



**HAL**  
open science

# A model of plasmid-bearing, plasmid-free competition in a chemostat

Tewfik Sari, Mohamed Dellal

► **To cite this version:**

Tewfik Sari, Mohamed Dellal. A model of plasmid-bearing, plasmid-free competition in a chemostat. 2024. hal-04514636

**HAL Id: hal-04514636**

**<https://hal.inrae.fr/hal-04514636>**

Preprint submitted on 21 Mar 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# A model of plasmid-bearing, plasmid-free competition in a chemostat

Tewfik Sari<sup>1</sup> and Mohamed Dellal<sup>2,3</sup>

<sup>1</sup> ITAP, Univ Montpellier, INRAE, Institut Agro, Montpellier, France  
tewfik.sari@inrae.fr

<sup>2</sup> Ibn Khaldoun University, 14000, Tiaret, Algeria

<sup>3</sup> LDM, Djillali Liabès University, 22000, Sidi Bel Abbès, Algeria

**Abstract.** In this paper we consider a competition model between plasmid-bearing and plasmid-free organisms in a chemostat that incorporates both general response functions, distinct yields and distinct removal rates. The model with identical removal rates and identical yields was studied in the existing literature. The object of this paper is to provide the local analysis of the model in the case of different removal rates and distinct yields. In this case the conservation law fails. The operating diagram giving the asymptotic behaviour of the model with respect of the operating parameters is also presented.

**Keywords:** Chemostat · Competition · Plasmid · Operating diagram.

## 1 Introduction

Genetically modified organisms are frequently used to produce other substances. Alteration is achieved by introducing DNA into the cell in the form of a plasmid. The genetically modified organism (the plasmid-bearing) is in competition with the plasmid-free organism. The chemostat is a common model in microbial ecology. It is used as an ecological model of a simple lake, as a model of waste-treatment, and as a model for commercial production of fermentation processes [3, 4, 7, 11].

The following model of competition between plasmid-bearing and plasmid-free organisms in a chemostat based on the mass balances of the organisms was proposed by Stephanopoulos and Lapidus [12]

$$\begin{aligned} S' &= (S^0 - S)D - \frac{1}{\gamma}f_1(S)x_1 - \frac{1}{\gamma}f_2(S)x_2, \\ x_1' &= [(1 - q)f_1(S) - D]x_1, \\ x_2' &= [f_2(S) - D]x_2 + qf_1(S)x_1. \end{aligned} \tag{1}$$

$S(t)$ ,  $x_1(t)$  and  $x_2(t)$  are the concentrations of nutrient, plasmid-bearing organisms and plasmid-free organisms at time  $t$ , respectively. The probability that a plasmid is lost in reproduction is represented by  $q$  and hence  $0 < q < 1$ . The specific growth rates of plasmid-bearing and plasmid-free organisms are  $f_1(S)$  and  $f_2(S)$ , respectively. The consumption rates are  $\frac{1}{\gamma}f_i(S)$  where  $\gamma$  is the

yield constant, assumed to be the same for both populations. Note that in this model the total biomass  $b = S + x_1/\gamma + x_2/\gamma$  satisfies the differential equation  $b' = D(S^0 - b)$  and using the theory of asymptotically autonomous systems the study of the system can be reduced to the invariant set  $S + x_1/\gamma + x_2/\gamma = S^0$ . Since the reduced system is two dimensional it is possible to obtain global asymptotic stability results.

Using index theory arguments, Stephanopoulos and Lapidus [12] determined the local stability analysis in the case where the growth rates are uninhibited, of the form  $f_i(S) = \frac{m_i S}{K_i + S}$  or inhibited of the form  $f_i(S) = \frac{m_i S}{K_i + S + S^2/K_I}$ . Hsu, Waltman and Wolkowicz [5] considered (1) in the case of arbitrary uninhibited growth rates and obtained global results. However, the validity of the assumption in (1) that all the removal rates are equal is not valid if a competitor death rate is significant. For this reason Li, Kuang and Smith [6] considered the model

$$\begin{aligned} S' &= (S^0 - S)D - \frac{1}{\gamma}f_1(S)x_1 - \frac{1}{\gamma}f_2(S)x_2, \\ x_1' &= [(1 - q)f_1(S) - D_1]x_1, \\ x_2' &= [f_2(S) - D_2]x_2 + qf_1(S)x_1. \end{aligned} \quad (2)$$

where  $D_1$  and  $D_2$  are not necessarily equal to  $D$ . In this case the model cannot be reduced to a two dimensional model. Our aim in this paper is to consider the more realistic situation where also the yields are not necessarily equal:

$$\begin{aligned} S' &= (S^0 - S)D - \frac{1}{\gamma_1}f_1(S)x_1 - \frac{1}{\gamma_2}f_2(S)x_2, \\ x_1' &= [(1 - q)f_1(S) - D_1]x_1, \\ x_2' &= [f_2(S) - D_2]x_2 + qf_1(S)x_1. \end{aligned} \quad (3)$$

The parameters  $\gamma_1$  and  $\gamma_2$  are the growth yield coefficients, representing the conversion of nutrients to biomass. They are not assumed to be the same for both populations. We construct the operating diagram of the system, that is the bifurcation diagram with respect of the operating parameters. The operating parameters are  $S^0$ , the input concentration of the nutrient and  $D$ , the dilution rate of the chemostat. This diagram is very useful to understand the model when the biological parameters are fixed and the operating parameters are varied, as they are the most easily manipulated parameters in a chemostat. This diagram is very important to understand the model from the mathematical and biological point of view. It is often built in the literature [1–3, 8–10].

## 2 Results

We consider the system (3) and we assume that

**H1**  $f_i(S)$  are continuously differentiable, with  $f_i(0) = 0$  and  $f_i'(S) > 0$  for all  $S > 0$ .

**H2**  $D_i = \alpha_i D + \varepsilon_i$  for  $i = 1, 2$  where  $\varepsilon_i$  is the specific death rate of  $x_i$  and  $\alpha_i \in (0, 1]$  is a parameter allowing us to decouple the Hydraulic Retention Time,  $\text{HRT} = 1/D$  and the Solid Retention Time  $\text{SRT} = 1/(\alpha_i D)$

The positivity and boundedness of the solutions of the system (3) is proved as in [6, Section 2].

## 2.1 Existence and stability of equilibria

We need to define the break-even concentrations  $\lambda_1$  and  $\lambda_2$ .

$$\lambda_1 = f_1^{-1}\left(\frac{D_1}{1-q}\right), \text{ if } D_1 \in [0, (1-q)f_1(\infty)) \quad (\lambda_1 = \infty \text{ if } D_1 \geq f_1(\infty)), \quad (4)$$

$$\lambda_2 = f_2^{-1}(D_2), \text{ if } D_2 \in [0, f_2(\infty)) \quad (\lambda_2 = \infty \text{ if } D_2 \geq f_2(\infty)). \quad (5)$$

We have the following result.

