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Modeling the Production of Genetically Modified Organisms in a Chemostat

Neli S. Dimitrova

A dynamical model of plasmid-bearing, plasmid-free competition in the chemostat with uncertain inhibitory kinetics is studied. Based on a feedback control, an extremum seeking model-based algorithm is designed and applied to maximize the concentration of the plasmid-bearing organisms in real time. Computer simulation is carried out to demonstrate the theoretical results.

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1. Introduction.

The chemostat is widely used for model-based tests and experiments in microbial biology. In recent years it is also exploited for laboratory production of substances by genetically altered organisms. The modification is typically obtained by introducing a DNA molecule into the cell in the form of a plasmid. These plasmid-bearing organisms are then grown in the chemostat. During the reproductive process, however, the plasmid might be lost, resulting in the appearance of a second competitor, the plasmid-free organism [2], [3].

We consider the following model describing the competition between plasmid-bearing and plasmid-free organisms in the chemostat [2], [3], [5]

$$
\begin{aligned}
\dot{s} &= D(s^0 - s) - x_1\mu_1(s) - x_2\mu_2(s) \\
\dot{x}_1 &= x_1\left((1-q)\mu_1(s) - D\right) \\
\dot{x}_2 &= x_2(\mu_2(s) - D) + q x_1\mu_1(s),
\end{aligned}
\tag{1}
$$

where

$s(t)$ is the substrate concentration
$x_1(t)$ is the concentration of the plasmid-bearing organism
$x_2(t)$ is the concentration of the plasmid-free organism
s^0 is the input substrate concentration
D is the dilution rate in the chemostat
$\mu_i(s)$ are the specific growth rate (uptake) functions, $i = 1, 2$
q is the plasmid loss probability, $0 < q < 1$.

For biological reasons, it is assumed that $0 \le s \le s^0$ is fulfilled. In what follows we shall assume that s^0 is fixed and D is the control variable.

In [2], [3] this model is studied under the following assumptions on the uptake functions:

(i) $\mu_i(s)$ are monotone increasing, continuously differentiable and bounded with $\mu_i(0) = 0$, $\mu_i(s) > 0$ for all $s > 0$, $i = 1, 2$;
(ii) the graphs of $(1-q)\mu_1(s)$ and $\mu_2(s)$ intersect once at a point $s^* \in (0, s^0)$, i. e. $(1-q)\mu_1(s^*) = \mu_2(s^*) = D^*$.
(iii) $(1-q)\mu_1(s) < \mu_2(s)$ for all $s \in (0, s^*)$ and $(1-q)\mu_1(s) > \mu_2(s)$ for all $s > s^*$.

Growth rate functions satisfying *(i)* are known as model functions of Holling type II; they have the form

$$\mu_i(s) = \frac{m_i s}{a_i + s} \quad \text{or} \quad \mu_1(s) = \frac{m_i s^2}{a_i + s^2}.$$

Condition *(iii)* means that for high substrate concentration ($s > s^*$) the plasmid-bearing organism is a superior competitor, while for low levels ($s < s^*$) the plasmid-free organism is superior. If the plasmid-free organism is a superior competitor, then this leads to the extinction of the plasmid-bearing species [2], which is an undesirable outcome of the process. Practical experiments show [5] that under certain conditions the plasmid-bearing organism can be a superior competitor and thus coexistence of both organisms is possible. In fact, it is not possible to achieve total efficiency of the chemostat by excluding the plasmid-free organisms [2], [3]. The best that can be done is to operate the chemostat at a state, where both populations survive. As it is shown in [2], [3] this is possible if the control input D is chosen very carefully. However, in the case when the uptake functions $\mu_1(s)$ and $\mu_2(s)$ are not exactly known but only bounds for them are available, coexistence might be lost leading to extinction of the plasmid-bearing organism. The latter drawback can be overcome by implementing the dilution rate D as a feedback law, depending on the system states, i. e. $D = D(s, x_1, x_2)$; then the model (1) takes the form

$$\begin{aligned} \dot{s} &= D(s, x_1, x_2) \cdot (s^0 - s) - x_1\mu_1(s) - x_2\mu_2(s) \\ \dot{x}_1 &= x_1\left((1-q)\mu_1(s) - D(s, x_1, x_2)\right) \\ \dot{x}_2 &= x_2\left(\mu_2(s) - D(s, x_1, x_2)\right) + qx_1\mu_1(s). \end{aligned} \tag{2}$$

Definition 1.1 [3]. A positive dynamic system is permanent if there exists a compact set K, $K \subset int\,(R^3_+)$, which attracts all solutions starting in $int\,(R^3_+)$.

Theorem 1.1 [3]. *For any* $\epsilon \in (0, D^*)$ *and* $k > \hat{k}$ *with* $\hat{k} = \dfrac{D^* - \epsilon}{s^0 - s^*}$ *define the following feedback*

$$D(x_1, x_2) = k(x_1 + x_2) + \epsilon. \tag{3}$$

Then the system (2) *is permanent. Moreover, there exists an equilibrium point* $P = (s^p, x_1^p, x_2^p) > 0$ *which is globally asymptotically stable for (2) with the feedback (3) with respect to initial conditions* $(s(0), x_1(0), x_2(0)) > 0$.

