

Plasmid-bearing, plasmid-free organisms competing for two complementary nutrients in a chemostat [☆]

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Received 1 August 2001; received in revised form 7 January 2002; accepted 15 March 2002

Dedicated to Professor Paul Waltman on the occasion of his seventieth birthday

Abstract

A model of competition for two complementary nutrients between plasmid-bearing and plasmid-free organisms in a chemostat is proposed. A rigorous mathematical analysis of the global asymptotic behavior of the model is presented. The work extends the model of competition for a single-limited nutrient studied by Stephanopoulos and Lapidus [Chem. Engng. Sci. 443 (1988) 49] and Hsu, Waltman and Wolkowicz [J. Math. Biol. 32 (1994) 731].

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AMS: 92A15; 92A17; 34C15; 34C35

Keywords: Chemostat; Competition for two complementary nutrients; Plasmid bearing; Plasmid free; Competitive exclusion; Coexistence; Dulac criterion; Green's theorem

1. Introduction

Genetically altered organisms are used in industry to manufacture a desired product, for example, a pharmaceutical. The alteration is accomplished by introducing DNA into the cell, frequently in the form of a plasmid. Plasmids contain bits of DNA which exist separately from the chromosome and replicate independently; the plasmid codes for the added production. The

[☆] Research Supported by National Council of Science, Republic of China.

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burden imposed on the cell by the task of production can result in the genetically altered (the plasmid-bearing) organism being a less able competitor than the plasmid-free organism. Unfortunately, the plasmid can be lost in the reproductive process; that is, it may not be passed to the daughter cells, producing a plasmid-free organism (the ‘wild’ type). Since commercial production can take place on a scale of many generations, it is important to understand the asymptotic behavior of this system. A model of competition for a single-limit nutrient between plasmid-bearing and plasmid-free organisms in a chemostat was proposed by Stephanopoulos and Lapidus [9], who give a local analysis of various cases. A global analysis of the behavior of system trajectories was presented in [4]. There are other models of plasmid loss (and conjugation), for example, Levin and Stewart [7] and Macken et al. [8]. In this paper, we consider the competition for two complementary nutrients between plasmid-bearing and plasmid-free organisms in the chemostat. Complementary nutrients are those of different essential substances which are metabolically independent requirements for growth, such as a carbon source and a nitrogen source for bacterium, or silica and phosphorus for a diatom. Leon and Tumpson [6] proposed a mathematical model of two species competing for two complementary resources in a chemostat. Cheng et al. [1] gave a complete mathematical analysis for the model. It was shown that, as in the classical Lotka–Volterra two species competition model, there are four possible outcomes, including the case in which winning depends on the initial abundance of the competitors. In [5] Li et al. extend the model to the distributed delay. Hsu and Waltman studied the inhibition effects on plasmid populations in [3].

In Section 2 we present the model. The principal differences from the model in [1] center on the parameter q , $0 < q < 1$, the fraction of plasmid-bearing organism converting into plasmid-free organism. As in the paper [4], biologically it is reasonable to assume for the nutrients S and R , plasmid-bearing and plasmid-free organisms have the same yield constants y_s and y_r respectively. In Section 3, we reduce the system of four equations to a system of two equations by using the theory of asymptotically autonomous systems [11] (or Appendix F in [10]). Then we apply Green’s Theorem to show the two dimensional system has no periodic orbits (The proof is deferred to Appendix A). The difference between the proof in this paper and that in [4] is the equations in this paper is not differentiable everywhere. We need to apply Dulac’s criterion with care. In Section 4, we classify all the rest points of the four dimensional system by a local stability analysis. In Section 5 we combine the results in Sections 3 and 4 to give a complete classification of the global asymptotic behavior of the solutions of governing equations. Section 6 is the discussion section where we give the biological interpretations for the mathematical results. Some operating diagrams are shown for practical uses. We also discuss the experiment and the mathematical model in [2]. The more demanding proofs and computations are deferred to Appendix A.

2. The model

We use the standard chemostat notations in [1,10]. In [1] we consider species x_1 and x_2 competing exploitatively for two complementary nutrients S and R . The model took the form

$$\begin{aligned}
 \frac{dS}{dt} &= (S^{(0)} - S)D - \frac{x_1}{y_{s1}}f_1(S, R) - \frac{x_2}{y_{s2}}f_2(S, R), \\
 \frac{dR}{dt} &= (R^{(0)} - R)D - \frac{x_1}{y_{r1}}f_1(S, R) - \frac{x_2}{y_{r2}}f_2(S, R), \\
 \frac{dx_1}{dt} &= (f_1(S, R) - D)x_1, \\
 \frac{dx_2}{dt} &= (f_2(S, R) - D)x_2, \\
 S(0) &\geq 0, \quad R(0) \geq 0, \quad x_1(0) \geq 0, \quad x_2(0) \geq 0,
 \end{aligned}
 \tag{2.1}$$

where

$$\begin{aligned}
 f_1(S, R) &= \min \left(\frac{m_{s1}S}{K_{s1} + S}, \frac{m_{r1}R}{K_{r1} + R} \right), \\
 f_2(S, R) &= \min \left(\frac{m_{s2}S}{K_{s2} + S}, \frac{m_{r2}R}{K_{r2} + R} \right).
 \end{aligned}
 \tag{2.2}$$

The constants $S^{(0)}$ and $R^{(0)}$ are the input concentration for the nutrients S and R respectively. D is the dilution rate of the chemostat. $m_{si}, m_{ri}, K_{si}, K_{ri}, i = 1, 2$ are the maximal growth of i th competitor and the Michaelis–Menten (or half-saturation) constants with respect to nutrients S and R alone. $y_{si}, y_{ri}, i = 1, 2$ are the yield constants with respect to nutrients S and R . The growth rate $f_i(S, R), i = 1, 2$, takes the form (2.2). For complementary nutrients S and R , the per capita consumption rate of whichever nutrient is currently limiting growth is identical to the one-nutrient per capita consumption rate for the appropriate nutrient. The per capita consumption rate of the non-limiting nutrient is proportional to the per capita consumption rate of the limiting nutrient. We note that when a species is S -limited, its per capita consumption rate of R is independent of the concentration of R ; whereas, when the species is R -limited, its per capita consumption rate of S is independent of the concentration of S .

In [4] we consider the case plasmid-bearing organism x_1 and plasmid-free organism x_2 compete exploitatively for a single-limit nutrient S . The equations took the form

$$\begin{aligned}
 \frac{dS}{dt} &= (S^{(0)} - S)D - \frac{x_1}{y_{s1}}g_1(S) - \frac{x_2}{y_{s2}}g_2(S), \\
 \frac{dx_1}{dt} &= ((1 - q)g_1(S) - D)x_1, \\
 \frac{dx_2}{dt} &= (g_2(S) - D)x_2 + qg_1(S)x_1, \\
 S(0) &\geq 0, \quad x_1(0) \geq 0, \quad x_2(0) \geq 0,
 \end{aligned}
 \tag{2.3}$$

where

$$g_1(S) = \frac{m_{s1}S}{K_{s1} + S}, \quad g_2(S) = \frac{m_{s2}S}{K_{s2} + S}.$$

$q, 0 < q < 1$ is the fraction constant of plasmid-bearing population converting into plasmid-free population during the replication. Biologically we may assume $y_{s1} = y_{s2} = y_s$. Now we combine the

models (2.1) and (2.3) for the competition of plasmid-bearing and plasmid-free organisms for two complementary nutrients. The equations take the form

$$\begin{aligned}
 \frac{dS}{dt} &= (S^{(0)} - S)D - \frac{x_1}{y_s} f_1(S, R) - \frac{x_2}{y_s} f_2(S, R), \\
 \frac{dR}{dt} &= (R^{(0)} - R) - \frac{x_1}{y_r} f_1(S, R) - \frac{x_2}{y_r} f_2(S, R), \\
 \frac{dx_1}{dt} &= ((1 - q)f_1(S, R) - D)x_1, \\
 \frac{dx_2}{dt} &= (f_2(S, R) - D)x_2 + qf_1(S, R)x_1, \\
 S(0) &\geq 0, \quad R(0) \geq 0, \quad x_1(0) \geq 0, \quad x_2(0) \geq 0,
 \end{aligned}
 \tag{2.4}$$

where $f_1(S, R), f_2(S, R)$ satisfy (2.2) and both organisms x_1 and x_2 have the same yield constants with respect to nutrient S and R .

We shall analyze the behavior of solutions of (2.4) in order to answer the biological question, under what conditions will neither, one or both species survive or die out? We also seek to determine the limiting behavior of the surviving organisms and the nutrients.