**Theorem 1.** *The system (3) can have up to three equilibria:*

- *The washout equilibrium  $E_1 = (S^0, 0, 0)$  of extinction of both organisms.*
- *The equilibrium involving plasmid-free organisms but no plasmid-bearing organisms, denoted  $E_2 = (\lambda_2, 0, x_2)$ , where  $\lambda_2$  is defined by (5) and*

$$x_2 = \frac{D\gamma_2}{D_2}(S^0 - \lambda_2). \quad (6)$$

- *The mixed culture equilibrium  $E_c = (S^*, x_1^*, x_2^*)$ , where  $S^* = \lambda_1$  is defined by (4) and*

$$x_1^* = \frac{\frac{D\gamma_1(1-q)}{D_1}(D_2 - f_2(\lambda_1))(S^0 - \lambda_1)}{D_2 - \left(1 - q\frac{\gamma_1}{\gamma_2}\right)f_2(\lambda_1)}, \quad x_2^* = \frac{q\gamma_1 D(S^0 - \lambda_1)}{D_2 - \left(1 - q\frac{\gamma_1}{\gamma_2}\right)f_2(\lambda_1)}. \quad (7)$$

*The conditions of existence and local stability of these equilibria are given in Table 1, where  $F$  is defined by*

$$F(S^0, D) = (a_1 + c_3)(a_1c_3 + a_3c_1) + a_1a_2b_1 - a_2b_3c_1. \quad (8)$$

*where  $a_i$ ,  $b_i$  and  $c_i$  are defined by*

$$\begin{aligned} a_1 &= D + \frac{1}{\gamma_1}f_1'(\lambda_1)x_1^* + \frac{1}{\gamma_2}f_2'(\lambda_1)x_2^*, \\ a_2 &= (1-q)f_1'(\lambda_1)x_1^*, \quad a_3 = f_2'(\lambda_1)x_2^* + qf_1'(\lambda_1)x_1^*, \\ b_1 &= \frac{1}{\gamma_1}\frac{D_1}{1-q}, \quad b_3 = \frac{qD_1}{1-q}, \quad c_1 = \frac{1}{\gamma_2}f_2(\lambda_1), \quad c_3 = D_2 - f_2(\lambda_1). \end{aligned} \quad (9)$$

*where  $x_1^*$  and  $x_2^*$  are given by (7).*

**Table 1.** Existence and local stability of equilibria of (3) where  $\lambda_1$ ,  $\lambda_2$  and  $F(D, S^0)$  are defined by (4), (5) and (8) respectively.

Equilibria	Existence	Local stability
$E_1$	Always	$S^0 < \min(\lambda_1, \lambda_2)$
$E_2$	$\lambda_2 < S^0$	$\lambda_2 < \lambda_1$
$E_c$	$\lambda_1 < \min(S^0, \lambda_2)$	$F(S^0, D) > 0$

*Proof.* The proof is given in Appendix A.1.

We now consider in more detail the stability of  $E_c$ .

**Proposition 2** *We have the following sufficient conditions of stability of  $E_c$ .*

1. *If  $D_1 \leq D$ , then  $E_c$  is stable if it exists. In particular, when the removal rates are equal, then  $E_c$  is stable, if it exists.*
2. *As it exists  $E_c$  is LES if  $DD_1 + q\frac{\gamma_1}{\gamma_2}D_2(D - D_1) \geq 0$ .*
3. *Let  $\varepsilon = (1 - q\frac{\gamma_1}{\gamma_2}\alpha_2)\varepsilon_1 + q\frac{\gamma_1}{\gamma_2}(1 - \alpha_1)\varepsilon_2$ ,  $\alpha = \alpha_1 + q\frac{\gamma_1}{\gamma_2}\alpha_2(1 - \alpha_1)$  and  $\Delta = \varepsilon^2 + 4\alpha q\frac{\gamma_1}{\gamma_2}\varepsilon_1\varepsilon_2$ . Let  $D_0 = \frac{\sqrt{\Delta} - \varepsilon}{2\alpha}$ . If  $D \geq D_0$ , then  $E_c$  is stable if it exists.*

*Proof.* The proof is given in Appendix A.2.

Since in [6]  $D$  is normalized to 1 and  $\gamma_1 = \gamma_2$ , the sufficient condition in item 2 of Prop. 2 is equivalent to the condition (3.15) in [6].

## 2.2 Operating diagram

The effect of the operating conditions on the asymptotic behavior of the system can be summarized with the aid of the operating diagram. The operating diagram has the operating parameters  $S^0$  and  $D$  as its coordinates and the various regions defined in it correspond to qualitatively different asymptotic behaviors. In order to construct the operating diagram of (3), one needs to determine and compute the boundaries of regions of this diagram, i.e. to compute the parameters values at which a qualitative change in the asymptotic behavior of the system (3) occurs. Since we want to study the behavior of the system as a function of operating parameters, it is preferable to use the notations

$$\lambda_1(D_1) = f_1^{-1}\left(\frac{D_1}{1-q}\right) \quad \text{and} \quad \lambda_2(D_2) = f_2^{-1}(D_2),$$

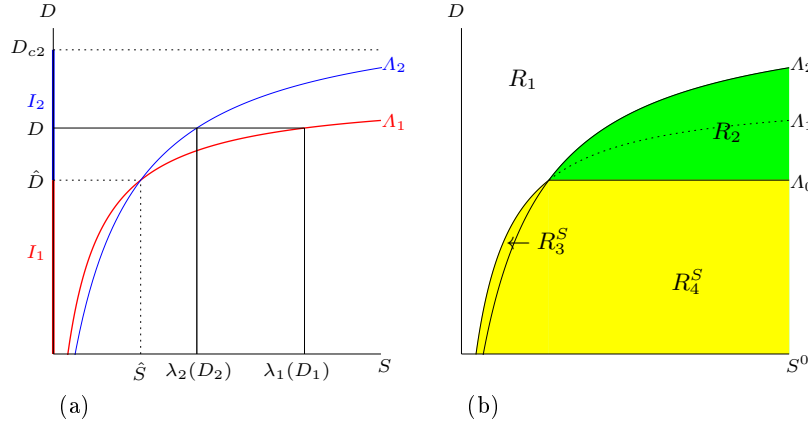
for the break-even concentrations  $\lambda_1$  and  $\lambda_2$ , defined by (4) and (5), respectively. Also recall that  $D_1$  and  $D_2$  are given by **H2**. From Table 1 we deduce that, among these boundaries, are the curves  $A_1$  and  $A_2$  defined by

$$\begin{aligned} A_1 &= \{(S^0, D) : S^0 = \lambda_1(\alpha_1 D + \varepsilon_1)\}, \\ A_2 &= \{(S^0, D) : S^0 = \lambda_2(\alpha_2 D + \varepsilon_2)\}. \end{aligned} \tag{10}$$

