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Competition between plasmid-bearing and plasmid-free organisms in a chemostat with an inhibitor

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Abstract. A model of competition in the chemostat with an inhibitor is combined with a model of competition in the chemostat between plasmid-bearing and plasmid-free organism to produce a model that more closely approximates the way chemostat-like devices are used in biotechnology. The asymptotic behavior of the solutions of the resulting system of nonlinear differential equations is analyzed as a function of the relevant parameters. The techniques are those of dynamical systems although perturbation techniques are used when the parameter reflecting plasmid-loss is small.

1 Introduction

The chemostat is a model for the manufacture of products by genetically altered organisms. The new product is coded by the insertion of a plasmid, a piece of genetic material, into the cell. This genetic material is reproduced when the cell divides. The organism carrying the plasmid, the plasmid-bearing organism, is likely to be a lesser competitor than one without, the plasmid-free organism, because of the added load on its metabolic machinery. The survival of the organism without the plasmid, reduces the efficiency of the production process, and, if it is a sufficiently better competitor, eliminates the altered organisms from the chemostat, halting production. Unfortunately, a small fraction of the plasmids are lost during reproduction, introducing the plasmid-free organisms into the chemostat. To compensate for this, an additional piece of genetic material is added to the plasmid, one that codes for resistance to an inhibitor (an antibiotic) and the inhibitor is added to the feed bottle of the chemostat. A very complete description of the chemostat and various models

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can be found in [SW]; see also the survey articles [FS], [W]. Plasmid models are discussed in [SL], [SSD], [MLW], [LuoH], [RD], [Si], [SLa], [LH], [HW], and [HWW].

A mathematical model of competing plasmid-bearing and plasmid-free competition was proposed by [SLa] where a local analysis was given. A global analysis of the behavior of the model equations was given in [HWW]. Although the basic model was described by a system of three nonlinear differential equations, the three dimensional system was reduced to a two dimensional one through a conservation principle inherent in the chemostat. A model of the chemostat with an inhibitor was proposed by [LH], and a computer simulation of various cases performed. A more complete mathematical analysis was given in [HW]. In this case the model consists of four nonlinear differential equations and the usual chemostat reduction still leaves a three dimensional system. That system, however, was competitive and the general theory of competitive systems could be applied. In particular the three dimensional Poincaré-Bendixson theory of Hirsch [H] and Smith [S1] could be applied.

In this paper, we put the two models together. A chemostat model is proposed which includes (i) the plasmid and its loss and (ii) the inhibitor and its effect on one of the populations. The same reduction to a system of one dimension less occurs as above; however, the resulting three dimensional nonlinear system of ordinary differential equations is not competitive, so the theory for such systems is not helpful to the analysis.

2 The model

The model presented here is an amalgamation of two chemostat models, one concerning the competition of plasmid-bearing and plasmid-free organisms and one concerning the competition of two organisms in the presence of an inhibitor affecting one. We describe the two models separately before combining them.

Stephanopoulis and Lapidus proposed a model of plasmid-bearing, plasmid-free competition of the form

$$\begin{aligned} S' &= (S^{(0)} - S)D - f_1(S)\frac{x_1}{\gamma} - f_2(S)\frac{x_2}{\gamma} \\ x_1' &= x_1(f_1(S)(1-q) - D) \\ x_2' &= x_2(f_2(S) - D) + qx_1 f_1(S) \\ S(0) &= S_0 \geqq 0, \quad x_i(0) > 0,\ i = 1,2 \end{aligned} \tag{2.1}$$

where S is the limiting nutrient, x_1 is the plasmid-bearing organism, x_2 is the plasmid-free organism, γ is a yield constant and q is a parameter which reflects the loss of the plasmid. The function $x_i f_i(S)$ is the nutrient uptake and growth is presumed proportional to uptake. $S^{(0)}$ is the input concentration of the

nutrient and D is the washout rate; $S^{(0)}$ and D are under the control of the experimenter. The system (2.1) can be reduced to a two dimensional system that has no limit cycles [HWW], and all solutions tend to equilibria. A typical $f(S)$ would be of the form $m_i S/(a_i + S)$ but other functions are also important. Note that the cell that looses the plasmid (x_1) reappears as an x_2. This small change from the standard chemostat destroys the strict competitiveness of the system.