3. Reduction to a two dimensional system

Let $\Sigma_1(t) = S^{(0)} - S(t) - x_1(t)/y_s - x_2(t)/y_s$ and $\Sigma_2(t) = R^{(0)} - R(t) - x_1(t)/y_r - x_2(t)/y_r$. Then the system (2.4) may be written equivalently as

$$\begin{aligned}
 \frac{d\Sigma_1}{dt} &= -D\Sigma_1, \\
 \frac{d\Sigma_2}{dt} &= -D\Sigma_2, \\
 \frac{dx_1}{dt} &= \left((1 - q)f_1\left(S^{(0)} - \Sigma_1 - \frac{x_1}{y_s} - \frac{x_2}{y_s}, R^{(0)} - \Sigma_2 - \frac{x_1}{y_r} - \frac{x_2}{y_r}\right) - D \right) x_1, \\
 \frac{dx_2}{dt} &= \left(f_2\left(S^{(0)} - \Sigma_1 - \frac{x_1}{y_s} - \frac{x_2}{y_s}, R^{(0)} - \Sigma_2 - \frac{x_1}{y_r} - \frac{x_2}{y_r}\right) - D \right) x_2 \\
 &\quad + qf_1\left(S^{(0)} - \Sigma_1 - \frac{x_1}{y_s} - \frac{x_2}{y_s}, R^{(0)} - \Sigma_2 - \frac{x_1}{y_r} - \frac{x_2}{y_r}\right) x_1, \\
 S^{(0)} - \Sigma_1(0) - \frac{x_1(0)}{y_s} - \frac{x_2(0)}{y_s} &\geq 0, \quad R^{(0)} - \Sigma_2(0) - \frac{x_1(0)}{y_r} - \frac{x_2(0)}{y_r} \geq 0, \\
 x_1(0) &\geq 0, \quad x_2(0) \geq 0, \quad 0 < q < 1.
 \end{aligned}
 \tag{3.1}$$

Clearly $\lim_{t \rightarrow \infty} \Sigma_1(t) = 0, \lim_{t \rightarrow \infty} \Sigma_2(t) = 0$, and so the omega limit set of any solution of (2.4) is contained in the set

$$\Omega^4 = \{(S, R, x_1, x_2) | S \geq 0, R \geq 0, x_1 \geq 0, x_2 \geq 0, \Sigma_1 = 0, \Sigma_2 = 0\}.
 \tag{3.2}$$

The limiting system, obtained by restricting the initial conditions to the set Ω^4 , is

$$\begin{aligned} \frac{dx_1}{dt} &= \left((1 - q)f_1 \left(S^{(0)} - \frac{x_1}{y_s} - \frac{x_2}{y_s}, R^{(0)} - \frac{x_1}{y_r} - \frac{x_2}{y_r} \right) - D \right) x_1, \\ \frac{dx_2}{dt} &= \left(f_2 \left(S^{(0)} - \frac{x_1}{y_s} - \frac{x_2}{y_s}, R^{(0)} - \frac{x_1}{y_r} - \frac{x_2}{y_r} \right) - D \right) x_2 \\ &\quad + qf_1 \left(S^{(0)} - \frac{x_1}{y_s} - \frac{x_2}{y_s}, R^{(0)} - \frac{x_1}{y_r} - \frac{x_2}{y_r} \right) x_1. \end{aligned} \tag{3.3}$$

These equations, of course, are restricted to the region

$$\Omega = \{(x_1, x_2) | x_1 \geq 0, x_2 \geq 0, x_1 + x_2 \leq \min(y_s S^{(0)}, y_r R^{(0)})\}.$$

The boundary of Ω satisfies the following properties

$$\begin{aligned} (x_1 + x_2)(\tau) &= \min(y_s S^{(0)}, y_r R^{(0)}) \\ \text{for some } \tau \geq 0 &\Rightarrow (x_1 + x_2)'(\tau) = -D(x_1 + x_2)(\tau) \leq 0, \end{aligned} \tag{3.4}$$

$$x_1(\tau) = 0 \quad \text{for some } \tau \geq 0 \Rightarrow x_1'(\tau) = 0, \tag{3.5}$$

$$x_2(\tau) = 0 \quad \text{for some } \tau \geq 0 \Rightarrow x_2'(\tau) = qx_1(\tau)f_1 \left(S^{(0)} - \frac{x_1}{y_s}, R^{(0)} - \frac{x_1}{y_r} \right) \geq 0. \tag{3.6}$$

Therefore, Ω is a positively invariant region. Similar arguments show that Ω^4 defined in (3.2) is positively invariant.

We shall proceed by first to determine the dynamics on the two dimensional globally attracting set Ω . To justify our conclusions for arbitrary initial conditions for the full four dimensional system (2.4), we will use the theory of asymptotically autonomous systems (see Appendix F in [10] or [11]).

First for simplicity we rewrite the system (3.3) in equivalent form:

$$\begin{aligned} \frac{dx_1}{dt} &= x_1((1 - q) \min(p_1(S), q_1(R)) - D) = g_1(x_1, x_2), \\ \frac{dx_2}{dt} &= x_2(\min(p_2(S), q_2(R)) - D) + qx_1 \min(p_1(S), q_1(R)) = g_2(x_1, x_2), \end{aligned} \tag{3.7}$$

where

$$p_i(S) = \frac{m_{si}S}{K_{si} + S}, \quad q_i(R) = \frac{m_{ri}R}{K_{ri} + R}, \quad i = 1, 2, \tag{3.8}$$

$$S = S^{(0)} - \frac{1}{y_s}(x_1 + x_2), \quad R = R^{(0)} - \frac{1}{y_r}(x_1 + x_2). \tag{3.9}$$

From (3.9) we have the following relations between $x_1 + x_2$ and S and R ,

$$\begin{aligned} x_1 + x_2 &= y_s(S^{(0)} - S) = y_r(R^{(0)} - R), \\ R &= \frac{y_s}{y_r}(S - S^{(0)}) + R^{(0)} \equiv h(S). \end{aligned} \tag{3.10}$$

Set

$$Q_1(S) = q_1(h(S)) = q_1(R), \quad Q_2(S) = q_2(h(S)) = q_2(R). \tag{3.11}$$

Obviously, we have $p'_i(S) > 0$, $p''_i(S) < 0$, $Q'_i(S) > 0$, $Q''_i(S) < 0$, for $i = 1, 2$.

Remark 3.1. We note that from (3.8), (3.10) and (3.11), it follows that for $i = 1, 2$

$$p_i(S) - Q_i(S) = \frac{m_{si}S}{K_{si} + S} - \frac{m_{ri}R}{K_{ri} + R} = 0$$

if and only if

$$m_{si}K_{ri}S - m_{ri}K_{si}R + (m_{si} - m_{ri})RS = 0. \tag{3.12}$$

Obviously, from (3.10) and (3.12) the equation $p_i(S) = Q_i(S)$ has at most two roots.

The main result of this section can be stated in the following. The proof is deferred to Appendix A.

Theorem 3.1. *System (3.7) has no periodic solutions.*

4. Rest points of Ω^4 and their local stability

We use the following notation for the relevant rest points of system (2.4). We say that a rest point of (2.4) does not exist if any one of its components is negative. Since $\lim_{t \rightarrow \infty} \Sigma_1(t) = 0$, and $\lim_{t \rightarrow \infty} \Sigma_2(t) = 0$, any rest point $\bar{E} = (\bar{S}, \bar{R}, \bar{x}_1, \bar{x}_2)$ of (2.4) must satisfy

$$y_s \bar{S} + \bar{x}_1 + \bar{x}_2 = y_s S^{(0)}, \tag{4.1}$$

$$y_r \bar{R} + \bar{x}_1 + \bar{x}_2 = y_r R^{(0)}, \tag{4.2}$$

or

$$\bar{S} = \frac{y_s S^{(0)} - (\bar{x}_1 + \bar{x}_2)}{y_s},$$

$$\bar{R} = \frac{y_r R^{(0)} - (\bar{x}_1 + \bar{x}_2)}{y_r}.$$

The washout rest point is denoted by $E_0 = (S^{(0)}, R^{(0)}, 0, 0)$ and it always exists. There are two possible rest points involving plasmid-free organisms but no plasmid-bearing organisms. In order to describe these two rest points, we introduce the following important parameters:

$$\lambda_{s1} = \frac{K_{s1}D}{(1 - q)m_{s1} - D}, \quad \lambda_{r1} = \frac{K_{r1}D}{(1 - q)m_{r1} - D},$$

$$\lambda_{s2} = \frac{K_{s2}D}{m_{s2} - D}, \quad \lambda_{r2} = \frac{K_{r2}D}{m_{r2} - D},$$

$$C = \frac{y_s}{y_r}, \quad T_i = \frac{R^{(0)} - \lambda_{ri}}{S^{(0)} - \lambda_{si}}, \quad i = 1, 2.$$

Note that $p_1(\lambda_{s1}) = 0$, $p_2(\lambda_{s2}) = 0$, $q_1(\lambda_{r1}) = 0$, $q_2(\lambda_{r2}) = 0$. In order to present the biological meaning of parameters T_i , and C , we assume $x_1(t) \equiv 0$ and rewrite T_2 , C as

$$C = \frac{1/y_r}{1/y_s}, \quad T_2 = \frac{(R^{(0)} - \lambda_{r2})D}{(S^{(0)} - \lambda_{s2})D}.$$

When only species 2 is present, T_2 represents the ratio of the steady-state nutrient regeneration rates at equilibrium under consumption by species 2. λ_{s2} and λ_{r2} are the equilibrium concentrations of resources S and R , respectively, under steady-state consumption by species 2. The parameter C represents the fixed yield ratio for species 2 growing on resources S and R . The units of $(1/y_r)$ are (units R consumed/unit species 2 produced); thus C is the ratio (units R consumed/units S consumed) per unit of species 2 produced.

