



# Competition between plasmid-bearing and plasmid-free organisms in selective media

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**Abstract**—The use of selective media is important in bioreactor problems. While the selective component of the medium (an antibiotic, for example) may be added from an external source, there seems to be an advantage from having the selective process generated internally. Models for two common ways of achieving selective media are considered. In the first, proposed originally by Sardonini and DiBiasio, the plasmid-free organism is auxotrophic for a metabolite which is produced by the plasmid bearing organism in excess. For this model we are able to characterize completely the global behavior of solutions, completing that theory. In the second, the plasmid-bearing organism devotes a portion of its resources to producing a toxin to the plasmid-free organism. Such a model was proposed by Chao and Levin and the model considered here is a slight variant of theirs. Again, the global asymptotic behavior of the model as a function of the parameters is obtained. Copyright © 1996 Elsevier Science Ltd

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## 1. INTRODUCTION

The ability to manufacture desired products through genetically altered organisms represents one of the major developments in biotechnology. The genetic alteration commonly takes place through the insertion of a plasmid to code for the production of the desired protein. Although the plasmid reproduces when the cell divides, it can happen that the plasmid is not passed to the daughter cell thereby introducing the plasmid-free organism into the process. Since the plasmid-free organism does not carry the added metabolic burden imposed by the plasmid, it is potentially a better competitor. The study of mathematical models for the competition between plasmid-free and plasmid-bearing populations has recently been a problem of considerable interest (Hsu *et al.*, 1994, 1995; Levin, 1988; Lenski and Hattingh, 1986; Luo and Hsu, 1995; Macken *et al.*, 1994; Ryder and DiBiasio, 1984; Simonson, 1991; Stephanopoulos and Lapidus, 1988). The general subject of microbial competition has been reviewed in Fredrickson and Stephanopoulos (1981). To avoid ‘capture’ of the process by the plasmid-free organism, selective media are used for the culture. The most obvious of these techniques is to induce antibiotic resistance into the cell on the same plasmid that

codes for the production and to introduce an antibiotic into the medium. A mathematical model of the chemostat with an inhibitor has been studied in Hsu and Waltman (1992), while the system with plasmids as well as the inhibitor has been studied in Hsu *et al.* (1995). For large scale production systems, the use of large quantities of an antibiotic may be undesirable either on environmental or cost grounds.

The alternative would be to use a medium where the selective pressure is generated in the system itself. Laboratory experiments exist taking advantage of both commensalism and inhibition to preserve the plasmid-bearing organism. Both commensalism and inhibition also occur in nature, and models for both are discussed and analyzed below in the context of a chemostat. The resulting model in each case is a set of four non-linear, ordinary differential equations. The conservation normally present in chemostat-like models (indeed, perhaps the defining feature of a chemostat model) reduces this to three non-linear equations in the usual way. Even three non-linear differential equations can have solutions with extremely complicated behavior and be resistant to analysis. We will show, however, that the asymptotic behavior of both models can be analyzed by a further reduction in the dimension of the system.

The organization of the paper is as follows. In Section 2 we describe the standard model for the simple chemostat as a model of a bio-reactor and describe the modification necessary for the introduction

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of commensalism through a needed metabolite and for the introduction of inhibition by the emission of a toxin. In Section 3 we analyze the commensal model and in Section 4, the inhibitory model. Section 5 discusses the results and alternative ways to model. Appendix A contains some technical information on a convergence result used in reducing the problem to a tractable, lower-dimensional system.

## 2. THE MODELS

The models we consider are based on the chemostat. The equations governing the ideal chemostat are well known and the derivation may be found in almost any biotechnology text (for example, Shuler and Kargi, 1992), or, in detail, in Smith and Waltman (1995). We give a brief review here to motivate the changes we must make to consider plasmid models in selective media.

The chemostat can be viewed as three connected vessels. The first (the feed bottle) contains all of the nutrients needed by the microorganism in a medium with one, hereafter simply called the nutrient, in shortest supply. The medium is pumped at a constant rate into the second vessel, the culture vessel, which contains one or more microorganisms. The contents of the culture vessel is pumped at a constant rate into the third vessel, the overflow or collection vessel, keeping the volume of the culture vessel constant. The culture vessel is well mixed and all relevant parameters (temperature, pH, etc.) are controlled.

The rate of change of the nutrient in the culture vessel can be expressed as a function of input, outflow, and consumption while that of the organism can be expressed in terms of growth and outflow. The consumption term is based on experimental evidence and is expressed in Michaelis–Menten or Monod form. If  $S(t)$  denotes the concentration of the nutrient at time  $t$  and  $x(t)$  denotes the concentration of the organism, the consumption term at time  $t$  is assumed to be of the form

$$\frac{mS(t)x(t)}{a + S(t)}$$

$m$  is the maximal growth rate and  $a$  is the Michaelis–Menten (or half saturation constant). Both can be measured in the laboratory. If one assumes that growth is proportional to the nutrient uptake, the differential equations take the form

$$\begin{aligned} S' &= (S^{(0)} - S)D - x \frac{f(S)}{\gamma} \\ x' &= x(f(S) - D), \quad ' = \frac{d}{dt} \end{aligned}$$

where  $S^{(0)}$  is the input concentration,  $D$  is the dilution rate (flow rate divided by volume),  $\gamma$  is a yield constant, and  $f(S) = mS/(a + S)$ . With two competitors and the same assumptions, the equations become

$$S' = (S^{(0)} - S)D - x_1 \frac{f_1(S)}{\gamma_1} - x_2 \frac{f_2(S)}{\gamma_2}$$

$$x_i' = x_i(f_i(S) - D), \quad i = 1, 2$$

where  $f_i(S) = m_i S/(a_i + S)$ .

Our interest focuses on the case where one organism,  $x_1$ , is plasmid-bearing but the plasmid can be lost in reproduction, resulting in a plasmid-free organism  $x_2$ . If the constant  $q$  is the fraction of plasmids lost, the modified equations become

$$\begin{aligned} S' &= (S^{(0)} - S)D - x_1 \frac{f_1(S)}{\gamma} - x_2 \frac{f_2(S)}{\gamma} \\ x_1' &= x_1(f_1(S)(1 - q) - D) \\ x_2' &= x_2(f_2(S) - D) + qx_1 f_1(S). \end{aligned} \quad (1)$$

We have simply attributed the quantity  $qx_1 f_1(S)$ , a fraction of the growth, to the plasmid-free organism, and, since the two organisms are assumed to be the same except for the plasmid, set the yield constants to be equal. These equations appear in Stephanopoulos and Lapidus (1988), and have been investigated in Hsu *et al.* (1994).

From the chemical engineering standpoint, the consumption of nutrient by the plasmid-free organism represents a loss of production in the bio-reactor. Moreover, the plasmid-free organism is unencumbered by the added metabolic load the plasmid imposes, and thus may be a better competitor and eliminate the desired organism. The obvious choice is to alter the medium in such a way as to favor the plasmid-bearing organism. An example of this noted above would be to encode antibiotic resistance on the plasmid and put an antibiotic into the feed bottle. Models in this direction have been studied in Lenski and Hattingh (1986), Hsu and Waltman (1992), and Hsu *et al.* (1995). A more desirable (and perhaps more cost effective in the production stage) solution would be to have the regulation occur ‘naturally’ in the culture vessel. We focus on two models where this is known to occur. From the standpoint of the system designer (who creates/selects the organisms), one of these can be regarded as ‘defensive’ (staving off elimination) and the other offensive (inhibiting the competition).