Lenski and Hattingh [LH] considered the effect of an inhibitor on two populations and proposed a model of the form (ignoring the yield constant)

$$\begin{aligned} S' &= (S^{(0)} - S)D - \frac{m_1 x_1 S}{a_1 + S} - \frac{m_2 x_2 S}{a_2 + S} e^{-\mu p} \\ x_1' &= x_1 \left(\frac{m_1 S}{a_1 + S} - D \right) \\ x_2' &= x_2 \left(\frac{m_2 S}{a_2 + S} e^{-\mu p} - D \right) \\ p' &= (p^{(0)} - p)D - \frac{\delta x_1 p}{K + p} \end{aligned} \tag{2.2}$$

$$S(0) \geqq 0,\ x_i(0) > 0,\ p(0) \geqq 0, \quad i = 1, 2\ .$$

The parameters are the same as before but a new substance, the inhibitor, denoted by p, appears. The inhibitor is input into the chemostat at a constant concentration and at the same flow rate as the nutrient. The inhibitor is taken up by the x_1 population without harm (in a Michaels Menten way) but the presence of the inhibitor affects the growth rate of the population x_2.

As noted in the introduction, we wish to combine the two – in particular, the effect of the inhibitor and the plasmid loss. The model takes the form

$$\begin{aligned} S' &= (S^{(0)} - S)D - f_1(S)\frac{x_1}{\gamma} - e^{-\mu p} f_2(S)\frac{x_2}{\gamma}\ . \\ x_1' &= x_1[(1 - q)f_1(S) - D] \\ x_2' &= x_2[e^{-\mu p} f_2(S) - D] + q f_1(S) x_1 \\ p' &= (p^{(0)} - p)D - \frac{\delta p}{K + p} x_1 \end{aligned} \tag{2.3}$$

$$S(0) \geqq 0,\ p(0) \geqq 0,\ x_i(0) > 0, \quad i = 1, 2\ ,$$

with $f_i(S) = \frac{m_i S}{a_i + S}$, $i = 1, 2$.

If, in (2.3), $p^{(0)} = p(0) = 0$, then $p(t) \equiv 0$ and (2.3) reduces to (2.1). If $q = 0$, (2.3) reduces to (2.2). Moreover, if both sets of conditions hold, then (2.3)

becomes the "standard" chemostat

$$
\begin{aligned}
S' &= (S^{(0)} - S)D - f_1(S)\frac{x_1}{\gamma} - f_2(S)\frac{x_2}{\gamma} \\
x_1' &= x_1[f_1(S) - D] \\
x_2' &= x_2[f_2(S) - D] \\
S(0) &\geqq 0,\ x_i(0) > 0, \quad i = 1,2\ . \\
f_i(S) &= \frac{m_i S}{a_i + S}, \quad i = 1,2\ .
\end{aligned}
\tag{2.4}
$$

The behavior of (2.4) may be summarized as follows [SW]:

i) If $m_i \leqq D$, $\lim_{t\to\infty} x_i(t) = 0$, $i = 1,2$ independent of competition

ii) If $m_i > D$, define $\lambda_i = \frac{a_i D}{m_i - D}$. If $\lambda_i \geqq S^{(0)}$, $\lim_{t\to\infty} x_i(t) = 0$, $i = 1,2$, independent of competition.

iii) If $0 < \lambda_1 < \lambda_2 < S^{(0)}$, then

$$
\begin{aligned}
\lim_{t\to\infty} x_1(t) &= \lambda_1 \\
\lim_{t\to\infty} x_2(t) &= 0\ .
\end{aligned}
$$

It is convenient to scale the variables in (2.2) as follows:

$$
\bar{S} = \frac{S}{S^{(0)}}, \qquad \bar{p} = \frac{p}{p^{(0)}}, \qquad \bar{x}_i = \frac{x_i}{\gamma S^{(0)}}\ ,
$$

$$
\tau = Dt, \qquad \bar{m}_i = \frac{m_i}{D}, \qquad \bar{a}_i = \frac{a_i}{S^{(0)}}\ ,
$$

$$
\bar{\delta} = \frac{\gamma\delta S^{(0)}}{D}, \qquad \bar{K} = \frac{K}{p^{(0)}}
$$

The new system (dropping the bars) becomes

$$
\begin{aligned}
S' &= 1 - S - f_1(S)x_1 - \mathrm{e}^{-\mu p} f_2(S)x_2 \\
x_1' &= x_1[(1-q)f_1(S) - 1] \\
x_2' &= x_2[\mathrm{e}^{-\mu p} f_2(S) - 1] + q f_1(S)x_1 \\
p' &= 1 - p - \frac{\delta p}{K + p}x_1\ .
\end{aligned}
\tag{2.5}
$$

The parameters have changed their meaning but the system is mathematically cleaner. This is the system we investigate.

