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Feedback control for chemostat models

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Abstract. It is shown that a chemostat with two organisms can be made coexistent by means of feedback control of the dilution rate. Remaining freedom in the feedback law can be used to guarantee robustness or improve particular performance indices. Unfortunately a topological property prevents coexistence by feedback control for chemostats with more than two organisms. We apply our results to control bioreactors aimed at producing commercial products through genetically altered organisms. In all our results the coexistence takes its simplest form: a global asymptotically stable equilibrium point in the interior of the non-negative orthant.

1. Introduction

The chemostat is a benchmark model in microbial ecology, used for a wide variety of systems ranging from lakes, waste-water treatment, to reactors for commercial production of substances by genetically altered organisms. It has the advantage of being easily implementable in a laboratory and hence the model has been subject to extensive tests and experiments. It may be used to model the competition of several organisms for a single nutrient source. A classical result is the ‘competitive exclusion principle’ [22], stating that in the long run only one organism survives while the others die out. There is a large literature devoted to modifying the chemostat model to ensure coexistence of the organisms. These are based on suitable manipulation of the chemostat’s operating parameters, the dilution rate [24, 2] or the input nutrient concentration [24, 9, 5], or by dropping the well-mixed hypothesis [23, 16]. If instead of being constant, an operating parameter is a periodically varying function of time, coexistence may occur (see [22] for a review).

In this paper we take a different approach based on ideas from control theory. Two control principles studied in control theory are *open-loop control* and *feedback control*. For results based on the former we refer to [18]. We consider the dilution rate as a feedback control variable, keeping the input nutrient concentration at a fixed value. It is shown that if the dilution rate depends affinely on the concentrations of two competing organisms, coexistence may be achieved as a globally asymptotically stable equilibrium point in the interior of the non-negative orthant. The dilution rate is proportional to the speed of the pump supplying the chemostat

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with the medium which contains the input nutrient. If accurate measurements of the concentrations of the competing organisms are available, this implies that a simple affine control algorithm would allow automated coexistence in the chemostat. In addition we show that our feedback approach is robust, a feature which open-loop control methods lack. Actually this difference holds for far more general problems (e.g. stabilization problems) than the one considered here and in part explains the success of feedback control when compared to open loop control paradigms. Also our method is constructive as an explicit feedback law will be provided whereas only existence of an open-loop control is shown in [18]. Unfortunately we show that coexistence is not achievable by means of feedback control of the dilution rate for a chemostat with more than two competing organisms due to a topological obstruction, while open-loop controls which do achieve this goal are shown to exist in [18].

We apply our results to control bioreactors aimed at producing a commercial product using genetically altered organisms. Organisms are typically altered by insertion of a small circle of DNA (a plasmid) coding for the production of the product and perhaps other useful attributes. Unfortunately, these plasmids may be unevenly divided between daughter cells during cell division leading occasionally to the reappearance of the wild-type organism containing no plasmid. Therefore, one is forced to deal with (i.e., feed) two organisms—the genetically altered one producing useful product and the wild-type which is useless for the intended purpose. Several control strategies have been employed to keep the wild-type organism, typically a strong competitor against the altered strain, to acceptably low levels, each with its own set of disadvantages. For example, the plasmid may also code for resistance to an antibiotic which is then added to the chemostat to select for the resistant, product-producing, strain [14, 17, 12]. In a similar strategy, the plasmid may also code for resistance to a growth inhibitor, supplied in the feed, for which the wild-type is not resistant [10, 13, 12]. Or, the plasmid may code both for production of and resistance to a toxin for which the wild-type is susceptible [15, 12]. While these strategies work to control the wild-type organism, the antibiotic or toxin may contaminate the product requiring an expensive separation process. We propose an alternative control strategy which requires no chemical agents and expensive post-production separation process. It does, however, require fast and accurate measurements of cell densities in the effluent in order to implement. It will be shown that feedback control can be used to control wild-type population levels and that it achieves superior robustness properties.

In section 2 we introduce the chemostat model. Controlling the chemostat by means of the dilution rate is discussed in section 3. Section 4 contains the main result on coexistence of two organisms by means of feedback control of the dilution rate. In section 5 it is shown that feedback control of the dilution rate is futile for more than two organisms due to a topological obstruction and we apply our results for control of bioreactors in section 6. Section 7 contains the proofs.

2. Model of the chemostat

The model of a chemostat is given by the following set of differential equations:

$$\begin{aligned}\dot{S} &= D(S^0 - S) - \sum_{i=1}^2 \frac{x_i}{\gamma_i} f_i(S) \\ \dot{x}_i &= x_i(f_i(S) - D), \quad i = 1, 2\end{aligned}\tag{1}$$

where

1. $S(t)$ is the concentration of nutrient and $x_i(t)$ is the concentration of organism i in the chemostat at time t .
2. D is the *dilution rate* of the chemostat (or equivalently, $1/D$ is the *residence time* of a molecule inside the chemostat) and S^0 is the concentration of the input nutrient. The constants γ_i are *yield constants*.
3. The functions f_i are called *uptake functions* and satisfy the following:
 - (regularity) $f_i : \mathbb{R}_+ \rightarrow \mathbb{R}_+$ is continuously differentiable and $f_i(0) = 0$.
 - (monotonicity) f_i is monotonically increasing, i.e. $f_i' > 0$ for all $S \in \mathbb{R}_+$.

A minimal requirement for any model of a chemostat is that the state components S , x_1 and x_2 only take non-negative values for all times $t \geq 0$. It can be shown that system (1) satisfies this requirement. It is convenient to pass to non-dimensional variables $\bar{S} := S/S^0$, $\bar{x}_i := x_i/(\gamma_i S^0)$:

$$\begin{aligned}\dot{\bar{S}} &= D(1 - \bar{S}) - \sum_{i=1}^2 \bar{x}_i f_i(S^0 \bar{S}) \\ \dot{\bar{x}}_i &= \bar{x}_i(f_i(S^0 \bar{S}) - D), \quad i = 1, 2\end{aligned}$$

or, after dropping the bars and writing $f_i(S)$ instead of $f_i(S^0 \bar{S})$ (note that both functions are qualitatively the same and therefore the ‘new’ $f_i(S)$ also satisfy regularity and monotonicity properties):

$$\begin{aligned}\dot{S} &= D(1 - S) - \sum_{i=1}^2 x_i f_i(S) \\ \dot{x}_i &= x_i(f_i(S) - D), \quad i = 1, 2\end{aligned}\tag{2}$$

The equilibrium points of system (2) are:

$$E_0 := (1, 0, 0), \quad E_1 := (\lambda_1, 1 - \lambda_1, 0) \text{ and } E_2 := (\lambda_2, 0, 1 - \lambda_2)\tag{3}$$

where the λ_i – which are assumed to be different – are implicitly defined as follows:

$$f_i(\lambda_i) = D, \quad i = 1, 2\tag{4}$$

The principal result concerning the chemostat is the so-called ‘competitive exclusion principle’ and can be stated as follows, see [22]:

Theorem 1. *If $0 < \lambda_1 < 1$ and if $\lambda_1 < \lambda_2$ then E_1 is a globally asymptotically stable equilibrium point of system (2) with respect to all initial conditions in the set $\{(S, x_1, x_2) \in \mathbb{R}_+^3 \mid x_i > 0, i = 1, 2\}$.*

Informally, the exclusion principle states that when two organisms compete for a single nutrient, one of the organisms is doomed in the long run and loses the competition. This principle also holds in case there are multiple organisms competing for the nutrient, see also [22].

3. Controlling the chemostat by means of the dilution rate

In view of the exclusion principle, one might wonder whether it is possible to change the long term behavior of the chemostat and make the organisms coexist. The obvious parameters that are manipulable by the operator running the chemostat are the dilution rate D and the input nutrient concentration S^0 . In this paper we will assume that S^0 is fixed and D is manipulable. The intuition behind the idea of manipulating the dilution rate to achieve coexistence is outlined next.

