\mu_1, \mu_2)x - (D_1 + D_2)x \\
\dot{s}_1 &= -y_1\mu_1(s_1)x + D_1 s_{1in} - (D_1 + D_2)s_1 \\
\dot{s}_2 &= -y_2\mu_2(s_2)x + D_2 s_{2in} - (D_1 + D_2)s_2 \\
\dot{v} &= (D_1 + D_2)v = F_1 + F_2
\end{aligned}
\tag{1}
$$

where f, the specific growth rate, is usually a sum or product of its arguments. Additionally, the state variables are x biomass concentration, s_i concentrations of limiting substrates in the tank, v volume. Functions μ_i are specific consumption rates. The parameters y_i are yield coefficients. The other two parameters s_{iin} are the limiting substrate concentrations in the corresponding feeding flow. Finally, the D_i are equal to the ratios F_i/v.

The substrates may play different roles (see Zinn [2004]). For example in two common cases:

(1) Both substrates are carbon sources and contribute both to growth and production.
(2) One substrate is a carbon source mainly affecting growth and the other one a nitrogen source affecting production and product characteristics [1].

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[1] Some authors defend in this case the yield coefficients depend on the s_1/s_2 ratio. See Zinn [2004] again

In either case there are mainly two goals from the biologists point of view:

(1) It is desirable to keep a given specific growth rate $f = \mu_{ref}$ corresponding to a desired physiological state at which the microorganism behaves optimally with respect to production, does not produce inhibiting products, etc. This translates into the tracking of a given trajectory for total biomass. In particular

$$x(t)v(t) = x_0 v_0 e^{\mu_{ref} t} \qquad (2)$$

which is (theoretically) unbounded. Volume goes to infinity, biomass follows a bounded trajectory adjusting to a logistic function and only the substrates may stabilize around a point. Hence, the convenience of partial stability concepts.

(2) It has been reported in for example Kellerhals [1999] and Xu [2005] that in many instances the ratio s_1/s_2 affects the product characteristics, e.g. in PHB production the bioplastic physical properties.

Both goals could be achieved fixing the s_i concentrations in the broth. Although, due to modelling errors and model changes with time, the "right" to keep μ_{ref} may deviate significantly from the precalculated ones. Thus, for better performance and security an adaptation of the reference concentrations s_{ir} may be necessary. This will be one of the future lines and for the time being ideal conditions will be assumed.

3. CONTROL LAW

The control law to be suggested is an extension of the closed-loop exponential feeding law for one-limiting substrate systems published in Pico-Marco [2006], Pico-Marco [2005] and H. De Battista [2006]. It has the form:

$$\begin{aligned} F_1 &= \lambda_1 xv \\ F_2 &= \lambda_2 xv \end{aligned} \qquad (3)$$

Provided the equations

$$\begin{aligned} \mu_1(s_1) &= \mu_{1r} \\ \mu_2(s_2) &= \mu_{2r} \end{aligned} \qquad (4)$$

have a satisfactory solution s_{1r}, s_{2r} the equations for the substrates in (1) become

$$\begin{aligned} \dot{s_1} &= (-y_1\mu_1 + \lambda_1 s_{1in} - (\lambda_1 + \lambda_2)s_1)x \\ \dot{s_2} &= (-y_2\mu_2 + \lambda_2 s_{2in} - (\lambda_1 + \lambda_2)s_2)x \end{aligned} \qquad (5)$$

and it is possible to find values λ_1, λ_2 for the given partial equilibrium solving

$$\begin{aligned} 0 &= -y_1\mu_{1r} + \lambda_1 s_{1in} - (\lambda_1 + \lambda_2)s_{1r} \\ 0 &= -y_2\mu_{2r} + \lambda_2 s_{2in} - (\lambda_1 + \lambda_2)s_{2r} \end{aligned} \qquad (6)$$

where λ_1, λ_2 are positive numbers for s_{1in}, s_{2in} big enough [2]. It is clear from the deduction that for any initial conditions such that $s_{10} = s_{1r}, s_{20} = s_{2r}$, this control law will keep the system in a submanifold Z^* such that $f = \mu_{ref}$ since $\dot{s_1} = \dot{s_2} = 0$. Hence, it is an invariant control. A more formal test can be produced along the lines in Pico-Marco [2006] checking the solution of the equation

$$\frac{\partial \varphi}{\partial X}f(X) + \frac{\partial \varphi}{\partial X}g(X)u(X) = 0 \qquad X \in Z^* \qquad (7)$$