3 Simplification

Define $\Sigma(t) = 1 - x_1(t) - x_2(t) - S(t)$. Then the system (2.5) may be written

$$\begin{aligned} \Sigma' &= -\Sigma \\ x_1' &= x_1[(1-q)f_1(1-x_1-x_2-\Sigma) - 1] \\ x_2' &= x_2[e^{-\mu p}f_2(1-x_1-x_2-\Sigma) - 1] + qx_1f_1(1-x_1-x_2-\Sigma) \\ p' &= 1 - p - \frac{\delta p}{K+p}x_1 . \end{aligned} \tag{3.1}$$

Clearly $\lim_{t\to\infty}\Sigma(t) = 0$ and the convergence is exponential. One may consider the last three equations with $\Sigma(t) = \Sigma(0)e^{-t}$ as an asymptotically autonomous system. The limiting system then is

$$\begin{aligned} x_1' &= x_1[(1-q)f_1(1-x_1-x_2) - 1] \\ x_2' &= x_2[e^{-\mu p}f_2(1-x_1-x_2) - 1] + qx_1f_1(1-x_1-x_2) \\ p' &= 1 - p - \frac{\delta p}{K+p}x_1 \end{aligned} \tag{3.2}$$

$$x_i(0) > 0,\ p(0) \geqq 0,\ 0 < x_1 + x_2 < 1 .$$

The region $\Gamma = \{(x_1, x_2, p) | 0 < x_1 + x_2 < 1,\ 1 > p \geqq 0\}$ is positively invariant. The conditions for the dynamics of (3.2) to be the same as that of (3.1) is given in the work of Thieme [Th1]. Clearly the global attractor for (3.1) lies in the hyperplane $\Sigma = 0$, where (3.2) holds.

For the problem to be interesting it is necessary that the x_1-population survive. We have already noted that we may assume that $0 \leqq p(t) \leqq 1$. Solutions of (3.2) satisfy

$$\begin{aligned} x_1' &= x_1[(1-q)f_1(1-x_1-x_2) - 1] \\ x_2' &\geqq x_2[e^{-\mu}f_2(1-x_1-x_2) - 1] . \end{aligned} \tag{3.3}$$

This system may be compared to the competitive system

$$\begin{aligned} z_1' &= z_1[(1-q)f_1(1-z_1-z_2) - 1] \\ z_2' &= z_2[e^{-\mu}f_2(1-z_1-z_2) - 1] \end{aligned} \tag{3.4}$$

(3.4) may be viewed as a chemostat with m_1 replaced by $(1-q)m_1$ and m_2 replaced by $e^{-\mu}m_2$. Thus for z_1 to survive in the chemostat, one needs $m_1(1-q) > 1$ and $0 < \frac{a_1}{m_1(1-q)-1} < 1$. As one would expect for q large $(1 > q \geqq 1 - 1/m_1)$ nothing interesting happens in (3.4). Similarly, if $q < 1 - 1/m_1$ but $\frac{a_1}{m_1(1-q)-1} > 1$, $z_1(t)$ also tends to zero. This last condition can be expressed as $(1-q)f_1(1) < 1$.

Since the system (3.4) is competitive, it is a generalized Kamke system with the order being that generated by using the fourth quadrant in the

plane as a cone. Specifically, if $\lim_{t\to\infty} z_1(t) = 0$, then $\lim_{t\to\infty} x_1(t) = 0$. [SW, Appendix B] or [S3]. Hence we shall assume that

$$(1-q)f_1(1) > 1\,. \tag{3.5}$$

(Note this encompasses both conditions.) What happens to $x_2(t)$ when $\lim_{t\to\infty} x_1(t) = 0$ similarly depends on whether $f_2(1) < e^{\mu}$. If so, $\lim_{t\to\infty} x_2(t) = 0$ and both competitors wash out of the chemostat. If $f_2(1) > e^{\mu}$, then $\lim_{t\to\infty} x_2(t) = x_2^* > 0$. Of course, $\lim_{t\to\infty} p(t) = 1$ when $\lim_{t\to\infty} x_1(t) = 0$. For the remainder of the paper, (3.5) will be a standard hypothesis.