By comparing T_2 with C , we can determine whether species 2 is S -limited or R -limited. This is because C represents the invariant ratio in which the essential nutrients R and S are consumed by species 2, whereas T_2 represents the ratio in which these same resources are being externally regenerated under steady-state consumption pressure from species 2. Therefore, if $T_2 > C$, the growth rate of species 2 is S -limited because S is regenerating at a steady-state rate slower than R with respect to the required consumption ratio for species 2. Similarly, if $T_2 < C$, the growth rate of species 2 will be R -limited (see [1]).

Definition 4.1. For $i = 1, 2$, if $T_i > C$, we say that species x_i is S -limited. Similarly, if $T_i < C$, we say that species x_i is R -limited.

From the fourth equation of (2.4), it is obvious that there is no rest point with plasmid-bearing organisms and zero plasmid-free organism. Let $E_2 = (\widehat{S}, \widehat{R}, 0, \widehat{x}_2)$, be a rest point without plasmid-bearing organisms. There are two possible such rest points, namely, $E_{2s} = (\lambda_{s2}, \widehat{R}, 0, \widehat{x}_2)$, $\widehat{S} = \lambda_{s2}$, $\widehat{R} > \lambda_{r2}$ and $E_{2r} = (\widehat{S}, \lambda_{r2}, 0, \widehat{x}_2)$, $\widehat{S} > \lambda_{s2}$, $\widehat{R} = \lambda_{r2}$.

Lemma 4.1. Assume $0 < \lambda_{s2} < S^{(0)}$, $0 < \lambda_{r2} < R^{(0)}$.

- (i) $T_2 > C$ (i.e., species x_2 is S -limited) if and only if E_{2s} exists,
- (ii) $T_2 < C$ (i.e., species x_2 is R -limited) if and only if E_{2r} exists.

Proof. Obviously from (4.1) and (4.2), the positive component of (E_2) , \widehat{x}_2 and \widehat{S} , \widehat{R} satisfy

$$y_s \widehat{S} + \widehat{x}_2 = y_s S^{(0)}, \quad y_r \widehat{R} + \widehat{x}_2 = y_r R^{(0)}.$$

Hence we have the relation

$$\widehat{R} = \frac{y_s}{y_r} (\widehat{S} - S^{(0)}) + R^{(0)}, \tag{4.3}$$

or

$$\widehat{S} = \frac{y_r}{y_s} (\widehat{R} - R^{(0)}) + S^{(0)}. \tag{4.4}$$

Then it follows that

$$(i) \quad T_2 > C \iff \frac{R^{(0)} - \lambda_{r2}}{S^{(0)} - \lambda_{s2}} > \frac{y_s}{y_r} \iff \frac{y_s}{y_r}(S^{(0)} - \lambda_{s2}) < R^{(0)} - \lambda_{r2} \iff \\ \hat{R} = \frac{y_s}{y_r}(\lambda_{s2} - S^{(0)}) + R^{(0)} > \lambda_{r2} \iff E_{2s}$$

exists, and

$$(ii) \quad T_2 < C \iff \frac{R^{(0)} - \lambda_{r2}}{S^{(0)} - \lambda_{s2}} < \frac{y_s}{y_r} \iff \frac{y_s}{y_r}(\lambda_{s2} - S^{(0)}) < \lambda_{r2} - R^{(0)} \iff \\ \hat{S} = \frac{y_r}{y_s}(\lambda_{r2} - R^{(0)}) + S^{(0)} > \lambda_{s2} \iff E_{2r}$$

exists. \square

Next we consider the existence and uniqueness of positive rest point. Let $E_c = (S_c, R_c, x_{1c}, x_{2c})$ be a positive equilibrium (i.e., $S_c, R_c, x_{1c}, x_{2c} > 0$). Then E_c satisfies

$$(1 - q)f_1(S_c, R_c) = D, \\ [f_2(S_c, R_c) - D] \left(\frac{x_{2c}}{x_{1c}} \right) + qf_1(S_c, R_c) = 0, \\ y_s S_c + x_{1c} + x_{2c} = y_s S^{(0)}, \\ y_r R_c + x_{1c} + x_{2c} = y_r R^{(0)}. \tag{4.5}$$

Let $\xi = x_{1c} + x_{2c}$ and $\eta = x_{2c}/x_{1c}$. Then we have

$$f_1 \left(\frac{y_s S^{(0)} - \xi}{y_s}, \frac{y_r R^{(0)} - \xi}{y_r} \right) = \frac{D}{1 - q}, \tag{4.6}$$

and

$$\left[f_2 \left(\frac{y_s S^{(0)} - \xi}{y_s}, \frac{y_r R^{(0)} - \xi}{y_r} \right) - D \right] \eta + \frac{q}{1 - q} D = 0. \tag{4.7}$$

From (4.7) we have

$$\eta = \frac{-\left(\frac{q}{1-q}D\right)}{f_2 \left(\frac{y_s S^{(0)} - \xi}{y_s}, \frac{y_r R^{(0)} - \xi}{y_r} \right) - D}. \tag{4.8}$$

The necessary and sufficient condition for the existence and uniqueness of positive rest point E_c is that there exists a unique ξ , $0 < \xi < \min\{y_s S^{(0)}, y_r R^{(0)}\}$ satisfying (4.6) and the η defined in (4.8) is positive. Obviously from (4.8), $\eta > 0$ if and only if

$$f_2 \left(\frac{y_s S^{(0)} - \xi}{y_s}, \frac{y_r R^{(0)} - \xi}{y_r} \right) - D < 0. \tag{4.9}$$

From (2.2), the LHS of (4.6) is a positive, monotonically decreasing of ξ for $0 < \xi < \min(y_s S^{(0)}, y_r R^{(0)})$. Eq. (4.6) has a unique positive solution ξ if $0 < \lambda_{s1} < S^{(0)}$, $0 < \lambda_{r1} < R^{(0)}$. To

find the positive rest point $E_c = (S_c, R_c, x_{1c}, x_{2c})$, from the first equation of (4.5), there are two cases:

Case 1: $S_c = \lambda_{s1}, R_c > \lambda_{r1}$.

Then $\lambda_{s1} = (y_s S^{(0)} - \xi)/y_s$ and $R_c = (y_r R^{(0)} - \xi)/y_r > \lambda_{r1}$ imply $C = y_s/y_r < T_1 = (R^{(0)} - \lambda_{r1})/(S^{(0)} - \lambda_{s1})$ or x_1 is S -limited. Then condition (4.9) implies

$$\lambda_{s1} < \lambda_{s2} \quad \text{or} \quad R_c < \lambda_{r2}. \tag{4.10}$$

Case 2: $S_c > \lambda_{s1}, R_c = \lambda_{r1}$

Then $\lambda_{r1} = (y_r R^{(0)} - \xi)/y_r$ and $S_c = (y_s S^{(0)} - \xi)/y_s > \lambda_{s1}$ imply $C > T_1$ or x_1 is R -limited. Then condition (4.9) implies:

$$\lambda_{r1} < \lambda_{r2} \quad \text{or} \quad S_c < \lambda_{s2}. \tag{4.11}$$

In Appendix A, we introduce the variational matrices of the system (2.4) at equilibria E_0, E_{2s}, E_{2r} and E_c and analyze their local stability. The reason we do the stability analysis of the original system (2.4) instead of the limiting system (3.3) is that it is easier to obtain stability criteria making more biological sense for various cases. The results of stability analysis are following:

(i) $E_0 = (S^{(0)}, R^{(0)}, 0, 0)$ is asymptotically stable if

$$(1 - q)f_1(S^{(0)}, R^{(0)}) - D < 0, \quad \text{and} \quad f_2(S^{(0)}, R^{(0)}) - D < 0. \tag{4.12}$$

(ii) $E_{2s} = (\lambda_{s2}, \widehat{R}, 0, \widehat{x}_{2s})$ is asymptotically stable if

$$(1 - q)f_1(\lambda_{s2}, \widehat{R}) - D < 0, \tag{4.13}$$

or equivalently,

$$\lambda_{s2} < \lambda_{s1} \quad \text{or} \quad \widehat{R} < \lambda_{r1}.$$

(iii) $E_{2r} = (\widehat{S}, \lambda_{r2}, 0, \widehat{x}_{2r})$ is asymptotically stable if

$$(1 - q)f_1(\widehat{S}, \lambda_{r2}) - D < 0, \tag{4.14}$$

or equivalently,

$$\widehat{S} < \lambda_{s1} \quad \text{or} \quad \lambda_{r2} < \lambda_{r1}.$$

(iv) $E_c = (S_c, R_c, x_{1c}, x_{2c})$ is asymptotically stable if

$$f_2(S_c, R_c) - D < 0, \tag{4.15}$$

i.e.,

$$S_c < \lambda_{s2} \quad \text{or} \quad R_c < \lambda_{r2}.$$

Since (4.15) is precisely (4.9), we conclude that if (E_c) exists then (E_c) is asymptotically stable. In the following lemma, we relate the existence of coexistence state (E_c) to the instability of (E_2) . The proof is deferred to Appendix A.

Lemma 4.2. E_c exists if and only if E_2 is unstable.