**Remark 1** *From the definitions of  $\lambda_1$  and  $\lambda_2$ , the curves  $A_1$  and  $A_2$  are also given by  $A_1 = \{(S^0, D) : D = \frac{(1-q)f_1(S^0) - \varepsilon_1}{\alpha_1}\}$ ,  $A_2 = \{(S^0, D) : D = \frac{f_2(S^0) - \varepsilon_2}{\alpha_2}\}$ .*

In addition, Table 1 allows us to deduce that the values of the dilution rate for which  $\lambda_1(D_1) = \lambda_2(D_2)$  play a major role in the condition of existence and stability of the equilibria. For this purpose, we define the following sets of dilution rates, see Figs. 1(a) and 2(a).

$$\begin{aligned} I_1 &= \{D \in [0, D_{c1}) : \lambda_1(D_1) < \lambda_2(D_2)\}, \\ I_2 &= \{D \in [0, D_{c2}) : \lambda_2(D_2) < \lambda_1(D_1)\}. \end{aligned} \tag{11}$$



**Fig. 1.** (a) Sets  $I_1$  (in red) and  $I_2$  (in blue) of the  $D$  axis defined by (11):  $I_1 = (0, \hat{D})$  and  $I_2 = (\hat{D}, D_{c2})$ . (b) Operating diagram when the subset  $I_c$  of  $I_1$  defined by (19) is empty ( $R_3^U$  and  $R_4^U$  regions do not exist).

where the critical values  $D_{c1}$  and  $D_{c2}$  are defined by  $D_{c1} := \frac{(1-q)f_1(\infty)-\varepsilon_1}{\alpha_1}$  and  $D_{c2} := \frac{f_2(\infty)-\varepsilon_2}{\alpha_2}$ , respectively. Consider the curves  $A_1$  and  $A_2$  given by (10). Let  $(\hat{S}, \hat{D})$ , be an intersection point, if it exists. The case with only one intersection point is illustrated in Figs. 1(a) or 2(a). Other cases, with two intersection points or no intersection point will be illustrated in Section 3, by choosing particular growth functions, see Table 4. We define the following curves, see Figs. 1(b) and 2(b).

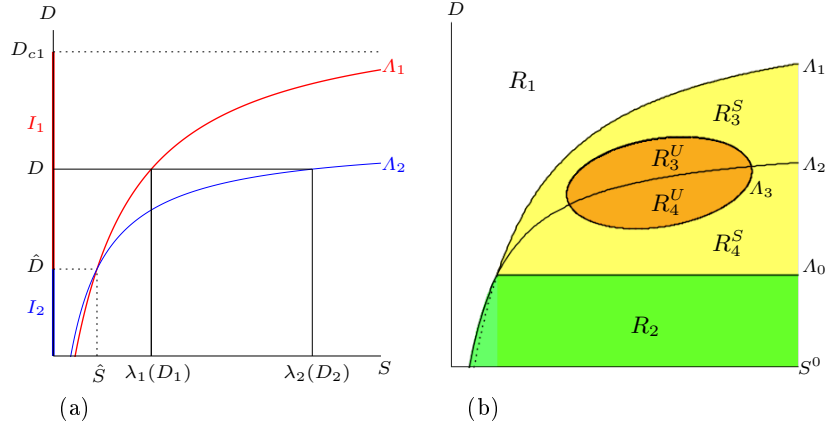
$$\begin{aligned} A_0 &= \{(S^0, D) : D = \hat{D} \text{ and } S^0 \geq \hat{S}\}, \\ A_3 &= \{(S^0, D) : F(S^0, D) = 0\}. \end{aligned} \quad (12)$$

Note that there are as many  $A_0$  curves as there are intersection points  $(\hat{S}, \hat{D})$  of the curves  $A_1$  and  $A_2$ . Using Lemma 5, the  $A_3$  curve is a set of closed disjoint simple curves.

The curves  $A_k$ ,  $k = 0, 1, 2, 3, 4$ , separate the set of operating parameters  $S^0 > 0$  and  $D > 0$  in at most six regions labelled  $R_1$ ,  $R_2$ ,  $R_3^S$ ,  $R_3^U$ ,  $R_4^S$  and  $R_4^U$  and defined in Table 2 and called hereafter the *regions of the operating diagram*. Note that  $R^U = R_3^U \cup R_4^U$  is the region of instability of  $E_c$  defined by (21). These regions are illustrated in Figs. 1(b) and 2(b) and in Section 3.

**Remark 2** *The region  $R_3^U$  and  $R_4^U$  may be empty, as in Fig. 1(b), meaning that  $E_c$  is stable if it exists. See also Figs. 4 and 5 in Section 3. If  $I_c$  is not empty, then at least one of the  $R_3^U$  or  $R_4^U$  regions is not empty, as in Fig. 2(b). In this figure we assume that  $I_c$  is an open interval. Hence, according to Lemma 5, the region  $R^U = R_3^U \cap R_4^U$  is homomorphic to the unit ball.*

The  $R_2$  region may be empty, meaning that equilibrium  $E_2$  does not exist, see Fig. 5(a). The  $R_4^S$  region may be empty, meaning that equilibrium  $E_c$  does



**Fig. 2.** (a) Sets  $I_1$  (in red) and  $I_2$  (in blue) of the  $D$  axis defined by (11):  $I_1 = (\hat{D}, D_{c1})$  and  $I_2 = (0, \hat{D})$ . (b) Operating diagram when the subset  $I_c$  of  $I_1$  defined by (19) is not empty (At least one of the  $R_3^U$  or  $R_4^U$  regions exists).

not exist, see Fig. 5(b). The regions  $R_2$ ,  $R_3^S$  and  $R_4^S$  can be not connected. We have the following result.

**Table 2.** Definitions of the regions of the operating diagram of (3)

Region
$R_1 = \{S^0 < \min(\lambda_1(D_1), \lambda_2(D_2))\}$
$R_2 = \{D \in I_2 \text{ and } S^0 > \lambda_2(D_2)\}$
$R_3^S = \{D \in I_1, \lambda_2(D_2) \geq S^0 > \lambda_1(D_1) \text{ and } F(D, S^0) > 0\}$
$R_3^U = \{D \in I_1, \lambda_2(D_2) \geq S^0 > \lambda_1(D_1) \text{ and } F(D, S^0) < 0\}$
$R_4^S = \{D \in I_1, S^0 > \lambda_2(D_2) > \lambda_1(D_1) \text{ and } F(D, S^0) > 0\}$
$R_4^U = \{D \in I_1, S^0 > \lambda_2(D_2) > \lambda_1(D_1) \text{ and } F(D, S^0) < 0\}$

**Proposition 3** *The asymptotic behavior of (3) in the regions of the operating diagram, defined in Table 2, is depicted in Table 3.*

*Proof.* The proof is given in Appendix A.3.