In the first case, suppose that the plasmid-free organism is auxotrophic for a metabolite  $M$  which is produced by the plasmid-bearing organism in excess. Then eq. (1) must be modified to account for the added metabolite. Such a model for the growth of *Saccharomyces cerevisiae* was proposed by Sardonini and DiBiasio (1987). They propose a model and carry out the relevant experiments along with a steady-state analysis. The model equations they propose take the form

$$\begin{aligned} \frac{dS}{dt} &= (S^{(0)} - S)D - \frac{f_1(S)x_1}{\gamma} - \frac{f_2(S,M)x_2}{\gamma} \\ \frac{dx_1}{dt} &= (1 - q)f_1(S)x_1 - Dx_1 \\ \frac{dx_2}{dt} &= f_2(S,M)x_2 - Dx_2 + qf_1(S)x_1 \\ \frac{dM}{dt} &= kf_1(S)x_1 - \frac{f_2(S,M)}{y}x_2 - DM \end{aligned} \quad (2)$$

with

$$f_2(S, M) = \frac{m_2 S}{a_2 + S} \frac{M}{K + M}.$$

$y$  is an additional yield constant. [These equations also appear in Shuler and Kargi (1992, p. 420).]

In Section 3, we give a mathematical analysis of the asymptotic behavior of solutions of system (2) with a more general form for  $f_2(S, M)$ . The intuition is clear: if the plasmid-free organism eliminates the plasmid-bearing organism, it also eliminates its own supply of the metabolite  $M$ . Thus, one anticipates that both will coexist or both will be eliminated from the chemostat (or bio-reactor). This is the 'defensive' model referred to above.

The other alternative occurs when the plasmid-bearing organism devotes a portion of its resource towards the production of an inhibitor to the plasmid-free organism. Chao and Levin (1981) and Levin (1988) document the evolution of 'anticompetitor toxins'. They study competition between sensitive and resistant strains of *E. coli* in both structured and mass habitats. Levin (1988) writes chemostat equations in the same spirit as those below. Their studies are relevant to questions of the evolution of temperate bacteriophages, Stewart and Levin (1973).

Consider the basic plasmid model (1) and introduce an inhibitor (toxin)  $P$  produced by the plasmid-bearing organism. The production of the inhibitor comes at a cost in reproduction, modeled by removing a fraction of the nutrient uptake and attributing that amount of consumption to the production of the inhibitor. The equations take the form

$$\begin{aligned} S' &= (S^{(0)} - S)D - x_1 \frac{f_1(S)}{\gamma} - x_2 e^{-\mu P} \frac{f_2(S)}{\gamma} \\ x_1' &= x_1 [(1 - q - k)f_1(S) - D] \\ x_2' &= x_2 [f_2(S)e^{-\mu P} - D] + qx_1 f_1(S) \\ P' &= kx_1 f_1(S) - DP. \end{aligned} \quad (3)$$

The term  $e^{-\mu P}$  represents the effect of the inhibitor, this form having been used by Lenski and Hattingh (1986);  $k$  is the fraction of consumption devoted to the production of the inhibitor. Section 4 deals with a mathematical analysis of the asymptotic behavior of solutions of a generalization of this system.

Both models (2) and (3) consist of four-dimensional systems of non-linear ordinary differential equations. Given the wide range of possible behavior patterns for such system (for example, even three dimensions is enough for chaotic behavior) it is most unusual that global mathematical results are possible.

Finally, as a general hypothesis, we will assume that all rest points are hyperbolic. This is usually reflected in the use of strong inequalities in theorem hypotheses. Since the parameters are measured quantities, they are not known to the precision required to generate a non-hyperbolic case.

### 3. THE DEFENSIVE MODEL

In this section, we analyze a generalization of the system (2). It is not necessary to restrict ourselves to Monod (or Michaelis-Menten) kinetics. We can restrict the functional responses  $f_1(S)$  and  $f_2(S, M)$  to  $C^1$  functions satisfying

- (i)  $f_1(0) = 0$ ,  $f_1'(S) > 0$ ,
- (ii)  $f_2(0, M) = f_2(S, 0) = 0$ ,  $\partial f_2 / \partial S > 0$ ,  $\partial f_2 / \partial M > 0$ .

This includes

$$\begin{aligned} f_1(S) &= \frac{m_1 S}{a_1 + S} \\ f_2(S, M) &= \frac{m_2 S}{a_2 + S} \frac{M}{K + M} \end{aligned} \quad (4)$$

the model of Sardonini and DiBiasio discussed in Section 2.

First, it is convenient to work with dimensionless variables. To achieve this, scale the variables by

$$\bar{S} = \frac{S}{S^{(0)}}, \quad \bar{x}_i = \frac{x_i}{\gamma S^{(0)}}, \quad \bar{M} = \frac{M}{S^{(0)}} \quad \tau = Dt.$$

Let

$$\bar{f}_1(\bar{S}) = \frac{f_1(S^{(0)} \bar{S})}{D} = \frac{f_1(S)}{D}$$

and

$$\bar{f}_2(\bar{S}, \bar{M}) = \frac{f_2(S^{(0)} \bar{S}, S^{(0)} \bar{M})}{D} = \frac{f_2(S, M)}{D}.$$

[Note that in the special case of eq. (2), the terms in  $S^{(0)}$  divide out and  $a$  and  $m$  have changed their biological meaning.] One can also view this scaling as relating the variables to the environment of the reactor,  $S^{(0)}$ ,  $D$ , the two variables under control of the experimenter.

Then, the system can be written (dropping all of the bars) as

$$\begin{aligned} S' &= (1 - S) - f_1(S)x_1 - f_2(S, M)x_2 \\ x_1' &= [(1 - q)f_1(S) - 1]x_1 \\ x_2' &= f_2(S, M)x_2 - x_2 + qf_1(S)x_1 \\ M' &= kf_1(S)x_1 - \frac{f_2(S, M)}{y}x_2 - M \end{aligned} \quad (5)$$

$$S(0) \geq 0, \quad x_1(0) > 0, \quad x_2(0) \geq 0, \quad M(0) \geq 0.$$

Although the system (5) is four-dimensional, we show that it is asymptotic to a two-dimensional system. The theory for this is contained in the work of Thieme (1992), but Appendix A is sufficient for our purposes. First let  $\Sigma = 1 - S - x_1 - x_2$ . The system (5) can be rewritten as

$$\begin{aligned} \Sigma' &= -\Sigma \\ x_1' &= x_1 [(1 - q)f_1(1 - x_1 - x_2 - \Sigma) - 1] \\ x_2' &= x_2 [f_2(1 - x_1 - x_2 - \Sigma) - 1] \\ &\quad + qx_1 f_1(1 - x_1 - x_2 - \Sigma) \\ M' &= kf_1(1 - x_1 - x_2 - \Sigma) \\ &\quad - \frac{f_2(1 - x_1 - x_2 - \Sigma, M)}{y}x_2 - M. \end{aligned} \quad (6)$$

Clearly,  $\Sigma(t) = \Sigma(0)e^{-t}$  or  $\lim_{t \rightarrow \infty} \Sigma(t) = 0$  and the last three equations of eq. (6) can be viewed as an asymptotically autonomous system with limiting system (note the exponential rate of convergence).

$$\begin{aligned} x_1' &= x_1[(1-q)f_1(1-x_1-x_2)-1] \\ x_2' &= x_2[f_2(1-x_1-x_2, M)-1] \\ &\quad + qx_1f_1(1-x_1-x_2) \\ M' &= kx_1f_1(1-x_1-x_2) \\ &\quad - x_2 \frac{f_2(1-x_1-x_2, M)}{y} - M. \end{aligned} \quad (7)$$

It is easy to see that  $S, x_1, x_2$  remain non-negative if the initial conditions are non-negative. That  $\lim_{t \rightarrow \infty} \Sigma(t) = 0$  shows that the solutions are bounded and that one should restrict consideration of (7) to the region  $x_1 + x_2 < 1$ . It is also easy to see, once  $f_1$  and  $f_2$  are bounded, that  $M$  remains bounded (although we do not yet have an explicit bound). Moreover, if  $M(0) = 0$ , and  $x_1 + x_2 < 1$ ,  $M' > 0$  ( $f_2(S, 0) = 0$ ). Thus,  $M$  cannot become negative and the original system is dissipative.