4 Principal results

The system (3.2) has a rest point at $E_0 = (0, 0, 1)$ where both competitors wash out of the system. The condition (3.5) makes E_0 unstable. There can be a rest point $E_1 = (0, x_2^*, 1)$ where x_2^* is a positive root of

$$e^{-\mu} f_2(1-z) = 1\,.$$

This can be written as

$$x_2^* = 1 - f_2^{-1}(e^{\mu})$$

or in terms of the scaled system parameters as

$$x_2^* = \frac{m_2 e^{-\mu} - (1+a_2)}{m_2 e^{-\mu} - 1}\,.$$

Thus, $f_2(1) > e^{\mu}$ guarantees the existence of E_1; the reversal of the inequality precludes the existence of E_1. If $f_2(1) > e^{\mu}$, the stable manifold of E_0 consists of the p-axis (the set $x_1 = x_2 = 0$). All trajectories in this set except the rest point are unbounded. If $f_2(1) < e^{\mu}$, the stable manifold of E_0 lies the $x_2 - p$ plane (the set $x_1 = 0$). Again, all non-trivial orbits in the positive quadrant of this plane are unbounded. The Butler-McGehee Theorem [SW, p. 12] then insures that no trajectory with $x_i(0) > 0$, $i = 1, 2$, has E_0 as an omega limit point.

Define λ_1^*, λ_2^* by

$$f_1(\lambda_1^*) = \frac{1}{1-q}\,, \qquad f_2(\lambda_2^*) = e^{\mu}\,.$$

Since population x_1 carries the plasmid (and manufactures the product), the following theorem corresponds to the extinction of the plasmid-bearing organism.

Theorem 4.1 *Let* (3.5) *hold and suppose that* $f_2(1) > e^{\mu}$. *If* $\lambda_2^* < \lambda_1^*$, *then* E_1 *is globally asymptotically stable* (*for* Γ).

Proof. Divide the relevant portion of the positive cone into three disjoint regions:

$$\Omega = \{(x_1, x_2, p) |\ 1 - x_1 - x_2 = \lambda_1^*\},$$

$$\Omega^+ = \{(x_1, x_2, p) |\ 1 - x_1 - x_2 > \lambda_1^*\},$$

$$\Omega^- = \{(x_1, x_2, p) |\ 1 - x_1 - x_2 < \lambda_1^*\},$$

In the region Ω^+, $x_1'(t) > 0$ for any trajectory. Hence, if a trajectory $\gamma(t) = (x_1(t), x_2(t), p(t))$ remains in Ω^+ (a bounded region) then $\lim_{t-\infty} x_1(t) = c > 0$. The quantity in square brackets on the right hand side of the x_1-equation in (3.2) is integrable (in t) since the above limit exists and is uniformly continuous (since the f_i's are continuously differentiable), and so has limit zero as $t \to \infty$. Thus, $\lim_{t\to\infty} x_1'(t) = 0$ or $\lim_{t\to\infty}[1 - x_1(t) - x_2(t)] = \lambda_1^*$. One always has $p' \leqq 1 - p$ and if $x_1(t) \to 0$, then one has $p' \geqq 1 - \varepsilon - p$ for every $\varepsilon > 0$. Hence, $\lim_{t\to\infty} p(t) = 1$. We seek to show that under the assumption that the trajectory γ remains in Ω^+ for all large time, $x_2(t)$ is unbounded, and thus reach a contradiction.

Since $\lambda_2^* < \lambda_1^*$, $f_2(\lambda_1^*) > f_2(\lambda_2^*) = e^{\mu}$. Hence, for $\varepsilon > 0$ and sufficiently small,

$$f_2(\lambda_1^* - \varepsilon) > e^{\mu(1+\varepsilon)}$$

Fix such an $\varepsilon > 0$. For t large, $1 - x_1(t) - x_2(t) > \lambda_1^* - \varepsilon$ and $p(t) < 1 + \varepsilon$. Hence for t sufficiently large

$$\begin{aligned} x_2'(t) &= x_2[e^{-\mu p} f_2(1 - x_1 - x_2) - 1] + q f_1(1 - x_1 - x_2) x_1 \\ &> x_2[e^{-\mu(1+\varepsilon)} f_2(\lambda_1^* - \varepsilon) - 1] \\ &\geqq \alpha x_2 \end{aligned}$$

where $\alpha > 0$. Thus $x_2(t)$ is unbounded, contrary to the assumption that the trajectory lies in Ω^+ for all future time.

Suppose a trajectory lies in Ω^- for all $t \geqq t_0$. Then $x_1'(t) < 0$ and $\lim_{t\to\infty} x_1(t) = c \geqq 0$ (since the wedge $x_1 \geqq 0, x_2 \geqq 0$, is positively invariant). If $c > 0$, then one argues as above that $\lim_{t\to\infty} x_1'(t) = 0$ or $\lim_{t\to\infty}[1 - x_1(t) - x_2(t)] = \lambda_1^* > \lambda_2^*$. Previous arguments imply that $x_2(t)$ is unbounded, a contradiction. Thus, every omega limit point of a trajectory that remains in Ω^- is of the form $(0, \hat{x}_2, \hat{p})$. The only invariant set in the interior of this face is $(0, x_2^*, 1)$ and the theorem is established for such trajectories. (We remind the reader that we have already noted the E_0 cannot be in the omega limit set.)