[2] In practice this requirement does not pose any problem

where X represents the whole state, f, g the function vectors appearing when (1) is written in affine form, and Z^* is the goal manifold given by

$$\varphi := \left\{ \begin{aligned} & x - \frac{\mu_{ref}}{\lambda_1 + \lambda_2} - (x_0 v_0 - \frac{\mu_{ref}}{\lambda_1 + \lambda_2}v_0)\frac{1}{v} = 0 \\ & s_1 - s_{1r} = 0 \\ & s_2 - s_{2r} = 0 \end{aligned} \right\} \qquad (8)$$

Using, for example Maple$^{\mathrm{TM}}$, it turns out the above suggested control is the solution for adequate λ_i values.

4. PARTIAL STABILITY

Partial stability is defined as the stability of dynamic systems with respect **not** to all but just to a given part of the state variables (see Vorotnikov [2002]). Fed-batch bioreactors correspond to the case of stability of **partial** equilibrium positions which is defined as follows:

"Let there be given a nonlinear system of ordinary differential equations

$$\begin{aligned} \dot{y} = Y(t, y, z) \quad \dot{z} = Z(t, y, z) \\ Y(t, 0, z) \equiv 0 \end{aligned} \qquad (9)$$

The set $y = 0$ of system (9) is said to be stable, if for any numbers $\epsilon > 0$, $t_0 \geq 0$, there is a number $\delta(\epsilon, t_0) > 0$ such that from $\| y_0 \| < \delta$, $\| z_0 \| < \infty$ it follows that $\| y(t; t_0, x_0) \| < \epsilon$ for all $t > t_0$."

This is the PSt-problem w.r.t. that part of the variables of the original system (or the corresponding perturbed motion system) for which this system has an equilibrium position. **Partial** equilibrium positions of this kind (also termed a **balanced motion**) are invariant sets of the system. Hence, it is actually the problem of stability of sets that is analyzed in this case.

In the following, a global analysis is carried out following Chellaboina [2002] due to its simplicity, although it could have been set up in the more general framework of Sun [2002] for y-stability under arbitrary z-perturbations. Both Monod and Haldane-like kinetic functions are considered.

Now, in order to introduce the theorem to be used in the stability proof, consider the nonlinear autonomous dynamical system (see Chellaboina [2002])

$$\begin{aligned} \dot{x_1} &= f_1(x_1, x_2), \quad x_1(0) = x_{10} \\ \dot{x_2} &= f_2(x_1, x_2), \quad x_2(0) = x_{20} \end{aligned} \qquad (10)$$

where $t \in I_{x_0}, x_1 \in D \subseteq R^{n_1}$, D is an open set with $0 \in D$, $x_2 \in R^{n_2}$ and

$$f_1 : D \times R^{n_2} \longrightarrow R^{n_1} \qquad (11)$$

is such that $\forall x_2 \in R^{n_2}$

$$f_1(0, x_2) = 0. \qquad (12)$$

and $f_1(., x_2)$ is locally Lipschitz in x_1. Also

$$f_2 : D \times R^{n_2} \longrightarrow R^{n_2} \qquad (13)$$

is such that for every $x_1 \in D, f_2(x_1, .)$ is locally Lipschitz in x_2 and $I_{x_0} \equiv [0, \tau_{x_0}), 0 < \tau_{x_0} \leq \infty$, is the maximal interval of existence for the solution $(x_1(t), x_2(t)), t \in I_{x_0}$. Under the above assumptions the solution exists and is unique over I_{x_0}.

Stability with respect to x_1 of the system defined above can be defined as:

" The nonlinear dynamical system (10) is Lyapunov stable w.r.t. x_1 if, for every $\epsilon > 0$ and $x_{20} \in R^{n_2}$, there exists $\delta(\epsilon, x_{20}) > 0$ such that $\| x_{10} \| < \delta$ implies that $\| x_1 \| < \epsilon$ for all $t \geq 0$."

Definition which corresponds with the notion of partial equilibria. In order to analyze partial stability, the following results from Chellaboina [2002] are used:

Theorem 1. Consider system (10), then if there exists a continuously differentiable function $V : D \times R^{n_2} \mapsto R$ and a class k function $\alpha(.)$ such that

$$
\begin{aligned}
V(0, x_2) &= 0 \quad x_2 \in R^{n_2} \\
\alpha(\| x_1 \|) &\leq V(x_1, x_2) \quad (x_1, x_2) \in D \times R^{n_2} \\
\dot{V}(x_1, x_2) &\leq 0 \quad (x_1, x_2) \in D \times R^{n_2}
\end{aligned}
\quad (14)
$$

Then system (10) is Lyapunov stable with respect to x_1.