5. Global analysis of (2.4)

As we note in Section 3, the governing system (2.4) of four equations can be reduced to the limiting system (3.3) of two equations by the theory of asymptotic autonomous system. We have shown that there is no periodic orbit for the system (3.3) in global attracting region Ω . Thus from Poincaré–Bendixson Theorem any trajectory converges to the unique locally asymptotic rest point. In Section 4, we find all possible rest points for the governing system (2.4) and classify them by local stability analysis. The asymptotic stability of the rest points of (2.4) automatically implies that of the corresponding rest points of the limiting system (3.3). In the following we state and prove our main results.

Theorem 5.1. *Let $(S(t), R(t), x_1(t), x_2(t))$ be a solution of (2.4) with $x_1(0) > 0, x_2(0) > 0$. Then*

- (i) *if $f_1(S^{(0)}, R^{(0)}) < D/(1 - q)$ and $f_2(S^{(0)}, R^{(0)}) < D$, then*

$$\lim_{t \rightarrow \infty} (SR(t), R(t), x_1R(t), x_2R(t)) = E_0 = (S^{(0)}, R^{(0)}, 0, 0),$$
- (ii) *if $f_1(S^{(0)}, R^{(0)}) < D/(1 - q)$ and $f_2(S^{(0)}, R^{(0)}) > D$, then*
 - (a) $\lim_{t \rightarrow \infty} (S(t), R(t), x_1(t), x_2(t)) = E_{2s} = (\lambda_{s2}, \widehat{R}, 0, \widehat{x}_{2s})$ *if $T_2 > C$ i.e., x_2 is S -limited,*
 - (b) $\lim_{t \rightarrow \infty} (S(t), R(t), x_1(t), x_2(t)) = E_{2r} = (\widehat{S}, \lambda_{r2}, 0, \widehat{x}_{2r})$ *if $T_2 < C$ i.e., x_2 is R -limited,*
- (iii) *if $f_1(S^{(0)}, R^{(0)}) > D/(1 - q)$ and $f_2(S^{(0)}, R^{(0)}) < D$, then*

$$\lim_{t \rightarrow \infty} (S(t), R(t), x_1(t), x_2(t)) = E_c,$$
- (iv) *if $f_1(S^{(0)}, R^{(0)}) > D/(1 - q)$ and $f_2(S^{(0)}, R^{(0)}) > D$ then*
 - (a) $\lim_{t \rightarrow \infty} (S(t), R(t), x_1(t), x_2(t)) = E_{2s}$ *or* E_{2r} , *depending on x_2 is S -limited or R -limited, if $f_2(S_c, R_c) - D > 0$ where $S_c = \lambda_{s1}, R_c = (y_s/y_r)(\lambda_{s1} - S^{(0)}) + R^{(0)}$ or $R_c = \lambda_{r1}, S_c = (y_r/y_s)(\lambda_{r1} - R^{(0)}) + S^{(0)}$,*
 - (b) $\lim_{t \rightarrow \infty} (S(t), R(t), x_1(t), x_2(t)) = E_c$ *if $f_2(S_c, R_c) - D < 0$.*

Proof. From the first two equations of (2.4) and differential inequalities, we have for any $\varepsilon > 0$, $S(t) \leq S^{(0)} + \varepsilon, R(t) \leq R^{(0)} + \varepsilon$, for $t \geq T_\varepsilon$ for some $T_\varepsilon > 0$. Since $(1 - q)f_1(S^{(0)}, R^{(0)}) - D < 0$, there exists $\varepsilon > 0$ sufficiently small such that $(1 - q)f_1(S^{(0)} + \varepsilon, R^{(0)} + \varepsilon) - D < 0$. From the third equation of (2.4), it follows that

$$\begin{aligned} x_1(t) &= x_1(T_\varepsilon) \exp \left(\int_{T_\varepsilon}^t [(1 - q)f_1(S(\tau), R(\tau)) - D] d\tau \right) \\ &\leq x_1(T_\varepsilon) \exp [((1 - q)f_1(S^{(0)} + \varepsilon, R^{(0)} + \varepsilon) - D)(t - T_\varepsilon)]. \end{aligned}$$

Hence $\lim_{t \rightarrow \infty} x_1(t) = 0$. By the boundedness of the solutions, we will have $\lim_{t \rightarrow \infty} qf_1(S(t), R(t))x_1(t) = 0$. Since $f_2(S^{(0)}, R^{(0)}) - D < 0$ from the fourth equation of (2.4), it follows that $\lim_{t \rightarrow \infty} x_2(t) = 0$. From (3.1) $\lim_{t \rightarrow \infty} S(t) = S^{(0)}$ and $\lim_{t \rightarrow \infty} R(t) = R^{(0)}$. Thus we complete the proof of (i).

For part (ii), $(1 - q)f_1(S^{(0)}, R^{(0)}) - D < 0$, implies $\lim_{t \rightarrow \infty} x_1(t) = 0$. We claim that $\limsup_{t \rightarrow \infty} x_2(t) > 0$. If on the contrary, $\lim_{t \rightarrow \infty} x_2(t) = 0$, then it follows that $\lim_{t \rightarrow \infty} S(t) = S^{(0)}$

and $\lim_{t \rightarrow \infty} R(t) = R^{(0)}$. Then from the fourth equation of (2.4), we have $x_2'(t) \geq (f_2(S, R) - D)x_2$ and the hypothesis $f_2(S^{(0)}, R^{(0)}) > D$ implies $x_2(t)$ becomes unbounded as $t \rightarrow \infty$. This is a contradiction to the boundedness of the solutions. Since $\limsup_{t \rightarrow \infty} x_2(t) > 0$, there exists $\{t_n\}$ such that $(S(t_n), R(t_n), x_1(t_n), x_2(t_n)) \rightarrow (\tilde{S}, \tilde{R}, 0, \tilde{x}_2)$, $\tilde{S} \geq 0$, $\tilde{R} \geq 0$, $\tilde{x}_2 \geq 0$. The solution $(S(t), R(t), x_2(t))$ of the subsystem obtained by setting $x_1(t) \equiv 0$ in (2.4) with initial condition $S(0) = \tilde{S}$, $R(0) = \tilde{R}$, $x_2(0) = \tilde{x}_2$ satisfies $\lim_{t \rightarrow \infty} (S(t), R(t), x_2(t)) = (\lambda_{s2}, \tilde{R}, \hat{x}_{2s})$ if x_2 is S -limited and $\lim_{t \rightarrow \infty} (S(t), R(t), x_2(t)) = (\tilde{S}, \lambda_{r2}, \hat{x}_{2r})$ if x_2 is R -limited. From the invariance of ω -limit set, the asymptotic stability of $E_2(E_2 = E_{2s}$ or $E_2 = E_{2r})$ implies the global stability of E_2 . Thus we complete the proof of part (ii).

For part (iii), we note that $\limsup_{t \rightarrow \infty} x_1(t) > 0$. Otherwise, if $\lim_{t \rightarrow \infty} x_1(t) = 0$, then $f_2(S^{(0)}, R^{(0)}) < D$ implies $\lim_{t \rightarrow \infty} x_2(t) = 0$, $\lim_{t \rightarrow \infty} S(t) = S^{(0)}$ and $\lim_{t \rightarrow \infty} R(t) = R^{(0)}$. From $f_1(S^{(0)}, R^{(0)}) > D/(1 - q)$, $x_1(t)$ becomes unbounded as $t \rightarrow \infty$. This is a contradiction. From the fourth equation of (2.4) and $\limsup_{t \rightarrow \infty} x_1(t) > 0$, it follows that $\limsup_{t \rightarrow \infty} x_2(t) > 0$. By the theory of asymptotic autonomous system and the limiting system (3.3), we obtain $\lim_{t \rightarrow \infty} (S(t), R(t), x_1(t), x_2(t)) = E_c$.

For part (iv), the hypothesis $f_1(S^{(0)}, R^{(0)}) > D/(1 - q)$, and $f_2(S^{(0)}, R^{(0)}) > D$ imply $0 < \lambda_{s1}, \lambda_{s2} < S^{(0)}$, and $0 < \lambda_{r1}, \lambda_{r2} < R^{(0)}$. If $f_2(S_c, R_c) - D > 0$, then from Lemma 4.2, E_2 is asymptotically stable and the positive rest point E_c does not exist. Then $\lim_{t \rightarrow \infty} (S(t), R(t), x_1(t), x_2(t)) = E_2$. Thus we complete the proof of (a). Similarly if $f_2(S_c, R_c) - D < 0$, then the rest point E_2 is unstable and the positive rest point E_c exists and is asymptotically stable. Hence $\lim_{t \rightarrow \infty} (S(t), R(t), x_1(t), x_2(t)) = E_c$. \square

6. Discussion

We have considered competition for two complementary nutrients between plasmid-bearing and plasmid-free organisms in a chemostat. This problem is important in biotechnology for the study of plasmid stability where the effects of plasmid loss in genetically altered organisms (the plasmid-free organism is presumably the better competitor) is studied. We have established the global asymptotic stability of the solutions of (2.4) in Theorem 5.1. Every solution of (2.4) tends as $t \rightarrow \infty$ to one of the three steady states, the washout state $E_0 = (S^{(0)}, R^{(0)}, 0, 0)$, the plasmid-bearing extinction state $E_2 = (\tilde{S}_2, \tilde{R}_2, 0, \hat{x}_2)$ and the coexistence state $E_c = (S_c, R_c, x_{1c}, x_{2c})$. The state $E_2 = E_{2s} = (\lambda_{s2}, \tilde{R}, 0, \hat{x}_{2s})$ if x_2 is S -limited and $E_2 = E_{2r} = (\tilde{S}, \lambda_{r2}, 0, \hat{x}_{2r})$ if x_2 is R -limited. In the following Table 1 we list the existence conditions for the rest points and indicate their global stability by enclosing them in a box.