Suppose now that a trajectory intersects the surface Ω at a point $(\bar{x}_1, \bar{x}_2, \bar{p}) = (x_1(t_0), x_2(t_0), p(t_0))$. The outward unit normal to this hyperplane is $\frac{1}{\sqrt{2}}(1, 1, 0)$. The projection of the vector field onto the normal at a point of Ω is (since $x_1' = 0$ there)

$$\begin{aligned} x_2'(t_0) &= \bar{x}_2[e^{-\mu\bar{p}} f_2(1 - \bar{x}_1, -\bar{x}_2) - 1] > \bar{x}_2[e^{-\mu} f_2(\lambda_1^*) - 1] \\ &\quad + q\bar{x}_1 f_1(1 - \bar{x}_1 - \bar{x}_2) > 0 \end{aligned}$$

since $\lambda_2^* < \lambda_1^*$. Therefore, the trajectory crosses Ω from Ω^+ to Ω^- at every point of Ω. Hence, it remains in Ω^- for $t \geqq t_0$. This completes the proof.

Theorem 4.2 *Assume* (3.5) *holds and that* $f_2(1) < e^{\mu}$. *Then* (3.2) *has a unique positive equilibrium.*

Proof. The proof is a computation to show that the right hand side of (3.2) set equal to zero has a positive solution; that is, we find a root of

$$\begin{aligned} (1-q)f_1(1-x_1-x_2)-1&=0\\ x_2[e^{-\mu p}f_2(1-x_1-x_2)-1]+qf_1(1-x_1-x_2)x_1&=0\\ 1-p-\frac{\delta p}{k+p}x_1&=0 \end{aligned} \tag{4.1}$$

The first equation says that a solution must satisfy

$$1-x_1-x_2=\lambda_1^* \tag{4.2}$$

where λ_1^* was defined in (4.1). Moreover, one can solve the third equation for p in terms of x_1 as

$$p=g(x_1) \tag{4.3}$$

with $g(0)=1$ and $g'(x_1)<0$ for $x_1>0$. (g is given by the quadratic formula.) One needs then to find a positive solution $x=x_1^*$ of $F(x)=0$ where

$$F(x)=[e^{-\mu g(x)}f_2(\lambda_1^*)-1][1-x-\lambda_1^*]+qf_1(\lambda_1^*)x$$

Since

$$F(0)=[e^{-\mu}f_2(\lambda_1^*)-1][1-\lambda_1^*]\,,$$

then $F(0)<0$ because $f_2(1)<e^{\mu}$. Moreover,

$$F(1-\lambda_1^*)=qf_1(\lambda_1^*)(1-\lambda_1^*)>0\,.$$

Hence, there is a root x_1^* between zero and $1-\lambda_1^*$ by continuity. We seek to show that it is unique.

Such a root defines p by $p=g(x_1^*)$ and $x_2^*=1-x_1^*-\lambda_1^*>0$. One computes

$$\begin{aligned} F'(x)=&-[e^{-\mu g(x)}f_2(\lambda_1^*)-1]+[1-x-\lambda_1^*]\cdot\\ &\cdot[-e^{-\mu g(x)}f_2(\lambda_1^*)\mu g'(x)]+q\,f_1(\lambda_1^*)\,. \end{aligned}$$

Using the equation, at a rest point, yields

$$x_2^*[1-e^{-\mu g(x_1^*)}f_2(\lambda_1^*)]=qf_1(\lambda_1^*)x_1^*\,.$$

Hence

$$F'(x_1^*)=\frac{qf_1(\lambda_1^*)}{1-\lambda_1^*-x_1^*}(1-\lambda_1^*)-(1-\lambda_1^*-x_1^*)f_2(\lambda_1^*)e^{-\mu g(x_1^*)}\mu g'(x_1^*)>0$$

and both terms are positive ($g'(x)<0$).

Table 4.1

	$f_2(1) < e^{\mu}$	$f_2(1) > e^{\mu}$
$f_1(1) < \frac{1}{1-q}$	E_0 is a global attractor	E_1 is a global attractor
$f_1(1) > \frac{1}{1-q}$	E_c exists	$1 > \lambda_2^* > \lambda_1^* > 0 - E_c$ exists
		$1 > \lambda_1^* > \lambda_2^* > 0 - E_1$ is a global attractor.

Essentially, the same proof establishes the following result.

