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Model of Bacteria Mutualism in a Chemostat: Analysis and Optimization with Interval Detector

Juan Carlos Arceo, Olivier Bernard, and Jean-Luc Gouzé

Abstract—This work focuses on a model of two bacteria growing and exchanging nutrients in a chemostat. Bacterial uptake rate is described via Michaelis-Menten equations; we assume a constant yield and metabolite production directly proportional to bacterial growth. We analyse the model via a reduced order system, then, conditions to determine existence and global stability of the equilibria are given in terms of the dilution rate. Finally, bacterial productivity is maximized; an interval detector is designed to estimate this productivity and an optimization strategy for periodic dilution rates is proposed.

Index Terms—Bacteria, Interval detector, Metabolite cross-feeding, Mutualism, Optimization

I. INTRODUCTION

Symbiosis is a concept employed to describe organisms living in a close association [1]; mutualism is a type of symbiosis that has been described as the interaction of species in which they obtain benefits from each other [2], [3]. In [4] an instance of mutualism (syntrophy) was modeled and analysed, in the syntrophic case at least one of the organisms can grow without the other. Examples of mutualism are found in plants and seed dispersers [5], or metabolic cross-feeding [6], defined as the interaction in which a bacteria consumes the metabolite produced by another bacteria. In this article we present the model, analysis and optimization of two bacteria under metabolic cross-feeding in a continuous chemostat.

We focus on a generic structure that characterizes a loop in an ecosystem. The structure is very general and can represent at least partially systems including such a loop: primary producers of detritic matter recycled by bacteria into nutrients [7]; oxygen produced by phytoplankton is consumed by bacteria, and it produces CO₂ to feed the phytoplankton [8].

Our contributions consist in 1) proposing a new model for mutualism 2) studying its global stability, 3) an application of monotone systems theory to derive interval detectors 4) the optimization of bacterial productivity via signal averaging.

The manuscript is organized as follows: section II presents a model for mutualistic interaction among two bacteria in a chemostat; in section III the model solutions are analysed, a model reduction is performed by means of the mass conservation principle, then, its steady-state, local and global stability are studied in terms of the chemostat dilution rate; in section IV the optimal dilution rate and substrate production term that maximizes bacterial productivity at steady-state are computed; in section V an interval detector is designed to estimate upper and lower bounds of the unmeasured states; in section VI an approach for steady-state optimization using the interval detector is presented; finally, in section VII a conclusion is given, as well as perspectives for future works are established.

II. SYSTEM DESCRIPTION AND MODELING

In this section we describe the system to be modeled. First, consider the chemostat [9] whose scheme is shown in Fig. 1; the tank volume is $V$ (m³), the inflow and outflow are denoted $F(t)$ (volume/time), the dilution rate is obtained by dividing the flow by the tank volume $d(t)=F(t)/V$ (1/time); the inflow contains two nutrients whose concentration are $s_1^n$ and $s_2^n$, moreover, there are two bacteria population inside the bioreactor $x_1$ and $x_2$ (mass/volume). Time dependency of the variables is dropped thereafter for notation simplicity. We assume that the bacteria $x_1$ is growth-limited by the substrate $s_1$, and it produces substrate $s_2$ while growing; the bacteria $x_2$ is growth-limited by the substrate $s_2$, and while it grows it produces the substrate $s_1$. The interaction among bacteria and substrates inside the chemostat has been illustrated in Fig. 2, and their rate of change (Δ) can be described by:

\[ \Delta = \text{input}_1 - \text{uptake}_{x_1} + \text{production}_{x_2} - \text{outflow}_1, \]

\[ \Delta = \text{growth}_{x_1} - \text{outflow}_1, \]

\[ \Delta = \text{input}_2 - \text{uptake}_{x_2} + \text{production}_{x_1} - \text{outflow}_2, \]

\[ \Delta = \text{growth}_{x_2} - \text{outflow}_2, \]