Now let  $z(t) = M(t) + (1/y)x_2(t) - [(ky+q)/(1-q)]/[x_1(t)/y]$ . Then a direct computation allows one to rewrite (7) as

$$\begin{aligned} z' &= -z \\ x_1' &= x_1[(1-q)f_1(1-x_1-x_2)-1] \\ x_2' &= x_2 \left[ f_2 \left( 1-x_1-x_2, z - \frac{1}{y}x_2 + \frac{ky+q}{1-q} \frac{x_1}{y} \right) - 1 \right] \\ &\quad + qx_1f_1(1-x_1-x_2). \end{aligned} \quad (8)$$

As above,  $z(t) = z(0)e^{-t}$  or  $\lim_{t \rightarrow \infty} z(t) = 0$  and the convergence is exponential. Hence, one may view the last two equations in eqs (8) as a two-dimensional, asymptotically, autonomous system with limiting system

$$\begin{aligned} x_1' &= x_1[(1-q)f_1(1-x_1-x_2)-1] \\ x_2' &= x_2 \left[ f_2 \left( 1-x_1-x_2, -\frac{x_2}{y} + \frac{ky+q}{1-q} \frac{x_1}{y} \right) - 1 \right] \\ &\quad + qx_1f_1(1-x_1-x_2) \end{aligned} \quad (9)$$

confined to the region

$$\Omega = \left\{ (x_1, x_2) \mid x_1 \geq 0, x_2 \geq 0, x_1 + x_2 \leq 1, \frac{ky+q}{1-q} x_1 - x_2 > 0 \right\}.$$

Figure 1 illustrates the region  $\Omega$ , a region bounded by two lines and the  $x_1$ -axis. This region is shown below to be positively invariant and is the region where system (9) is meaningful. The omega limit sets of all positive trajectories of eqs (5) lie here.

To avoid a great deal of surplus notation, we write eqs (9) as

$$\begin{aligned} x_1' &= [(1-q)f_1(S)-1]x_1 = g_1(x_1, x_2) \\ x_2' &= (f_2(S, M)-1)x_2 + qf_1(S)x_1 = g_2(x_1, x_2) \end{aligned}$$

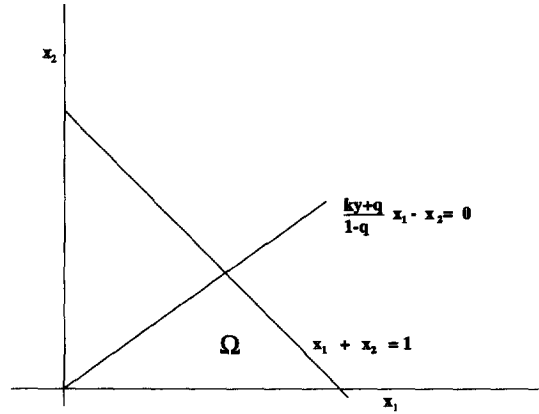


Fig. 1. The region  $\Omega$ : the domain of the limiting system.

$$\begin{aligned} S &= 1 - x_1 - x_2 \\ M &= \frac{ky+q}{1-q} \frac{x_1}{y} - \frac{1}{y} x_2. \end{aligned} \quad (10)$$

It is the reduced system that we analyze. One way to interpret this reduction is that, since the system is dissipative, there is a global attractor which must be on the intersections of the sets (in  $R^4$ ) given by

$$x_1 + x_2 + S = 1, \quad \frac{ky+q}{1-q} \frac{x_1}{y} - \frac{1}{y} x_2 - M = 0$$

and solutions on the attractor satisfy eqs (10).

To verify that the region  $\Omega$  is positively invariant, one must check the flow on the boundaries. First of all,  $(dx_2/dt)|_{x_2=0} = qf_1(1-x_1)x_1 > 0$  if  $x_1 > 0$ . Further,  $(1-x_1(t)-x_2(t))|_{x_1+x_2=1, x_1 \geq 0, x_2 \geq 0} = -x_1 - x_2 < 0$ . Finally, let  $M(t) = [(ky+q)/(1-q)][x_1(t)/y] - x_2(t)/y$ . Then  $M'(t)|_{M=0} > 0$ . On all three pieces of the boundary, the vector field points into  $\Omega$  except at the rest point at the origin.

The variational matrix for (10) at the origin has the form

$$J = \begin{bmatrix} m_{11} & 0 \\ m_{21} & m_{22} \end{bmatrix}$$

so the two eigenvalues  $\mu_1, \mu_2$  are the diagonal elements. A direct computation yields

$$\begin{aligned} m_{11} &= (1-q)f_1(1) - 1 \\ m_{22} &= -1. \end{aligned}$$

The eigenvector corresponding to  $\mu_2$  lies along the  $x_2$ -axis and is not relevant. The eigenvector corresponding to  $\mu_1$  lies along the line  $[q/(1-q)]x_1 - x_2 = 0$  and, hence, points into  $\Omega$ . Thus, the origin is asymptotically stable if  $f_1(1) < 1/(1-q)$  and unstable if  $f_1(1) > 1/(1-q)$ .

The next question is whether there are any additional fixed points in  $\Omega$ . To find them, one must first solve

$$(1-q)f_1(1-x_1-x_2) - 1 = 0.$$

If  $f(1) \leq 1/(1-q)$ , this equation has no solution with  $0 < x_1 + x_2 < 1$ . Thus,  $f(1) > 1/(1-q)$  is a necessary condition for the existence of at least one additional rest point.

The condition is also sufficient. As noted, the region  $\Omega$  is positively invariant under the flow. Let  $T(t)(x_0, y_0)$  denote the mapping which takes the point  $(x_0, y_0)$  to the point  $(x(t), y(t))$  on the unique trajectory through  $(x_0, y_0)$ . Then,  $T(t)\Omega \subset \Omega$ . The ejective fixed point theorem (Browder, 1965, Theorem 2) yields the existence of a non-ejective fixed point, and  $f(1) > 1/(1-q)$  makes the origin ejective. Hence, there is an additional rest point  $(x_1^*, x_2^*)$ .

Uniqueness unfortunately requires a computation. If we denote by  $\lambda_1$ ,  $0 < \lambda_1 < 1$  the unique value such that  $f_1(\lambda_1) = 1/(1-q)$ , then one has at once that

$$1 - x_1^* - x_2^* = \lambda_1$$

or

$$x_2^* = 1 - \lambda_1 - x_1^*.$$

Thus,  $x_1^*$  must be a root of

$$F(x_1) = \left[ f_2 \left( \lambda_1, \frac{ky+q}{1-q} \frac{x_1}{y} - \frac{1}{y} (1-x_1-\lambda_1) \right) - 1 \right]$$

$$\times (1-x_1-\lambda_1) + qf_1(\lambda_1)x_1 = 0.$$

We establish uniqueness by showing that if there is a point  $x_1$  such that  $F(x_1) = 0$ , then  $F'(x_1) > 0$ . A straightforward computation yields

$$\begin{aligned} F'(x_1) &= qf_1(\lambda_1) - [f_2(\lambda_1, M) - 1] \\ &\quad + (1-x_1-\lambda_1) \frac{\partial f_2}{\partial M}(\lambda_1, M) \\ &\quad \times \left[ \frac{ky+q}{y(1-q)} + \frac{1}{y} \right]. \end{aligned} \quad (11)$$

However,  $F(x_1) = 0$  implies that

$$f_2(\lambda_1, M) - 1 = \frac{-qf_1(\lambda_1)x_1}{1-x_1-\lambda_1} < 0.$$

Hence, the terms in  $F'(x_1)$  are positive or  $F'(x_1) > 0$ . This establishes uniqueness. The following lemma summarizes the discussion.