Corollary 2. Consider system (10). If there exists a continuously differentiable, positive definite function $V : D \mapsto R$ such that

$$
V'(x_1)f_1(x_1, x_2) \leq 0, \quad (x_1, x_2) \in D \times R^{n_2} \quad (15)
$$

then system (10) is Lyapunov stable with respect to x_1, uniformly in x_{20}.

For system (1) and control (3), the following system is obtained

$$
\begin{aligned}
\dot{x} &= f(\mu_1, \mu_2)x - (\lambda_1 + \lambda_2)x^2 \\
\dot{s_1} &= (-y_1\mu_1(s_1) + \lambda_1 s_{1in} - (\lambda_1 + \lambda_2)s_1)x \\
\dot{s_2} &= (-y_2\mu_2(s_2) + \lambda_2 s_{2in} - (\lambda_1 + \lambda_2)s_2)x \\
\dot{v} &= (\lambda_1 + \lambda_2)xv
\end{aligned}
\quad (16)
$$

For system (16) it is possible to apply partial stability successively, first with respect to s_1 and then to s_2. This is so because, as seen in expressions (16), there is no case in which say s_2 should follow a given trajectory or reach a given point for s_1 to reach s_{1r}. There is no direct interaction between the substrates and biomass is always a positive common factor in the $\dot{s_i}$ equations. Thus, affecting performance but not stability.

Since the analysis is the same for each substrate, let us illustrate it using s_1. System (16) is reordered as follows:

$$
\begin{aligned}
f_1 &:= \dot{s_1} = (-y_1\mu_1(s_1) + \lambda_1 s_{1in} - (\lambda_1 + \lambda_2)s_1)x \\
f_2 &:= \left\{ \begin{array}{l} \dot{x} = f(\mu_1, \mu_2)x - (\lambda_1 + \lambda_2)x^2 \\ \dot{s_2} = (-y_2\mu_2(s_2) + \lambda_2 s_{2in} - (\lambda_1 + \lambda_2)s_2)x \\ \dot{v} = (\lambda_1 + \lambda_2)xv \end{array} \right\}
\end{aligned}
\quad (17)
$$

with $x_1 = (s_1)$ and $x_2 = (x, s_2, v)$. Whenever $s_1 = s_{1r}$ we have

$$
f_1 = 0 \quad \forall x_2 \quad (18)
$$

A candidate partial lyapunov function is

$$
V = \frac{1}{2}(s_1 - s_{1r})^2 \quad (19)
$$

Then

$$
\begin{aligned}
\dot{V} &= (s_1 - s_{1r})\dot{s_1} = \\
&= x(s_1 - s_{1r})(\lambda_1 s_{1in} - (\lambda_1 + \lambda_2)s_1 - y_1\mu_1(s_1))
\end{aligned}
\quad (20)
$$

with $y_1 = const > 0$ and $x > 0$. Clearly, whenever $s_1 > s_{1r}$ the curve defined by $y_1\mu_1(s_1)$ must be over the straight line defined by