In [2] the authors did the experiment for the competition of plasmid-bearing and plasmid-free organisms for two complementary nutrients. They also formulated a mathematical model and did computer simulation to explain the experimental data. They used a leucine auxotroph strain of *Saccharomyces cerevisiae* (plasmid-free organism) and its recombinants (plasmid-bearing organism) competing for two complementary nutrients, sucrose and yeast extract. Sucrose is a carbon resource and yeast extract is a nitrogen resource. Since the recombinants enjoyed a growth rate advantage over the plasmid-free cell at critically low yeast extract concentration, a two-stage cultivation strategy was design in order to create a yeast extract limited environment so that plasmid-free cells could not grow and overtake the recombinants. The cells were cultivated in selected media (i.e. sucrose) in the first stage, and then transferred continuously to the second

Table 1

| Case | Criteria for existence of rest points and global stability of boxed rest point | | Rest Points |
|------|--|---------------------|---------------------|
| 1 | $f_1(S^{(0)}, R^{(0)})(1 - q) < D, f_2(S^{(0)}, R^{(0)}) < D$ | | $\{E_0\}$ |
| 2 | $f_1(S^{(0)}, R^{(0)})(1 - q) > D, f_2(S^{(0)}, R^{(0)}) < D$ | | $\{E_0, E_c\}$ |
| 3a | $f_1(S^{(0)}, R^{(0)})(1 - q) < D, f_2(S^{(0)}, R^{(0)}) > D$ | $T_2 > C$ | $\{E_0, E_{2s}\}$ |
| 3b | $f_1(S^{(0)}, R^{(0)})(1 - q) < D, f_2(S^{(0)}, R^{(0)}) > D$ | $T_2 < C$ | $\{E_0, E_{2r}\}$ |
| 4a | $f_1(S^{(0)}, R^{(0)})(1 - q) > D, f_2(S^{(0)}, R^{(0)}) > D$ | $f_2(S_c, R_c) > D$ | $\{E_0, E_2\}$ |
| 4b | $f_1(S^{(0)}, R^{(0)})(1 - q) > D, f_2(S^{(0)}, R^{(0)}) > D$ | $f_2(S_c, R_c) < D$ | $\{E_0, E_2, E_c\}$ |

In Cases 4a, 4b, E_2 is either E_{2s} or E_{2r} depending on that x_2 is S -limited or R -limited. We note that in Cases 4a, 4b, $S_c = \lambda_{s1}, R_c = (y_s/y_r)(\lambda_{s1} - S^{(0)})$ if x_1 is S -limited and $R_c = \lambda_{r1}, S_c = (y_r/y_s)(\lambda_{r1} - R^{(0)}) + S^{(0)}$ if x_1 is R -limited.

stage where the media was enriched by feeding yeast extract. This strategy resulted in a stable existence of recombinant cells, which stabilized around 60% at steady state during the tested period of cultivation. Their mathematical model can be written as follows:

Stage I: In first reactor, we have following equations:

$$\begin{aligned}
 \frac{dS_1}{dt} &= (S_1^{(0)} - S_1)D_1 - \frac{x_1}{\eta_{S_1}}g_1(S_1) - \frac{y_1}{\eta_{S_1}}g_2(S_1), \\
 \frac{dx_1}{dt} &= [(1 - q_1)g_1(S_1) - D_1]x_1, \\
 \frac{dy_1}{dt} &= [g_2(S_1) - D_1]y_1 + q_1g_1(S_1)x_1,
 \end{aligned}
 \tag{6.1}$$

where $S_1(t)$ is the concentration of sucrose; $g_1(S_1) = m_1S_1/(K_1 + S_1), g_2(S_1) = m_2S_1/(K_2 + S_1)$ are the specific growth rate of plasmid-bearing and plasmid-free cells respectively; $x_1(t), y_1(t)$ are the concentration of plasmid-bearing, plasmid-free organisms respectively; $0 < q_1 < 1$ is the probability of plasmid-free cell emergence; $D_1 = f_1/V_1$ is the dilution rate where f_1 is the flow rate, V_1 is the volume of the first reactor; η_{S_1} is the yield constant.

Stage II: In the second reactor, we have following equations:

$$\begin{aligned}
 \frac{dS}{dt} &= \frac{f_1S_1(t)}{V_2} + \frac{f_2S^{(0)}}{V_2} - \frac{(f_1 + f_2)S(t)}{V_2} - \frac{x_2}{\eta_S}\mu_1(S, R) - \frac{y_2}{\eta_S}\mu_2(S, R), \\
 \frac{dR}{dt} &= \frac{f_2R^{(0)}}{V_2} - \frac{(f_1 + f_2)R(t)}{V_2} - \frac{x_2}{\eta_R}\mu_1(S, R) - \frac{y_2}{\eta_R}\mu_2(S, R), \\
 \frac{dx_2}{dt} &= \frac{f_1x_1(t)}{V_2} + [(1 - q_2)\mu_1(S, R) - D_2]x_2, \\
 \frac{dy_2}{dt} &= \frac{f_1y_1(t)}{V_2} + [\mu_2(S, R) - D_2]y_2 + q_2\mu_1(S, R)x_2,
 \end{aligned}
 \tag{6.2}$$

where $S(t), R(t)$ are the concentration of sucrose and yeast extract respectively; $x_2(t), y_2(t)$ are the concentration of plasmid-bearing and plasmid-free organisms respectively; $f_2, V_2, D_2 = f_2/V_2$ are the flow rate, volume and dilution rate; $0 < q_2 < 1$ is the probability of plasmid-free cell emergence; $\mu_1(S, R)$ and $\mu_2(S, R)$ are the specific growth rate of plasmid-bearing and plasmid-free organisms respectively.

We note that in [2] $\mu_1(S, R)$ and $\mu_2(S, R)$ take the form

$$\mu_1(S, R) = \left(0.25 + \frac{m_r R}{K_r + R} \right) \left(\frac{S}{K_s + S} \right)$$

and

$$\mu_2(S, R) = \left(\frac{m_r R}{K_r + R} \right) \left(\frac{S}{K_s + S} \right)$$

instead of the form we gave in (2.2).

Obviously the mathematical model (6.1) and (6.2) is different from our model (2.4). It is an interesting problem and we shall study it in the near future.

In the governing system (2.4) we have three operating parameters $S^{(0)}, R^{(0)}$ and D . In the following operation diagram, we vary the input concentrations $S^{(0)}, R^{(0)}$ as operating parameters and fix the dilution rate D and other parameters with $\lambda_{s1}, \lambda_{s2}, \lambda_{r1}, \lambda_{r2} > 0$. We note that the conditions in Table 1 can be listed as follows:

$$\begin{aligned} f_1(S^{(0)}, R^{(0)})(1 - q) < D & \text{ iff } S^{(0)} < \lambda_{s1} \text{ or } R^{(0)} < \lambda_{r1}, \\ f_1(S^{(0)}, R^{(0)})(1 - q) > D & \text{ iff } S^{(0)} > \lambda_{s1} \text{ or } R^{(0)} > \lambda_{r1}, \\ f_2(S^{(0)}, R^{(0)}) < D & \text{ iff } S^{(0)} < \lambda_{s2} \text{ or } R^{(0)} < \lambda_{r2}, \\ f_2(S^{(0)}, R^{(0)}) > D & \text{ iff } S^{(0)} > \lambda_{s2} \text{ or } R^{(0)} > \lambda_{r2}, \\ f_2(S_c, R_c) < D & \text{ iff } S_c < \lambda_{s2} \text{ or } R_c < \lambda_{r2}. \end{aligned} \tag{6.3}$$

There are four possible cases and from (6.3) their operating diagrams can be plotted as follows.

Here I is the region of washout states. II_s, II_r are the regions of S -limited and R -limited plasmid-free state respectively. III is the coexistence region. The lines $L_i, i = 1, 2, 3, 4$ have same slope $C = y_s/y_r$.

For Case A: $\lambda_{s2} < \lambda_{s1}, \lambda_{r2} < \lambda_{r1}$, the plasmid-free organism has a smaller break-even concentrations for both of the nutrients S and R and it is a better competitor for both nutrients and wins the competition over the plasmid-bearing organism. Hence in Fig. 1(A) we have two regions I and II in the $S^{(0)} - R^{(0)}$ parameter space.

For Case B: $\lambda_{s1} < \lambda_{s2}, \lambda_{r1} < \lambda_{r2}$, the plasmid-bearing organism has a smaller break-even concentrations for both of the nutrients S and R and hence it survives in the competition. Since there is a fraction $q, 0 < q < 1$ of plasmid-bearing organisms converting into plasmid-free organisms during the replication, for $S^{(0)} > \lambda_{s1}, R^{(0)} > \lambda_{r1}$ we have a coexistence region III in $S^{(0)} - R^{(0)}$ parameter space (operating diagram see Fig. 1(B)).