**Lemma 3.1.** *A necessary and sufficient condition for the existence of a non-trivial rest point  $(x_1^*, x_2^*)$  of eqs (10) is that  $f(1) > 1/(1-q)$ . There is at most one such interior rest point.*

The local stability of the interior rest point is determined by the eigenvalues of the variational matrix

$$J = \begin{bmatrix} m_{11} & m_{12} \\ m_{21} & m_{22} \end{bmatrix}.$$

A straightforward calculation yields that

$$m_{11} = x_1^*(1-q)f_1'(S^*) < 0$$

$$m_{12} = x_1^*(1-q)f_1'(S^*) < 0$$

$$\begin{aligned} m_{21} &= x_2^* \left[ -\frac{\partial f_2}{\partial S}(S^*, M^*) + \frac{\partial f_2}{\partial M}(S^*, M^*) \cdot \frac{ky+q}{y(1-q)} \right] \\ &\quad + qf_1(S^*) - qf_1'(S^*)x_1^* \end{aligned}$$

$$m_{22} = [f_2(S^*, M^*) - 1] + x_2^* \left[ -\frac{\partial f_2}{\partial S}(S^*, M^*) \right.$$

$$\left. - \frac{1}{y} \frac{\partial f_2}{\partial M}(S^*, M^*) \right] - qf_1'(S^*)x_1^*$$

$$= \frac{-qf_1(S^*)x_1^*}{x_2^*} + x_2^* \left[ -\frac{\partial f_2}{\partial S}(S^*, M^*) \right.$$

$$\left. - \frac{1}{y} \frac{\partial f_2}{\partial M}(S^*, M^*) \right] - qf_1'(S^*)x_1^* < 0.$$

Thus, one has at once that  $m_{11} + m_{22} < 0$ . We compute the sign of the determinant of  $J$ ,  $D = m_{11}m_{22} - m_{12}m_{21}$ . Since  $m_{11} = m_{12} < 0$ ,  $D = m_{11}(m_{22} - m_{21})$ .  $D$  will be positive if and only if  $m_{21} > m_{22}$ . We simply check this inequality. To simplify the notation, we use

$$S^* = 1 - x_1^* - x_2^*, \quad M^* = \frac{ky+q}{1-q} \frac{x_1^*}{y} - \frac{x_2^*}{q}.$$

Is

$$\begin{aligned} &-\frac{qf_1(S^*)x_1^*}{x_2^*} + x_2^* \left[ -\frac{\partial f_2}{\partial S}(S^*, M^*) \right. \\ &\quad \left. - \frac{1}{y} \frac{\partial f_2}{\partial M}(S^*, M^*) \right] - qf_1'(S^*)x_1^* \\ &< x_2^* \left[ -\frac{\partial f_2}{\partial S}(S^*, M^*) + \frac{\partial f_2}{\partial M}(S^*, M^*) \cdot \frac{k+q}{1-q} \right] \\ &\quad + qf_1(S^*) - qf_1'(S^*)x_1^*? \end{aligned}$$

If one cancels equal terms from each side, the question is whether

$$\begin{aligned} &-\frac{qf_1(S^*)x_1^*}{x_2^*} - \frac{x_2^*}{y} \frac{\partial f_2}{\partial M}(S^*, M^*) \\ &< \frac{x_2^*}{y} \frac{\partial f_2}{\partial M}(S^*, M^*) \left( \frac{ky+q}{1-q} \right) + qf_1(S^*)? \end{aligned}$$

This clearly holds since the left-hand side is negative and the right-hand side is positive. Thus, one has

**Lemma 3.2.** *If the interior equilibrium exists, it is locally asymptotically stable.*

The problem now is to show that the stability is global. The next lemma excludes the possibility of limit cycles for system (10).

**Lemma 3.3.** *System (10) has no periodic trajectories.*

**Proof:** We apply the Dulac criterion (Andronov *et al.*, 1973) with  $h(x_1, x_2) = 1/(x_1 x_2)$ . Compute

$$\begin{aligned} & \frac{\partial}{\partial x_1} [hg_1] + \frac{\partial}{\partial x_2} [hg_2] \\ &= -(1-q) \frac{1}{x_2} f_1'(1-x_1-x_2) - \frac{1}{x_1 x_2^2} q f_1(S) x_1 \\ & \quad - \frac{1}{x_1} q f_1'(S) x_1 - \frac{1}{x_1} \frac{\partial f_2}{\partial S} - \frac{1}{y} \frac{\partial f_2}{\partial M} \frac{1}{x_1} - \frac{1}{x_2} \\ & \quad \times q f_1'(1-x_1-x_2) < 0. \end{aligned}$$

Using the Dulac criterion, one can conclude that there are no limit cycles.

**Theorem 3.1.** *If  $f(1) < 1/(1-q)$ , then all solutions of system (10) with initial conditions in  $\Omega$  satisfy*

$$\lim_{t \rightarrow \infty} x_1(t) = 0$$

$$\lim_{t \rightarrow \infty} x_2(t) = 0.$$

*If  $f(1) > 1/(1-q)$ , then all non-trivial solutions satisfy*

$$\lim_{t \rightarrow \infty} x_1(t) = x_1^*$$

$$\lim_{t \rightarrow \infty} x_2(t) = x_2^*$$

where  $(x_1^*, x_2^*)$  is the unique interior rest point of system (10).

**Proof:** The proof follows directly from the Poincaré-Bendixson Theorem (Levinson, 1955), given Lemmas 3.1–3.3.

The results of Appendix A allow the same conclusion for the larger system — if  $f_1(1) > 1/(1-q)$ , all trajectories of eqs (5) will tend to a rest point. The precise coordinates have to be recovered from the asymptotics, for example,  $S^* = 1 - x_1^* - x_2^*$  and  $M^* = [(ky + q)/(1-q)] x_1^* - x_2^*/y$ . These quantities are positive by constraints on the domain in which system (10) was considered.

#### 4. THE OFFENSIVE MODEL

In this Section, we analyze a generalization of (3). We will assume only that the functions  $f_i(S)$  are  $C^1$  functions which satisfy  $f_i(0) = 0$  and  $f_i'(S) > 0$ ,  $i = 1, 2$ . As before, the variables are first scaled to non-dimensional ones. To achieve this let

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

$$\tau = Dt, \quad \bar{\mu} = \mu \gamma S^{(0)}.$$

Define the functions  $\bar{f}_i(\bar{S})$  by

$$\bar{f}_i(\bar{S}) = \frac{f_i(S^{(0)} \bar{S})}{D}.$$

We remind the reader that for Michaelis-Menten kinetics, this redefines the basic coefficients  $m_i$  and  $a_i$ ,  $i = 1, 2$ . Then, dropping the bars, system (3) may be

written as

$$\begin{aligned} S' &= 1 - S - x_1 f_1(S) - x_2 e^{-\mu P} f_2(S) \\ x_1' &= x_1 [(1-q-k)f_1(S) - 1] \\ x_2' &= x_2 [f_2(S) e^{-\mu P} - 1] + q x_1 f_1(S) \\ P' &= k x_1 f_1(S) - P. \end{aligned} \quad (12)$$

If the new variable  $\Sigma = 1 - S - x_1 - x_2 - P$  is introduced, system (12) can be written as the equivalent system

$$\begin{aligned} \Sigma' &= -\Sigma \\ x_1' &= x_1 [(1-q-k)f_1(1-x_1-x_2-P-\Sigma) - 1] \\ x_2' &= x_2 [f_2(1-x_1-x_2-P-\Sigma) e^{-\mu P} - 1] \\ & \quad + q x_1 f_1(1-x_1-x_2-P-\Sigma) \\ P' &= k x_1 f_1(1-x_1-x_2-P-\Sigma) - P. \end{aligned} \quad (13)$$

Since  $\Sigma(t) = \Sigma(0) e^{-t}$ , one can regard the last three equations in system (13) as an asymptotically autonomous system with limiting system

$$\begin{aligned} x_1' &= x_1 [(1-q-k)f_1(1-x_1-x_2-P) - 1] \\ x_2' &= x_2 [f_2(1-x_1-x_2-P) e^{-\mu P} - 1] \\ & \quad + q x_1 f_1(1-x_1-x_2-P) \\ P' &= k x_1 f_1(1-x_1-x_2-P) - P. \end{aligned} \quad (14)$$