$$
r = \lambda_1 s_{1in} - (\lambda_1 + \lambda_2)s_1 \quad (21)
$$

and viceversa. This will be always the case for Monod-like kinetic functions, see figure 1. In the Haldane case there may be several intersection points, see figure 2. Consequently, depending on the initial conditions, substrate concentrations may evolve towards undesired values.
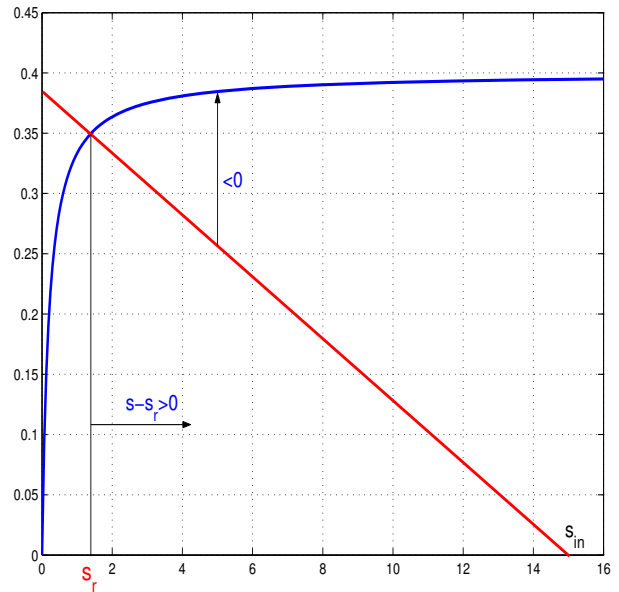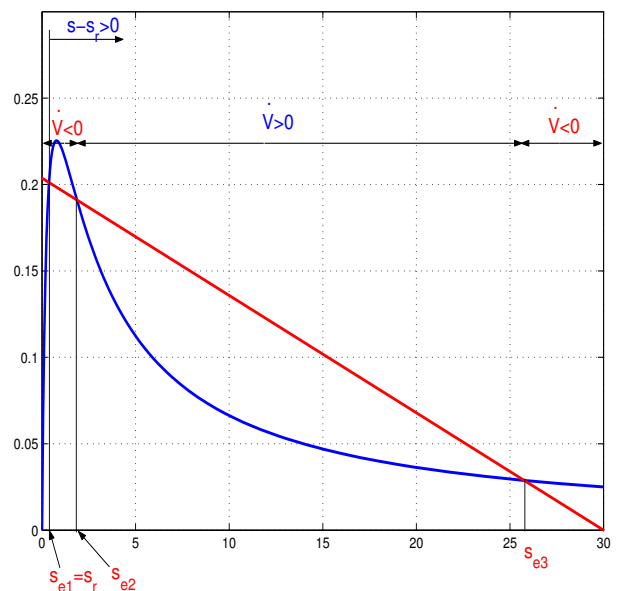

Fig. 1. Line 21 on top of a Monod-like curve

Fig. 2. Line 21 on top of a Haldane-like curve

The stability region for the partial equilibrium (s_{1r}, s_{2r}) is determined by the cartesian product of the intervals for each substrate s_i obtained with the previous analysis.

5. SIMULATIONS

To test the behaviour of the controller described in this paper, the model used in Chan [2003] has been simulated. Model parameters are shown in the following table:

$\mu_{m1} = 0.5$	$\mu_{m2} = 0.5$
$K_{s1} = 0.5$	$K_{s2} = 0.5$
$K_{i1} = 2$	$K_{i2} = 2$
$Y_1 = 2$	$Y_2 = 2$
$s_{1in} = 20$	$S_{2in} = 20$

and four scenarios have been tried:

(1) Perfect modeling: The model parameters used for the controller design are the same as the original one. The initial substrate concentrations start the system to the left of the maximum growth rate of the Haldane functions. So, in this case, growth rate increases with the substrate concentration.

(2) Perfect modeling. But in this case the initial substrate concentrations (both) are to the right of the maximum value. In this case, an increase of the concentration produces a decrease in the growth rate.

(3) Model errors in one of the Haldane functions. $\mu_{m1} = 0.6, K_{s1} = 0.3, K_{i1} = 1$. The initial conditions are the same as in case 1.

(4) Model error in one of the yield coefficient ($Y_1 = 0.6$) and in the Haldane function (case 3). Initial conditions as in case 1

The table below briefly gives the initial values used in the experiments.

All figures		$x_0 = 0.5$	
All figures		$V_0 = 0.2$	
Figures 1,3 and 4		$s_{10} = 1$	$s_{20} = 0.5$
Figure 2		$s10 = 5$	$s20 = 4$

Figures 3 to 6 show the simulation results. In all cases, the growth rate and the substrates reach a steady state, but not the biomass and volume (not shown), as expected. Furthermore, when there are no model errors, the growth rate reaches the reference specification independently of the initial values of the substrates. It must be remarked that the controller only uses the biomass concentration and the volume. In figure 4 it is shown how the controller can reduce the substrate when the system starts to the right of the maximum of the growth rate functions and it can reach the stability point to the left of the maximum: the reference value can be next to the maximum value and the system will remain stable. Also, the controller has low sensitivity to changes in the specific growth rate function as can be seen in figure 5. But, yield coefficient errors produce moderate errors.