We will assume throughout the rest of this paper that the graphs of the uptake functions f_i , $i = 1, 2$ are as depicted in Figure 1. Therefore we introduce the following standing hypothesis:

H The graphs of the functions f_1 and f_2 intersect once at \tilde{S} :

$$f_1(\tilde{S}) = f_2(\tilde{S}) = \tilde{D} \quad (5)$$

where $\tilde{S} \in (0, 1)$. For all $S \in (0, \tilde{S})$ the inequality $f_1(S) > f_2(S)$ holds, while for all $S > \tilde{S}$, $f_1(S) < f_2(S)$ holds. Moreover $f_2'(\tilde{S}) > f_1'(\tilde{S})$.

It follows from theorem 1 that for low values of the dilution rate ($D < \tilde{D}$) such as D_a , organism 1 wins the competition and for higher values ($D > \tilde{D}$, but $D < D_{\max}$) such as D_b , organism 2 wins the competition.

If the goal is coexistence it is therefore tempting to vary the dilution rate between low and high values. The variation is achievable in different ways:

1. One way is to make the dilution rate a *time-dependent function*. A particular case occurs for *periodically varying dilution rates*: $D \equiv D(t) = D(t + T)$ for all $t \in \mathbb{R}$ and for some $T > 0$. It has been shown in [22] that in this case it is indeed possible to make both organisms coexist. Coexistence is expressed by the existence of a globally attracting periodic solution of system (2) which lies in $\text{int}(\mathbb{R}_+^3)$.
2. In this paper we propose a different approach. Instead of letting the dilution rate depend on *time*, we let it depend on the *state* of the chemostat:

$$D \equiv D(S, x_1, x_2) \quad (6)$$

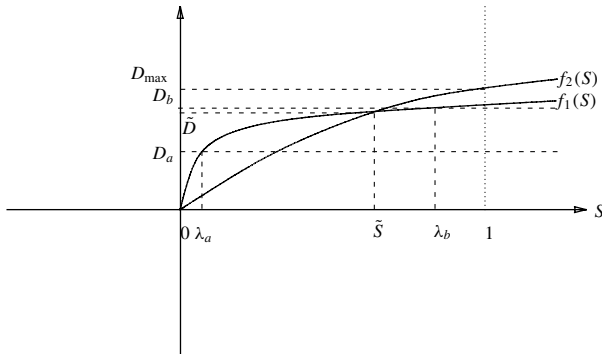


Fig. 1. Different dilution rates result in different winners.

where $D(S, x_1, x_2)$ is some function defined on \mathbb{R}_+^3 . Of course this function should only take non-negative values for obvious physical reasons (dilution rates cannot be negative), implying that $D(S, x_1, x_2) : \mathbb{R}_+^3 \rightarrow \mathbb{R}_+$.

This approach amounts to interpreting the dilution rate as a *state feedback control variable*: Its value at a certain moment, depends on the current state of the chemostat.

Remark 1. We comment briefly on implications of using state feedback.

A mathematical simplification might be anticipated when using state feedback: Periodically varying dilution rates result in a periodically varying chemostat, while state feedback leads to a time-independent chemostat.

On the other hand there is a technological problem related to using feedback control. The current state has to be known to determine the current dilution rate. But it might be difficult to measure all state variables in an operating chemostat. For instance, it may not be reasonable to assume that a measurement of the nutrient concentration is available. We show below that D may be chosen to depend only on x_1 and x_2 and not on S . In general the search for or design of feedback laws depending on *all* state variables should not be considered as useless, even if it is not possible to measure all state variables. Suppose that a feedback law has been found which requires the full state of the system, but that it is impossible to obtain measurements of say the concentration of the nutrient. The solution to this problem proposed in control theory is then to find a so-called *observer*. This is a dynamical system which produces an estimate of the nutrient concentration based on the available information of the state of the system. Provided an observer can be found, the next step is then to use this estimate in the original feedback law instead of the actual value. Of course the challenge is then to prove that the desired goal -which may be to make the system stable or coexistent for instance- is still achieved.

4. Permanence by means of feedback control

Although we have been using the term coexistence rather informally in the previous sections and equated it with the survival of all organisms in the long run, we will instead use the well-known concept of *permanence*, see e.g. [8], in the rest of this paper. But first we define positive systems. Consider a system $\dot{x} = f(x)$ where $x \in \mathbb{R}^n$ and f is sufficiently smooth such that existence and uniqueness of solutions is guaranteed. A (forward) solution starting in x_0 at $t = 0$ is denoted by $x(t, x_0)$ and is defined on its maximal (forward) interval of existence $\mathcal{I}(x_0)$ ($\mathcal{I}^+(x_0)$). The system is called positive if \mathbb{R}_+^n is a forward invariant set for it, i.e. if

$$\forall x_0 \in \mathbb{R}_+^n : x(t, x_0) \in \mathbb{R}_+^n, \forall t \in \mathcal{I}^+(x_0).$$

Definition 1. A positive system $\dot{x} = f(x)$ is permanent if there exists a compact set K , $K \subset \text{int}(\mathbb{R}_+^n)$, which attracts all solutions starting in $\text{int}(\mathbb{R}_+^n)$, i.e.

$$\forall x_0 \in \text{int}(\mathbb{R}_+^n), \exists T(x_0) : x(t, x_0) \in K, \forall t \geq T(x_0). \quad (7)$$

Permanence might come in different forms. The simplest manifestation occurs if a positive system possesses an equilibrium point in $\text{int}(\mathbb{R}_+^n)$ which is globally asymptotically stable (GAS) with respect to initial conditions in $\text{int}(\mathbb{R}_+^n)$.

Motivated by the discussion in the previous section, we formulate the following problem:

Permanence Problem: Find – if possible – a feedback $D : \mathbb{R}_+^3 \rightarrow \mathbb{R}_+$ such that the following system:

$$\begin{aligned} \dot{S} &= D(x)(1 - S) - \sum_{i=1}^2 x_i f_i(S) \\ \dot{x}_i &= x_i(f_i(S) - D(x)), \quad i = 1, 2 \end{aligned} \quad (8)$$

where $x := (S, x_1, x_2)^T$, is permanent.

Now we are in a position to state the main result of this section.

Theorem 2. *Pick any ϵ in the interval $[0, \tilde{D})$. Then the permanence problem is solvable by the following affine feedback:*

$$D(x) = k_1 x_1 + k_2 x_2 + \epsilon \quad (9)$$

if the gains $k_i \geq 0$, $i = 1, 2$ satisfy the following inequalities:

$$k_2 < \tilde{k} < k_1 \quad (10)$$

where $\tilde{k} := \frac{\tilde{D} - \epsilon}{1 - \delta}$ and where $k_2 > 0$ if $\epsilon = 0$.

In particular there exists an equilibrium point $x^e \in \text{int}(\mathbb{R}_+^3)$ which is GAS for system (8) with feedback (9) with respect to initial conditions in $\text{int}(\mathbb{R}_+^3)$.

Conversely, if system (8) with a nonnegative affine feedback $D(x) = k_1 x_1 + k_2 x_2 + \epsilon$ possesses a unique equilibrium point in $\text{int}(\mathbb{R}_+^3)$ which is locally asymptotically stable, then the feedback parameters satisfy $\epsilon < \tilde{D}$ and (10).

Remark 2. Notice that with feedback (9), no knowledge on the nutrient concentration $S(t)$ is needed to determine the current dilution rate $D(x(t))$.

On the other hand the concentration $x_1(t)$ and, in general also $x_2(t)$, have to be known to determine the dilution rate. Only if $k_2 = 0$ – which is an acceptable choice if $\epsilon > 0$ since it is in accordance with (10) – is it not necessary to determine x_2 .

Moreover since (10) implies that $k_1 \neq k_2$, any device used to monitor the concentrations of organisms (with the intention of using them to determine the current value of the dilution rate) must be capable of distinguishing between them. For methods used in practice to measure concentrations we refer to [6, 11, 1].