**Remark 3** *The  $R_1$  region corresponds to case I of [6]. The  $R_2$  region corresponds to case II or IVa of [6]. More precisely, the part of the  $A_1$  curve, corresponding to  $D \in I_2$ , separates the  $R_2$  region (colored in green) in two regions corresponding to cases II and IVa of [6], respectively. These two regions do not differ in any way with regard to the asymptotic behavior of the system, as shown*

**Table 3.** Existence and stability of equilibria of (3) in the regions of the operating diagram. The last column indicate the color in which the region is depicted in the figures.

Region	$E_1$	$E_2$	$E_c$	Color
$R_1$	S			White
$R_2$	U	S		Green
$R_3^S$	U		S	Yellow
$R_3^U$	U		U	Orange
$R_4^S$	U	U	S	Yellow
$R_4^U$	U	U	U	Orange

in [6, Table 1]. The  $R_3 = R_3^S \cup R_3^U$  region corresponds to case III of [6]. The  $R_4 = R_4^S \cup R_4^U$  region corresponds to case IVb of [6].

The operating diagrams shown in Figs. 1 and 2 are given only as illustrative examples, showing that our analysis gives a complete description of the behavior of the system for a large class of growth functions. Notice that for plotting operating diagrams we must choose the growth function  $f_1$  and  $f_2$  in (3), and fix the values of the biological parameters. We illustrate this in the next section for some examples that have been considered in the literature.

### 3 Applications to Monod growth functions

To plot the regions of an operating diagram, it is necessary to fix the biological parameters in (3). In the following figures we use the Monod growth functions  $f_1(S) = \frac{m_1 S}{K_1 + S}$  and  $f_2(S) = \frac{m_2 S}{K_2 + S}$ . We consider the biological parameters values given in Appendix C.

#### 3.1 The positive equilibrium is stable if it exists

The operating diagram shown in Fig. 4 corresponds to the biological parameters values used in [6, Fig. 6.1], with  $S^0 = D = 1$ . The curves  $A_1$  and  $A_2$  cross twice. The positive equilibrium  $E_c$  can exist only if  $0 < D < \hat{D}_1$  or  $\hat{D}_2 < D < D_{c1}$ , where  $\hat{D}_1 \approx 0.431$ ,  $\hat{D}_2 \approx 2.444$  and  $D_{c1} = 3.5$ . Using Proposition 2 and Proposition 4 we see that  $E_c$  is stable as soon as it exists. Therefore the regions  $R_3^U$  and  $R_4^U$  are empty, see Fig. 4.

**Remark 4** *The operating point  $(S^0, D) = (1, 1)$  belongs to the green region, so that  $E_2$  is stable, in accordance with [6, Fig. 6.1]. The additional information provided by the operating diagram is that  $E_c$  can exist if the operating parameters are chosen in the yellow regions.*

The operating diagram shown in Fig. 5(a) corresponds to the biological parameters values used in [6, Fig. 6.3], with  $S^0 = D = 1$ . The curve  $A_1$  is above



$A_2$  so that  $E_c$  can exist if  $0 < D < D_{c1}$ , with  $D_{c1} = 3.4$ . We can see that  $E_c$  is stable as soon as it exists. Therefore the regions  $R_3^U$  and  $R_4^U$  are empty, see Fig. 5(a).

**Remark 5** *The operating point  $(S^0, D) = (1, 1)$  belongs to the yellow region, so that  $E_c$  is stable, in accordance with [6, Fig. 6.3]. The additional information provided by the operating diagram is that  $E_c$  is always stable if it exists and  $E_2$  can never be stable for the biological parameter values under consideration (the green region is empty).*

The operating diagram shown in Fig. 5(b) corresponds to the biological parameters values used in [6, Fig. 6.2], with  $S^0 = D = 1$ . The curve  $A_1$  is below  $A_2$  so that  $E_c$  cannot exist.

**Remark 6** *The operating point  $(S^0, D) = (1, 1)$  belongs to the green region, so that  $E_2$  is stable, in accordance with [6, Fig. 6.1]. The additional information provided by the operating diagram is that  $E_c$  can never exist for the biological parameter values under consideration (the yellow region is empty).*

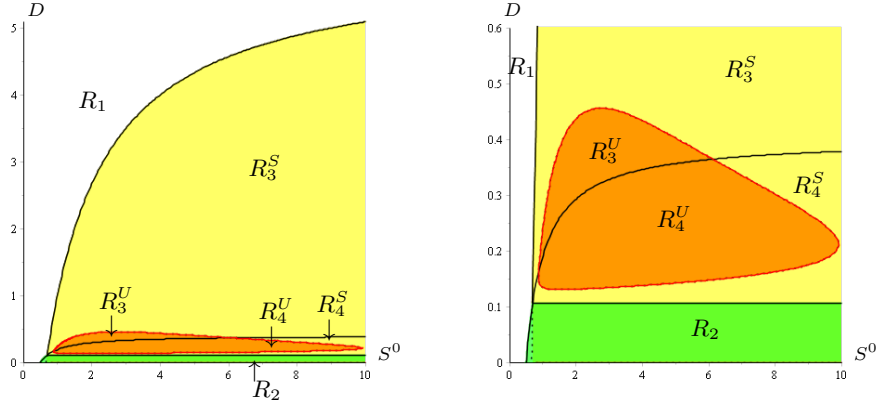
### 3.2 The positive equilibrium can be unstable

The operating diagram shown in Fig. 3 corresponds to an example for which  $E_c$  can be unstable. According to Proposition 4, the interval  $I_c$  defined by (19) is not empty. We see that  $I_c = (D_-, D_+)$ , where  $D_- \approx 0.122$ ,  $D_+ \approx 0.456$ . Therefore, for  $D \in I_c$ , the positive equilibrium  $E_c$  is unstable if and only if  $F_1(D) < S^0 < F_2(D)$  where  $F_1(D)$  and  $F_2(D)$  are given by (20). The operating diagram, given in Fig. 3, shows that both regions  $R_3^U$  and  $R_4^U$  are not empty. Note that in this case we have  $D_0 = 2$ . The sufficient condition of stability of  $E_c$  given in item 3 of Proposition 2 shows that  $E_c$  is stable if  $D > D_0 := 2$ , in agreement with Fig. 3, which shows that the orange region of  $E_c$  instability lies in the region defined by  $0.1 < D < 0.5$ .

