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

Tewfik Sari, Mohamed Dellal

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

Tewfik Sari[1] and Mohamed Dellal[2,3]

[1] ITAP, Univ Montpellier, INRAE, Institut Agro, Montpellier, France
tewfik.sari@inrae.fr
[2] Ibn Khaldoun University, 14000, Tiaret, Algeria
[3] 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 Stephanopoulis and Lapidus [12]

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

$S(t)$, $x_1(t)$ 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 γ 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 the 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, Stephanopoulis 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

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

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:

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

The parameters γ_1 and γ_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 fxed 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 ε_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, HRT $= 1/D$ and the Solid Retention Time 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 λ_1 and λ_2.

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

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

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 λ_2 is defined by* (5) *and*

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

- *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

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

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

Table 1. Existence and local stability of equilibria of (3) where λ_1, λ_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 λ_1 and λ_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 Λ_1 and Λ_2 defined by

$$\begin{aligned} \Lambda_1 &= \{(S^0, D) : S^0 = \lambda_1(\alpha_1 D + \varepsilon_1)\}, \\ \Lambda_2 &= \{(S^0, D) : S^0 = \lambda_2(\alpha_2 D + \varepsilon_2)\}. \end{aligned} \tag{10}$$

Remark 1 *From the definitions of λ_1 and λ_2, the curves Λ_1 and Λ_2 are also given by $\Lambda_1 = \{(S^0, D) : D = \frac{(1-q)f_1(S^0)-\varepsilon_1}{\alpha_1}\}$, $\Lambda_2 = \{(S^0, D) : D = \frac{f_2(S^0)-\varepsilon_2}{\alpha_2}\}$.*

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).

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

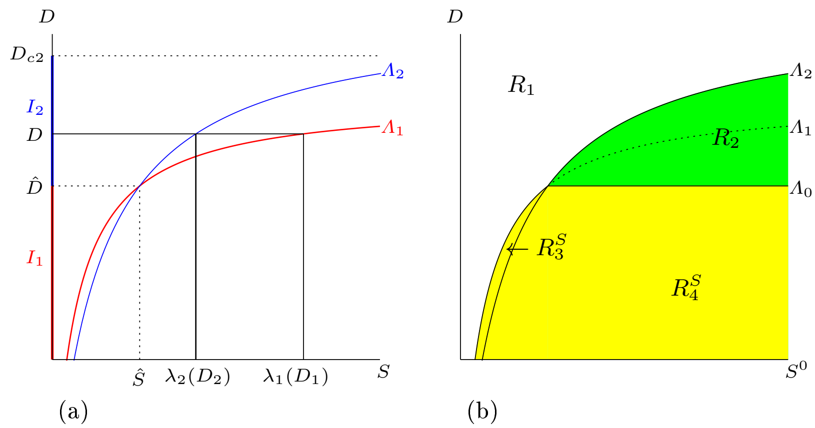

Fig. 1. (a) Sets I_1 (in red) and I_2 (in blue) of the D axis defined by (11): $I_1 = (0, \hat{D})$ and $I_2 = (\hat{D}, D_{c2})$. (b) Operating diagram when the subset I_c of I_1 defined by (19) is empty (R_3^U and R_4^U regions do not exist).

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 Λ_1 and Λ_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).

$$\begin{aligned} \Lambda_0 &= \{(S^0, D) : D = \hat{D} \text{ and } S^0 \geq \hat{S}\}, \\ \Lambda_3 &= \{(S^0, D) : F(S^0, D) = 0\}. \end{aligned} \tag{12}$$

Note that there are as many Λ_0 curves as there are intersection points $(\hat{S}, \hat{D})$ of the curves Λ_1 and Λ_2. Using Lemma 5, the Λ_3 curve is a set of closed disjoint simple curves.

The curves Λ_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 homemorphic 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

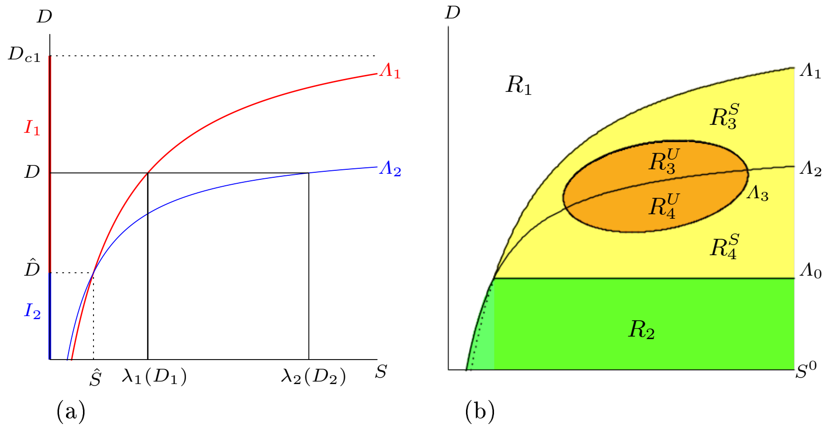

Fig. 2. (a) Sets I_1 (in red) and I_2 (in blue) of the D axis defined by (11): $I_1 = (\hat{D}, D_{c1})$ and $I_2 = (0, \hat{D})$. (b) Operating diagram when the subset I_c of I_1 defined by (19) is not empty (At least one of the R_3^U or R_4^U regions exists).

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 Λ_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 Λ_1 and Λ_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 Λ_1 is above

Λ_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 Λ_1 is below Λ_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 winch 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.