As before, we must justify the reduction and this is contained in Appendix A. Introduce the new variable  $\Gamma = P - c x_1$  in (14) where  $c = k/(1-q-k)$ . Then (14) may be written as

$$\begin{aligned} \Gamma' &= -\Gamma \\ x_1' &= x_1 [(1-q-k)f_1(1-(1+c)x_1-x_2-\Gamma) - 1] \\ x_2' &= x_2 [f_2(1-(1+c)x_1-x_2-\Gamma) e^{-\mu(\Sigma+c x_1)} - 1] \\ & \quad + q x_1 f_1(1-(1+c)x_1-x_2-\Gamma) \end{aligned}$$

with limiting system

$$\begin{aligned} x_1' &= x_1 [(1-q-k)f_1(1-(1+c)x_1-x_2) - 1] \\ x_2' &= x_2 [f_2(1-(1+c)x_1-x_2) e^{-c \mu x_1} - 1] \\ & \quad + q x_1 f_1(1-(1+c)x_1-x_2). \end{aligned} \quad (15)$$

The variables are constrained to be in  $\Omega = \{(x_1, x_2) | x_i \geq 0, i = 1, 2, (1+c)x_1 + x_2 \leq 1, c = k/(1-q-k)\}$ . It is the system (15) that we analyze. Note that the region  $\Omega$  is positively invariant under the solution map for system (15).  $E_0 = (0, 0)$  is a rest point of system (15). The variational matrix about  $E_0$  takes the form

$$J = \begin{bmatrix} m_{11} & 0 \\ m_{21} & m_{22} \end{bmatrix} \quad (16)$$

so  $\mu_1 = m_{11}$  and  $\mu_2 = m_{22}$  are eigenvalues. A direct computation yields

$$\begin{aligned} \mu_1 &= (1-q-k)f_1(1) - 1 \\ \mu_2 &= f_2(1) - 1. \end{aligned}$$

**Remark:** The origin is an attractor if  $f_1(1) < 1/(1 - q - k)$  and  $f_2(1) < 1$ . The origin is unstable if either of these inequalities is reversed.

If  $f_2(1) > 1$ , denote by  $\lambda_2$  the unique number such that  $f_2(\lambda_2) = 1$ . This is the 'break-even' level for  $x_2$  if  $x_1$  were not present.  $E_1 = (0, 1 - \lambda_2)$  is also a rest point. The variational matrix about  $E_1$  is also of the form given by (16) and the eigenvalues are given by

$$\mu_1 = (1 - q - k)f_1(\lambda_2) - 1$$

$$\mu_2 = -(1 - \lambda_2)f_2'(\lambda_2).$$

The eigenvector corresponding to  $\mu_2$  lies along the  $x_2$ -axis ( $x_1 = 0$ ) is an invariant set for the flow corresponding to system (15). Thus, the stable manifold of  $E_1$  is at least one-dimensional and  $E_1$  will be an attractor if  $f_1(\lambda_2) < 1/(1 - q - k)$ .

**Theorem 4.1.** If  $f_1(1) < 1/(1 - q - k)$  and  $f_2(1) < 1$ , the origin is a global attractor (for  $\Omega$ ). If  $f_2(1) > 1$  and  $f_1(\lambda_2) < 1/(1 - q - k)$ ,  $E_1$  is an attractor. If there are no interior rest points,  $E_1$  is a global attractor of the interior of  $\Omega$ .

**Proof:** If  $f_1(1) < 1/(1 - q - k)$ , every rest point is of the form  $(0, z)$ . If  $f_2(1) < 1$ , then  $z = 0$  and the origin is the only rest point and is a local attractor. Without an interior rest point there are no periodic orbits. Hence, the Poincaré-Bendixson Theorem completes the proof of the first statement.

If  $f_2(1) > 1$ , the origin is unstable and  $E_1$  exists and is a local attractor since  $f_1(\lambda_2) < 1/(1 - q - k)$  by hypothesis. It remains to show that no orbit with initial conditions in  $\Omega$  tends to the origin (i.e.  $E_1$  is a global attractor of  $\Omega$ ). Choose  $\varepsilon > 0$  such that  $f_2(1 - (2 + c)\varepsilon)e^{-\mu\varepsilon} > 1$ . (This is possible by continuity.)

If  $\lim_{t \rightarrow \infty} x_i(t) = 0$ ,  $i = 1, 2$ , then for  $t$  sufficiently large,  $x_2'(t) > 0$ , a contradiction. (Alternatively, one could use the Butler McGehee lemma to exclude the origin as an omega limit point.) The Poincaré-Bendixson Theorem completes the proof.

If there is an interior rest point, periodic orbits might exist. The next lemma eliminates this possibility.

**Lemma 4.1.** The system (15) has no periodic orbits in  $\Omega$ .

**Proof:** The proof makes use of the Dulac criterion (Andronov *et al.*, 1973). (See Smith and Waltman, 1995, for applications.) Think of the system as

$$x_1' = g_1(x_1, x_2)$$

$$x_2' = g_2(x_1, x_2)$$

and compute the divergence of the vector field  $(\beta g_1, \beta g_2)$  with  $\beta = 1/x_1 x_2$ . A direct computation

gives this quantity as

$$-\frac{(1+c)(1-q-k)}{x_2} f_1'(1 - (1+c)x_1 - x_2)$$

$$-\frac{1}{x_1} f_2'(1 - (1+c)x_1 - x_2) e^{-c\mu x_1}$$

$$-\frac{q}{x_2^2} f_1(1 - (1+c)x_1 - x_2)$$

$$-\frac{q}{x_2} f_1'(1 - (1+c)x_1 - x_2) < 0.$$

The Dulac criterion then excludes any periodic orbits in the interior of  $\Omega$ .

The rest point  $E_1$  represents the total exclusion of the plasmid-bearing organism and, thus, is an undesirable state from the standpoint of a bio-reactor. No product is manufactured (and nutrient is consumed). There is no exclusionary state for the plasmid-free organism except total washout of both since the plasmid may be lost from a plasmid-bearing organism producing a plasmid-free organism. Thus, the question of the existence of interior rest points is paramount.

An interior rest point must satisfy

$$f_1(1 - (1+c)x_1 - x_2) = \frac{1}{1 - q - k}.$$

We have at once the following:

**Remark:** If  $f_1(1) \leq 1/(1 - q - k)$ , there is no interior rest point.

Thus, we can limit ourselves to the case that

$$f_1(1) > \frac{1}{1 - q - k}.$$

since Theorem 4.1 applies if this inequality is reserved. (We exclude the non-hyperbolic case  $f_1(1) = 1/(1 - q - k)$ .)

If  $f_1(1) > 1/(1 - q - k)$ , define  $\lambda_1$  as the unique value of  $z$  such that

$$f_1(z) = \frac{1}{1 - q - k}.$$

(We tacitly assume  $\lambda_1 \neq \lambda_2$ .) An interior rest point must be on the line segment

$$(1+c)x_1 + x_2 = 1 - \lambda_1, (x_1, x_2) \in \overset{\circ}{\Omega}$$

and the  $x_1$  coordinate must be a root of

$$F(x) = [1 - \lambda_1 - (1+c)x] [f_2(\lambda_1) e^{-c\mu x} - 1] + \frac{qx}{1 - q - k}, \quad 0 < x < \frac{1 - \lambda_1}{1 + c}. \quad (17)$$

One has at once that

$$F'(x) = -(1+c) [f_2(\lambda_1) e^{-c\mu x} - 1] + [1 - \lambda_1 - (1+c)x] (c\mu f_2(\lambda_1) e^{-c\mu x}) + \frac{q}{1 - q - k}$$

and

$$F''(x) = 2c\mu(1+c)f_2(\lambda_1)e^{-c\mu x} + c^2\mu^2 \\ \times [1 - \lambda_1 - (1+c)x]e^{-c\mu x} f_2(\lambda_1) > 0. \quad (18)$$

Thus, any zero of  $F'(x)$  is a local minimum. Finally, note that

$$F(0) = (1 - \lambda_1)(f_2(\lambda_1) - 1) \quad (19)$$

and

$$F\left(\frac{1 - \lambda_1}{1 + c}\right) = \frac{q(1 - \lambda_1)}{(1 + c)(1 - q - k)} > 0. \quad (20)$$

**Theorem 4.2.** *If  $f_2(\lambda_1) < 1$ ,  $f_1(1) > 1/(1 - q - k)$ , there exists a unique interior rest point,  $E^*$ , which is a global attractor.*

**Proof:** In view of the hypothesis, one has that

$$F(0) = (1 - \lambda_1)(f_2(\lambda_1) - 1) < 0.$$

Equation (20) implies the existence of at least one zero of  $F(x)$  and (18) implies exactly one. Hence, there is a unique interior rest point  $(x_1^*, x_2^*)$ .