Remark 3. Recall that for obvious physical reasons, the dilution rate $D(x)$ should take non-negative values for all $x \in \mathbb{R}_+^3$. Since $\epsilon \geq 0$ and $k_i \geq 0$, $i = 1, 2$, this condition is indeed satisfied. Moreover, recalling that the dilution rate is proportional to the speed of the pump which fills the chemostat, this feedback exhibits a

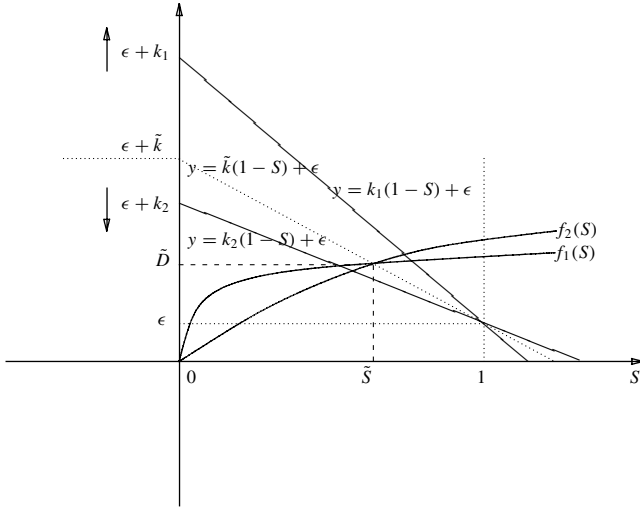


Fig. 2. Bound for k_1 and k_2 .

desirable property: If the concentrations of the organisms inside the chemostat are low (x_1 and x_2 are small), then the dilution rate and thus the speed of the pump is low. On the contrary, if these values are high, then the pump speed is also high.

Remark 4. It is instructive to consider the equations governing the species concentrations of the system resulting from the insertion of the feedback law (9) into equations (8):

$$\dot{x}_i = x_i(f_i(S) - k_1x_1 - k_2x_2 - \epsilon), \quad i = 1, 2$$

Together with an equation for S with constant dilution rate and with terms representing nutrient recycling of dead biomass, this model is similar to that studied in e.g. [4, 19] where terms like $-k_1x_1 - k_2x_2$ have been interpreted as representing intra- and inter-specific “interference” competition. There, it is appropriate to double index the k as k_{ij} . In this sense it is possible to rephrase the resulting action of our feedback law into a form which is more familiar to mathematical biologists. Similarly it may inform control theorists of the physical meaning of their feedback control laws for these models.

Remark 5. A simple graphical interpretation for the inequalities (10) follows from Figure 2. Denoting the vertical axis in this figure as y , the intersection of the line $y = k_1(1 - S) + \epsilon$, respectively $y = k_2(1 - S) + \epsilon$, and the y -axis should be above, respectively below, the intersection of the line $y = \tilde{k}(1 - S) + \epsilon$ and the y -axis.

Remark 6. We emphasize that the constraints (10) on the feedback gains entail some freedom for the feedback law (9). This freedom might be used for different purposes:

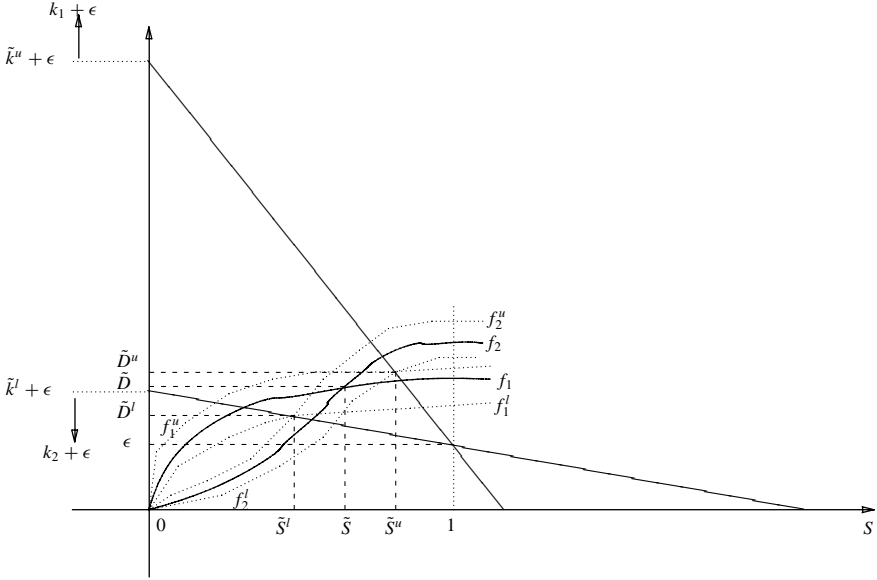


Fig. 3. Robustness.

1. Robustness

In many applications the uptake functions are not exactly known, and only bounds of these functions are available. Denote the upper bounds by $f_i^u(S)$ and the lower bounds by $f_i^l(S)$, see Figure 3:

$$\forall S \in \mathbb{R}_+ : f_i^l(S) \leq f_i(S) \leq f_i^u(S), \quad i = 1, 2 \quad (11)$$

It is natural to assume that these bounds also satisfy regularity and monotonicity conditions.

An important consequence of not knowing the uptake functions exactly is that the value \tilde{S} , needed in the construction of the feedback which achieves permanence, is not known either. Instead, only a compact interval $I := [\tilde{S}^l, \tilde{S}^u]$ (which is assumed to be a subset of $(0, 1)$) is known to contain \tilde{S} , see also Figure 3. The limits of the interval I are implicitly given by the following equalities:

$$\begin{aligned} f_1^l(\tilde{S}^l) &= f_2^u(\tilde{S}^l) \quad (:= \tilde{D}^l) \\ f_1^u(\tilde{S}^u) &= f_2^l(\tilde{S}^u) \quad (:= \tilde{D}^u) \end{aligned} \quad (12)$$

and we assume (this assumption might be relaxed, but we prefer not do to so because it would not change the argument) that:

$$\begin{aligned} \forall S \in (0, \tilde{S}^l) : f_2^u(S) &< f_1^l(S) \\ \forall S > \tilde{S}^u : f_1^u(S) &< f_2^l(S) \end{aligned}$$

This lack of exact information on the uptake functions might endanger the capability of the feedback law (9) to render the system (8) permanent. However, it turns out that if (10) are replaced by:

$$k_1 > \tilde{k}^u \text{ and } k_2 < \tilde{k}^l \quad (13)$$

where $\tilde{k}^u := \frac{\tilde{D}^u - \epsilon}{1 - \tilde{S}^u}$ and $\tilde{k}^l := \frac{\tilde{D}^l - \epsilon}{1 - \tilde{S}^l}$, then the feedback law (9) with $k_i \geq 0$, $i = 1, 2$, still solves the permanence problem. The price paid for this robustness property is a smaller range of feedback gains which is immediate from a comparison of (10) and (13).

2. Performance indices

The remaining freedom might also be used to modify particular performance indices, associated to the behavior of the controlled chemostat.

An obvious performance index is the ratio of the values of both organisms at the interior equilibrium point x^e . The proof of theorem 2 will reveal that this ratio is given by:

$$\frac{x_1^e}{x_2^e} = \frac{\tilde{k} - k_2}{k_1 - \tilde{k}} \quad (14)$$

showing that *any value* can be assigned to it without violating the gain constraints (10).

Remark 7. Next we give an intuitive explanation of why theorem 2 holds. First recall from the discussion in the previous section that if D is set to a low value, then organism 1 wins the competition because the single-organism equilibrium point corresponding to organism 1 is globally asymptotically stable. Similarly, if D equals a high value, then the single-organism equilibrium point corresponding to organism 2 is globally asymptotically stable and organism 2 wins.