So, even though the controller shows good properties, some further work must be done to solve the sensitivity to yield coefficient errors. This could be accomplished with an adaptive controller that can change the λ_i values to adapt to yield coefficient errors or changes. Some ideas used in Pico-Marco [2005] could be extended to the multisubstrate fermentation.
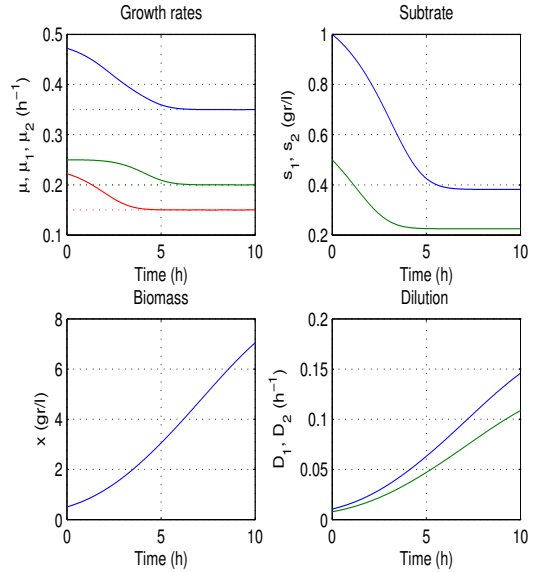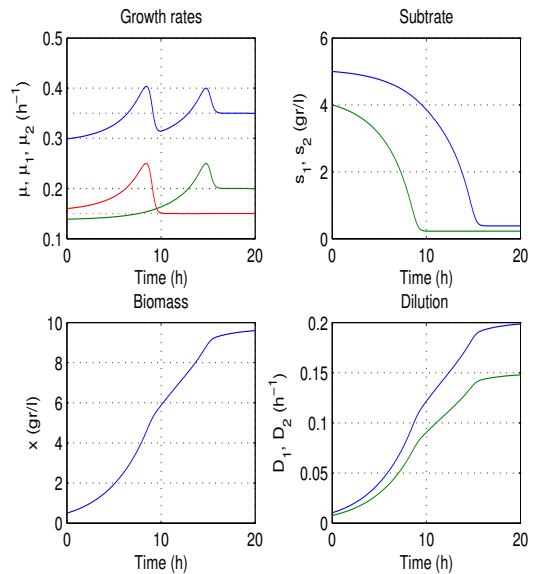

Fig. 3. Case 1

Fig. 4. Case 2

6. CONCLUSIONS

The extended closed-loop exponential feeding law introduced in the paper is very promising for practical applications given the results in simulations, the generality of the model and the fact that only biomass and volume must be measured. On the other hand, it assumes an ideal model. Consequently, for refined regulation of both the specific growth rate and the substrates ratio, an additional structure would be needed to adapt both the reference substrate values and the controller λ-parameters. These developments will be studied in the future.
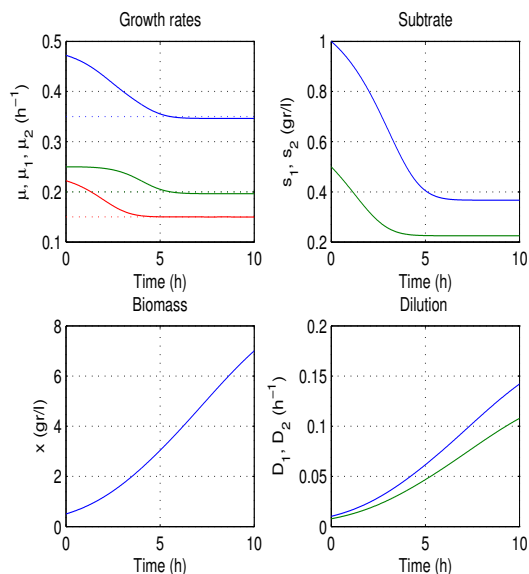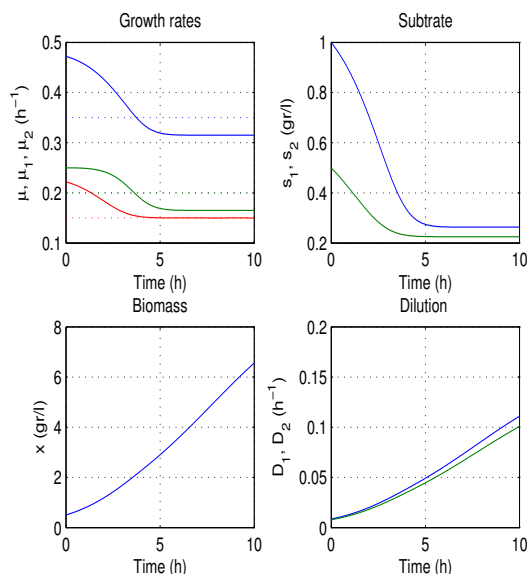
Fig. 5. Case 3

Fig. 6. Case 4

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