where $\text{input}_1 = ds_1^n$ and $\text{input}_2 = ds_2^n$ are the inflow of growth-limiting substrates, the $\text{outflow}$ terms indicate the flow of substances out of the tank. The substrate consumed by the bacteria $x_1$ and $x_2$ are uptake $x_1 = \frac{m_1 x_1}{\gamma_1 (s_1^n + s_2^n)}$, and uptake $x_2 = \frac{m_2 x_2}{\gamma_2 (s_1^n + s_2^n)}$, respectively; these are Monod functions, also known as Michaelis-Menten equations [9], where $m_i$, $a_i$ and $\gamma_i$ denote the maximum growth rate, half-saturation constant and

\[ F(t) \]

input flow

\[ (s_1^n, s_2^n) \]

output flow

\[ F(t) \]

\[ \text{Bacteria 1} (x_1) \]

\[ \text{Bacteria 2} (x_2) \]

Fig. 1. Continuous stirred tank bioreactor with two bacteria.
yield constant for the bacteria $i$, with $i \in \{1, 2\}$, respectively. The yield constants are the mass relation between formed organism and consumed substrate, $\gamma_i = \frac{\text{mass of } s_i \text{ formed}}{\text{mass of } x_i \text{ consumed}}$. Bacterial growth is the product of substrate consumption and its yield constant, i.e., $\text{growth } x_i = \frac{m_{x_i}}{m_{s_i}} x_i$ and growth $x_2 = \frac{m_{x_2}}{m_{s_2}} x_2$.

The production $x_1 = \beta_1 \left( \frac{m_{x_1} x_1}{s_1 + a_1} \right)$ term indicates the production of $s_2$ by the bacteria $x_1$ and production $x_2 = \beta_2 \left( \frac{m_{x_2} x_2}{s_2 + a_2} \right)$ indicates the production of $s_1$ by the bacteria $x_2$, where $\beta_1 > 0$ and $\beta_2 > 0$. Combining the previous as indicated in (1) yields:

$$
\begin{align*}
\dot{s}_1 &= ds_1 - \frac{m_{x_1} x_1}{s_1 + a_1} + \beta_2 \frac{m_{x_2} x_2}{s_2 + a_2} - ds_1, \\
\dot{s}_2 &= ds_2 - \frac{m_{x_2} x_2}{s_2 + a_2} + \beta_1 \frac{m_{x_1} x_1}{s_1 + a_1} - ds_2, \\
\dot{x}_1 &= \frac{m_{x_1} x_1}{s_1 + a_1} - dx_1, \\
\dot{x}_2 &= \frac{m_{x_2} x_2}{s_2 + a_2} - dx_2.
\end{align*}
$$

Now, we proceed to study the properties of the model.

### III. MODEL ANALYSIS

#### A. Positive invariance

The model solutions must be coherent; in our case the states correspond to concentrations; thus, they must be nonnegative. First, consider the bacteria trajectory at the border of the positive orthant, that is, $x_1 = 0$ and $x_2 = 0$ in (2); which yields $\dot{x}_1 = 0$ and $\dot{x}_2 = 0$. Therefore, if the bacteria concentrations were initialized with a nonnegative value their solution will be nonnegative, $x_1(0) \geq 0$ and $x_2(0) \geq 0$ implies $x_1(t) \geq 0$ and $x_2(t) \geq 0, \forall t \geq 0$. Now, if the substrates are $s_1 = 0$ and $s_2 = 0$ in (2) gives $\dot{s}_1 = ds_1^0 + \beta_2 \left( \frac{m_{x_2} x_2}{s_2 + a_2} \right)$ and $\dot{s}_2 = ds_2^0 + \beta_1 \left( \frac{m_{x_1} x_1}{s_1 + a_1} \right)$; so, if they are nonnegative their solution is nonnegative, $s_1(0) \geq 0$ and $s_2(0) \geq 0$ implies $s_1(t) \geq 0$ and $s_2(t) \geq 0, \forall t \geq 0$.