Although the single-organism equilibrium points depend on the choice of D , they should be unstable to achieve permanence. To destabilize the single-organism equilibrium point corresponding to organism 1, D should take large values in a neighborhood of this equilibrium point (for if it was small, one would expect it to be stable). Similarly, to destabilize the equilibrium point corresponding to organism 2, D should take small values in a neighborhood of it. Both conditions are satisfied for the linear feedback (9) in case (10) holds: Close to the single-organism equilibrium point corresponding to organism 1, the dilution rate is close to $k_1 x_1 + \epsilon$, which is large if k_1 is large. A similar argument shows that at the other single-organism equilibrium point, the dilution rate is small if k_2 is small.

Remark 8. The simplest and easiest feedback to implement would be to let $k_1 = k_2 = k$ so that $D(x) = k(x_1 + x_2) + \epsilon$ requiring no distinction between organisms by the observer. In fact, just this system, with $\epsilon = 0$ and piecewise linear uptake functions, was numerically simulated in [3], where it is pointed out that a flow reactor with such negative feedback on the dilution rate is commonly used to regulate population size and is called a turbidostat. The turbidostat may be useful

for pure culture population regulation but it cannot maintain permanence for two distinct competing species. Indeed, coexistence equilibria can only exist if k takes a particular value, namely, $k = \frac{\tilde{D}-\epsilon}{1-\tilde{S}}$, determined by the point of intersection of the growth curves and by the supplied nutrient concentration (here scaled to unity), and in that case there is a line of equilibria given by $x_1 + x_2 = 1 - \tilde{S}$. Random effects can then be expected to eventually eliminate one of the competitors. However, as pointed out by [3], extinction may take a very long time.

5. Multiple organisms

We have shown that a chemostat with two competing organisms can be made permanent by means of feedback control of the dilution rate. Unfortunately this is no longer true for chemostats with more than two competitors. The reason for this failure is one of topological nature: A permanent chemostat should possess at least one equilibrium point in the interior of the positive orthant. But no matter what feedback law is chosen for the dilution rate, chemostats with more than two competing species (generically) cannot satisfy this property as we show next.

The (non-dimensional) model for a chemostat with multiple, say n , competitors is readily obtained from the two competitors model and takes the following form:

$$\begin{aligned}\dot{S} &= D(x)(1 - S) - \sum_{i=1}^n x_i f_i(S) \\ \dot{x}_i &= x_i(f_i(S) - D(x)), \quad i = 1, \dots, n\end{aligned}\tag{15}$$

where $x := (S, x_1, \dots, x_n)^T$ is the state vector consisting of the concentrations of the nutrient and the n organisms. As before the n uptake functions f_i are assumed to satisfy regularity and monotonicity conditions and the problem is to find a suitable feedback function for the dilution rate $D : \mathbb{R}_+^{n+1} \rightarrow \mathbb{R}_+$ ensuring permanence for the resulting chemostat.

Notice that if $n \geq 3$ the graphs of the uptake functions in general do not have a nontrivial common intersection point, unless in the (biologically unlikely and unrealistic) case that the following set of nonlinear equations has a solution $\tilde{S} \in \mathbb{R}_+^n$ with $\tilde{S} \neq 0$:

$$f_1(S) = f_2(S) = \dots = f_n(S)\tag{16}$$

On the other hand, the existence of a solution $\tilde{S} \in \mathbb{R}_+^n$ with $\tilde{S} \neq 0$ for the equations (16) is a necessary condition for the existence of an equilibrium point of system (15) in $\text{int}(\mathbb{R}_+^{n+1})$, irrespective of the choice for the feedback function $D(x)$. And it follows from a generalization of theorem 13.3.1 in [8] (in [8] the result is only proved for so-called Kolmogorov systems, but a careful reading of the proof reveals that is still valid for arbitrary positive systems) that in turn this is a necessary condition for permanence of system (15).

Summarizing, if $n \geq 3$, it is in general not possible to find a feedback function for the dilution rate which ensures permanence of the resulting system (15).

6. Application: Producing genetically altered organisms

A potential application area of feedback controlled chemostats is the commercial production of substances using genetically altered organisms in continuous culture. The alteration is obtained by inserting DNA in a cell in the form of a plasmid. These plasmid-bearing organisms are then grown in a bioreactor, modeled by a chemostat. Unfortunately the plasmid might be lost in the reproduction process, resulting in the appearance of a second competitor, the plasmid-free organism. It was shown in [12] that if the plasmid-free organism is a superior competitor (being a superior competitor can be made mathematically precise), it takes over the bioreactor and leads to the extinction of the plasmid-bearing organism. This should of course be prevented by all means. It is sometimes argued that the plasmid-free organism *is* a superior competitor because it is not burdened by the extra load of the plasmid. But if this is true, then there is no hope for efficient long-term production of the plasmid-bearing organisms in view of the mentioned extinction result. Moreover, there have been reports that under certain conditions the plasmid-bearing competitor is the superior competitor, see [25] and cited references therein. Coexistence of both organisms seems to be the best possible outcome. It has indeed been shown in [12] that this can be achieved. In this section we show that permanence is also achievable by feedback control and leads to superior robustness properties compared to the case where the dilution rate is constant.

First we introduce a modified chemostat model to describe the competition between plasmid-bearing and plasmid-free organisms:

$$\begin{aligned}\dot{S} &= D(S^0 - S) - \sum_{i=1}^2 \frac{x_i}{\gamma_i} f_i(S) \\ \dot{x}_1 &= x_1((1 - q)f_1(S) - D) \\ \dot{x}_2 &= x_2(f_2(S) - D) + qx_1 f_1(S)\end{aligned}\tag{17}$$

where $S(t)$ is the concentration of the nutrient and $x_1(t)$, respectively $x_2(t)$, is the concentration of the plasmid-bearing organism, respectively plasmid-free organism. The parameters appearing in (17) have the same meaning as in (1) and the uptake functions f_i , $i = 1, 2$, satisfy regularity and monotonicity conditions as before. The parameter q in (17) represents the probability that the plasmid is lost in the reproduction process and, being a probability, satisfies:

$$0 < q < 1$$

Passing to non-dimensional variables (recall that input nutrient concentration S^0 is assumed to be a constant) yields the following model:

$$\begin{aligned}\dot{S} &= D(1 - S) - \sum_{i=1}^2 x_i f_i(S) \\ \dot{x}_1 &= x_1((1 - q)f_1(S) - D) \\ \dot{x}_2 &= x_2(f_2(S) - D) + qx_1 f_1(S)\end{aligned}\tag{18}$$

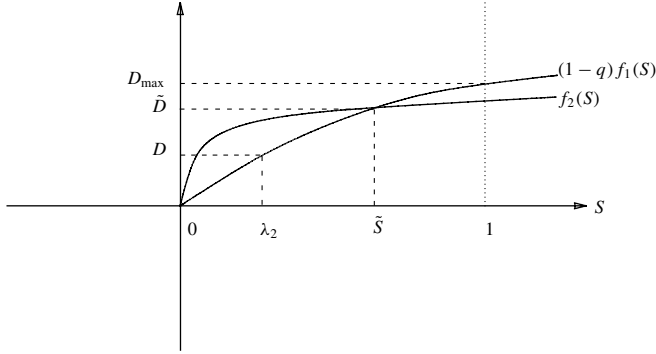


Fig. 4. Plasmid-bearing $((1 - q)f_1)$, plasmid-free (f_2) uptake functions.

The following standing hypothesis concerning the uptake functions is similar to **H** and illustrated in Figure 4:

H' The graphs of the functions $(1 - q)f_1$ and f_2 intersect once at \tilde{S} :

$$(1 - q)f_1(\tilde{S}) = f_2(\tilde{S}) = \tilde{D} \quad (19)$$

where $\tilde{S} \in (0, 1)$. For all $S \in (0, \tilde{S})$ the inequality $(1 - q)f_1(S) < f_2(S)$ holds, while for all $S > \tilde{S}$, $(1 - q)f_1(S) > f_2(S)$ holds.

Notice that **H'** implies that for high nutrient levels ($\tilde{S} < S$), the plasmid-bearing organism is a superior competitor, while for low levels ($\tilde{S} > S$), the plasmid-free organism is.