$E_0$  is unstable. If  $E_1$  exists, then since

$$f_2(\lambda_1) < 1 = f_2(\lambda_2)$$

it follows that  $\lambda_1 < \lambda_2$ . Hence

$$f_1(\lambda_2) > f_1(\lambda_1) = \frac{1}{1 - q - k}$$

by monotonicity, so  $E_1$  is unstable (a saddle). The Butler–McGehee Theorem (Smith and Waltman, 1995, p. 12), shows that neither  $E_0$  nor  $E_1$  can be an omega limit point on a trajectory with initial conditions in  $\Omega$ . The Poincaré–Bendixson Theorem shows that all orbits tend to  $(x_1^*, x_2^*)$  as  $t$  tends to infinity.

It remains only to consider the case  $f_2(\lambda_1) > 1$ . As we have noted, the plasmid-free organism is likely a better competitor if there were no inhibition. The parameter  $\mu$  correlates with the effectiveness of the inhibitor. It can happen, of course, that the plasmid-free organism is a better competitor even at the maximum attainable level of inhibition. This is the content of the next statement which is intended as motivation for the next principal result.

**Remark:** *If  $f_2(\lambda_1) > e^{c\mu[(1 - \lambda_1)/(1 + c)]}$ , the only rest points are  $E_0$  and  $E_1$ .  $E_1$  is a global attractor of  $\Omega$ .*

**Proof:** Since  $f_2(1) > 1$  follows from monotonicity, the origin is unstable and  $E_1$  exists. Both terms on the right-hand side of (17) are positive, so there is no zero and, hence, no interior equilibrium.

From the hypothesis, it follows that  $f_2(\lambda_1) > 1 = f_2(\lambda_2)$  or  $\lambda_1 > \lambda_2$ . Hence,  $f_1(\lambda_1) > f_1(\lambda_2)$  or  $(1 - q - k)f_1(\lambda_2) < 1$ . Thus,  $E_1$  is a local attractor. That it is a global attractor follows from the Poincaré–Bendixson Theorem.

Note that  $\lambda_2 < \lambda_1$  reflects that fact that without the inhibitor,  $x_2$  is a better competitor. The remark gives a sufficient condition since the hypothesis (crudely)

makes both terms in eq. (17) positive. To seek a better solution, we need to account for the parameter  $\mu$  in a more delicate way. Before stating the result, we remind the reader that the existence and (local) stability of  $E_1$  is independent of the parameter  $\mu$  since  $x_1 = 0$  there.

**Theorem 4.3.** *If  $f_1(1) > 1/(1 - q - k)$  and  $f_2(\lambda_1) > 1$ , there exists a unique number  $\mu^*$  such that*

(i) *if  $\mu < \mu^*$  there are no interior rest points and  $E_1$  is a global attractor*

(ii) *if  $\mu > \mu^*$  there are exactly two interior rest points  $E^* = (x_1^*, x_2^*)$  and  $\hat{E} = (\hat{x}_1, \hat{x}_2)$ .  $E_1$  and one of  $(E^*, \hat{E})$  are local attractors.*

**Proof:**  $F(0) > 0$ ,  $F[(1 - \lambda_1)/(1 + c)] > 0$ , and  $F''(x) > 0$ ,  $0 < x < (1 - \lambda_1)/(1 + c)$ . Hence, there is no root, a double root or exactly two roots of  $F(x) = 0$  in  $I = (0, (1 - \lambda_1)/(1 + c))$ . Suppose first that there are no roots. Then  $F(x) > 0$  for all  $x$ .

Since  $f_2(1) > f_2(\lambda_1) > 1$ ,  $E_0$  is unstable (a repeller) and  $E_1$  exists. Since  $f_2(\lambda_1) > 1 = f_2(\lambda_2)$  it follows that  $\lambda_1 > \lambda_2$  and that  $E_1$  is locally stable. Since there are no other rest points, the Poincaré–Bendixson Theorem implies that all trajectories converge to  $E_1$ .

Suppose there were exactly two roots of  $F(x) = 0$  in  $I$ ,  $E^*$  and  $\hat{E}$ . Since  $E_1$  is a local attractor and is not a global one, its basin of attraction,  $\beta$ , has a boundary  $\partial\beta$  which is invariant.  $\partial\beta \cap \Omega$  must contain a rest point, other than the origin, by the Poincaré–Bendixson Theorem. This must be one of  $E^*$  or  $\hat{E}$  and that rest point is necessarily unstable. The other rest point is outside the basin of attraction (if it is hyperbolic, our generic assumption).  $\partial\beta$  separates  $\Omega$  and so the Poincaré–Bendixson Theorem says that the remaining rest point must attract all trajectories with initial conditions in  $\Omega/\beta$  (where  $\beta$  is the closure of  $\beta$ ).

There remains to show the existence of a unique  $\mu^*$  which separates the two cases.

As already noted, the function  $F(x)$  in (17) is convex and, hence, has at most one critical point which must be a minimum. For each  $\mu > 0$ , let  $F_\mu(x)$  be defined by (17). Then define  $g(x) = \lim_{\mu \rightarrow \infty} F_\mu(x) = \lambda_1 - 1 + (1 + c + qf_1(\lambda_1))x$  for  $x \in I$ . For fixed  $x$  the convergence is monotone. Since  $g(x) < 0$  for  $0 < x < \{(1 - \lambda_1)/[1 + c + qf_1(\lambda_1)]\}$ , for sufficiently large  $\mu$ ,  $F_\mu(x)$  has two zeros on  $J = (0, (1 - \lambda_1)/[1 + c + qf_1(\lambda_1)])$ . Moreover, if  $\mu_1 < \mu_2$  and  $F_{\mu_1}(x)$  has two zeros on  $J$ , so does  $F_{\mu_2}(x)$ . Also, if  $F_{\mu_2}(x)$  has no zero on  $J$ , then  $F_{\mu_1}(x)$  has no zeros. Hence,

$$A = \{\mu \mid \mu > 0, F_\mu(x) \text{ has two zeros on } J\}$$

and

$$B = \{\mu \mid \mu > 0, F_\mu(x) \text{ has no zeros on } J\}$$

are open disjoint sets. Let  $\mu^* = \sup B$ .  $F_{\mu^*}(x)$  has exactly one zero on  $J$ . This is the  $\mu^*$  of the theorem. By monotonicity, it is unique.

Again, the material in the appendix shows that similar conclusions hold for the original system. See the remarks at the end of Section 3.



Table 1

$f_1(1) < \frac{1}{1-q-k}$	$f_2(1) < 1$		$E_0$ is a global attractor
	$f_2(1) > 1$		$E_1$ is a global attractor
$f_1(1) > \frac{1}{1-q-k}$	$f_2(\lambda_1) < 1$	$\mu < \mu^*$	$E^*$ is a global attractor
	$f_2(\lambda_1) > 1$	$\mu > \mu^*$	$E_1$ is a global attractor
			bistable attractors

The conclusions of this section are illustrated in Table 1.

The reader will note that we have not used the exponential character of the term  $e^{-\mu P}$  which expresses the effect of the inhibitor. The term  $e^{-\mu P}$  can be replaced by a general function  $h(\mu, P)$  which is continuously differentiable and which satisfies:

- (i)  $h(\mu, 0) = h(0, P) = 1$ ;
- (ii) for  $\mu > 0, P > 0, \partial h / \partial \mu < 0, \partial h / \partial P < 0$ ;
- (iii)  $h(\mu, P)$  is convex in  $P$ ;
- (iv) for fixed  $P > 0, \lim_{\mu \rightarrow \infty} h(\mu, P) = 0$ .