#### B. Model reduction

Chemostat systems must satisfy the mass conservation principle, and a common approach is to reduce the order of the model [10], it can be either to avoid numerical errors in simulation or to simplify its analysis [11]. First define $\dot{\tilde{x}} = [s_1 \quad x_1 \quad s_2 \quad x_2]^T$, and rewrite (2) in the form $\dot{\tilde{x}} = \text{K} \cdot (\tilde{x}) + \text{K} \cdot (\tilde{x})_i:

$$
\begin{pmatrix}
\tilde{s}_1 \\
\tilde{x}_1 \\
\tilde{s}_2 \\
\tilde{x}_2
\end{pmatrix} =
\begin{pmatrix}
\frac{1}{\gamma_1} & 0 & \beta_2 & 0 \\
0 & \frac{1}{\gamma_2} & 0 & \beta_1 \\
\beta_2 & 0 & \frac{1}{\gamma_1} & 0 \\
0 & \beta_1 & 0 & \frac{1}{\gamma_2}
\end{pmatrix}
\begin{pmatrix}
s_1 \\
x_1 \\
s_2 \\
x_2
\end{pmatrix} +
\begin{pmatrix}
\gamma_1 & 0 & 0 & -\gamma_1 \beta_2 \\
0 & \gamma_2 & -\gamma_2 \beta_1 & 0 \\
0 & 0 & \gamma_1 & 0 \\
0 & 0 & 0 & \gamma_2
\end{pmatrix}
\begin{pmatrix}
\tilde{s}_1 \\
\tilde{x}_1 \\
\tilde{s}_2 \\
\tilde{x}_2
\end{pmatrix}
$$

where $K$ is the stoichiometric matrix, with $\text{rank}(K) = m < n$, and $n$ is the number of states. Then, find a matrix $N$ in the left-kernel of $K$, i.e., $3N \neq 0 : NK = 0$; a possible choice is $N = [\gamma_1 1 0 -\gamma_1 \beta_2]$, and define the variables $z = N \tilde{x}$:

$$
z_1 = \gamma_1 s_1 + x_1 - \beta_2 \gamma_1 x_1, \quad z_2 = x_2 + \gamma_2 s_2 - \beta_1 \gamma_2 x_1.
$$

Their dynamics are $\dot{z} = N \tilde{x} = NK(x) + N \text{K} \cdot (\tilde{x})_i$, yields to $\dot{z}_1 = d \left( \gamma_1 s_1 + x_2 - \beta_2 \gamma_1 x_1 \right)$ and $\dot{z}_2 = d \left( s_2 + \beta_1 x_1 - \beta_2 \gamma_2 x_1 \right)$, substitute $\beta_1 \gamma_2 x_1 = x_2 - 2 \gamma_2 s_2$ and $\beta_1 \gamma_2 x_1 = x_2 - x_2 - 2 \gamma_2 s_2$, to get $\dot{z}_1 = -d x_1 - \beta_2 \gamma_1 x_1$, $\dot{z}_2 = -d x_2 - d \gamma_2 s_2$.

Now, rewrite the substrates as $s_1 = \beta_2 x_2 + \frac{\gamma_2 s_2}{\beta_1}$ and $s_2 = \beta_2 x_2 + \frac{\gamma_2 s_2}{\beta_1}$, $\frac{\gamma_2 s_2}{\beta_1}$. After the two pseudo first integral described by $z_1$ and $z_2$ in (5) have converged to their equilibria we can substitute $z_1 = \gamma_1 s_1$ and $z_2 = \gamma_2 s_2$ in (5) to obtain

$$
\dot{x}_1 = -\left( \frac{m_1}{\beta_2 x_2 + \frac{s_1}{\gamma_2} + a_1} \right) - d x_1, \quad \dot{x}_2 = -\left( \frac{m_2}{\beta_1 x_1 + \frac{s_2}{\gamma_2} + a_2} \right) - d x_2,
$$

which describe the chemostat behavior after an initial transient. In the next subsection we will analyse their equilibrium points.