6.1. Constant dilution rate

If the dilution rate D is constant, then the possible equilibrium points of system (18) are given by:

$$E_0 := (1, 0, 0), \quad E_2 := (\lambda_2, 0, 1 - \lambda_2) \quad \text{and} \quad E^* := (\lambda^*, x_1^*, x_2^*) \quad (20)$$

The washout equilibrium point E_0 always exists, while E_2 (an equilibrium point involving only plasmid-free organisms and no plasmid-bearing organisms) exists only if the following nonlinear equation:

$$f_2(S) = D \quad (21)$$

possesses a solution $S \equiv \lambda_2 > 0$. Obviously, this depends on the function f_2 and the value of D , see Figure 4. Similarly, the equilibrium point E^* only exists if an appropriate nonlinear set of equations is solvable and possesses a solution in $\text{int}(\mathbb{R}_+^3)$.

More importantly, notice that no matter what value D takes, there is no equilibrium point corresponding to plasmid-bearing organisms only. This is different from the classical chemostat model we discussed before. It also implies that it

is not possible to operate the reactor corresponding to the chemostat model (18) at a desired state where only plasmid-bearing and no plasmid-free organisms are present. The best one can hope for then is to operate it at the equilibrium point E^* , if it exists of course. It turns out that this is indeed possible if the dilution rate is carefully chosen as we show next by recalling one of the principal results in [12]. In the statement of this result we will need the constant D_{\max} which is defined as the value of $(1 - q)f_1(1)$.

Theorem 3. *If $D > D_{\max}$, then E_0 is a GAS equilibrium point of system (18) with respect to all initial conditions in the set $P := \{(S, x_1, x_2) \in \mathbb{R}_+^3 \mid x_i > 0, i = 1, 2\}$.*

If $D \in (\tilde{D}, D_{\max})$, then E^ is a GAS equilibrium point of system (18) with respect to all initial conditions in the set P .*

If $D \in (0, \tilde{D})$, then E_2 is a GAS equilibrium point of system (18) with respect to all initial conditions in the set P .

Theorem 3 states that if the dilution rate D is constant, then washout occurs if D is too high and loss of plasmid-bearing organism if D is too low. Only for moderate values of D , permanence is possible and the operator running the bioreactor should be very careful choosing D .

6.2. Feedback control

If instead of taking constant values for the dilution rate, we allow it to depend on the state, system (18) is modified to:

$$\begin{aligned} \dot{S} &= D(x)(1 - S) - \sum_{i=1}^2 x_i f_i(S) \\ \dot{x}_1 &= x_1((1 - q)f_1(S) - D(x)) \\ \dot{x}_2 &= x_2(f_2(S) - D(x)) + qx_1 f_1(S) \end{aligned} \quad (22)$$

where $D(x)$ is a feedback function, defined for all $x = (S, x_1, x_2) \in \mathbb{R}_+^3$ and taking values in \mathbb{R}_+ . The obvious problem is to find – if possible – a suitable feedback function $D(x)$ such that the resulting chemostat model (22) is permanent.

We are ready to state the main result of this section.

Theorem 4. *Pick any ϵ in the interval $(0, \tilde{D})$. Then system (22) with the following affine feedback:*

$$D(x) = k(x_1 + x_2) + \epsilon \quad (23)$$

is permanent if k satisfies the following inequality:

$$k > \tilde{k} \quad (24)$$

where $\tilde{k} := \frac{\tilde{D} - \epsilon}{1 - \tilde{S}}$.

In particular there exists an equilibrium point $x^e \in \text{int}(\mathbb{R}_+^3)$ which is GAS for system (22) with feedback (23) with respect to initial conditions in $\text{int}(\mathbb{R}_+^3)$.

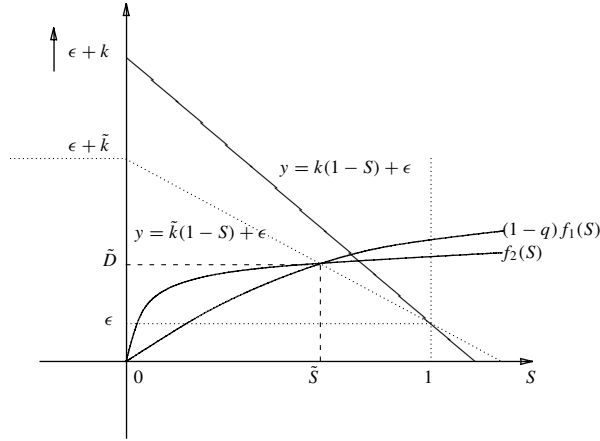


Fig. 5. Bound for k .

Remark 9. As before for the classical chemostat, the feedback (23) is independent of the nutrient concentration $S(t)$. In control theory the feedback law (23) with constraint (24) is sometimes called a ‘high gain feedback law’.

A comparison of feedback (9) with feedback (23) reveals that there is only one gain k , instead of two gains k_1 and k_2 . There are three reasons for restricting the number of gain constants here:

1. First, it is possible to achieve permanence of the plasmid model (18) by means of a single gain, while this was not possible for the classical chemostat model (8), see the inequalities (10) which imply that $k_1 \neq k_2$.
2. A single gain constant k offers the advantage of cheaper implementation for the feedback (23). Indeed, in this case $D(x)$ depends only on the *sum* of the concentrations of x_1 and x_2 and not on their respective values; the observer need not distinguish one strain from another – a potentially difficult and expensive process. This contrasts to the case of the classical chemostat model, see remark 2, where a more expensive implementation is necessary.
3. A single gain results in a simplification of the analysis of the resulting model.

Remark 10. As before a simple graphical interpretation can be given for the inequality (24), see Figure 5: The intersection of the line $y = k(1 - S) + \epsilon$, and the y -axis should be above the intersection of the line $y = \tilde{k}(1 - S) + \epsilon$ and the y -axis.

Remark 11. Next we outline why feedback control might be advantageous as a control strategy. The reason is the superior robustness property achieved with feedback control, compared to the situation where the dilution rate is constant.

Suppose that the functions $(1 - q)f_1$ and f_2 are not known exactly, but only bounds are available which we denote as $(1 - q)f_1^l, (1 - q)f_1^u, f_2^l$ and f_2^u , see Figure 6. These functions are assumed to satisfy regularity and monotonicity properties

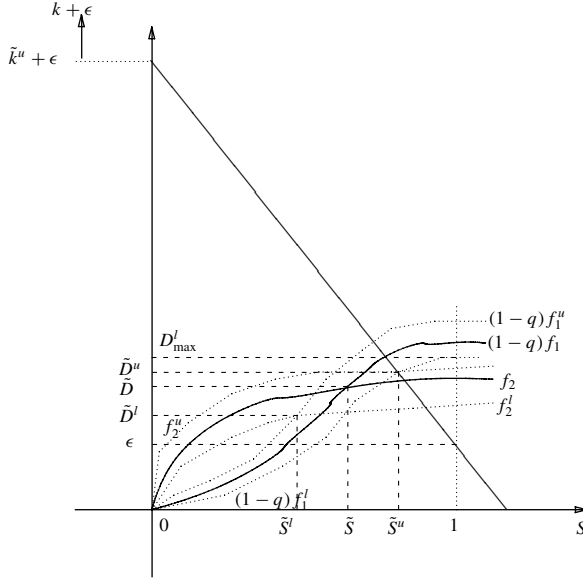


Fig. 6. Robustness.

and, being bounds for $(1 - q)f_1$ and f_2 , satisfy the following:

$$\begin{aligned} \forall S \in \mathbb{R}_+ : (1 - q)f_1^l(S) &\leq (1 - q)f_1(S) \leq (1 - q)f_1^u(S) \\ \forall S \in \mathbb{R}_+ : f_2^l(S) &\leq f_2(S) \leq f_2^u(S) \end{aligned} \quad (25)$$