For Case C: $\lambda_{s1} < \lambda_{s2}, \lambda_{r2} < \lambda_{r1}$, plasmid-bearing organism x_1 is a better competitor with respect to nutrient S while plasmid-free organism x_2 is a better competitor with respect to nutrient R . In

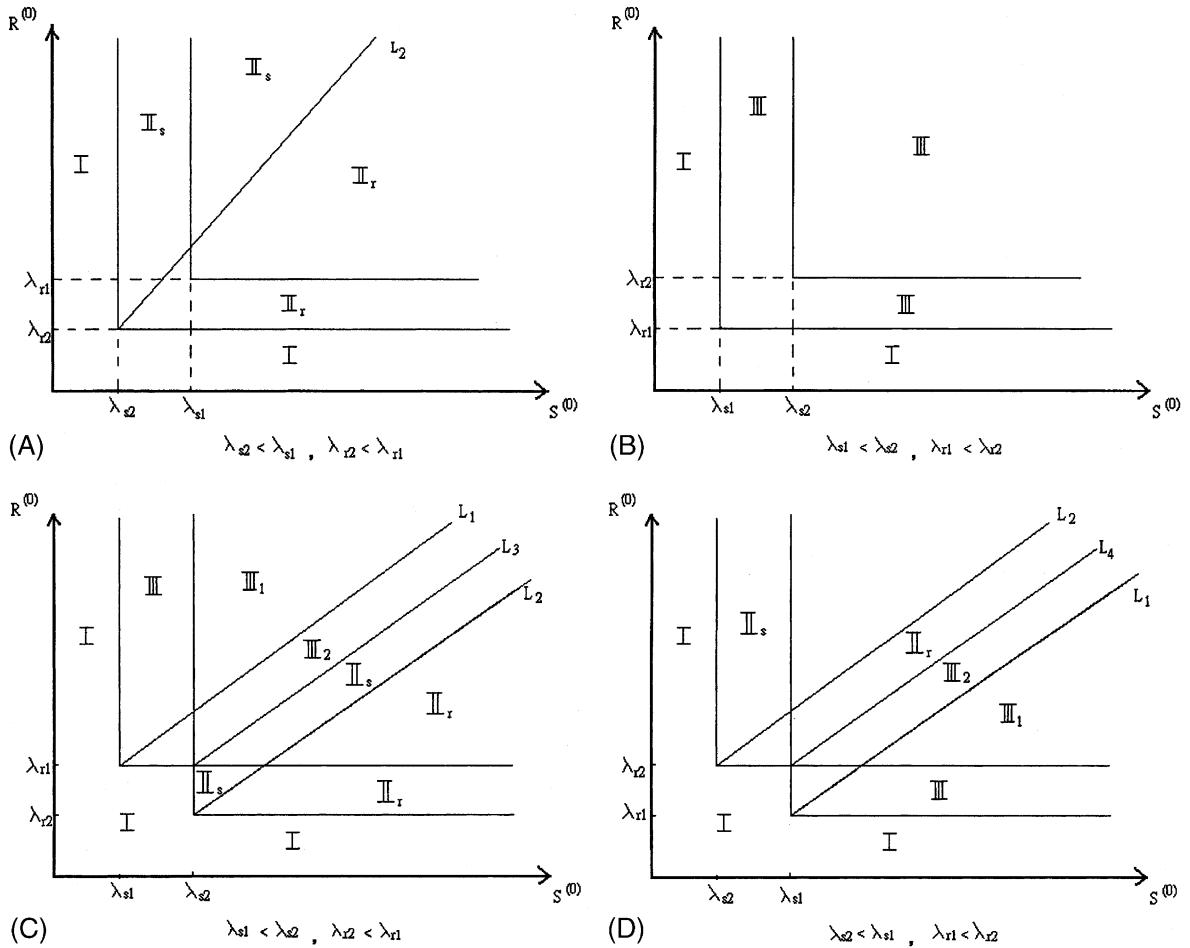


Fig. 1.

the coexistence region III₁, organisms x_1 and x_2 are both S -limited. If there is no plasmid loss i.e., $q = 0$, then from [1] organism x_1 should win the competition. Since a fraction $q > 0$ of organism x_1 converts into organism x_2 , it follows that organism x_1 and x_2 coexists. In coexistence region III₂, and plasmid free region II_s, organism x_1 is R -limited and organism x_2 is S -limited. If $q = 0$, then from [1] the competition outcomes should depend on initial populations. Since $0 < q < 1$, the coexistence equilibrium E_c exists in the region III₂ while E_c does not exist in the region II_s. In the region II_r organism x_1 and x_2 are R -limited. Then organism x_1 goes to extinction because organism x_2 is a better competitor for nutrient R (operating diagram see Fig. 1(C)).

For Case D: $\lambda_{s2} < \lambda_{s1}, \lambda_{r1} < \lambda_{r2}$, organism x_1 is a better competitor with respect to nutrient R and organism x_2 is a better competitor with respect to nutrient S . In the coexistence region III₁, organism x_1 and x_2 are both R -limited. Organism x_1 is S -limited and organism x_2 is R -limited in the region III₂ and II_r. In the region II_s, organism x_1 and x_2 are both S -limited. Similar biological interpretations as in Case C can be given in Case D (operating diagram see Fig. 1(D)).

Acknowledgements

The authors would like to express their thanks to Professor I-Ming Chu of the Department of Chemical engineering, Tsing Hua University, Taiwan, for posing the problems considered here and for the stimulated discussions.

Appendix A. Proofs and computations

Proof of Theorem 3.1. First we note that if $p_1(S) = Q_1(S)$ or $p_2(S) = Q_2(S)$ for some $0 < S < S^{(0)}$, then the right hand side of the system (3.7) is continuous but not differentiable at the line $x_1 + x_2 = y_s(S^{(0)} - S)$. From Remark 3.1, for $i = 1, 2$ the curves $z = p_i(S)$ and $z = Q_i(S)$ may not intersect or intersects at one point or intersects at two points. Hence there are nine possible cases. In Case A, for $i = 1, 2$, we assume $p_i(S)$ and $Q_i(S)$ do not intersect in the interval $(0, S^{(0)})$. In case B we assume $p_i(S)$ and $Q_i(S)$ intersect at some point in $(0, S^{(0)})$ for some $i = 1, 2$. There are eight cases in case B; however their proofs are similar and for simplicity we only consider the case where for $i = 1, 2$, $p_i(S)$ and $Q_i(S)$ intersect at one point in $(0, S^{(0)})$.

Case A: We assume $p_i(S) = Q_i(S)$, has no intersection points, for $i = 1, 2$. As in [4] we apply the Dulac criterion with the auxiliary function

$$B(x_1, x_2) = \frac{1}{x_1 x_2},$$

to the vector field given by (3.7). In this case, There are four possible cases for the system (3.7). For simplicity, we only consider the case $p_1(S) < Q_1(S)$, $Q_2(S) < p_2(S)$ on $(0, S^{(0)})$. Then the system (3.7) becomes

$$\frac{dx_1}{dt} = x_1((1 - q)p_1(S) - D), \quad \frac{dx_2}{dt} = x_2(Q_2(S) - D) + qx_1 p_1(S),$$

where

$$S = S^{(0)} - \frac{1}{y_s}(x_1 + x_2).$$

An easy computation yields

$$\frac{\partial(B(x_1, x_2)g_1(x_1, x_2))}{\partial x_1} + \frac{\partial(B(x_1, x_2)g_2(x_1, x_2))}{\partial x_2} = -\frac{q}{x_2^2}p_1(S) - \frac{1}{x_2} \frac{1}{y_s} p_1'(S) - \frac{1}{x_1} \frac{1}{y_s} Q_2'(S) < 0.$$

We complete the proof of the Case A and hence there are no non-trivial periodic solutions.

Case B: We assume that for $i = 1, 2$, $p_i(S)$ and $Q_i(S)$ intersect exactly at one point $\widehat{S}_i \in (0, S^{(0)})$. Depending on $q_i(h(0)) = q_i(R^{(0)} - (y_s/y_r)S^{(0)}) > 0$ or $q_i(h(0)) < 0$, we have either $Q_i(S) > p_i(S)$ for $0 < S < \widehat{S}_i$, $Q_i(S) < p_i(S)$ for $S > \widehat{S}_i$, $i = 1, 2$ or $p_i(S) > Q_i(S)$ for $0 < S < \widehat{S}_i$, $p_i(S) < Q_i(S)$ for $S > \widehat{S}_i$, $i = 1, 2$. (See Fig. 2.)