### C. Equilibrium points

This subsection is dedicated to obtain the equilibrium points of the reduced order model. For notation simplification consider $\xi_1 = m_1 - d$, $\xi_2 = m_2 - d$, $\xi_3 = \xi_1 \xi_2 - da_1$, $\xi_4 = \xi_2 s_2 - da_2$, and $\xi_5 = 1 - \gamma_1 \gamma_2 \beta_1 \beta_2$. The equilibrium points are at the intersection of the nullclines (6); these are:

$$
\begin{align*}
\dot{x}_1 &= \frac{m_1}{\beta_2 x_2 + \frac{s_1}{\gamma_2} + a_1} - d, \\
\dot{x}_2 &= \frac{m_2}{\beta_1 x_1 + \frac{s_2}{\gamma_2} + a_2} - d.
\end{align*}
$$

1) The trivial case ($x_1^* = 0, x_2^* = 0$), known as washout. 2) The case ($x_1^* \neq 0, x_2^* = 0$) yields to $x_1^* = \gamma_1 \left( s_1 + a_1 \right)$, it exists if $d < m_1 a_1$ holds. 3) The case ($x_1^* = 0, x_2^* \neq 0$) it gives $x_2^* = \gamma_2 s_2 - \frac{da_2}{m_2 a_2}$, and exists if $d < m_2 a_2$ holds.

4) The interior equilibrium ($x_1^* \neq 0, x_2^* \neq 0$) is located at

$$
\begin{align*}
x_1^* &= \frac{1}{\xi_3} \left( s_1 + a_1 \right), \\
x_2^* &= \frac{1}{\xi_4} \left( s_2 + a_2 \right).
\end{align*}
$$

It will exist if $\gamma_1 \gamma_2 \beta_1 \beta_2 < 1$ and $d < \min \left( \frac{m_1 a_1}{\omega_1}, \frac{m_2 a_2}{\omega_2}, \frac{m_1 a_2}{\omega_1 + \omega_2}, \frac{m_2 a_1}{\omega_1 + \omega_2} \right)$.
hold, with \( \omega_{11} = \frac{(m_1+m_2)(s_1^n + \beta_2 \gamma_1 s_2^n) + a_1 m_1 s_1 + a_2 s_2}{a_1 s_1^n + (a_2 + a_1^n) s_2^n \gamma_2}, \)
\[ \omega_{21} = -\frac{(m_1+m_2)(s_1^n + \beta_1 \gamma_1 s_2^n) + a_2 m_2 s_2 + a_1 s_1}{a_2 s_2^n + (a_1 + a_2^n) s_1^n \gamma_1}, \]
\[ \omega_{12} = \frac{a_1 s_1^n + (a_2 + a_1^n) s_2^n \beta_1 \gamma_1}{a_2 s_2^n + (a_1 + a_2^n) s_1^n \beta_2 \gamma_2}. \]

From the previous we can see that the interior equilibrium point (peristence case) might exist even if one of the input substrates is equals to zero, \( s_1^n = 0, s_2^n = 0 \). Moreover, the bacteria concentration higher in the case of coexistence compared to their isolation value [5]. The steady-state for \( d = 0 \) is out of the scope for this manuscript because it does not corresponds to a continuous bioreactor, but to a batch culture. Each equilibrium point of the reduced model (6) corresponds to a unique point of the original (2) obtained by substituting \( s_1^n, s_2^n, x_1^* \) and \( x_2^* \) into \( s_1^* = \beta_2 x_2^* + \frac{x_1^*-x_1}{\gamma_1} \) and \( s_2^* = \beta_1 x_1^* + \frac{x_2^*-x_2}{\gamma_2} \).