Furthermore we assume that instead of \tilde{S} , only a compact interval $I := [\tilde{S}^u, \tilde{S}^l]$ (which is assumed to be a subset of $(0, 1)$) is known to contain \tilde{S} , where the bounds of I are implicitly given by:

$$\begin{aligned} f_2^l(\tilde{S}^l) &= (1 - q)f_1^u(\tilde{S}^l) \quad (:= \tilde{D}^l) \\ f_2^u(\tilde{S}^u) &= (1 - q)f_1^l(\tilde{S}^u) \quad (:= \tilde{D}^u) \end{aligned} \quad (26)$$

and where it is assumed that:

$$\begin{aligned} \forall S \in (0, \tilde{S}^l) : (1 - q)f_1^u(S) &< f_2^l(S) \\ \forall S > \tilde{S}^u : f_2^u(S) &< (1 - q)f_1^l(S) \end{aligned}$$

If the dilution rate is constant, it can be shown that permanence is achievable if $D \in (\tilde{D}^u, D_{\max}^l)$, where $D_{\max}^l := (1 - q)f_1^l(1)$. Dilution rates outside this interval do not guarantee permanence. If \tilde{D}^l and D_{\max}^l are very close to each other, the operator who is controlling the chemostat should be very careful in setting the dilution rate.

On the contrary if the dilution rate is implemented as a feedback law as in (23), it can be shown that permanence is achieved if $\epsilon \in (0, \tilde{D}^l)$ and $k > \tilde{k}^u := \frac{\tilde{D}^u - \epsilon}{1 - \tilde{S}^u}$. In other words, if the gain is chosen high enough, permanence is achieved.

Remark 12. We have assumed throughout this section that hypothesis \mathbf{H}' was satisfied, implying that the plasmid-free (plasmid-bearing) organism is the superior competitor for low (high) nutrient concentrations. An equally interesting case occurs when the opposite holds, in which case the assumption should be changed to the following:

\mathbf{H}'' The graphs of the functions $(1 - q)f_1$ and f_2 intersect once at \tilde{S} :

$$(1 - q)f_1(\tilde{S}) = f_2(\tilde{S}) = \tilde{D} \quad (27)$$

where $\tilde{S} \in (0, 1)$. For all $S \in (0, \tilde{S})$ the inequality $(1 - q)f_1(S) > f_2(S)$ holds, while for all $S > \tilde{S}$, $(1 - q)f_1(S) < f_2(S)$ holds.

A similar permanence result can be obtained by means of suitably chosen affine feedback laws. Since the proof of this result is very similar to that of theorem 4, we have decided to omit it here.

Finally, in theory there are two remaining cases. One is where the plasmid-bearing competitor is always the superior competitor. This is an (biologically) unrealistic assumption. The other case is the opposite situation, where the plasmid-free competitor is always the superior competitor. But we already mentioned in the beginning of this section that in this case there is no hope of achieving permanence. Indeed, a necessary condition for permanence is that at least for some nutrient concentrations, the plasmid-bearing competitor is superior.

7. Proofs

7.1. Classical chemostat model

We start by proving that all solutions of a chemostat controlled by an affine feedback law for the dilution rate are bounded and that they converge to a particular subset in \mathbb{R}_+^3 .

Proposition 1. *If $\epsilon > 0$ and $k_i \geq 0$, $i = 1, 2$, or if $\epsilon = 0$ and $k_i > 0$, $i = 1, 2$ then all solutions of system (8) with feedback (9) starting in \mathbb{R}_+^3 are bounded. If $x(t) := (S(t), x_1(t), x_2(t))$ is a solution starting in \mathbb{R}_+^3 , then*

$$\lim_{t \rightarrow +\infty} S(t) + x_1(t) + x_2(t) = 1 \quad (28)$$

Proof. Define the function $V : \mathbb{R}_+^3 \rightarrow \mathbb{R}_+$ as follows:

$$V(x) := \frac{1}{2}(S + x_1 + x_2 - 1)^2 \quad (29)$$

It follows from the system equations (8) that:

$$\dot{V} = -2D(x)V(x) \quad (30)$$

Case 1. $\epsilon > 0$.

Observe that $D(x) > 0$ for all $x \in \mathbb{R}_+^3$ because $k_i \geq 0$, $i = 1, 2$ and $\epsilon > 0$. This implies that $\dot{V} \leq 0$ for all $x \in \mathbb{R}_+^3$. Consequently, all solutions of system (8) with feedback (9) in \mathbb{R}_+^3 are bounded since V is radially unbounded in \mathbb{R}_+^3 . Furthermore,

Lasalle's invariance principle implies that all solutions converge to the largest invariant set E contained in the set $M := \{x \in \mathbb{R}_+^3 \mid \dot{V} = 0\}$. As before $D(x) > 0$ for $x \in \mathbb{R}_+^n$ and therefore (30) implies that $M = \{x \in \mathbb{R}_+^3 \mid V(x) = 0\}$ or:

$$\lim_{t \rightarrow +\infty} d(x(t), M) = 0 \quad (31)$$

where $d(a, A)$ is the (Euclidean) distance between the point a and the set A . The proof is concluded by observing that (31) and (28) are equivalent.

Case 2. $\epsilon = 0$.

We will prove that since $k_i > 0$, $i = 1, 2$ all solutions of system (8) with feedback (9) starting in \mathbb{R}_+^3 but not on the S -axis are bounded and if $x(t) := (S(t), x_1(t), x_2(t))$ is such a solution, then (28) holds. Consider (30) but observe that $D(x)$ is only nonnegative instead of positive in \mathbb{R}_+^3 . Lasalle's invariance principle learns that solutions converge to the largest invariant set contained in the union of the S -axis and the set $\{x \in \mathbb{R}_+^3 \mid V(x) = 0\}$. We show next that no point of the S -axis can be the ω limit point of a solution which is not initiated on the S -axis (observe that the S -axis is an invariant set consisting of equilibrium points).

If $S(t_0) = r$ at some instant t_0 where $r \geq 0$ is sufficiently small then $\dot{S} = \sum_{i=1,2} (k_i(1-r) - f_i(r))x_i$ which is positive if $(x_1, x_2) \neq (0, 0)$. Thus there exists a real number $r_0 > 0$ such that all solutions not initiated on the S -axis satisfy $S(t) > r_0$ for sufficiently large t . We show next that for sufficiently large t , $D(t) > p_0$ -where p_0 is some positive real number- holds for all solutions not initiated on the S -axis. Pick any p satisfying $f_i(r_0) > p > 0$, $i = 1, 2$. Then if $D(t_1) = p$ at some instant t_1 , $\dot{D} \geq D \min(f_i(r_0) - p) > 0$ and thus there exists $p_0 > 0$ such that the above claim holds. This concludes the proof that no point of the S -axis is an ω limit point and therefore (28) holds. \square

The proof of the previous result motivates the definition of the following set:

$$\Omega := \{x \in \mathbb{R}_+^3 \mid S + x_1 + x_2 = 1\} \quad (32)$$

According to proposition 1 the ω -limit set of every solution of system (8) and feedback (9) belongs to the set Ω , which is an invariant set for the system. It may be conjectured that the asymptotic behavior of solutions on the set Ω also determines the asymptotic behavior of solutions of the original system. Although this is not true in general, we will show later that in this case it is true.