## 4 Discussion

In the figures presenting operating diagrams, a region is coloured according to the color in Table 3. Each color corresponds to different asymptotic behaviors:

- White for the washout of both plasmid-free and plasmid bearing organisms, that is, the equilibrium  $E_1$  is locally stable, which occurs only in region  $R_1$ .
- Green for the washout of plasmid bearing organisms while plasmid free organisms are maintained, that is, the equilibrium  $E_2$  is locally stable, which occurs only in region  $R_2$ .
- Yellow for the local stability of the equilibrium  $E_c$ , where both plasmid bearing organisms and plasmid free organisms are maintained. This behavior occurs in regions  $R_3^S$  and  $R_4^S$ . These regions differ only by the existence or not of the unstable equilibrium  $E_2$  of washout of plasmid bearing organisms.



**Fig. 3.** The operating diagram of (3). The biological parameter values are given Table 4. The positive equilibrium can be unstable.

- Orange for the instability of equilibrium  $E_c$ . This behavior occurs in regions  $R_3^U$  and  $R_4^U$ . These regions differ only by the existence or not of the unstable equilibrium  $E_2$ . In these regions plasmid bearing organisms and plasmid free organisms are maintained around a stable limit cycle.

It is worth noting that, from an experimental point of view, it is necessary to operate the chemostat in order to avoid the white region ( $E_1$  is LAS) and the green region ( $E_2$  is LAS). Yellow regions ( $E_c$  is LAS) are the “target” operating regions, as they correspond to the stability of the equilibrium where the plasmid bearing organisms survive. Orange regions ( $E_c$  exists but is unstable) are also permitted but if the system is operated in these regions then the plasmid bearing organisms survive along a cycle.

Let us see what happens if  $S^0$  is fixed and  $D$  is gradually decreased. For example, if  $S^0 = 8$  then as  $D$  decreases, there is a bifurcation from the white region  $R_1$  where the washout equilibrium is stable to the yellow region  $R_4^S$  where the coexistence equilibrium becomes stable, see Fig. 3. Decreasing  $D$  further, the next bifurcation is to the orange region  $R_4^U$ , where  $E_c$  becomes unstable. Decreasing  $D$  further, the next bifurcation is to the yellow region  $R_3^S$  where  $E_c$  becomes stable. We see numerically that two Hopf bifurcation occur when the operating point  $(S^0, D)$  crosses the boundary curve  $A_3$  for  $D = D_{crit}^j$ ,  $j = 1, 2$ .

Decreasing  $D$  further, the next bifurcation is to the green region  $R_2$  where the equilibrium with extinction of the plasmid bearing bacteria is stable. Thus, in this case, the sequence of bifurcations as  $D$  decreases, results in the stability passing from  $E_1$  to  $E_c$  to a more complex attractor, to  $E_c$ , to  $E_2$  and remaining there, see Fig. 3. We see numerically that the attractor in the region  $R_4^U$  is stable limit cycle, at least when  $S^0 = 8$ .

## A Proofs

### A.1 Proof of Theorem 1

The washout equilibrium is  $E_1 = (S^0, 0, 0)$ . This equilibrium always exists.

There is only one possible equilibrium involving plasmid-free organisms but no plasmid-bearing organisms, denoted  $E_2 = (S_2, 0, x_2)$ , where  $x_2 > 0$ . Therefore  $f_2(S) = D_2$ , so that  $S_2 = \lambda_2$ , where  $\lambda_2$  is defined by (5). In addition, we have  $x_2 = \frac{D\gamma_2}{D_2}(S^0 - \lambda_2)$ , which proves (6). This equilibrium exists if and only  $x_2 > 0$  which is equivalent to  $S^0 > \lambda_2$ . This is the condition of existence of  $E_2$ , given in Table 1.

The mixed culture equilibrium is denoted  $E_c = (S^*, x_1^*, x_2^*)$ , where  $x_1^* > 0$  and  $x_2^* > 0$ . Therefore  $f_1(S^*) = \frac{D_1}{1-q}$ , so that  $S^* = \lambda_1$ , where  $\lambda_1$  is defined by (4). A straightforward computation shows that  $x_1^*$  and  $x_2^*$  are given by (7).

- In the case when  $1 - q\gamma_1/\gamma_2 \leq 0$ , the denominator of  $x_2^*$  is positive. Hence, for  $x_2^* > 0$  to hold, the numerator must also be positive, i.e.  $S^0 - \lambda_1 > 0$ . But  $x_1^*$  has the same denominator, and so the numerator of  $x_1^*$  must also be positive. This is true if and only if  $D_2 - f_2(\lambda_1) > 0$ . So  $x_1^*$  and  $x_2^*$  are both positive if and only if  $S^0 - \lambda_1 > 0$  and  $D_2 - f_2(\lambda_1) > 0$ .

- In the case when  $1 - q\gamma_1/\gamma_2 > 0$ , in order for  $x_2^*$  to be positive, we must have either  $D_2 - (1 - q\gamma_1/\gamma_2)f_2(\lambda_1) > 0$  and  $S^0 - \lambda_1 > 0$  or  $D_2 - (1 - q\gamma_1/\gamma_2)f_2(\lambda_1) < 0$  and  $S^0 - \lambda_1 < 0$ . Note that  $D_2 - (1 - q\gamma_1/\gamma_2)f_2(\lambda_1) < 0$  implies  $D_2 - f_2(\lambda_1) < 0$ . Hence, if  $D_2 - (1 - q\gamma_1/\gamma_2)f_2(\lambda_1) < 0$  and  $S^0 - \lambda_1 < 0$  then  $x_1^* < 0$ . On the other hand, if  $D_2 - (1 - q\gamma_1/\gamma_2)f_2(\lambda_1) > 0$  and  $S^0 - \lambda_1 > 0$ , in order for  $x_1^*$  to be positive, we must have  $D_2 - f_2(\lambda_1) > 0$ . Therefore, both  $x_1^*$  and  $x_2^*$  are positive if and only if  $D_2 - f_2(\lambda_1) > 0$  and  $S^0 - \lambda_1 > 0$ .

Hence, we proved in both cases that  $E_c$  exists if and only if  $S^0 > \lambda_1$  and  $D_2 > f_2(\lambda_1)$ . Since  $f_2$  are increasing and  $f_2(\lambda_2) = D_2$ , the condition  $D_2 > f_2(\lambda_1)$  is equivalent to  $\lambda_2 > \lambda_1$ . Hence that  $E_c$  exists if and only if  $S^0 > \lambda_1$  and  $\lambda_2 > \lambda_1$ , which is the condition of existence of  $E_c$  given in Table 1.