It is shown in [3], that the feedback (3) ensures permanence of (2) also in the case of uncertain uptake functions if ϵ and k are chosen in a proper way.

According to Theorem 1.1, it is not possible to achieve total efficiency (exclusion of plasmid-free organisms) since there is no attracting solution with $x_2(t) = 0$ and $x_1(t) > 0$. The open problem posed in [2] is how to optimize the location of the point P to maximize the concentration of the plasmid-bearing organisms.

In this paper we solve the latter problem under more general assumptions on the uptake functions; we consider the case when at least one of $\mu_1(s)$ and $\mu_2(s)$ is of Holling type III, i. e. it exhibits inhibition, and the graphs of $(1-q)\mu_1(s)$ and $\mu_2(s)$ intersect at two points. Assuming further, that $\mu_1(s)$ and $\mu_2(s)$ are uncertain but bounded, Theorem 1.1 is modified so that to guarantee permanence of the closed-loop system in this case. A model-based extremum seeking algorithm is applied to maximize the concentration of the plasmid-bearing organisms in real time.

2. The model with non-monotone uptake functions

We consider now the model (1) under the following assumptions on the uptake functions μ_1 and μ_2:

(A1) $\mu_i(s)$ are continuously differentiable,
$\mu_i(0) = 0$ and $\mu_i(s) > 0$ for $s > 0, i = 1, 2$
(A2) $\mu_2(s)$ is monotone increasing for $0 < s < s^0$
(A3) $\mu_1(s)$ has a maximum at a unique point $s^m \in (0, s^0)$
(A4) $(1-q)\mu_1(s)$ and $\mu_2(s)$ intersect twice at points s_1 and s_2,
$s_1 < s^m < s_2 < s^0$ and
$(1-q)\mu_1(s_1) = \mu_2(s_1) = D_1$
$(1-q)\mu_1(s_2) = \mu_2(s_2) = D_2$
(A5) $\mu_2(s) > (1-q)\mu_1(s)$ for all $s \in (0, s_1) \cup (s_2, s^0)$ and
$\mu_2(s) < (1-q)\mu_1(s)$ for all $s \in (s_1,\ s_2)$.

Denote $D_{\max} = \max\{(1-q)\mu_1(s^m),\ \mu_2(s^0)\}$; it follows from (A4) that $D_1 < D_2 < D_{\max}$ and $D_2 < \mu_2(s^0)$.

Figure 1 visualizes the uptake functions satisfying the above assumptions.

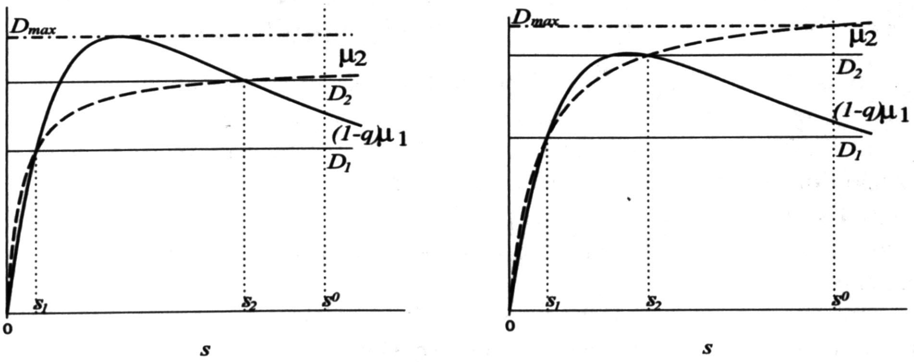

Figure 1: The uptake functions $(1-q)\mu_1(s)$ and $\mu_2(s)$

Equilibrium points for constant dilution rate. Let us consider the dilution rate D is a parameter (not depending on the time t). The equilibrium points are solutions of the following system of algebraic equations

$$D(s^0 - s) - x_1\mu_1(s) - x_2\mu_2(s) = 0$$

$$
\begin{aligned}
&x_1\left((1-q)\mu_1(s)-D\right)=0\\
&x_2(\mu_2(s)-D)+qx_1\mu_1(s)=0.
\end{aligned}
$$

Obviously, the equilibrium point

$$E_0=(s^0,\ 0,\ 0)$$

always exists; it is called a wash-out steady state. If $x_1=0$, $x_2\neq 0$ hold true, and D is chosen so that the equation $\mu_2(s)=D$ has a (unique) solution $s=\alpha_2<s^0$, then a second equilibrium point is

$$E_2=(\alpha_2,0,s^0-\alpha_2).$$

When $x_1\neq 0$ and $x_2\neq 0$ are valid, the equation $(1-q)\mu_1(s)=D$ may possess at most two solutions (depending on the value of D), denoted by α_1 and β_1, such that $\alpha_1<\beta_1<s^0$ hold true. Then the equilibrium components for x_1 and x_2 are solutions of the following two linear systems

$$
\begin{aligned}
&\mu_1(\zeta)x_1+\mu_2(\zeta)x_2=D(s^0-\zeta)\\
&q\mu_1(\zeta)x_1+(\mu_2(\zeta)-D)x_2=0,\quad \zeta\in\{\alpha_1,\beta_1\}.
\end{aligned}
$$