$h(\mu, P) = e^{-\mu P}$  satisfies these conditions. Another example is  $h(\mu, P) = 1/(1 + \mu P)$ .

## 5. DISCUSSION

Since the plasmid-bearing organism can always lose the plasmid thereby, perhaps, creating a better competitor, the designer of a bio-reactor must contend with this reality. The obvious aid to the plasmid-bearing organisms is to alter the medium in such a way as not to allow the plasmid-bearing organism to be out-competed. Hence the idea of selective medium is introduced. A typical solution is to encode an antibiotic resistance in the plasmid and to put an antibiotic in the feed bottle. Thus, if the plasmid is lost, so is the antibiotic resistance. For long-running reactors, the cost of the antibiotic might be significant, and there are environmental concerns. A generally more desirable solution might be to create the selective medium from within.

In this paper, we have considered models for two ways of doing this. The first involves using an organism that is auxotrophic for a metabolite that is produced in the normal reproduction of the plasmid-bearing organism. Thus, if the plasmid free organism out-competes the plasmid-bearing one, it destroys its source of the metabolite. We have viewed this as a defensive strategy (from the standpoint of the engineer, not the organism). Another alternative is to have the plasmid-bearing organism produce a toxin against the plasmid-free one, at some expense, of course, to its own reproductive abilities. This, more aggressive strategy, we have viewed as an offensive strategy. Experiments exist in the literature showing both.

The chemostat models for each are formulated as four non-linear ordinary differential equations. By an appropriate change of variables, the system can be viewed as an asymptotically autonomous system where the limiting equations are two-dimensional. The theory for asymptotically autonomous equations

exists as does a very complete theory for two-dimensional autonomous differential equations. In the latter case, the Dulac criterion and the Poincaré-Bendixson Theorem are standard, powerful tools. In both cases the Dulac criterion allowed us to eliminate the possibility of limit cycles which are known to occur (in theory) for an external inhibitor (Hsu and Waltman, 1992). In the first (the defensive) case, we are able to give a complete answer to the asymptotic behavior of the model in terms of the parameters involved. We view this as the completion of the analysis of Sardonini and DiBiasio (1984). In the offensive case, we are able to give an explicit answer for a large region of the parameter space and show the existence of a critical parameter which determines the behavior in the remaining cases. While the critical value is not explicit for the general case, it could be numerically determined in any specific case. The most interesting result here was the conditions for the existence of two attractors, with the asymptotic behavior being dependent upon the initial conditions. One of the two attractors eliminates the plasmid-bearing organism. The user of the bio-reactor must begin his system in the proper region if he is to have a useful outcome. While it is theoretically possible to begin with only plasmid-bearing organisms and be in the domain of attraction of the coexistence steady state, this could be a problem in practice. The numerical solutions of Levin (1988, Fig. 1) illustrated the possibility (for a slightly different model) of the existence of two attractors. Again, we view the current work as a rigorous demonstration of this phenomenon and provide the relevant parameters regions for it to occur. The model is very close to that of Levin (1988).

In the inhibitor case, there is some choice of modeling strategies and we wished to comment on our approach. Obviously, the model should reduce the plasmid-free population when the inhibitor is present. One could view this as 'contact' between the inhibitor and the organisms which results in the death of the organisms. Such modeling is common in predator-prey modeling and in epidemic modeling where mass action terms are used. We have chosen to follow the work of Lenski and Hattingh (1986), who model the inhibitor as having a negative effect on the nutrient uptake—and hence on the reproduction (assumed proportional to consumption) of the organism, resulting in smaller numbers of the population. This preserves the conservation that is natural to the chemostat. The basic premise is that if all variables are taken into account (everything is expressed in nutrient equivalents), then the chemostat with no organism

and the chemostat with nutrient consumption would in sum have the same behavior. The organism is just another manifestation of the nutrient — at least in the limit. It is this conservation that allows us to reduce the dimension of the system.

The theorems have been stated in terms of scaled quantities; in particular, the variables under control,  $S^{(0)}$  and  $D$ , have been scaled out of the system. We now restate the results in terms of the original parameters and provide operating diagrams to display the regions of common behavior. The operating diagrams show the functional responses as being of Michaelis-Menten type but, of course, they are more general than this. The major result of Section 3, Theorem 3.1, provides only two regions. Region I gives total washout and occurs when

$$f_1(S^{(0)})(1 - q) < D.$$

Region II represents convergence to a coexistence steady state and occurs when

$$f_1(S^{(0)})(1 - q) > D.$$

This is illustrated in Fig. 2.

The material in Section 4 is more complex. Table 1 provides four possible regions. We show two representative cases. Two curves are plotted

$$L_1 : (1 - q - k)f_1(S^{(0)}) = D$$

and

$$L_2 : f_2(S^{(0)}) = D.$$

One way to view the curves is as plots of  $\lambda_1$  and  $\lambda_2$  for a given value of  $D$ . If the curves cross, as in Fig. 3, this is a common value—at this point the values of  $S^{(0)}$  and  $D$  make  $\lambda_1 = \lambda_2$ . Above the first

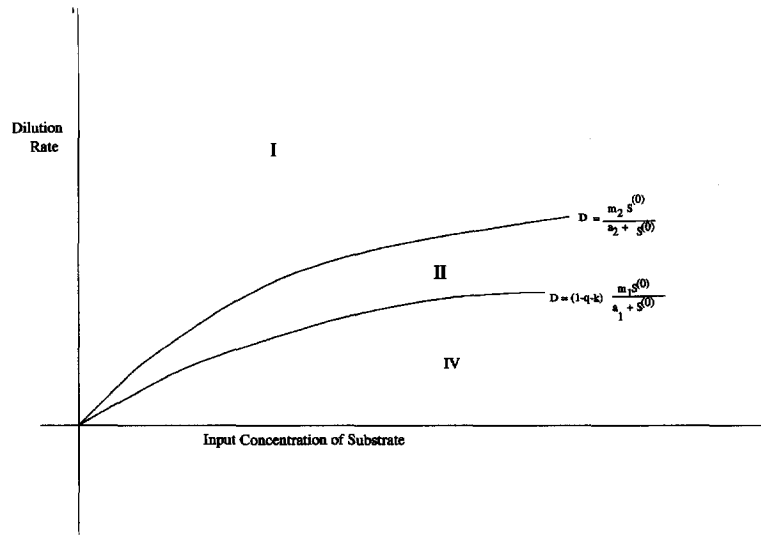


Fig. 2. Operating diagram for the defensive case.

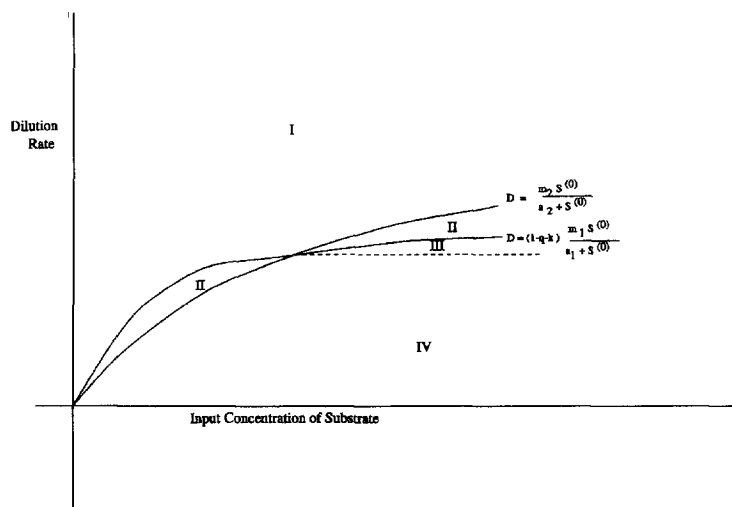


Fig. 3. Operating diagram for the offensive case with intersecting functional responses and  $\mu > \mu^*$ .