Now we investigate the local exponential stability of the equilibria by finding the eigenvalues of the associated Jacobian matrices. The Jacobian matrix of (3) takes the form

$$J = \begin{bmatrix} -D - \frac{1}{\gamma_1}f_1'(S)x_1 - \frac{1}{\gamma_2}f_2'(S)x_2 & -\frac{1}{\gamma_1}f_1(S) & -\frac{1}{\gamma_2}f_2(S) \\ (1-q)f_1'(S)x_1 & (1-q)f_1(S) - D_1 & 0 \\ qf_1'(S)x_1 + f_2'(S)x_2 & qf_1(S) & f_2(S) - D_2 \end{bmatrix} \quad (13)$$

At  $E_1 = (S^0, 0, 0)$ , the Jacobian matrix (13) is

$$J_1 = \begin{bmatrix} -D & -\frac{1}{\gamma_1}f_1(S^0) & -\frac{1}{\gamma_2}f_2(S^0) \\ 0 & (1-q)f_1(S^0) - D_1 & 0 \\ 0 & qf_1(S^0) & f_2(S^0) - D_2 \end{bmatrix} \quad (14)$$

The eigenvalues of (14) lie on the diagonal. They are all negative if and only if  $f_1(S^0) < \frac{D_1}{1-q}$  and  $f_2(S^0) < D_2$ . Since  $f_1$  and  $f_2$  are increasing,  $f_1(\lambda_1) = \frac{D_1}{1-q}$

and  $f_2(\lambda_2) = D_2$ , these conditions are equivalent to  $S^0 < \lambda_1$  and  $S^0 < \lambda_2$ , which the condition of stability of  $E_1$ , given in Table 1.

At  $E_2 = (\lambda_2, 0, x_2)$ , the Jacobian matrix (13) is

$$J_2 = \begin{bmatrix} -D - \frac{1}{\gamma_2} f_2'(\lambda_2) x_2 & -\frac{1}{\gamma_1} f_1(\lambda_2) & -\frac{1}{\gamma_2} D_2 \\ 0 & (1-q)f_1(\lambda_2) - D_1 & 0 \\ f_2'(\lambda_2) x_2 & q f_1(\lambda_2) & 0 \end{bmatrix} \quad (15)$$

The characteristic equation of  $J_2$  is given by

$$(z - (1-q)f_1(\lambda_2) + D_1)(z^2 + Bz + C) = 0$$

where  $B = D + \frac{1}{\gamma_2} f_2'(\lambda_2) x_2$  and  $C = \frac{1}{\gamma_2} D_2 f_2'(\lambda_2) x_2$ . The eigenvalues of  $J_2$  are  $(1-q)f_1(\lambda_2) - D_1$  and the roots of equation  $z^2 + Bz + C = 0$ . Since  $B > 0$  and  $C > 0$ , the real parts of the roots of the quadratic equation are negative. Therefore, the real parts of the eigenvalues of (15) are negative if and only if  $f_1(\lambda_2) < \frac{D_1}{1-q}$ . Since  $f_1$  is increasing and  $f_1(\lambda_1) = \frac{D_1}{1-q}$  this condition is equivalent to  $\lambda_2 < \lambda_1$ , which the condition of stability of  $E_2$ , given in Table 1.

At  $E_c$ , the Jacobian matrix (13) is

$$J_c = \begin{bmatrix} -a_1 & -b_1 & -c_1 \\ a_2 & 0 & 0 \\ a_3 & b_3 & -c_3 \end{bmatrix} \quad (16)$$

where  $a_i$ ,  $b_i$  and  $c_i$  are defined by (9). Clearly,  $a_i > 0$  for  $i = 1, 2, 3$ ,  $b_1 > 0$ ,  $b_3 > 0$  and  $c_1 > 0$ . In addition, if  $E_c$  exists then  $\lambda_2 > \lambda_1$ , and hence, since  $f_2$  is increasing, we have  $D_2 = f_2(\lambda_2) > f_2(\lambda_1)$ . Therefore,  $c_3 = D_2 - f_2(\lambda_1) > 0$ . The characteristic equation of  $J_c$  is given by

$$z^3 + (a_1 + c_3)z^2 + (a_1c_3 + a_3c_1 + a_2b_1)z + a_2(b_1c_3 + b_3c_1) = 0 \quad (17)$$

Since all the coefficients in (17) are positive, the Routh-Hurwitz criterion says that  $E_c$  will be LES if and only if

$$(a_1 + c_3)(a_1c_3 + a_3c_1 + a_2b_1) > a_2(b_1c_3 + b_3c_1).$$

This condition is equivalent to  $F(D, S^0) > 0$  where  $F(D, S^0)$  is given by (8), which the condition of stability of  $E_c$ , given in Table 1.

## A.2 Proof of Proposition 2

We have  $F(S^0, D) > A$  where  $A = a_1(a_3c_1 + a_2b_1) - a_2b_3c_1$ . From the definitions (9) of  $a_i$ ,  $b_i$  and  $c_i$  and using  $a_1 > D$  and  $a_3 > qf_1'(\lambda_1)x_1^*$ , we have

$$\begin{aligned} A &> D \left( \frac{q}{\gamma_2} f_1'(\lambda_1) x_1^* f_2(\lambda_1) + \frac{1}{\gamma_1} D_1 f_1'(\lambda_1) x_1^* \right) - \frac{q}{\gamma_2} D_1 f_1'(\lambda_1) x_1^* f_2(\lambda_1) \\ &= f_1'(\lambda_1) x_1^* \left[ D \left( \frac{q}{\gamma_2} f_2(\lambda_1) + \frac{1}{\gamma_1} D_1 \right) - \frac{q}{\gamma_2} D_1 f_2(\lambda_1) \right] \\ &= \frac{1}{\gamma_1} f_1'(\lambda_1) x_1^* \left[ DD_1 + q \frac{\gamma_1}{\gamma_2} f_2(\lambda_1) (D - D_1) \right] \end{aligned}$$

If  $D_1 \leq D$ , then  $A > 0$ , which proves item 1. Using  $D_2 > f_2(\lambda_1)$ , which is equivalent to the necessary condition  $\lambda_1 < \lambda_2$  for  $E_c$  to exist, if  $D_1 > D$ , then  $f_2(\lambda_1)(D - D_1) > D_2(D - D_1)$ . Therefore, the sufficient condition in item 2 of Prop. 2 is satisfied, then

$$DD_1 + q\frac{\gamma_1}{\gamma_2}f_2(\lambda_1)(D - D_1) > DD_1 + q\frac{\gamma_1}{\gamma_2}D_2(D - D_1) \geq 0,$$

so that  $A > 0$ , which proves item 2. For the proof of item 3, we replace  $D_1$  and  $D_2$  in the sufficient condition in item 2 of Prop. 2 by their expressions given in **H2**. We obtain

$$aD^2 + \varepsilon D - q\frac{\gamma_1}{\gamma_2}\varepsilon_1\varepsilon_2 \geq 0, \quad (18)$$

where  $\alpha$  and  $\varepsilon$  are as in the proposition. Hence (18) is satisfied if  $D \geq \frac{\sqrt{\Delta} - \varepsilon}{2a}$ , where  $\Delta$  is as in the proposition.