Denote

$$
\begin{aligned}
\Delta(\zeta)&=\mu_1(\zeta)\left((1-q)\mu_2(\zeta)-D\right)\\
\Delta_1(\zeta)&=(s^0-\zeta)D\left(\mu_2(\zeta)-D\right)\\
\Delta_2(\zeta)&=-(s^0-\zeta)\,D\,q\,\mu_1(\zeta),\qquad \zeta\in\{\alpha_1,\beta_1\}.
\end{aligned}
$$

Positive solutions for x_1 and x_2 exist if and only if

$$\Delta(\zeta)\neq 0 \text{ and } \mathit{sign}\,\Delta(\zeta)=\mathit{sign}\,\Delta_1(\zeta)=\mathit{sign}\,\Delta_2(\zeta),\quad \zeta\in\{\alpha_1,\beta_1\},$$

which is valid when

$$
\begin{aligned}
&\mu_2(\alpha_1)<D \quad\text{and}\quad \mu_2(\alpha_1)<\mu_1(\alpha_1)=\frac{D}{1-q},\\
&\mu_2(\beta_1)<D \quad\text{and}\quad \mu_2(\beta_1)<\mu_1(\beta_1)=\frac{D}{1-q}.
\end{aligned}
$$

Then there exist the steady states

$$E^e=\left(\alpha_1,\ \frac{\Delta_1(\alpha_1)}{\Delta(\alpha_1)},\ \frac{\Delta_2(\alpha_1)}{\Delta(\alpha_1)}\right),\qquad F^e=\left(\beta_1,\ \frac{\Delta_1(\beta_1)}{\Delta(\beta_1)},\ \frac{\Delta_2(\beta_1)}{\Delta(\beta_1)}\right).$$

As mentioned before, there is no equilibrium where only plasmid-bearing but no plasmid-free organisms are present (i. e. with $x_1 \neq 0$ and $x_2 = 0$ in the steady state components).

Define the set $\Omega = \{(s, x_1, x_2) \mid s \geq 0,\ x_1 > 0,\ x_2 > 0\}$.

The next theorem reports on stability of the equilibrium points.

Theorem 2.1 . *Under Assumptions (A1) to (A5), the following statements are true:*

(a) E^e *is asymptotically stable for* $D \in (D_1,\ D_2)$ *with respect to initial conditions in the set* Ω.

(b) E_0 *is asymptotically stable for* $D > D_{\max}$ *on* Ω.

(c) E_2 *is asymptotically stable on* Ω *for* $D < D_1$ *and if* $(1-q)\mu_1(s^m) < D < \mu_2(s^0)$.

(d) E_0 *and* E^e *are asymptotically stable on* Ω *for* $\mu_2(s^0) < D < (1-q)\mu_1(s^m)$.

(e) E_2 *and* E^e *are asymptotically stable on* Ω *for* $D_2 < D < \min\{(1-q)\mu_1(s^m),\ \mu_2(s^0)\}$.

(f) F^e *(if exists) is always asymptotically unstable on* Ω.

Proof. The proof follows from standard local analysis based on investigating the eigenvalues of the Jacobian matrix at the steady states and we omit it here. ■

Uncertain uptake functions. Usually the formulation of the uptake functions is based on experimental results with measurement errors. Therefore it is not possible to obtain an analytic form of the functions, but only some qualitative properties and quantitative bounds. Assume that bounds for $\mu_1(s)$ and $\mu_2(s)$ are available:

$$\mu_1(s) \in [\mu_1^-(s), \mu_1^+(s)], \quad \mu_2(s) \in [\mu_2^-(s), \mu_2^+(s)] \text{ for all } s > 0.$$

Let the boundary functions $\mu_i^-(s)$, $\mu_i^+(s)$ satisfy the following assumptions:

(B1) $\mu_i^-(s),\ \ \mu_i^+(s)$ are continuously differentiable,
$\mu_i^-(0) = 0,\ \ \mu_i^+(0) = 0$ and $\mu_i^-(s) > 0,\ \ \mu_i^+(0) > 0$ for $s > 0, i = 1, 2$;

(B2) $\mu_2^-(s)$ and $\mu_2^+(s)$ are monotone increasing for $0 < s < s^0$

(B3) $\mu_1^-(s)$ (resp. $\mu_1^+(s)$) has a maximum at a unique point s^{m^-} (resp. s^{m^+}) with $s^{m^-}, s^{m^+} \in (0, s^0)$.