Theorem 4.3 *Assume* (3.5) *holds and that* $1 > \lambda_2^* > \lambda_1^*$. *Then* (3.2) *has a unique positive equilibrium.*

Proof. We proceed as above except now one argues that

$$F(0) = [e^{-\mu} f_2(\lambda_1^*) - 1][1 - \lambda_1^*] < e^{-\mu} f_2(\lambda_2^*) - 1 = 0$$

since f_2 is monotone and $\lambda_1^* < \lambda_2^*$. Table 4.1 summarizes the results so far.

Let $E_c = (x_{1c}, x_{2c}, p_c) = (x_{1c}(q), x_{2c}(q), p_c(q))$ be the interior rest point. The Jacobian at E_c takes the form

$$J = \begin{pmatrix} m_{11} & m_{12} & 0 \\ m_{21} & m_{22} & m_{23} \\ m_{31} & 0 & m_{33} \end{pmatrix}$$

where

$$\begin{aligned}
m_{11} &= -x_{1c} f_1'(1 - x_{1c} - x_{2c})(1 - q) \\
m_{12} &= m_{11} \\
m_{21} &= -e^{-\mu p_c} x_{2c} f_2'(1 - x_{1c} - x_{2c}) + q f_1(1 - x_{1c} - x_{2c}) \\
&\quad - q f_1'(1 - x_{1c} - x_{2c}) x_{1c} \\
m_{22} &= -q f_1(1 - x_{1c} - x_{2c}) x_{1c} - e^{-\mu p_c} x_{2c} f_2'(1 - x_{1c} - x_{2c}) \\
&\quad - q x_{1c} f_1'(1 - x_{1c} - x_{2c}) \\
m_{23} &= -\mu e^{-\mu p_c} f_2(1 - x_{1c} - x_{2c}) x_{2c} \\
m_{31} &= -\frac{\delta p_c}{K + p_c} \\
m_{33} &= -1 - \frac{\delta K}{(K + p_c)^2} x_{1c}
\end{aligned}$$

$$m_{11} < 0, \quad m_{22} < 0, \quad m_{23} < 0, \quad m_{31} < 0 \quad \text{and} \quad m_{33} < 0.$$

The characteristic polynomial takes the form (after a long and tedious computation)

$$\lambda^3 + B_1\lambda^2 + B_2\lambda + B_3 = 0$$

where

$$B_1 = -m_{11} - m_{22} - m_{33} > 0$$

$$B_2 = m_{11}m_{22} - m_{12}m_{21} + m_{11}m_{33} + m_{22}m_{33}$$

$$B_3 = -m_{33}(m_{11}m_{22} - m_{12}m_{21}) - m_{12}m_{23}m_{31}$$

We note that

$$m_{12}m_{22} - m_{12}m_{21} = -m_{11}(-m_{22} + m_{21}) > 0.$$

To see this, we rewrite the bracket in terms of the original Jacobian entries to find that

$$\begin{aligned} m_{21} - m_{22} = {} & e^{-\mu p_c} f_2'(1 - x_{1c} - x_{2c})x_{2c} + q f_1(1 - x_{1c} - x_{2c}) \\ & - q f_1'(1 - x_{1c} - x_{2c})x_{1c} + q f_1(1 - x_{1c} - x_{2c})x_{1c} \\ & + e^{-\mu p_c} f_2'(1 - x_{1c} - x_{2c})x_{2c} + q x_{1c} f_1'(1 - x_{1c} - x_{2c}). \end{aligned}$$

Since the third and the last terms cancel, $m_{21} - m_{22} > 0$. This has the consequence that $B_2 > 0$ and $B_3 > 0$. Hence, one may apply the Routh Hurwitz criterion [C, p. 158] to conclude that all of the roots have negative real part if and only if $B_1B_2 > B_3$.

Theorem 4.4 *Suppose that E_c exists. If $B_1B_2 > B_3$, E_c is a local attractor. If $B_1B_2 < B_3$, E_c is unstable with a one dimensional stable manifold.*

Proof. Everything has been shown except the dimension of the stable manifold. Since the determinant of J (evaluated at E_c) is negative (expand along the last row to see this) there is always a negative eigenvalue.

The B's can be expressed in terms of the original parameters of the system and the location of the rest point. p_c and x_{2c} can be expressed in terms of x_{1c} quite simply, but finding x_{1c} presents a challenge. It would most likely have to be done numerically.