### A.3 Proof of Proposition 3

Recall that the conditions of existence and local stability of equilibria are given in Table 1. We give the proof for the regions  $R_4^S$  and  $R_4^U$ . If  $(S^0, D)$  belongs to one of these regions, then  $S^0 > \lambda_2(D_2) > \lambda_1(D_1)$ . Therefore  $S^0 < \min(\lambda_1(D_1), \lambda_2(D_2))$  is not satisfied, so that  $E_1$  is unstable. On the other hand,  $\lambda_2(D_2) < S^0$ , so that  $E_2$  exists and, since the condition  $\lambda_2(D_2) < \lambda_1(D_1)$  is not satisfied, it is unstable. Finally  $\lambda_1(D_1) < \min(S^0, \lambda_2(D_2))$ , so that  $E_c$  exists. It is stable in  $R_4^S$ , where  $F(S^0, D) > 0$ , and unstable in  $R_4^U$ , where  $F(S^0, D) < 0$ . The proofs for the other regions are similar and are left to the reader.

## B Instability of the positive equilibrium

We will now give a necessary and sufficient condition for the local stability of  $E_c$ , which is easier to handle than the condition  $F(S^0, D) > 0$  shown in Table 1. If we replace  $a_1, a_2$  and  $a_3$  by their expressions given by (9) and  $x_1^*$  by  $x_1^* = \frac{(1-q)c_3}{qD_1}x_2^*$  we see that  $F(S^0, D)$  is a polynomial in  $x_2^*$  whose coefficients are depending only in  $D$ . Indeed, we have  $F(S^0, D) = \frac{1}{q^2D_1^2}P(x_2^*)$ , where the polynomial  $P(X)$  is given by

$$P(X) = C_2(D)X^2 + C_1(D)X + C_0(D),$$

where

$$\begin{aligned} C_2(D) &= \left[ \frac{1-q}{\gamma_1}f_1'(\lambda_1)c_3 + \frac{q}{\gamma_2}f_2'(\lambda_1)D_1 \right] \left[ qf_2'(\lambda_1)D_1 \left( c_1 + \frac{1}{\gamma_2}c_3 \right) \right. \\ &\quad \left. + (1-q)f_1'(\lambda_1)c_3 \left( qc_1 + (1-q)b_1 + \frac{1}{\gamma_1}c_3 \right) \right], \\ C_1(D) &= qD_1 \left[ qD_1f_2'(\lambda_1) \left( \frac{1}{\gamma_2}c_3^2 + c_1c_3 + \left( c_1 + \frac{2}{\gamma_2}c_3 \right) D \right) \right. \\ &\quad \left. + (1-q)f_1'(\lambda_1)c_3 \left( \frac{1}{\gamma_1}c_3^2 + qc_1c_3 + \left( \frac{2}{\gamma_1}c_3 + (1-q)b_1 + qc_1 \right) D - (1-q)b_3c_1 \right) \right], \\ C_0(D) &= q^2DD_1^2c_3(c_3 + D), \end{aligned}$$

Note that  $C_0 > 0$  and  $C_2 > 0$  and there is only a negative term in  $C_1$ . Therefore it is easy to determine the sign of  $P(X)$  and hence the stability of  $E_c$ . Indeed  $P(X)$  can be negative for positives values of  $X$  if and only if there exists  $D$  such that  $C_1(D) < 0$  and  $\Delta(D) := C_1^2(D) - 4C_0(D)C_2(D) > 0$ . We define the set

$$I_c := \{D : \lambda_1 < \lambda_2, C_1(D) < 0 \text{ and } \Delta(D) > 0\}. \quad (19)$$

Note that  $I_c$  is an open set. If  $D \in I_c$  we denote by

$$X_1(D) = \frac{-C_1(D) - \sqrt{\Delta(D)}}{2C_2(D)} \text{ and } X_2(D) = \frac{-C_1(D) + \sqrt{\Delta(D)}}{2C_2(D)}$$

the roots of  $P(X)$ . They are real and positive. Let

$$F_i(D) = \lambda_1 + \frac{D_2 - \left(1 - q \frac{\gamma_1}{\gamma_2}\right) f_2(\lambda_1)}{q\gamma_1 D} X_i(D), \quad i = 1, 2. \quad (20)$$

We have the following result

**Proposition 4** *The coexistence equilibrium  $E_c$  can be unstable only if the set  $I_c$  defined by (19) is non empty. If this condition holds then  $E_c$  is unstable if and only if  $D \in I_c$  and  $F_1(D) < S^0 < F_2(D)$  where  $F_1(D)$  and  $F_2(D)$  are given by (20).*

*Proof.* The product  $C_0(D)/C_2(D)$  of the roots of  $P(X)$  is positive so that the roots can be real and positive if and only if  $C_1(D) < 0$  and  $\Delta(D) > 0$ , that is to say  $D \in I_c$ . If  $I_c$  is not empty and  $D \in I_c$  then  $P(X)$  has two positive roots denoted  $0 < X_1(D) < X_2(D)$ . Note that  $P(X) < 0$  if and only if  $X_1(D) < X < X_2(D)$ . Replacing  $X$  by  $x_2^*$  and using the formula (7) giving  $x_2^*$  with respect to the operating parameters  $S^0$  and  $D$ , we see that the condition  $X_1(D) < X < X_2(D)$  is equivalent to the condition  $F_1(D) < S^0 < F_2(D)$  where  $F_1(D)$  and  $F_2(D)$  are given by (20).

The following lemma states that  $F_1(D)$  and  $F_2(D)$  are equal on the boundary of  $I_c$ , so the region

$$R^U = \{(S^0, D) : D \in I_c \text{ and } F_1(D) < S^0 < F_2(D)\}, \quad (21)$$

where  $E_c$  is unstable, is an union of disjoint regions which are homeomorphic to the unit ball.

**Lemma 5** *Let  $(D_-, D_+)$  be a connected component of  $I_c$ . If  $D \in (D_-, D_+)$ , then  $F_1(D) < F_2(D)$ . Moreover  $F_1(D_-) = F_2(D_-)$  and  $F_1(D_+) = F_2(D_+)$ . Therefore, if  $I_c = (D_-, D_+)$  then  $R^U$  is homeomorphic to the unit ball.*