(B4) There exist compact intervals $[s_1] = [s_1^-, s_1^+] \subseteq (0, s^0]$

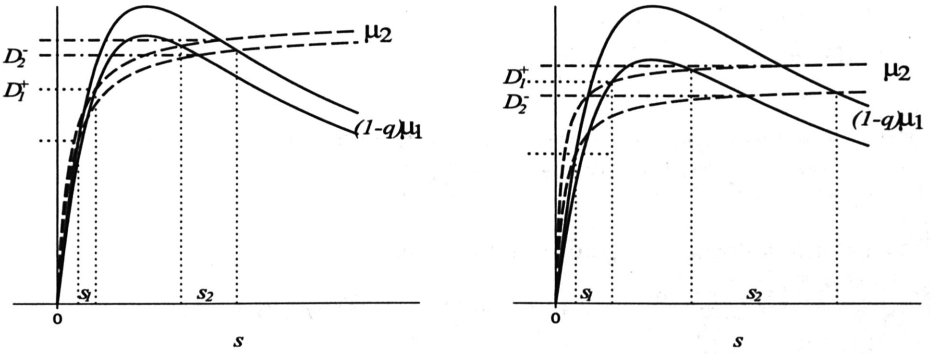

Figure 2: The uncertain uptake functions $(1-q)\mu_1(s)$ and $\mu_2(s)$

and $[s_2] = [s_2^-, s_2^+] \subseteq (0, s^0]$ such that $[s_1] \cap [s_2] = \emptyset$ and
$\mu_2^-(s_1^-) = (1-q)\mu_1^+(s_1^-)$, $\mu_2^+(s_1^+) = (1-q)\mu_1^-(s_1^+)$,
$\mu_2^+(s_2^-) = (1-q)\mu_1^-(s_2^-)$, $\mu_2^-(s_2^+) = (1-q)\mu_1^+(s_2^+)$.

$$\text{(B5)}\quad \begin{array}{ll} (1-q)\mu_1^+(s) < \mu_2^-(s) & \text{for all } s \in (0, s_1^-) \cup (s_2^+, s^0) \\ \mu_2^+(s) < (1-q)\mu_1^-(s) & \text{for all } s \in (s_1^+, s_2^-). \end{array}$$

Denote by $[D_1] = [D_1^-, D_1^+]$ and $[D_2] = [D_2^-, D_2^+]$ the projections of $[s_1]$ and $[s_2]$ respectively on the vertical axis.

If the dilution rate is constant, then it follows from Theorem 2.1, that persistence (i. e. coexistence of both organisms in the chemostat) is achievable if $[D_1] \cap [D_2] = \emptyset$ and $D \in \left(D_1^+, D_2^-\right)$, see Figure 2 (left plot). Dilution rates outside this interval do not guarantee persistence. If D_1^+ and D_2^- are very close to each other, the operator who is controlling the chemostat should be very careful in setting the dilution rate. Persistence is also not guaranteed in the case when $[D_1] \cap [D_2] \neq \emptyset$, i. e. if $D_1^+ > D_2^-$ and $D \in \left(D_2^-, D_1^+\right)$, see Figure 2 (right plot). Therefore, keeping the operating conditions s^0 and D constant and introducing uncertainty only in the uptake functions may lead to extinction of the plasmid-bearing organism or of both organisms. As we shall see in the next section, this drawback can be overcome by introducing feedback control.

3. Feedback control

Consider the model (2) where the dilution rate is implemented as a feedback law, depending on the system states, $D = D(s, x_1, x_2)$, assuming that $D(s, x_1, x_2)$ is defined for all $(s, x_1, x_2) \geq 0$ and $D(s, x_1, x_2) > 0$ holds true.

Denote by $s_*^m = \min\{s^{m^-}, s^{m^+}\}$.

Additionally to (B1)–(B5), let the following assumption is satisfied:

(B6) For any $\mu_1 \in [\mu_1]$, the derivative $\mu_1'(s)$ is enclosed by $(\mu_1^-)'(s)$ and $(\mu_1^+)'(s)$ for any $s \in [s_*^m, s^0]$.

Let the following inequality holds true:

$$\delta := D_1^- + (s^0 - s_1^-) \min \min_{s\in[s_*^m, s^0]} \{(\mu_1^-)'(s), (\mu_1^+)'(s)\} > 0. \tag{4}$$

Denote

$$\hat{D}_2 := \mu_2^+(s_2^-) = (1-q)\mu_1^-(s_2^-)$$

and define the constants

$$k_1 = \frac{D_1^+ - d}{s^0 - s_1^+}, \quad k_2 = \frac{\hat{D}_2 - d}{s^0 - s_2^-} \quad \text{for} \quad d \in (0, \delta). \tag{5}$$

Theorem 3.1. *Let the uncertain uptake functions* $[\mu_1]$ *and* $[\mu_2]$ *satisfy assumptions* (B1) *to* (B6). *For any* $d \in (0, \delta)$ *and any* $k \in (k_1, k_2)$ *define the following feedback*

$$D \equiv D(x_1, x_2) = k(x_1 + x_2) + d. \tag{6}$$

Then the closed-loop system (2) with the feedback (6) is permanent with respect to initial conditions $(s(0), x_1(0), x_2(0)) > 0$.

Proof. We shall show that for any $\mu_1 \in [\mu_1]$, $\mu_2 \in [\mu_2]$ there exists an equilibrium point $E_c = (s^c, x_1^c, x_2^c) > 0$ which is globally asymptotically stable for the closed-loop system (2) with the feedback (6). The proof is very similar to the proof of Theorem 1.1, see [3]. We present below the main and different steps of the proof for convenience. Let $\mu_1 \in [\mu_1]$, $\mu_2 \in [\mu_2]$ be any uptake functions. Define the function

$$\Sigma(s, x_1, x_2) = \frac{1}{2}(s + x_1 + x_2 - s^0)^2.$$

Then the derivative of $\Sigma(s, x_1, x_2)$ on any trajectory of (2) with the feedback (6) is

$$\dot{\Sigma}(s, x_1, x_2) = -D(x_1, x_2) \cdot \Sigma(s, x_1, x_2).$$

Since $D(x_1, x_2) > 0$ for all $(x_1, x_2) \geq 0$, it follows that $\dot{\Sigma}(s, x_1, x_2) \leq 0$. Lasalle's invariance principle implies that every solution of the closed-loop system converges to the largest invariant set, which is contained in the set $\{(s, x_1, x_2) \in$