5 The case of small q

If q is small, one anticipates that the behavior of the system (2.5) should be close to that of the chemostat with an inhibitor, but without the plasmid loss term. If (2.2) is reduced and scaled in a similar way to the reduction and scaling of (2.5) which produced (3.2), one obtains

$$\begin{aligned} x_1' &= x_1[f_1(1 - x_1 - x_2) - 1] \\ x_2 &= x_2[e^{-\mu p} f_2(1 - x_1 - x_2) - 1] \\ p' &= 1 - p - \frac{\delta p}{K + p} x_1. \end{aligned} \tag{5.1}$$

(5.1) was investigated in [HW].

The most interesting behavior for (5.1) was the existence of a stable limit cycle. Can that happen with (3.2)? That it can for small q is a standard result in perturbation theory ([CL], p. 352) provided one assumes the limit cycle for (5.1) is hyperbolic (has all but one multiplier inside the unit circle.) More information is contained in a theorem of Smith [S2], a special case of which is restated here for system (3.2) and (5.1).

Theorem 5.1 *Suppose* $y(t) = (\bar{x}_1(t), \bar{x}_2(t), \bar{p}(t))$ *is a periodic solution of* (5.1) *of period* w *which has two characteristic multipliers inside the unit circle. Then there is an* $q_0 > 0$ *and a neighborhood* W *of the orbit of* y, Γ, *such that* (3.2) *has a periodic solution* $x(t, q) = (x_1(t), x_2(t), p(t))$ *in* W *of period* $\omega^*(q)$, $|q| \leqq q_0$, $x(t, 0) = y(t), \omega^*(0) = \omega, x$ *and* w *continuous in* q *and in* $t, t \varepsilon R$, $|q| \leqq q_0$. *Moreover* $x(t, q)$ *is the unique periodic solution of* (3.2) *in* W *and it is asymptotically orbitally stable with asymptotic phase. If* M *is any compact set in the basin of attraction of* Γ, $B(\Gamma)$, *then there exists a positive number* $q_1 = q_1(M) \leqq \varepsilon_0$ *such that if* $\hat{z}(t)$ *is a solution of* (3.2) *with* $\hat{z}_0 = \hat{z}(0) \in M$, $0 < q \leqq q_1$, then

$$\lim_{t \to \infty} |\hat{z}(t) - x(t + \gamma, q)| = 0$$

for some $\gamma = \gamma(z_0)$.

The condition for the existence of an asymptotically stable periodic solution of (5.1) were given in [HW]. That the orbit is hyperbolic is a generic assumption. This theorem sets the tone for this section; one assumes the behavior of (5.1) and deduces a result for (3.2) for q small.

The possibility of limit cycles for (3.2) raises an interesting question for the effectiveness of production by genetically altered organisms. Is production higher with a limit cycle or with a stable equilibrium state? (We assume "production" is equivalent to average amount of plasmid bearing organisms.)

There is a corresponding theorem for rest points not formally stated but following from [S2] which we will denote as Theorem (5.1)′. The assumption is that the roots of the variational matrix at the rest point have negative real parts. We will be able to state slightly more than Theorem (5.1)′ when we know in addition that the rest point is the global attractor (of the interior in our case).

If E_c is hyperbolic and globally asymptotically stable for solutions of (5.1) in the interior of Γ (defined in Sect. 3), then it follows that the system (3.2) has a rest point, which we denote $E_c(q)$, that is (locally) asymptotically stable. Using Theorem (5.1)′ above and properties of global attractors, one can say more.

Theorem 5.2 *Suppose that all rest points of* (5.1) *are hyperbolic and* E_c *is a global attractor of all solutions of* (5.1) *in the interior of* Γ. *Then, for* q *sufficiently small,* $E_c(q)$ *is a global attractor of all solutions of* (3.2) *in the interior of* Γ.

Proof. Consider the region G in the open positive octant on the origin side of the plane $x_1 + x_2 = 1$. Suppose that (3.2) is uniformly persistent for $q \geqq 0$. Since all solutions of (3.2) eventually lie in the bounded set Γ, (3.2) has a global attractor A_q for every q. By Theorem 3.2 of [HW], the flow restricted to the

open region G has a global attractor in that region, which to conserve notation we continue to call A_q. (The system is dissipative, the rest points are hyperbolic, and there are no cycles on the boundary. The above cited theorem also had a hypothesis that the boundary was invariant but this is not used in the proof.) Let $B(E_c)$ be a ball about E_c which lies in the positive cone. Let M of the hypothesis of Theorem (5.1)′ be the closure of $B(E_c)$ and q_0 the corresponding value given in the conclusion. Then, for $0 < q \leqq q_0$, every trajectory with initial conditions in M, tends to $E_c(q)$. Let U be an open subset of M containing E_c. Since the global attractor is upper semi-continuous, there is a q_1, $0 < q_1 \leqq q_0$, such that $A_q \subset U, 0 < q < q_1$. For $0 < q < q_1$, every trajectory in the region is asymptotic to $A_q \subset U \subset M$ intersects M, and hence, by (5.1)′ converges to $E_c(q)$. (This of course includes points of A_q which makes $E_c(q)$ a global attractor of the interior of G).