D. Local stability of the bacteria concentration

Now we study the local stability of the equilibrium points. It can be done either by computing a linearization of the reduced order model (6) at the equilibrium points (Jacobian matrix) and examining its eigenvalues; or since (6) is a second order, another alternative is verifying that its trace and determinant are negative and positive, respectively. The Jacobian matrix is

\[ J|_{x_1,x_2} = \begin{bmatrix} \mu_1 - d - \left( \frac{s_1^n}{\gamma_1} \right) \mu_1' & \beta_2 x_2^* \beta_1 x_1^* \mu_1'' \\ \beta_1 x_1^* \beta_2 x_2^* \mu_2'' & \mu_2 - d - \left( \frac{s_2^n}{\gamma_2} \right) \mu_2' \end{bmatrix}, \]

where \( \mu_1 = \frac{m_1 (\beta_2 x_2^* + \beta_1 x_1^* - \frac{x_1^*+x_2^*}{\gamma_1})}{a_1 m_1 + a_1^n s_1^n + a_2 s_2^n} \), \( \mu_2 = \frac{m_2 (\beta_1 x_1^* - \frac{x_1^*+x_2^*}{\gamma_2})}{a_2 m_2 + a_2^n s_2^n + a_1 s_1^n} \),

\[ \mu_1' = \frac{-a_1^n + s_1^n + s_2^n}{a_1 m_1 + a_1^n s_1^n + a_2 s_2^n} \quad \text{and} \quad \mu_2' = \frac{-a_2^n + s_2^n + s_1^n}{a_2 m_2 + a_2^n s_2^n + a_1 s_1^n}. \]

The corresponding values for each case are:

1) The eigenvalues of the Jacobian matrix for the washout case are \( \sigma_1 = \frac{\mu_1'}{\mu_1''} \) and \( \sigma_2 = \frac{\mu_2'}{\mu_2''} \). Therefore, it is stable if \( d > \max \left( \frac{m_1 s_1^n + m_2 s_2^n}{a_1 s_1^n + a_2 s_2^n} \right) \) holds.

2) In the case \( (x_1^* \neq 0, x_2^* = 0) \) the trace and determinant are \( J_{11} + J_{22} \) and \( J_{11} J_{22} \), respectively, with \( J_{11} = \frac{4s_1^n}{\gamma_1 m_1} \) and \( J_{22} = \frac{m_2 s_2^n + a_2 s_2^n + a_2^n s_2^n}{a_2 s_2^n + a_1^n s_1^n} \), \( J_{11} J_{22} > 0 \) and \( J_{11} + J_{22} > 0 \) as \( d < \min \left( \frac{m_1 s_1^n}{a_1 m_1 + a_1^n s_1^n}, \frac{m_2 s_2^n}{a_2 m_2 + a_2^n s_2^n} \right) \) holds.

3) If we move from the point \( p^i \) to the point \( p^j \) we must cross two nullclines to get there \( (x_1 = 0 \text{ and } x_2 = 0) \); therefore, the sign of both time derivatives changes to \( x_1 < 0 \) and \( x_2 < 0 \), and the trajectory is indicated by \( p^j \).

4) In order to go from \( p^j \) to \( p^i \) we must cross the nullcline corresponding to \( x_2 = 0 \), therefore, there is a change of sign on \( x_1 \), which produces \( x_1 > 0 \) and \( x_2 < 0 \) as indicated by the direction of the trajectory in \( p^i \).

Notice that the nullcline \( x_1 = 0 \) can only be crossed by means of \( x_2 \) and vice-versa. Thus, any trajectory on the regions where the points \( p^2 \) or \( p^4 \) are located will be pushed to the regions of the points \( p^3 \) or \( p^1 \); the latter regions are invariant, that is, trajectories can not escape from them. After that the solution can only head towards the coexistence equilibrium. Then, we can conclude that the interior equilibrium is globally stable.