Without loss of generality we assume $\widehat{S}_1 < \widehat{S}_2$ and $q_i(h(0)) > 0$. We shall prove by contradiction. Suppose on the contrary there exists a periodic orbit C in the first quadrant of $x_1 - x_2$ plane. Since $q_i(h(0)) > 0$ (see Fig. 2(a)) and $\widehat{S}_1 < \widehat{S}_2$, if $S < \widehat{S}_1$, then $Q_1(S) > p_1(S)$ and $Q_2(S) > p_2(S)$; if

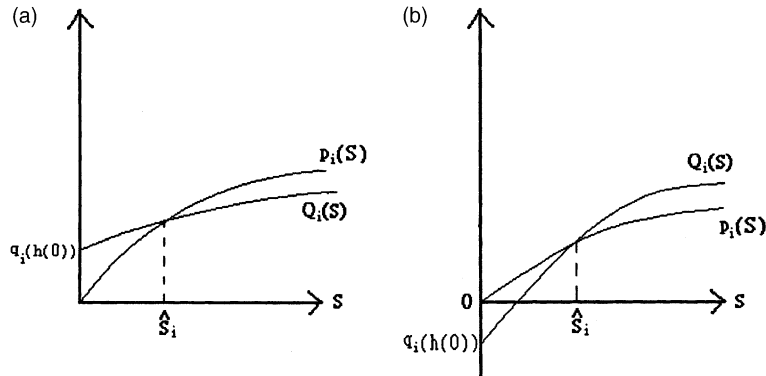


Fig. 2.

$\widehat{S}_1 < S < \widehat{S}_2$, then $p_1(S) > Q_1(S)$ and $Q_2(S) > p_2(S)$; if $S > \widehat{S}_2$ then $p_1(S) > Q_1(S)$ and $p_2(S) > Q_2(S)$. In $x_1 - x_2$ plane, from (3.10) $S < \widehat{S}_i$ is equivalent to $x_1 + x_2 > y_s(S^{(0)} - \widehat{S}_i)$, $i = 1, 2$. Define the regions

$$\begin{aligned}
 I_1 &= \{(x_1, x_2) : x_1 \geq 0, x_2 \geq 0, x_1 + x_2 > y_s(S^{(0)} - \widehat{S}_1)\}, \\
 I_2 &= \{(x_1, x_2) : x_1 \geq 0, x_2 \geq 0, y_s(S^{(0)} - \widehat{S}_1) > x_1 + x_2 > y_s(S^{(0)} - \widehat{S}_2)\}, \\
 I_3 &= \{(x_1, x_2) : x_1 \geq 0, x_2 \geq 0, x_1 + x_2 < y_s(S^{(0)} - \widehat{S}_2)\}.
 \end{aligned}$$

On the region I_1 , the system (3.7) becomes

$$\begin{aligned}
 x'_1 &= x_1((1 - q)p_1(S) - D), \\
 x'_2 &= x_2(p_2(S) - D) + qp_1(S)x_1.
 \end{aligned} \tag{A.1}$$

On the region I_2 , the system (3.7) becomes

$$\begin{aligned}
 x'_1 &= x_1((1 - q)Q_1(S) - D), \\
 x'_2 &= x_2(p_2(S) - D) + qQ_1(S)x_1.
 \end{aligned} \tag{A.2}$$

On the region I_3 , the system (3.7) becomes

$$\begin{aligned}
 x'_1 &= x_1((1 - q)Q_1(S) - D), \\
 x'_2 &= x_2(Q_2(S) - D) + qQ_2(S)x_1.
 \end{aligned} \tag{A.3}$$

If the periodic orbit C lies entirely in the region I_i for some $i = 1, 2, 3$, then from (A.1)–(A.3), it is easy to show as we did in Case A,

$$\frac{\partial(B(x_1, x_2)g_1(x_1, x_2))}{\partial x_1} + \frac{\partial(B(x_1, x_2)g_1(x_1, x_2))}{\partial x_2} = \begin{cases} -\frac{q}{x_2^2}p_1(S) - \frac{1}{x_2} \frac{1}{y_s} p'_1(S) - \frac{1}{x_1} \frac{1}{y_s} p'_2(S), & \text{on } I_1 \\ -\frac{q}{x_2^2}Q_1(S) - \frac{1}{x_2} \frac{1}{y_s} Q'_1(S) - \frac{1}{x_1} \frac{1}{y_s} p'_2(S), & \text{on } I_2 \\ -\frac{q}{x_2^2}Q_1(S) - \frac{1}{x_2} \frac{1}{y_s} Q'_1(S) - \frac{1}{x_1} \frac{1}{y_s} Q'_2(S), & \text{on } I_3 \end{cases} < 0. \tag{A.4}$$

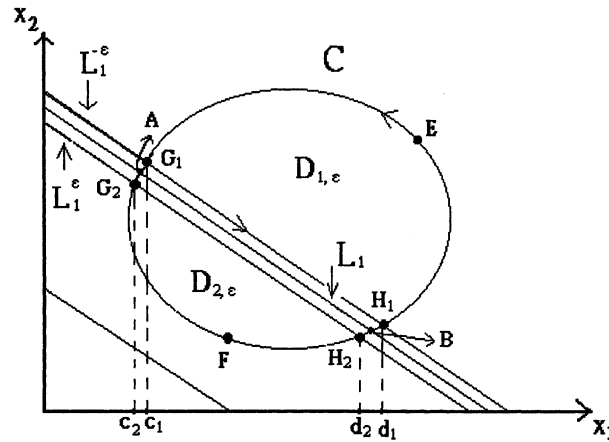


Fig. 3.

Then Dulac criterion leads to a contradiction. Now for simplicity we first consider a ‘typical’ case that the periodic orbit C satisfying $C \subseteq I_1 \cup I_2$ (see Fig. 3).

The other cases will be discussed and proved by similar arguments. Let $\varepsilon > 0$ be arbitrary small and consider the lines

$$L_1 : S = \widehat{S}_1, \quad \text{and} \quad L_1^{\pm\varepsilon} : S = \widehat{S}_1 \pm \varepsilon.$$

Since $g_1(x_1, x_2)$ and $g_2(x_1, x_2)$ are smooth at each point of C except point A and B , it follows that

$$\oint_C B(x_1, x_2)g_1(x_1, x_2) dx_2 - B(x_1, x_2)g_2(x_1, x_2) dx_1 = \int_{\widehat{BEA}} + \int_{\widehat{AFB}}.$$

Obviously

$$\int_{\widehat{BEA}} + \int_{\widehat{AFB}} = 0.$$

Hence

$$\oint_C = 0.$$

On the other hand

$$\begin{aligned} 0 &= \oint_C = \int_{\widehat{H_1EG_1}} + \int_{\widehat{G_1G_2}} + \int_{\widehat{G_2FH_2}} + \int_{\widehat{H_2H_1}} \\ &= \left[\int_{\widehat{H_1EG_1}} + \int_{\widehat{G_1H_1}} \right] + \left[\int_{\widehat{G_2FH_2}} + \int_{\widehat{H_2G_2}} \right] + \int_{\widehat{G_1G_2}} + \int_{\widehat{H_2H_1}} + \left[- \int_{\widehat{G_1H_1}} + \int_{\widehat{G_2H_2}} \right]. \end{aligned} \tag{A.5}$$

From Green’s Theorem and (A.4), we have

$$\begin{aligned} & \lim_{\varepsilon \rightarrow 0} \left(\int_{\widehat{H_1 E G_1}} + \int_{\widehat{G_1 H_1}} \right) (B g_1 dx_2 - B g_2 dx_1) \\ &= \lim_{\varepsilon \rightarrow 0} \int \int_{D_{1,\varepsilon}} \frac{\partial}{\partial x_1} (B g_1) + \frac{\partial}{\partial x_2} (B g_2) dx_1 dx_2 \\ &= \int \int_{D_1} \left[-\frac{q}{x_2^2} p_1(S) - \frac{1}{x_2} \frac{1}{y_s} p'_1(S) - \frac{1}{x_1} \frac{1}{y_s} p'_2(S) \right] dx_1 dx_2 < 0, \end{aligned}$$

$$\begin{aligned} & \lim_{\varepsilon \rightarrow 0} \left(\int_{\widehat{G_2 F H_2}} + \int_{\widehat{H_2 G_2}} \right) (B g_1 dx_2 - B g_2 dx_1) \\ &= \lim_{\varepsilon \rightarrow 0} \int \int_{D_{2,\varepsilon}} \frac{\partial}{\partial x_1} (B g_1) + \frac{\partial}{\partial x_2} (B g_2) dx_1 dx_2 \\ &= \int \int_{D_2} \left[-\frac{q}{x_2^2} Q_1(S) - \frac{1}{x_2} \frac{1}{y_s} Q'_1(S) - \frac{1}{x_1} \frac{1}{y_s} p'_s(S) \right] dx_1 dx_2 < 0, \end{aligned}$$

where D_1, D_2 are the regions bounded by closed curves \widehat{BEA} and \widehat{AFB} respectively. Obviously

$$\lim_{\varepsilon \rightarrow 0} \int_{\widehat{G_1 G_2}} = \lim_{\varepsilon \rightarrow 0} \int_{\widehat{H_2 H_1}} = 0.$$

The equations of $L_1^{-\varepsilon}$ and L_1^ε are $x_1 + x_2 = y_s(S^{(0)} - (\widehat{S}_1 - \varepsilon))$ and $x_1 + x_2 = y_s(S^{(0)} - (\widehat{S}_1 + \varepsilon))$ respectively. Hence

$$\begin{aligned} & \int_{\widehat{G_1 H_1}} B(x_1, x_2) g_1(x_1, x_2) dx_2 - B(x_1, x_2) g_2(x_1, x_2) dx_1 \\ &= - \int_{c_1}^{d_1} B(x_1, x_2) (g_1(x_1, x_2) + g_2(x_1, x_2)) dx_1 \\ &= - \int_{c_1}^{d_1} (x_1 p_1(S) + x_2 p_2(S) - D(x_1 + x_2)) B(x_1, x_2) dx_1 \\ &= - \int_{c_1}^{d_1} (x_1 p_1(\widehat{S}_1 - \varepsilon) + x_2 p_2(\widehat{S}_1 - \varepsilon) - D(y_s(S^{(0)} - \widehat{S}_1 + \varepsilon))) B(x_1, x_2) dx_1. \end{aligned}$$