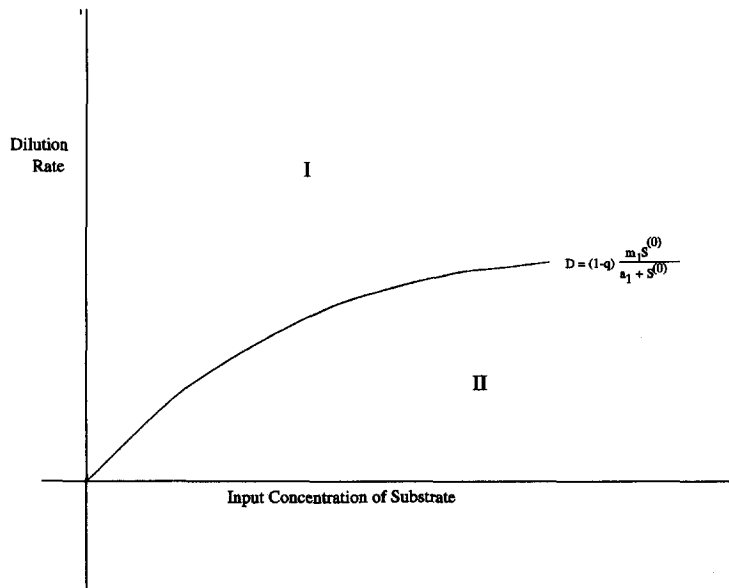


Fig. 4. Operating diagram for the offensive case with non-intersecting functional responses and  $\mu > \mu^*$ .

curve, the plasmid-bearing organisms wash out of the system; in particular there are no interior steady states. Above both curves, both organisms wash out. Hence, Region I illustrates the total washout that occurs when

$$(1 - q - k)f_1(S^{(0)}) < D$$

and

$$f_2(S^{(0)}) < D.$$

Region II represents the washout of only the plasmid-bearing organism. This occurs in two different scenarios. First, it can happen when the origin is unstable and there are no interior rest points (independent of  $\mu$ ), i.e. when exactly one of the above inequalities are violated: the other case is described below when the parameter  $\mu$  enters the discussion.

Region III represents the part of the parameter space where a unique interior rest point, called  $E^*$ , is the global attractor. This region must be below the curve  $L_1$  and in the region defined by

$$f_2(\lambda_1) > D.$$

The last condition is simply the region where  $\lambda_2 < \lambda_1$ . This is marked III in Fig. 3.

The bistable case, marked IV, requires that the parameter  $\mu$  be sufficiently large ( $\mu > \mu_*$ ), the region be below the curve  $L_1$ , and be in the region where  $\lambda_2 < \lambda_1$  (below the line indicated in Fig. 3). If the parameter  $\mu$  is not sufficiently large, the region marked IV becomes II.

Finally, the curves may not cross. We illustrate the case where the curve  $L_2$  is above  $L_1$  so  $\lambda_2 < \lambda_1$  for all values of the input concentration and the dilution rate. Thus it is always the case that  $\lambda_1 > \lambda_2$  and so the region below  $L_2$  is either II or IV depending on the size of  $\mu$ . We illustrate the case  $\mu > \mu^*$  in Fig. 4.

If the curves are ordered the other way, the lower region becomes III.

#### Acknowledgements

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#### NOTATION

$a$	Michaelis-Menten constant
$D$	dilution rate
$E_0$	rest point
$E_1$	rest point
$E^*$	rest point
$\tilde{E}$	rest point
$J$	variational matrix
$k$	fraction of consumption devoted to production of inhibitor
$m$	maximal growth rate
$M$	metabolite concentration
$M^*$	metabolite concentration at a rest point
$P$	inhibitor concentration
$P^*$	inhibitor concentration at a rest point
$q$	fraction of plasmids lost
$S$	nutrient concentration
$S^*$	nutrient concentration at a rest point
$S^{(0)}$	input nutrient concentration
$t$	time
$x_1$	plasmid-free organism concentration
$x_2$	plasmid-bearing organism concentration
$x_1^*$	plasmid-free organism concentration at a rest point
$x_2^*$	plasmid-bearing organism concentration at a rest point
$\beta$	basin of attraction

$\gamma$	yield constant
$\Gamma$	combination of dependent variables
$\Sigma$	combination of dependent variables
$\Omega$	region in $R^2$
$\tau$	dimensionless time
$\mu$	inhibition constant

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## APPENDIX A

In several cases, we were able to change variables and write the system under investigation as an asymptotically autonomous system of one dimension lower. The standard theory easily yields that the global attractor lies in a space of one dimension lower. However, it is not trivial that the dynamics are the same for the two systems even if the limit sets are the same. The original work in this direction is a paper of Markus (1953), which has been greatly improved in a recent paper of Thieme (1992). Thieme's result is very general but also difficult to explain briefly. The material that we present is a very special case of Thieme's work and the statement and proofs can be found in Appendix F of Smith and Waltman (1995).

Consider two systems of ordinary differential equations of the form

$$\begin{aligned} z' &= Az \\ y' &= f(y, z) \end{aligned} \tag{A1}$$

and

$$x' = f(x, 0) \tag{A2}$$

where

$$\begin{aligned} z \in R^m, \quad (y, z) \in D \subset R^n \times R^m, \\ x \in \Omega = \{x | (x, 0) \in D\} \subset R^n. \end{aligned}$$

It will be assumed that  $f$  is continuously differentiable,  $D$  is positively invariant for (A1), and that solutions of eq. (A1) are bounded. The following hypotheses will be needed.

(H1) All of the eigenvalues of  $A$  have negative real parts.

(H2) Equation (A2) has a finite number of rest points in  $\Omega$ , each of which is hyperbolic for eq. (A2). Denote these rest points by  $x_1, x_2, \dots, x_p$ .

(H3) The dimension of the stable manifold of  $x_i$  is  $n$  for  $1 \leq i \leq r$  and the dimension of the stable manifolds of  $x_j$  is less than  $n$  for  $j = r + 1, \dots, p$ . In symbols,  $\dim(M^+(x_i)) = n$ ,  $i = 1, \dots, r$ ,  $\dim(M^+(x_j)) < n$  for  $j = r + 1, \dots, p$ .

(H4)  $\Omega = \bigcup_1^p M^+(x_i)$ .

(H5) Equation (A2) does not have a cycle of rest points.

**Theorem A.1.** *Let (H1) – (H5) hold and let  $(y(t), z(t))$  be a solution of (A1). Then for some  $i$ ,*

$$\lim_{t \rightarrow \infty} (y(t), z(t)) = (x_i, 0).$$

In our case, the first equation in (A1) was one-dimensional, that is,  $A$  was a  $1 \times 1$  matrix with entry  $-1$ . We only considered limit sets that were hyperbolic rest points, so hypotheses (H1)–(H4) were automatically satisfied. In all cases where there was no interior rest point, (H5) was clearly satisfied. Moreover, a rest point that is an attractor or a repeller cannot be a part of a cycle. These observations are sufficient to apply Theorem A.1 to all of the reductions and to support convergence of all solutions of the four-dimen-

sional system to rest points on the basis of the convergence established for the two-dimensional system. The only troublesome case is the case of two stable equilibria in Section 4 (the last case). The origin can be connected to the interior unstable rest point which in turn is connected to  $E_1$  or the other interior rest point. However, no further connections are possible and no cycle exists.

We note, however, that the possibility remains for a solution of the higher-dimensional system to converge to a rest point which, for the corresponding two-dimensional system, is unstable. The totality of these stable manifolds (of rest points which are unstable for the two-dimensional limiting system) has (four-dimensional) Lebesgue measure zero. Except for trivial cases on the boundary (take  $x_1(0) = x_2(0) = 0$ ,  $S(0) > 0$ ,  $p(0) > 0$ ), this can only happen in the models being considered here in the last case of Section 4, for the interior, unstable rest point.