We have proven global stability for the reduced system in dimension two. Global stability for the full system (5) is easy to deduce by classical arguments of asymptotically autonomous systems. Considering the linear dynamics of the equations for \( (x_1, x_2) \), we can for example apply the Convergence Theorem of Appendix F in [9]. Moreover, we remark that the system (5) is a monotone and cooperative system (see section V); therefore, it has strong properties of convergence. It is easy to see that we would be able to study the same mutualism system in the case of \( N \)-bacteria forming a closed-loop to feed each other, using these properties of stability of monotone system. This cannot be detailed here due to lack of space.
Example 1. Consider the parameters $s_{1i}^{in}=0.4$ (g/l), $m_{i}=0.8$ (per hour), $a_{i}=0.5$ (g/l), $\gamma_{i}=0.75$, $\beta_{i}=0.12$ and $d=0.01$ (per hour), with $i \in \{1, 2\}$. The phase-portrait obtained is in Fig. 4. According to subsections III-D and III-D the interior equilibrium is $(x_{1}^{*}, x_{2}^{*})=(0.3245, 0.3245)$, while the isolated equilibria are $(x_{1iso}^{*}, x_{2iso}^{*})=(0.2953, 0.2953)$; it illustrates that concentrations in coexistence are higher than in isolation.

IV. STEADY-STATE OPTIMIZATION

Here we find the optimal dilution rate and metabolite production term to maximize the productivity of the chemostat.

A. Optimal dilution rate

First, let us define the bacterial productivity as the product of bacteria concentration at steady-state and the dilution rate $\alpha$. Optimal dilution rate $\alpha_{opt}$

\[ Y(d) = dx_{2}^{*} = \frac{\gamma_{1}}{\xi_{1}} \left( \frac{p_{2}(d)}{p_{2}(d)} \right) = \frac{\gamma_{1}}{\xi_{1}} \left( \frac{\delta_{1} s_{1}^{in} + \delta_{2} a_{1} s_{1}^{in} + \delta_{3} a_{1} s_{1}^{in}}{m_{1} + m_{2} + m_{3}} \right), \] (8)

with $\delta_{1} = a_{2} + s_{2}^{in} + (a_{1} + s_{1}^{in}) \beta_{1}$, $\delta_{2} = -(m_{1} + m_{2}) (s_{2}^{in} + \beta_{1} s_{1}^{in}) - m_{2} a_{1} \beta_{1}$, and $\delta_{3} = m_{2} m_{3} (s_{2}^{in} + \beta_{1} s_{1}^{in})$. The critical points of (8) can be obtained by solving

\[ \frac{\partial Y(d)}{\partial d} = \frac{\gamma_{1} p_{2}(d) a_{2}(d) a_{2}(d) a_{2}(d)}{\xi_{1} p_{2}(d) a_{2}(d) a_{2}(d)} = \delta_{1} p_{2}(d) \] (9)

d to determine if $d_{c}$ corresponds to a local maximum $\left( \frac{\partial Y(d)}{\partial d} < 0 \right)$ of (8), the sign of $\frac{\partial^{2} Y(d)}{\partial d^{2}}$ is determined by

\[ \frac{\partial^{2} Y(d)}{\partial d^{2}} = 4d^{3} + 3d \delta_{1} \delta_{1} + 2a_{2} \delta_{2} + \Delta_{y}. \] Finally, we must verify that $d_{c}$ satisfies the coexistence conditions in subsection III-C.

Example 2 (Optimal dilution). Consider the previous chemostat values and compute the solutions for $p_{3}(d)$, the critical dilution rates obtained were $d_{c}=[0.2037, 0.8, 1.3963]$. Only $d_{c}=0.2037$ satisfies the conditions for coexistence. Moreover, evaluating this critical dilution rate gives $\frac{\partial^{2} Y(d)}{\partial d^{2}}=-0.4240$. As indicated, $d_{c}$ corresponds to a local maximum. The bacterial productivity function $Y(d)$ for the mutualistic and isolated cases within an operational range $d \in [0.0001, 0.35]$ are shown in Fig. 5 as well as the optimal dilution rate computed. As foreseen in Fig. 5 the mutualistic case is more advantageous than the isolated, since higher concentrations can be achieved.