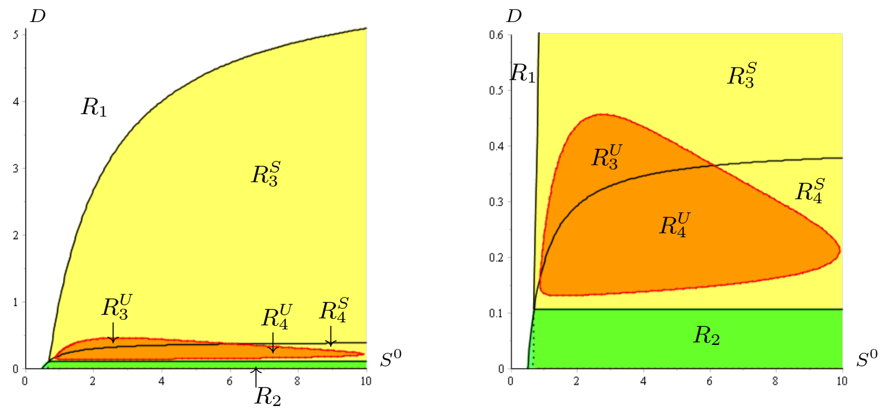

Fig. 3. The operating diagram of (3). The biological parameter values are given Table 4. The positive equilibrium can be unstable.

– 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_4^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 Λ_3 for $D = D^j_{crit}$, $j = 1, 2$.

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 λ_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 $E_c = (S^*, x_l^*, x_2^*)$, where $x_1^* > 0$ and $x_2^* > 0$. Therefore $f_1(S^*) = \frac{D_1}{1-q}$, so that $S^* = \lambda_1$, where λ_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

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

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

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

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

$$z^3 + (a_1 + c_3) z^2 + (a_1 c_3 + a_3 c_1 + a_2 b_1) z + a_2 (b_1 c_3 + b_3 c_1) = 0 \tag{17}$$

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

$$(a_1 + c_3)(a_1 c_3 + a_3 c_1 + a_2 b_1) > a_2 (b_1 c_3 + b_3 c_1).$$

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 $A = a_1(a_3 c_1 + a_2 b_1) - a_2 b_3 c_1$. From the definitions (9) of a_i, b_i and c_i and using $a_1 > D$ and $a_3 > q f_1'(\lambda_1) x_1^*$, we have

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

If $D_1 \le D$, 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 exists, 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

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

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

$$aD^2 + \varepsilon D - q\frac{\gamma_1}{\gamma_2}\varepsilon_1\varepsilon_2 \ge 0, \tag{18}$$

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

A.3 Proof of Proposition 3

Recall that the conditions if 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

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

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

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

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

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

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

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

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 $D = D_-$ of $D = D_+$, 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	ε_1	m_2	K_2	ε_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	

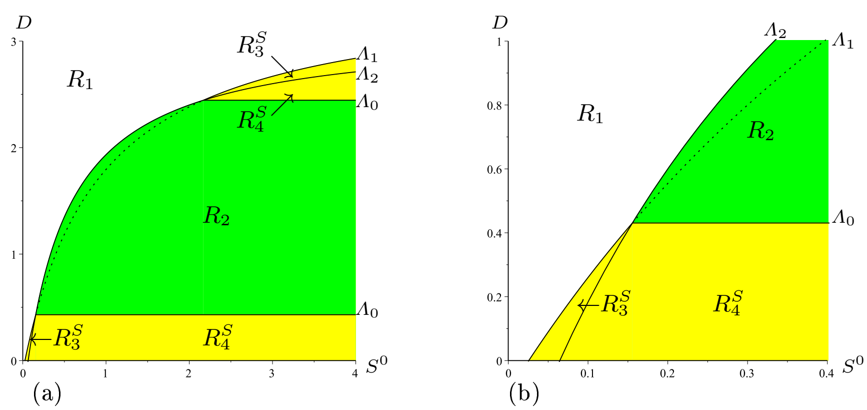

Fig. 4. The biological parameter values are given in Table 4. Curves Λ_1 and Λ_2 cross at $(\hat{S}_1, \hat{D}_1) = (0.156, 0.431)$ and $(\hat{S}_2, \hat{D}_2) = (2.169, 2.444)$. (a) The operating diagram. (b) A magnification showing the region R_3^S. The posisitive equilibrium is stable if it exists.

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.

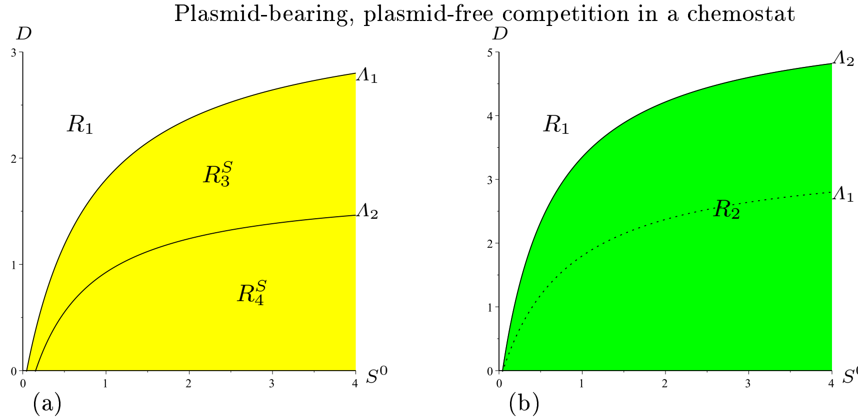

Fig. 5. The biological parameter values are given in Table 4. The curves Λ_1 and Λ_2 do not intersect. The posisitive equilibrium is stable if it exists.

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.