$R^3_+ \,|\, \dot{\Sigma}(s,x_1,x_2) = 0\}$, or equivalently to the set $\{(s,x_1,x_2) \in R^3_+ \,|\, \Sigma(s,x_1,x_2) = 0\}$. This implies that all solutions of (2), starting in R^3_+ are bounded and the following relation holds true

$$\lim_{t \to +\infty} (s(t) + x_1(t) + x_2(t)) = s^0.$$

The latter implies that the set

$$\Omega^r = \left\{(s,x_1,x_2) \in R^3_+ \mid s + x_1 + x_2 = s^0\right\}$$

is invariant for (2) and the ω-limit set of every solution of (2) belongs to Ω^r. Consider the restriction of the original system (2) with the feedback (6) on Ω^r:

$$\begin{aligned} \dot{x}_1 &= x_1\Big((1-q)\mu_1(s^0-x_1-x_2) - D(x_1,x_2)\Big) \\ \dot{x}_2 &= x_2\Big(\mu_2(s^0-x_1-x_2) - D(x_1,x_2)\Big) + qx_1\mu_1(s^0-x_1-x_2) \\ & \quad\; x_1(0) > 0, \;\; x_2(0) > 0, \;\; x_1(0) + x_2(0) \le s^0. \end{aligned} \tag{7}$$

System (7) possesses the following equilibrium points

$$E_0^r = (0,0), \quad E_2^r = (0, s^0 - s^c), \quad E_c^r = (x_1^c, x_2^c),$$

where s^c is defined as a solution of the equation

$$(1-q)\mu_1(s^c) = k(s^0 - s^c) + d, \tag{8}$$

and thus

$$x_1^c = \frac{(s^0 - s^c)((1-q)\mu_1(s^c) - \mu_2(s^c))}{\mu_1(s^c) - \mu_2(s^c))}, \qquad x_2^c = s^0 - s^c - x_1^c. \tag{9}$$

It can be directly checked that $s_1^+ < s^c < s_2^- < s_2^+ < s^0$ and therefore $x_1^c > 0$, $x_2^c > 0$ hold true. Denote the vector field in the right-hand side of (7) by $f(x_1,x_2)$. By calculating the Jacobian matrices of $f(x_1,x_2)$ at the three steady states, it follows that E_0^r is a repeller, E_2^r is a saddle, and E_c^r is an attractor. The Butler–McGehee theorem [4] implies that E_2^r does not belong to the limit set of solutions with positive initial conditions $x_i(0) > 0$, $i = 1,2$. Further, the existence of nontrivial periodic solutions for (7) can also be excluded by means of the Dulac's criterion [4]: the divergence of the vector field $\frac{1}{x_1x_2}f(x_1,x_2)$ is equal to

$$\begin{aligned} \operatorname{div} \frac{1}{x_1x_2} f(x_1,x_2) \;&=\; -\frac{1}{x_2}\left(\frac{d\mu_1}{ds}(s^0 - x_1 - x_2) + k\right) \\ &\quad\; -\frac{1}{x_1}\left(\frac{d\mu_2}{ds}(s^0 - x_1 - x_2) + k\right) - \frac{q\mu_1(s^0 - x_1 - x_2)}{x_2^2}. \end{aligned}$$

Using Assumption (B6), (4) and (5) we obtain consecutively

$$\begin{aligned}
&d < D_1^- + (s^0 - s_1^+)\min \min_{s\in[s_*^m, s^0]}\{(\mu_1^-)'(s), (\mu_1^+)'(s)\}\\
&D_1^- - d > -(s^0 - s_1^+)\min \min_{s\in[s_*^m, s^0]}\{(\mu_1^-)'(s), (\mu_1^+)'(s)\}\\
&\frac{D_1^- - d}{s^0 - s_1^+} > -\min \min_{s\in[s_*^m, s^0]}\{(\mu_1^-)'(s), (\mu_1^+)'(s)\}\\
&k > -\min \min_{s\in[s_*^m, s^0]}\{(\mu_1^-)'(s), (\mu_1^+)'(s)\} \geq -\mu_1'(s) \text{ for all } s \in [s_*^m, s^0].
\end{aligned}$$

The latter inequality, together with the fact that $\mu_1'(s) \geq 0$ for all $s \in [0, s_*^m]$ means that div $\frac{1}{x_1 x_2} f(x_1, x_2)$ is negative for all $(x_1, x_2) > 0$; this implies that limit cycles do not exist. Therefore, the equilibrium E_c^r is globally asymptotically stable.