B. Optimal substrate production

Optogenetic techniques [12] allow us to control the metabolite production in bacteria. Thus, now we assume that our variable of control is the metabolite production of bacteria $x_{1}$ and we want to find an optimal $\beta_{1}$ to maximize $\dot{Y}(\beta_{1}) = dx_{2}^{*} = \frac{\gamma_{1} p_{2}(d)}{p_{2}(d)}$, with $p_{1}(\beta_{1}) = \frac{d_{1} \gamma_{1} s_{1}^{in} - \frac{d_{2} \gamma_{1} s_{2}^{in}}{m_{2} - d} + \beta_{1} \gamma_{1} s_{1}^{in}}{(1-\beta_{1} \gamma_{1} s_{1}^{in})}$ and $p_{2}(\beta_{1}) = 1 - \beta_{1} \gamma_{1} s_{1}^{in}$. Computing $\frac{\partial Y(\beta_{1})}{\partial \beta_{1}} = \frac{dx_{2}^{*} (s_{1}^{in} - \frac{a_{1}}{m_{1}-d})}{(1-\beta_{1} \gamma_{1} s_{1}^{in})}$, we can see that the sign of this partial derivative is constant $\frac{\partial Y(\beta_{1})}{\partial \beta_{1}} > 0$. Thus, the maximum of the cost function $Y(\beta_{1})$ is determined by the maximum value of $\beta_{1}$ that we can provide.

V. INTERVAL DETECTOR

We assume that the initial conditions of the system $X(0)= [x_{1}(0) \ x_{2}(0) \ z_{1}(0) \ z_{2}(0)]^{T}$ are unknown and bounded by maximum and minimum known values, $X(0) \geq X(0) \geq X(0)$. A system is said observable when its states can be reconstructed from its output. Similarly, a system is detectable when unobservable states are stable. We will employ the concept of detectability, as well as other model properties to design an interval detector that estimates upper and lower bounds of the states. These bounds can be used to estimate variables of interest such as bacterial productivity $\dot{d_{x_{2}^{*}}}$. The system (5) is cooperative, which means that the off-diagonal terms of its Jacobian matrix $J$ are nonnegative, this matrix has the form

\[ J = \begin{bmatrix}
- \ J_{12} & \ J_{13} & 0 \\
- \ J_{13} & 0 & 0 \\
0 & 0 & - \ J_{24}
\end{bmatrix}, \]

where $J_{12} = \frac{a_{1} \delta_{1} \gamma_{1} s_{1}^{in} m_{2}}{(z_{1}-x_{1}+\gamma_{1}(a_{1}+\gamma_{2}x_{2}))^{2}}$. 

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Fig. 4. Phase portrait of the bacteria ($x_{1}-x_{2}$) for Example 1, nullclines for $\dot{x}_{1}=0$ and $\dot{x}_{2}=0$ are indicated in green and red, respectively. Trajectories from simulation with initial conditions (0.05, 0.35) and (0.35, 0.05).

Fig. 5. Productivity of $x_{2}$ in mutualism and isolated cases.
with \( (i, j) \in \{1, 2\} \), \( i \neq j \), where \((\bar{x}_i, \bar{z}_i)\) and \((\bar{x}_j, \bar{z}_j)\) corresponds to the upper and lower estimations of the bacteria concentration and auxiliary variables, respectively. We can obtain an asymptotic estimation of the states from it. In Fig. 6 the states time evolution and their bounds is shown, with a dilution rate \( d = 0.2037 \), the initial conditions for the system, upper and lower estimations were \( X(0) = [0.01 \ 0.05 \ 0.1555 \ 0.1991]^T \), \( \bar{X}(0) = [0.02 \ 0.06 \ 0.1721 \ 0.2157]^T \) and \( \bar{X}(0) = [0.005 \ 0.045 \ 0.1472 \ 0.1908]^T \), respectively.