Therefore we study the behavior of the original system restricted to the set Ω . The dynamics of this system is governed by the differential equation which results by substituting S by $1 - x_1 - x_2$ in the original system equations:

$$\begin{aligned} \dot{x}_1 &= x_1(f_1(1 - x_1 - x_2) - D(x)) \\ \dot{x}_2 &= x_2(f_2(1 - x_1 - x_2) - D(x)) \\ x_1(0) &\geq 0, \quad x_2(0) \geq 0 \text{ and } x_1(0) + x_2(0) \leq 1 \end{aligned} \quad (33)$$

where $D(x) = k_1x_1 + k_2x_2 + \epsilon$ as before. Notice that if $\epsilon \in [0, \tilde{D})$ and $k_i \geq 0$, $i = 1, 2$ are chosen to satisfy (10), then system (33) possesses four equilibrium

points:

$$E_0^r = (0, 0), E_1^r = (1 - \lambda_1, 0), E_2^r = (0, 1 - \lambda_2) \text{ and } E_3^r = (x_1^e, x_2^e) \quad (34)$$

where λ_i and x_i^e , $i = 1, 2$ are given by:

$$\begin{aligned} f_i(\lambda_i) &= k_i(1 - \lambda_i) + \epsilon, \quad i = 1, 2 \\ x_1^e &= \frac{1}{k_1 - k_2} (\tilde{D} - (k_2(1 - \tilde{S}) + \epsilon)) \\ x_2^e &= \frac{1}{k_1 - k_2} ((k_1(1 - \tilde{S}) + \epsilon) - \tilde{D}) \end{aligned} \quad (35)$$

The assumptions on ϵ and k_i , $i = 1, 2$ imply that $x_i^e > 0$ and thus that $E_3^r \in \text{int}(\mathbb{R}_+^2)$. Recall also that the values λ_i , $i = 1, 2$ can be easily found from the graphs in Figure 2. For example, λ_1 is the value of the concentration of the intersection point of the graph of f_1 and the line $k_1(1 - S) + \epsilon$.

The asymptotic behavior of system (33) is discussed in the following proposition.

Proposition 2. *If $\epsilon \in [0, \tilde{D})$ and if the gains $k_i \geq 0$, $i = 1, 2$ satisfy the inequalities (10), then E_3^r is a globally asymptotically stable equilibrium point for system (33) with respect to initial conditions satisfying $x_1(0) > 0$, $x_2(0) > 0$ and $x_1(0) + x_2(0) \leq 1$.*

Proof. The proof is based on the Poincaré-Bendixson theorem, which sums up the possible ω -limit sets of two-dimensional systems.

Denote the Jacobian matrices of the equilibrium points E_k^r , $k = 0, \dots, 3$ of system (33) by J_k and the spectrum of these matrices by $\sigma(J_k)$. Simple calculations and using the assumptions on ϵ and k_i , $i = 1, 2$, leads to the following conclusions:

$$\begin{aligned} \sigma(J_0) &= \{r_0^1, r_0^2\} \text{ with } r_0^j > 0, \quad j = 1, 2. \\ \sigma(J_1) &= \{r_1^1, r_1^2\} \text{ with } r_1^1 > 0 \text{ and } r_1^2 < 0. \\ \sigma(J_2) &= \{r_2^1, r_2^2\} \text{ with } r_2^1 > 0 \text{ and } r_2^2 < 0. \\ \sigma(J_3) &= \{r_3^1, r_3^2\} \text{ with } r_3^j < 0, \quad j = 1, 2. \end{aligned}$$

This means that E_0^r is a repellor, E_3^r is locally asymptotically stable and E_1^r and E_2^r are saddle points.

Consider a solution $(x_1(t), x_2(t))$ with $x_i(0) > 0$, $i = 1, 2$. A simple application of the Butler-McGehee theorem, see e.g. [22], shows that E_1^r and E_2^r cannot belong to the ω -limit set of this solution. The same is true for E_0^r since it is a repellor. Notice that system (33) is a *competitive system*, i.e. the off-diagonal entries of the Jacobian matrix in all points are negative or zero, implying that the system does not exhibit periodic solutions, see [7] or [21]. Therefore the only possible ω -limit set of $(x_1(t), x_2(t))$ is the equilibrium point E_3^r or a cycle containing only one equilibrium point which would necessarily be E_3^r . But since a cycle cannot contain a locally asymptotically stable equilibrium point, the ω -limit set of $(x(t), x_2(t))$ is E_3^r . This concludes the proof. \square

Finally we are in a position to prove theorem 2.

Proof of Theorem 2

First we introduce the following variable:

$$\Sigma := S + x_1 + x_2 - 1 \quad (36)$$

System (8) with feedback (9) takes the form:

$$\begin{aligned} \dot{x}_i &= x_i(f_i(1 - \Sigma - x_1 - x_2) - D(x)), \quad i = 1, 2 \\ \dot{\Sigma} &= -D(x)\Sigma \end{aligned} \quad (37)$$

where $\Sigma(0) \geq -1$ and $x_i(0) \geq 0$. From proposition (1) we obtain that $\lim_{t \rightarrow +\infty} \Sigma(t) = 0$ and that system (37) is uniformly bounded. On the set $\Omega := \{(x, \Sigma) \in \mathbb{R}_+^2 \times [-1, +\infty) \mid \Sigma = 0\}$ the dynamics of system (37) is given by system (33).

Notice that system (37) takes the form of system (44) and system (33) the form of system (45) in the Appendix. This is immediate in the case $\epsilon > 0$. If $\epsilon = 0$ it follows from the asymptotic inequality $D(t) > p_0$ for large t , which was shown in the proof of proposition 1. Relying on proposition 2 it can be checked that hypotheses **H1**–**H3** are true for system (33). Hypothesis **H4** holds since if system (33) would possess a cycle in Ω , only E_1^r and/or E_2^r could possibly belong to it. Indeed, it as been shown in the proof of proposition 2 that E_0^r is a repellor and E_3^r is locally asymptotically stable and clearly these equilibrium points cannot belong to a cycle. But since the stable manifolds of E_1^r and E_2^r are portions of the x_1 -, respectively x_2 -axis (and both axes are invariant sets for system (33)), they cannot be part of a cycle of equilibrium points either. Consequently theorem 5 can be applied to system (37). In particular it follows that almost all solutions of this system converge to the equilibrium point $(E_3^r, 0)$ where E_3^r is the asymptotically stable equilibrium point of system (33). The solutions which do not converge to this equilibrium point belong to the stable manifolds of the equilibrium points $(E_1^r, 0)$ and $(E_2^r, 0)$ (where E_2^r and E_3^r are the saddle points of system (33)), but these stable manifolds are subsets of the boundary faces $\{(x_1, x_2, \Sigma) \in \mathbb{R}^3 \mid x_2 = 0\}$, respectively $\{(x_1, x_2, \Sigma) \in \mathbb{R}^3 \mid x_1 = 0\}$, while we are only interested in solutions with initial condition satisfying $x_i(0) > 0$, $i = 1, 2$. These facts are easily rephrased for the original system (8), which concludes the first part of the proof.

The converse statement in the theorem follows from the following argument. The Jacobian matrix evaluated at the interior equilibrium point E_3^r :

$$J(E_3^r) = \begin{pmatrix} -\tilde{x}_1(f_1'(\tilde{S}) + k_1) & -\tilde{x}_1(f_1'(\tilde{S}) + k_2) \\ -\tilde{x}_2(f_2'(\tilde{S}) + k_1) & -\tilde{x}_2(f_2'(\tilde{S}) + k_2) \end{pmatrix}$$

is a Hurwitz matrix (it is always nonsingular by the last part of hypothesis **H**) only if $k_1 > k_2$. Since $k_i \geq 0$ it follows that $k_1 > 0$ and therefore the equalities $D(\tilde{x}_1, \tilde{x}_2) = k_1\tilde{x}_1 + k_2\tilde{x}_2 + \epsilon = \tilde{D}$ imply that $\epsilon < \tilde{D}$. Finally, the equilibrium point E_3^r belongs to $\text{int}(\mathbb{R}_+^2)$ only if (10) are satisfied which follows immediately from the expression (35).

7.2. Plasmid-bearing and plasmid-free competitors

The proof of theorem 4 is similar to the proof of theorem 2. Therefore we will not provide all details, but only focus on the differences.

First of all it is easy to prove that all solutions of system (22) with feedback (23) are bounded if $\epsilon > 0$ and $k \geq 0$, and that they converge to the set Ω (see (32)). The proof is based on the same arguments as the proof of proposition 1 and therefore omitted.