Denote $z = s + x_1 + x_2 - s^0$ and consider finally the system

$$\begin{aligned}
\dot{x}_1 &= x_1\Big((1-q)\mu_1(s^0{+}z{-}x_1{-}x_2) - D(x_1, x_2)\Big)\\
\dot{x}_2 &= x_2\Big(\mu_2(s^0{+}z{-}x_1{-}x_2) - D(x_1, x_2)\Big) + q x_1 \mu_1(s^0{+}z{-}x_1{-}x_2)\\
\dot{z} &= -D(x_1, x_2)\cdot z,
\end{aligned} \tag{10}$$

where $z(0) \geq -s^0$, $x_i(0) \geq 0$, $i = 1, 2$. We have that $\lim_{t\to\infty} z(t) = 0$ and that system (10) is uniformly bounded. Moreover, on the set $\{(x_1, x_2, z) \in R_+^2 \times [-s^0, +\infty) \mid z = 0\}$ the dynamics of (10) is given by system (7). The equilibrium points of system (10) are easily seen to be $(E_0^r, 0)$, $(E_2^r, 0)$ and $(E_c^r, 0)$. Finally, systems (10) and (7) satisfy the hypotheses of Theorem 5 in [3], see also Appendix F in [4]. This means that the equilibrium point $E_c = (s^c, x_1^c, x - 2^c)$ is globally asymptotically stable, which completes the proof. ■

4. Model-based extremum seeking

It follows from the proof of Theorem 3.1, that the feedback $D(x_1, x_2)$ can be presented in the form

$$D(s) = k(s^0 - s) + d \text{ with } k \in (k_1,\ k_2).$$

On Figure 3, the two dash-dot line segments present the boundary lines $k_1(s^0 - s) + d$ and $k_2(s^0 - s) + d$ for fixed d.

We shall use the freedom in choosing the slope $k \in (k_1, k_2)$ to maximize the concentration $x_1(t)$ of the plasmid-bearing organisms. From Theorem 3.1 we know that for *any* $d \in (0, \delta)$ and *any* $k \in (k_1, k_2)$ there exists a globally

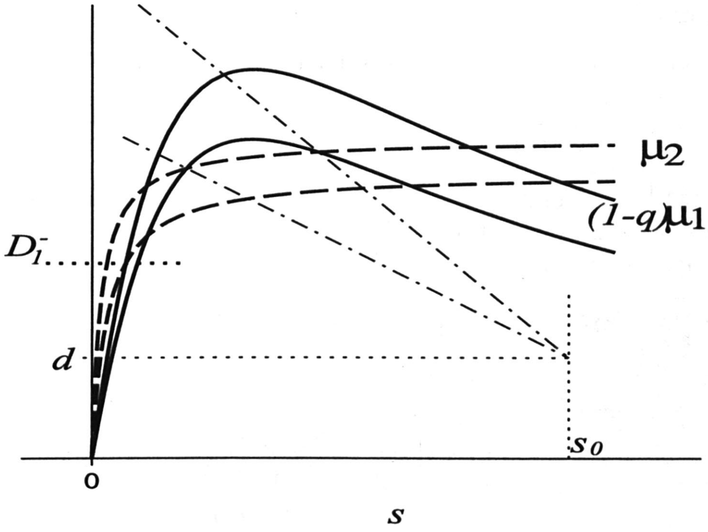

Figure 3: The feedback control law

asymptotically stable equilibrium of (7) with the feedback $D(x_1, x_2)$. Let us fix some $d \in (0, \delta)$, and denote this equilibrium by $E_c^r(k) = (x_1^c(k), x_2^c(k))$. Our goal is to stabilize the dynamics to a point $E_c^r(k^m) = (x_1^c(k^m), x_2^c(k^m))$ where $x_1^c(k^m)$ takes its maximal value. This is achieved by applying a numerical extremum seeking algorithm. Such algorithm was firstly proposed in [1] to solve another optimization problem in a bioreactor. Here the algorithm is adapted for this chemostat model. The main idea of the algorithm is based on the fact, that Theorem 3.1 is valid for any k from the known interval (k_1, k_2). Therefore we can construct in a proper way a sequence of points $k^0, k^1, k^2, \ldots$, that converges to the (unknown) point k^m, where $x_1^c(k^m)$ is maximal. The main steps of the algorithm are presented in the Appendix. The algorithm is executed in two stages: on the first stage, an interval $[K] = [K^-, K^+] \subset (k_1, k_2)$ is determined; such that $k^m \in [K]$; on the second stage the interval $[K]$ is refined by means of Fibonacci elimination techniques to obtain an interval $[K^m] \subset [K]$, which width is less than a user-defined tolerance $\varepsilon > 0$.

5. Numerical simulation

In the simulation process we consider two specific uptake functions:

$$\mu_1(s) = \frac{m_1 s}{(a_1 + s)(b_1 + s)}, \qquad \mu_2(s) = \frac{m_2 s}{a_2 + s}.$$

Obviously, $\mu_1(s)$ has a maximum at $s^m = \sqrt{a_1 b_1}$. The uncertainty in μ_1 and μ_2 is simulated by assuming that the coefficients m_i, a_i and b_1 are bounded within compact positive intervals:

$$m_i \in [m_i] = [m_i^-, m_i^+], \;\; a_i \in [a_i] = [a_i^-, a_i^+], \;\; i = 1, 2; \quad b_1 \in [b_1] = [b_1^-, b_1^+].$$