Since \( \underline{x}_2 \leq x_2 \leq \bar{x}_2 \) holds, we can compute upper and lower bounds of the productivity as \( \underline{d}_{x_2} \leq d_{x_2} \leq \bar{d}_{x_2} \), we will employ it to optimize the dilution rate in the following section.

**VI. ONLINE DILUTION OPTIMIZATION**

In this section we assume that the dilution rate is periodic, to our knowledge, an analytical solution for a periodic dilution rate it not available. Thus, here we will present different optimization strategies using the interval detector. Our approach is inspired by the extreme seeking algorithm [13].

**A. Average optimization**

Consider a periodic dilution rate \( d(t) = a (1+b \sin(\omega t)) \), where \( a > 0, \ 0 < b < 1 \) and \( \omega > 0 \). Then, assume the lower estimation of the bacteria is available, \( \underline{x}_2(t) \) and we want to maximize the average of the estimated productivity \( \text{Avg} \ (d(t) \underline{x}_2(t)) \) measured over a period \( T = \frac{1}{f}, \omega = 2\pi f \), with a sufficiently large initial averaging time \((t_0)\), that is, the system trajectories are near to a periodic regime, then, our objective function is \( \text{Avg} \ (d_{x_2}) = \frac{1}{T} \int_{t_0}^{t_0+T} d(t) \underline{x}_2(t) \ dt \).

Since the signal order is preserved, the system and the interval detector converge to the same periodic regime; so, it follows that \( \text{Avg} \ (d_{x_2}) \leq \text{Avg} \ (d_{\bar{x}_2}) \leq \text{Avg} \ (d_{\bar{x}_2}) \), holds.

**Example 3.** The parameters considered are \( a = 0.01, \ f = 0.005 \ \text{Hz} \) and \( b = 0.5 \), the evolution of the productivity is shown in Fig. 8a. The averages obtained considering \( a \in (0.001, 0.355) \) are in Fig. 8b (black line). Only \( \text{Avg} \ (d_{x_2}) \) is shown because the states and the estimated are in a periodic regime, so the signals average are overlapped.

**B. Heuristic optimization**

Here we present an heuristic method to optimize the dilution rate. Consider a periodic dilution rate \( d(t) = a_i (1+b \sin(\omega t)) \), where \( a_i > 0, \ 0 \leq b \leq 1 \) and \( \omega > 0 \) and follow this algorithm:

1) First, provide positive initial values for \( a_0 \) and \( \epsilon_0 \), then, measure the T-average bacterial production.
The mathematical model for two bacteria growing under cross-feeding interaction was presented, its steady-state and stability were studied. Through an analysis of the vector field trajectories we could conclude that when the interior equilibrium (stability case) exists it is globally stable. As in other mutualism cases the bacteria concentration in coexistence is higher than isolation. The optimization of biomass production for a bacteria of interest at steady-state was carried out.

Also, an interval detector was designed to compute upper and lower bounds of the trajectories. Here we assumed unknown initial conditions with known bounds and no measurements of the variables to estimate were required. The designed interval detector was employed to estimate the chemostat productivity, then, algorithms to optimize this productivity were presented. One of them based on the extremum seeking algorithm was adapted to periodic dilution rates, it is robust since is always looking for optimal values in a neighbourhood.

For the future of this work we can consider mutualism appearing in different scenarios such as algae and bacteria, that would require different models (like Droop [14] or Geider [15] models), but the analysis and optimization methods could be similar to the ones in this manuscript. Finally, it might be convenient improve the dilution rate update policy to take into account the signals provided by the interval detector. Additionally, we can consider uncertainties in the model parameters and take advantage of the Monod functions structure to build an interval detector to estimate bounds of the unmeasured states.

### REFERENCES