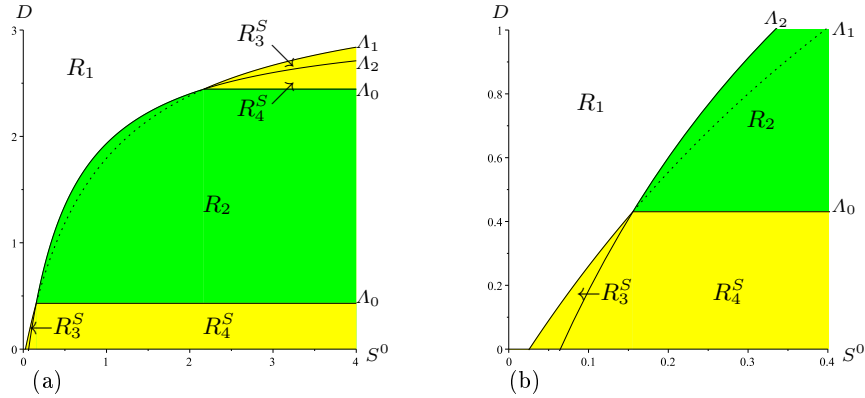
*Proof.* The boundary of  $I_c$  is defined by  $C_1(D) = 0$  or  $\Delta(D) = 0$ . If  $C_1(D_-) = 0$ , then, from the definition of  $\Delta(D)$ ,  $\Delta(D_-) < 0$ . Thus  $\Delta(D) < 0$  for  $D > D_-$  close to  $D_-$ , so  $D \notin I_c$ , a contradiction. Similarly, if  $C_1(D_+) = 0$ , then,  $\Delta(D_+) < 0$ . Thus  $\Delta(D) < 0$  for  $D < D_+$  close to  $D_+$ , so  $D \notin I_c$ , a contradiction. Therefore, if  $D = D_-$  or  $D = D_+$ , then  $\Delta(D) = 0$ . Hence, if  $D = D_-$  or  $D = D_+$ , then  $X_1(D) = X_2(D)$  and hence,  $F_1(D) = F_2(D)$ .

## C Biological parameters values

We give in Table 4 the biological parameter values used in the figures. Some of these values have already been considered in [6], and we have included them to illustrate the contribution of the operating diagram to the results of [6]. Other values have been chosen to illustrate interesting phenomena, such as the possibility of  $E_c$  being unstable.

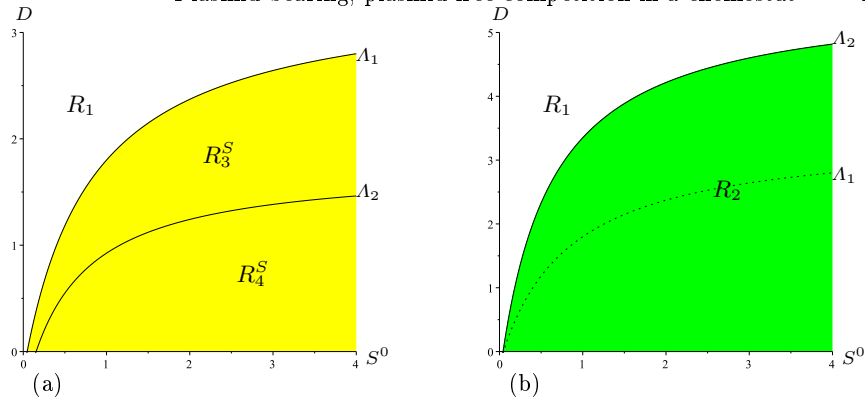
**Table 4.** Biological parameters values used in the figures (and  $\alpha_1 = \alpha_2 = 1$ ). The last column gives the threshold value  $D_0$  of item 3 in Proposition 2.

Figure	$m_1$	$K_1$	$\varepsilon_1$	$m_2$	$K_2$	$\varepsilon_2$	$q$	$D_0$	Figure of [6]
4	4	0.9	0.1	3.5	0.5	0.4	0.1	0.033	6.1
5(a)	4	0.8	0.2	2.2	0.6	0.45	0.1	0.041	6.3
5(b)	4	0.8	0.2	6	0.6	0.4	0.1		6.2
3	20	1	4	4.4	0.05	4	0.5	2	



**Fig. 4.** The biological parameter values are given in Table 4. Curves  $A_1$  and  $A_2$  cross at  $(\hat{S}_1, \hat{D}_1) = (0.156, 0.431)$  and  $(\hat{S}_2, \hat{D}_2) = (2.169, 2.444)$ . (a) The operating diagram. (b) A magnification showing the region  $R_3^S$ . The positive equilibrium is stable if it exists.

**Acknowledgments.** The authors thank the Algerian-Tunisian research project DGR-SDT/DGRS: “Mathematical ecology, modeling and optimization of depollution bioprocesses” and the Euro-Mediterranean research network TREASURE (Treatment and Sustainable Reuse of Effluents in semiarid climates, <http://www.inrae.fr/treasure>) for their support during the preparation of this work.



**Fig. 5.** The biological parameter values are given in Table 4. The curves  $\Lambda_1$  and  $\Lambda_2$  do not intersect. The positive equilibrium is stable if it exists.

## References

1. Abdellatif, N., Fekih-Salem, R. and Sari, T., Competition for a single resource and coexistence of several species in the chemostat, *Math. Biosci. Eng.* **13** (2016) 631–652.
2. Dellal, M., Lakrib, M. and Sari, T. The operating diagram of a model of two competitors in a chemostat with an external inhibitor, *Mathematical Biosciences*, **302** (2018) 27–45.
3. Harmand, J., Lobry, C., Rapaport, A. and Sari, T. *The chemostat: Mathematical theory of microorganism cultures* (2017) John Wiley & Sons.
4. Hoskisson, P. A and Hobbs, G. Continuous culture—making a comeback?, *Microbiology* **151** (2005) 3153–3159.
5. Hsu, S.B., Waltman, P. and Wolkowitz, G.S.K. Global analysis of a model of plasmid-bearing, plasmid-free competition in the chemostat, *J. Math. Biol.* **32** (1994) 731–742.
6. Li, B., Kuang, Y. and Smith, H.L., Competition between plasmid-bearing and plasmid-free microorganisms in a chemostat with distinct removal rates, *The Canadian Applied Mathematics Quarterly* **7** (1999) 251–281.
7. Monod, J. La technique de culture continue: théorie et applications, *Ann. Inst. Pasteur* **79** (1950) 390–410.
8. Mtar, T., Fekih-Salem, R. and Sari, T. Mortality can produce limit cycles in density-dependent models with a predator-prey relationship, *Discrete Contin. Dyn. Syst. Ser. B*, **27** (2022) 7445–7467.
9. Nouaoura, S., Fekih-Salem, R., Abdellatif, N. and Sari, T. Operating diagrams for a three-tiered microbial food web in the chemostat, *J. Math. Biol.* **85** (2022) 44.
10. Sari, T. Best Operating Conditions for Biogas Production in Some Simple Anaerobic Digestion Models., *Processes*, 2022, 10-258.
11. Smith, H.L. and Waltman, P. *The theory of the chemostat: dynamics of microbial competition*, (1995) Cambridge university press.
12. Stephanopoulos, G. and Lapidus, G., Chemostat dynamics of plasmid-bearing plasmid-free mixed recombinant cultures, *Chem. Eng. Sci.* **43** (1988) 49–57.