Then

$$\begin{aligned} [\mu_1](s) &= [\mu_1^-(s), \mu_1^+(s)] = \left[\frac{m_1^- s}{(a_1^+ + s)(b_1^+ + s)}, \frac{m_1^+ s}{(a_1^- + s)(b_1^- + s)}\right] \\ [\mu_2](s) &= [\mu_2^-(s), \mu_2^+(s)] = \left[\frac{m_2^- s}{a_2^+ + s}, \frac{m_2^+ s}{a_2^- + s}\right]. \end{aligned}$$

The boundary functions μ_i^- and μ_i^+, $i = 1, 2$, satisfy assumptions (B1) to (B3). It is straightforward to see that

$$\mu_1'(s) \in \left[(\mu_1^+)'(s), (\mu_1^-)'(s)\right] \quad \text{for} \quad s \in [s_*^m, s^0]$$

so that (B6) is also satisfied. To fulfill the assumptions (B4), (B5) as well as the conditions of Theorem 3.1, particular numerical values for the intervals $[m_i]$, $[a_i]$ and $[b_1]$ are considered.

Further we denote the feedback by $D_k = k(x_1 + x_2) + d$ to indicate explicitly the dependence of k; note that $d \in (0, \delta)$ is fixed. For simplicity, the globally asymptotically stable equilibrium, corresponding to k, is denoted by $x^c(k) = (x_1^c(k), x_2^c(k))$.

In the simulation process we proceed in the following way. At the initial time $t_0 = 0$ we take random values for the coefficients $m_i \in [m_i]$, $a_i \in [a_i]$ ($i = 1, 2$) and $b_1 \in [b_1]$. We apply the extremum seeking algorithm to stabilize the closed-loop system to the globally stable equilibrium, where the plasmid-bearing concentration is maximal. Then, at some time $t_1 > t_0$, we choose another set of random coefficient values and repeat the process. Figure 4 visualizes the time evolution of the state variables $x_1(t)$ and $x_2(t)$. The dash horizontal line segments go through the points $x_1^c(k^m)$ and $x_2^c(k^m)$ respectively. The vertical dot line segments mark the time moment t_1, when the new coefficients values are taken in a random way from the corresponding intervals.

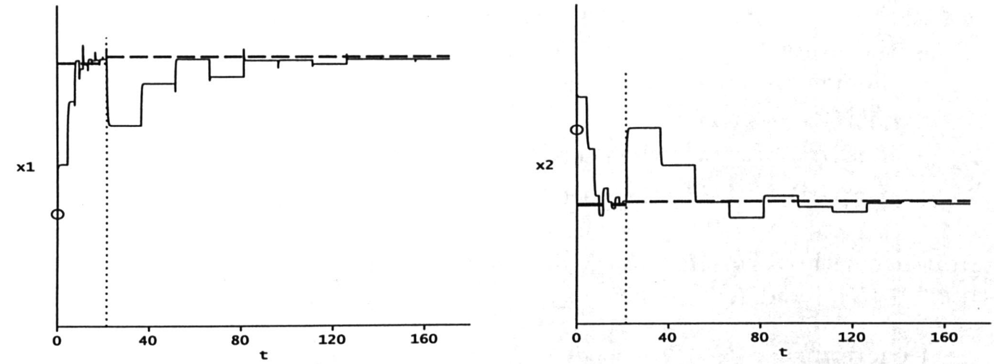

Figure 4: Time evolution of $x_1(t)$ (left) and $x_2(t)$ (right).

Appendix: The extremum seeking algorithm

We present here the main steps of the numerical extremum seeking algorithm. The steps are executed in the given order except as indicated by branching. We assume tolerances $\varepsilon > 0$, $\varepsilon_k > 0$ and $h > 0$ to be given.

I. Determine an interval $[K] = [K^-, K^+]$ such that $[K] \subset (k_1, k_2)$ and $k^m \in [K]$.

Step I.0. Choose $k^0 \in (k_1, k_2)$. Apply the feedback D_{k^0} to stabilize the system to $x^c(k^0)$. According to Theorem 3.1, there is a time moment t_0 such that $|x_1(t_0) - x_1^c(k^0)| < \varepsilon_k$; set $x_1^c(k^0) := x_1(t_0)$, $x_2^c(k^0) := x_2(t_0)$.

Step I.1. Set $\sigma := 1$, $k^1 := k^0 + \sigma h$. Apply the feedback D_{k^1} to stabilize the system to $x^c(k^1)$. According to Theorem 3.1, there is a time moment t_1 such that $|x_1(t_0) - x_1^c(k^1)| < \varepsilon_k$; set $x_1^c(k^1) := x_1(t_1)$, $x_2^c(k^1) := x_2(t_1)$.
If $x_1^c(k^1) > x_1^c(k^0)$ then goto Step I.3
else goto Step I.2.

Step I.2. Set $\sigma := -1$, $k^1 := k^0 + \sigma h$. Apply the feedback D_{k^1} to stabilize the system to $x^c(k^1)$. According to Theorem 3.1, there is a time moment t_1 such that $|x_1(t_1) - x_1^c(k^1)| < \varepsilon_k$; set $x_1^c(k^1) := x_1(t_1)$, $x_2^c(k^1) := x_2(t_1)$.
If $x_1^c(k^1) > x_1^c(k^0)$ then goto Step I.3.
If $x_1^c(k^1) \leq x_1^c(k^0)$ then set $h := h/2$;
  if $h \leq \varepsilon/2$ then set $[K^m] := [k^0 - \varepsilon, k^0 + \varepsilon]$; goto *III*;
  if $h > \varepsilon/2$ then goto Step I.1.