The second step is then to study the reduced system on Ω , which is given by the following differential equation:

$$\begin{aligned} \dot{x}_1 &= x_1((1-q)f_1(1-x_1-x_2) - D(x)) \\ \dot{x}_2 &= x_2(f_2(1-x_1-x_2) - D(x)) + qx_1f_1(1-x_1-x_2) \\ x_1(0) &\geq 0, \quad x_2(0) \geq 0 \text{ and } x_1(0) + x_2(0) \leq 1 \end{aligned} \quad (38)$$

where $D(x) = k(x_1 + x_2) + \epsilon$ as before. If $\epsilon \in (0, \tilde{D})$ and the gain k satisfies the condition (24) of theorem 4, then system (38) possesses three equilibrium points:

$$E_0^r = (0, 0), \quad E_2^r = (0, 1 - \lambda_2) \text{ and } E_3^r = (x_1^e, x_2^e) \quad (39)$$

where λ_2 is implicitly defined by:

$$f_2(\lambda_2) := k(1 - \lambda_2) + \epsilon \quad (40)$$

The value of λ_2 can be easily determined graphically, see Figure 7: For given values of ϵ and k (satisfying the conditions of theorem 4), the intersection of the line $y = k(1 - S) + \epsilon$ and the graph of $f_2(S)$ determine the value of λ_2 .

In (39), the values of x_i^e , $i = 1, 2$, are given by:

$$\begin{aligned} x_1^e &= \frac{(1 - \lambda^*)((1 - q)f_1(\lambda^*) - f_2(\lambda^*))}{f_1(\lambda^*) - f_2(\lambda^*)} \\ x_2^e &= \frac{(1 - \lambda^*)qf_1(\lambda^*)}{f_1(\lambda^*) - f_2(\lambda^*)} \end{aligned} \quad (41)$$

where λ^* is implicitly defined by:

$$(1 - q)f_1(\lambda^*) := k(1 - \lambda^*) + \epsilon \equiv D^* \quad (42)$$

As was the case for λ_2 , it is easy to determine λ^* and D^* graphically, see Figure 7. Since ϵ and k satisfy the conditions of theorem 4, it can be verified that the following holds:

$$\tilde{S} < \lambda^* < \lambda_2 < 1 \text{ and } D^* > \tilde{D} \quad (43)$$

This implies in particular that $x_i^e > 0$, $i = 1, 2$ and thus that $x^e \in \text{int}(\mathbb{R}_+^3)$ as claimed in the statement of theorem 4.

An important difference between the reduced system (38) and the reduced system (33) is that system (38) is not a competitive system. Recall that competitive planar systems cannot have nontrivial periodic solutions and that we used this to determine the asymptotic behavior of system (33). Fortunately we can also exclude the existence of nontrivial periodic solutions of system (38) by means of Dulac's criterion as we show next.

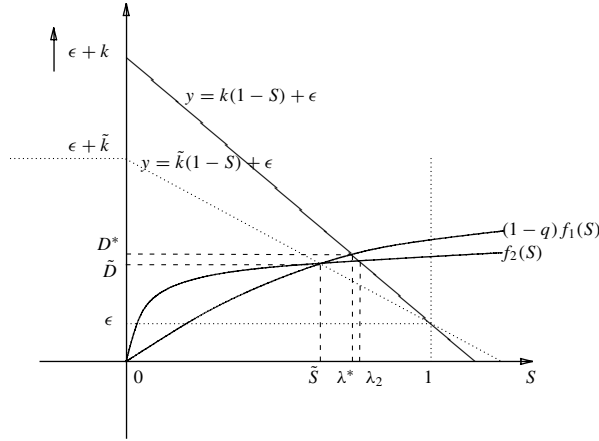


Fig. 7. Nutrient concentration values at equilibrium points.

Lemma 1. *If $k \geq 0$ then system (38) does not possess nontrivial periodic solutions.*

Proof. Denote the vector field in the right-hand side of system (38) by $F(x_1, x_2)$ and consider the divergence of the vector field $\frac{1}{x_1 x_2} F(x_1, x_2)$:

$$-\frac{1}{x_2} \left(\frac{\partial f_1}{\partial S} (1 - x_1 - x_2) + k \right) - \frac{1}{x_1} \left(\frac{\partial f_2}{\partial S} (1 - x_1 - x_2) + k \right) - \frac{q f_1 (1 - x_1 - x_2)}{x_2^2}$$

which is negative for all $x_i, i = 1, 2$ of interest. Dulac’s criterion for the exclusion of nontrivial periodic orbits is therefore satisfied which concludes the proof. \square

The asymptotic behavior of the reduced system (38) is then summarized as follows.

Proposition 3. *If ϵ and the gain k satisfy the conditions of theorem 4 then E_3^r is a GAS equilibrium point for system (38) with respect to initial conditions satisfying $x_1(0) > 0, x_2(0) > 0$ and $x_1(0) + x_2(0) \leq 1$.*

Proof. Since the proof is very similar to the proof of proposition 2 and we only provide a sketch of it. It is also based on the Poincaré-Bendixson theorem.

From a calculation of the Jacobian matrices at the equilibrium points of system (38) follows that E_0^r is a repeller, E_2^r is a saddle point and E_3^r is locally asymptotically stable. By lemma 1 system (38) does not possess nontrivial periodic solutions and the Butler-McGehee theorem shows that E_2^r does not belong to the limit set of solutions with initial conditions $x_i(0) > 0, i = 1, 2$. Therefore E_3^r is GAS and this concludes the proof. \square

Finally the proof of theorem 4 can be concluded with similar arguments used to finish the proof of theorem 2 (see the end of the previous subsection). In particular, theorem 5 is invoked, but since the details are similar as before, we omit the proof.

8. Appendix

In this section we state a convergence theorem.

Consider the following system:

$$\begin{cases} \dot{x} = f(x, y), & x \in \mathbb{R}^n \\ \dot{y} = -\gamma(x)y, & y \in \mathbb{R} \end{cases} \quad (44)$$

where $f : \mathbb{R}^{n+1} \rightarrow \mathbb{R}^n$ and $\gamma : \mathbb{R}^n \rightarrow \mathbb{R}_+ \setminus \{0\}$ are sufficiently smooth (say at least of class C^1). We assume that D is a forward invariant set for system (44) and henceforth restrict initial conditions to D . Moreover it is assumed that solutions of system (44) are *uniformly bounded*, i.e. there exists a compact subset of D into which all solutions enter at some time and remain.

Next consider the following system:

$$\dot{x} = f(x, 0) \quad (45)$$

where $x \in \Omega := \{x \in \mathbb{R}^n \mid (x, 0) \in D\} \subset \mathbb{R}^n$. For system (45) we introduce the following set of hypotheses:

H1 There are only a finite number, say p , equilibrium points in Ω and these are denoted as x_1, \dots, x_p .

H2 Denoting the stable manifold of equilibrium point x_j as $W^s(x_j)$, the following holds for the dimensions of these manifolds:

$$\begin{aligned} \forall j = 1, \dots, r &: \dim(W^s(x_j)) = n \\ \forall j = r + 1, \dots, p &: \dim(W^s(x_j)) < n \end{aligned}$$

for some $r \in \{1, 2, \dots, p\}$.

H3 $\bigcup_{j=1}^p W^s(x_j) = \Omega$.

H4 There are no cycles of equilibrium points in Ω .

The following result is then only a slight modification of theorem F.1 in [22].

Theorem 5. *If H1–H4 are true, then for some $i \in \{1, \dots, p\}$:*

$$\lim_{t \rightarrow +\infty} (x(t), y(t)) = (x_i, 0)$$

where $(x(t), y(t))$ is an arbitrary solution of system (44) in D . Moreover, $\bigcup_{j=r+1}^p \tilde{W}^s(x_j, 0)$ has Lebesgue measure zero, where $\tilde{W}^s(x_j, 0)$ is the stable manifold of equilibrium point $(x_j, 0)$ with respect to system (44).

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