To complete the proof one must show that the system is uniformly persistent.

Lemma 5.3 *If all rest points for* (3.2) *are hyperbolic,* (3.2) *is uniformly persistent for sufficiently small* $q \geqq 0$.

Proof. The statement of the lemma holds for $q = 0$ hypothesis. If E_1 exists ($f_2(1) > e^{\mu}$), for E_c to exist for $q = 0$, it must be the case (assuming hyperbolicity) that $f_2(1) > e^{\mu}$ and $0 < \lambda_1^* < \lambda_2^* < 1$, [HW]. (The reader is cautioned that there is a difference of notation between this paper and [HW].) As a consequence, when $q = 0$, the rest point E_1 is an attractor in the $x_1 = 0$ plane and repels the interior of the cone. The same remains true for q sufficiently small. E_0 is a repeller for all q.

Let $X_1 = \{(x_1, x_2, p) | 0 < p < 1, x_i > 0, \quad i = 1, 2, x_1 + x_2 < 1\}$ and let X_2 be the boundary in R^3. X_1 is forward invariant, X_2 is compact, and only the plane $x_1 = 0$ is invariant in X_2. Two rest points, E_0 and E_1, are in X_2 and each is a weak uniform repeller for X_1 under the current conditions (with q sufficiently small). The two rest points can be taken as the acyclic cover and now Theorem 4.5 of [Th2] completes the proof of the lemma.

If E_1 does not exist ($f_2(1) < e^{\mu}$), then the rest point E_0 has a two-dimensional stable manifold (the $x_1 = 0$ plane) and a one-dimensional unstable manifold which points into the cone. Thus, using the same X_1 and X_2 as above, E_0 is a uniform weak repeller of X_1. The lemma follows as above.

6 Discussion

This paper considers a chemostat model which incorporates features of a basic plasmid model of Stephanopoulis and Lapidus [SLa] and a model of the chemostat with an inhibitor proposed by Lenski and Hattingh [LH]. The key parameters differentiating the two are the proportion of plasmids lost in reproduction q, not present in the model in [LH] and the input inhibitor concentration $p^{(0)}$, not present in [SLa]. (When $p^{(0)} = 0$ we also set the initial concentration of the inhibitor to zero to avoid any consideration whatever.)

A mathematical analysis of the model in [LH] can be found in [HW] while the global analysis of the model of [SLa] in the case of Monod dynamics can be found in [HWW] and in the case of Andrews dynamics in [LuoH].

Plasmid models are important since products are manufactured by genetically altered organism but there is a tendency of the host cells to loose the plasmid and revert to their unaltered phenotype [RD] (the plasmid-free cells in the title of this paper.) One anticipates that the altered organism is a poorer competitor since growth and primary metabolism may be suppressed due to the artificially forced production [KR]. Simply, one anticipates that the plasmid-free organism is a better competitor, and, without the inhibitor, it might dominate the chemostat. This paper has focused on determining the asymptotic behavior as a function of the parameters of the organisms and the alteration of the parameters by the inhibitor. The analysis indicates where it is reasonable to operate the chemostat.

The mathematics is interesting because the planar techniques used in [HWW] are no longer available since the new system is inherently three dimensional. The properties of competitive three dimensional systems used in [HW] are no longer available because a positive value of q destroys the competitive nature of the problem. Although the global analysis is not complete, we have been able to give a global analysis for a significant portion of the parameter space. Moreover, when q is small, we have used perturbation techniques to show that the global results in [HW] carry over.

The nutrient itself can act as an inhibitor at high concentrations. This has been studied in the context of a plasmid model in [LuoH] and represents an entirely different phenomenon than that discussed here. In mathematical terms accounting for inhibition by the nutrient means changing the functional response rather than adding an additional substance to the model. Of course, one could do both and provide a model with double inhibition.

As noted above, when q is small, the results of [HW] apply. The most interesting of these was the presence of limit cycles for an open region of the parameter space. In this case it would be interesting to determine whether the mean concentration of plasmid-carrying cells in higher or lower than the case with a stable interior rest point. Might higher production be achieved in an oscillating rather than a steady state fermenter?

Finally we note that the perturbation results indicate that systems of differential equations sufficiently close to competitive ones have similar properties. Is there a general theorem in this direction?

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