Step I.3. Set $h := 2h$, $k^2 := k^1 + \sigma h$. Apply the feedback D_{k^2} to stabilize the system to $x^c(k^2)$. According to Theorem 3.1, there is a time moment t_2 such that $|x_1(t_2) - x_1^c(k^2)| < \varepsilon_k$; set $x_1^c(k^2) := x_1(t_2)$, $x_2^c(k^2) := x_2(t_2)$.

If $x_1^c(k^2) \leq x_1^c(k^1)$ then set $[K] = [K^-, K^+] := [k^0, k^2]$ and goto *II*.

If $x_1^c(k^2) > x_1^c(k^1)$ then set $k^0 := k^1$, $k^1 := k^2$; repeat this Step I.3.

II. Starting with $[K] = [K^-, K^+]$, determine an interval $[K^m] = [K^{m^-}, K^{m^+}]$ with $k^m \in [K^m]$ and $K^{m^+} - K^{m^-} \leq \varepsilon$.

Denote $k^{0^-} := K^-$, $k^{0^+} := K^+$, $\lambda := \dfrac{\sqrt{5}-1}{2}$; compute $\Delta_1 := k^{0^+} - k^{0^-}$.

Step II.0. Compute $\Delta_2 := (1 - \lambda)\Delta_1$, $p_0 := k^{0^-} + \Delta_2$, $q_0 := k^{0^+} - \Delta_2$.

Step II.1. Apply the feedback D_{p_0} to stabilize the system to $x^c(p_0)$. According to Theorem 3.1, there exists a moment of time $t_{p_0} > 0$ such that $|x_1(t_{p_0}) - x_1^c(p_0)| < \varepsilon_k$; set $x_1^c(p_0) := x_1(t_{p_0})$, $x_2^c(p_0) := x_2(t_{p_0})$.

Apply the feedback D_{q_0} to stabilize the system to q_0. According to Theorem 3.1, there exists a moment of time $t_{q_0} > 0$ such that $|x_1(t_{q_0}) - x_1^c(q_0)| < \varepsilon_k$; set $x_1^c(q_0) := x_1(t_{q_0})$, $x_2^c(q_0) := x_2(t_{q_0})$.

Step II.2. Set $\Delta_3 := q_0 - p_0$.

If $x_1^c(p_0) > x_1^c(q_0)$ then set $k^{1^-} := k^{0^-}$, $k^{1^+} := q_0$, $p_1 := k^{1^-} + \Delta_3$, $q_1 := p_0$;

If $x_1^c(p_0) \leq x_1^c(q_0)$ then set $k^{1^-} := p_0$, $k^{1^+} := k^{0^+}$, $p_1 := q_0$, $q_1 := k^{1^+} - \Delta_3$.

Compute $\Delta_1 := k^{1^+} - k^{1^-}$.

Step II.3. If $\Delta_1 \leq \varepsilon$ then set $[K^m] := [k^{1^-}, k^{1^+}]$; goto *III*.

If $\Delta_1 > \varepsilon$ then

- if $p_1 \geq q_1$ then set $k^{0^-} := k^{1^-}$, $k^{0^+} := k^{1^+}$ and goto Step II.0.
- if $p_1 < q_1$ then
  - if $x_1^c(p_0) > x_1^c(q_0)$ then apply the feedback D_{p_1} to stabilize the system to $x^c(p_1)$. According to Theorem 3.1, there exists a moment of time $t_{p_1} > 0$ such that $|x_1(t_{p_1}) - x_1^c(p_1)| < \varepsilon_k$; set $x_1^c(p_1) := x_1(t_{p_1})$, $x_2^c(p_1) := x_2(t_{p_1})$.
  - if $x_1^c(p_0) \leq x_1^c(q_0)$ then apply the feedback D_{q_1} to stabilize the system to $x^c(q_1)$. According to Theorem 3.1, there exists a moment of time $t_{q_1} > 0$ such that $|x_1(t_{q_1}) - x_1^c(q_1)| < \varepsilon_k$; set $x_1^c(q_1) := x_1(t_{q_1})$, $x_2^c(q_1) := x_2(t_{q_1})$.

```
    Set $p_0 := p_1$, $q_0 := q_1$, $k^{0^-} := k^{1^-}$, $k^{0^+} := k^{1^+}$,
    $x_1^c(p_0) := x_1^c(p_1)$, $x_1^c(q_0) := x_1^c(q_1)$;
    goto Step II.2.
III. STOP computations
```

Remark 1. At any step of the algorithm, the last computed values for x_1 and x_2 are used as initial conditions for the next step.

Remark 2. The algorithm can be implemented to work online. If the system states (measurements) change due to some parameter perturbations, then the algorithm starts to work from Stage I.

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Institute of Mathematics and Informatics
Bulgarian Academy of Sciences
Acad. G. Bonchev Str., Bl. 8
Sofia 1113, BULGARIA
E-MAIL: nelid@bio.bas.bg