Similarly, we have

$$\begin{aligned} & \int_{\widehat{G_2 H_2}} B(x_1, x_2) g_1(x_1, x_2) dx_2 - B(x_1, x_2) g_2(x_2, x_2) dx_1 \\ &= - \int_{c_2}^{d_2} (x_1 Q_1(\widehat{S}_1 + \varepsilon) + x_2 p_2(\widehat{S}_1 + \varepsilon) - D(y_s(S^{(0)} - \widehat{S}_1 - \varepsilon))) B(x_1, x_2) dx_1. \end{aligned}$$

Obviously

$$\lim_{\varepsilon \rightarrow 0} \left(\int_{\widehat{G_2 H_2}} - \int_{\widehat{G_1 H_1}} \right) = 0.$$

Hence as $\varepsilon \rightarrow 0$, the right-hand side of (A.5) is negative, and we obtain a contradiction.

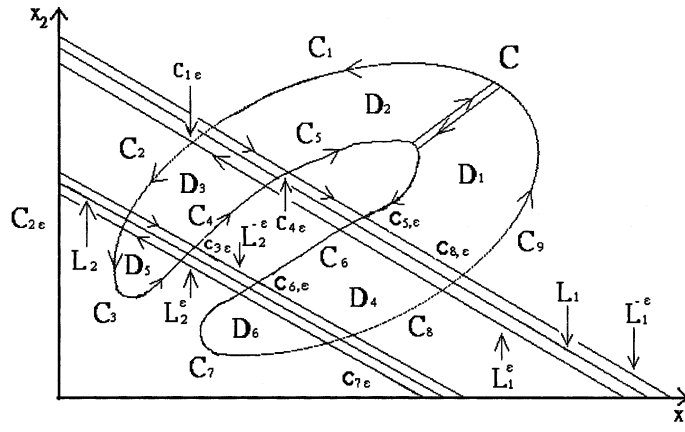


Fig. 4.

Now we assume the periodic orbit C intersects each region I_i , $i = 1, 2, 3$. The other cases can be proved by similar arguments. We omit the proofs. Let $\varepsilon > 0$ be arbitrary small and consider the lines (see Fig. 4):

$$L_1^{\pm\varepsilon} : S = \widehat{S}_1 \pm \varepsilon, \quad L_1 : S = \widehat{S}_1,$$

$$L_2^{\pm\varepsilon} : S = \widehat{S}_2 \pm \varepsilon, \quad L_2 : S = \widehat{S}_2.$$

As we did in the above ‘typical’ case,

$$\begin{aligned} 0 &= \oint_C Bg_1 dx_2 - Bg_2 dx_1 \\ &= \int \int_{D_1 \cup D_2} \left(-\frac{q}{x_2^2} p_1(S) - \frac{1}{x_2} \frac{1}{y_s} p_1'(S) - \frac{1}{x_1} \frac{1}{y_s} p_2'(S) \right) dx_1 dx_2 \\ &\quad + \int \int_{D_3 \cup D_4} \left(-\frac{q}{x_2^2} Q_1(S) - \frac{1}{x_2} \frac{1}{y_s} Q_1'(S) - \frac{1}{x_1} \frac{1}{y_s} p_2'(S) \right) dx_1 dx_2 \\ &\quad + \int \int_{D_5 \cup D_6} \left(-\frac{q}{x_2^2} Q_1(S) - \frac{1}{x_2} \frac{1}{y_s} Q_1'(S) - \frac{1}{x_1} \frac{1}{y_s} Q_2'(S) \right) dx_1 dx_2 < 0. \end{aligned}$$

Thus obtain a contradiction and complete the proof of Theorem 3.1.

Remark A.1. The proof of Theorem 3.1 would not work if the periodic orbit shown in Fig. 4 made infinitely many crossings. It cannot because of compactness and uniqueness of solutions.

We introduce the variational matrices of the system (2.4) at equilibria E_0 , E_{2s} , E_{2r} and E_c and analyze their local stability. The variational matrix M is

$$M(E) = \begin{bmatrix} -D - \frac{x_1}{y_s} \frac{\partial f_1}{\partial S} - \frac{x_2}{y_s} \frac{\partial f_2}{\partial S} & -\frac{x_1}{y_s} \frac{\partial f_1}{\partial R} - \frac{x_2}{y_2} \frac{\partial f_2}{\partial R} & -\frac{f_1}{y_s} & -\frac{f_2}{y_s} \\ -\frac{x_1}{y_r} \frac{\partial f_1}{\partial S} - \frac{x_2}{y_r} \frac{\partial f_2}{\partial S} & -D - \frac{x_1}{y_r} \frac{\partial f_1}{\partial R} - \frac{x_2}{y_r} \frac{\partial f_2}{\partial R} & -\frac{f_1}{y_r} & -\frac{f_2}{y_r} \\ (1-q)x_1 \frac{\partial f_1}{\partial S} & (1-q)x_1 \frac{\partial f_1}{\partial R} & (1-q)f_1 - D & 0 \\ x_2 \frac{\partial f_2}{\partial S} + qx_1 \frac{\partial f_1}{\partial S} & x_2 \frac{\partial f_2}{\partial R} + qx_1 \frac{\partial f_1}{\partial R} & qf_1 & f_2 - D \end{bmatrix}.$$

At washout state $E_0 = (S^{(0)}, R^{(0)}, 0, 0)$,

$$M(E_0) = \begin{bmatrix} -D & 0 & -\frac{f_1}{y_s} & -\frac{f_2}{y_s} \\ 0 & -D & -\frac{f_1}{y_r} & -\frac{f_2}{y_r} \\ 0 & 0 & (1-q)f_1 - D & 0 \\ 0 & 0 & qf_1 & f_2 - D \end{bmatrix}.$$

Obviously the eigenvalues of $M(E_0)$ are $-D$, $-D$, $(1-q)f_1(S^{(0)}, R^{(0)}) - D$, and $f_2(S^{(0)}, R^{(0)}) - D$. Hence E_0 is asymptotically stable if

$$(1-q)f_1(S^{(0)}, R^{(0)}) - D < 0, \quad \text{and} \quad f_2(S^{(0)}, R^{(0)}) - D < 0. \tag{A.6}$$

At $E_{2s} = (\lambda_{s2}, \hat{R}, 0, \hat{x}_{2s})$, where $\hat{R} > \lambda_{r2}$

$$M(E_{2s}) = \begin{bmatrix} -D - \frac{\hat{x}_{2s}}{y_s} \frac{\partial f_2}{\partial S} & -\frac{\hat{x}_{2s}}{y_s} \frac{\partial f_2}{\partial R} & -\frac{f_1}{y_s} & -\frac{D}{y_s} \\ -\frac{\hat{x}_{2s}}{y_r} \frac{\partial f_2}{\partial S} & -D - \frac{\hat{x}_{2s}}{y_r} \frac{\partial f_2}{\partial R} & -\frac{f_1}{y_r} & -\frac{D}{y_r} \\ 0 & 0 & (1-q)f_1 - D & 0 \\ \hat{x}_{2s} \frac{\partial f_2}{\partial S} & \hat{x}_{2s} \frac{\partial f_2}{\partial R} & qf_1 & 0 \end{bmatrix}.$$

The characteristic polynomial of the matrix $M(E_{2s})$ is

$$F(\lambda) = [(1-q)f_1 - D - \lambda] \det \begin{bmatrix} -D - \frac{\hat{x}_{2s}}{y_s} \frac{\partial f_2}{\partial S} - \lambda & -\frac{\hat{x}_{2s}}{y_s} \frac{\partial f_2}{\partial R} & -\frac{D}{y_s} \\ -\frac{\hat{x}_{2s}}{y_r} \frac{\partial f_2}{\partial S} & -D - \frac{\hat{x}_{2s}}{y_r} \frac{\partial f_2}{\partial R} - \lambda & -\frac{D}{y_r} \\ \hat{x}_{2s} \frac{\partial f_2}{\partial S} & \hat{x}_{2s} \frac{\partial f_2}{\partial R} & -\lambda \end{bmatrix}$$

$$\begin{aligned}
 &= [(1 - q)f_1 - D - \lambda] \det \begin{bmatrix} -D - \lambda & 0 & -\frac{1}{y_s}(D + \lambda) \\ 0 & -D - \lambda & -\frac{1}{y_r}(D + \lambda) \\ \hat{x}_{2s} \frac{\partial f_2}{\partial S} & \hat{x}_{2s} \frac{\partial f_2}{\partial R} & -\lambda \end{bmatrix} \\
 &= -((1 - q)f_1 - D - \lambda)(D + \lambda)^2 \left(\lambda + \left(\frac{\hat{x}_{2s}}{y_r} \frac{\partial f_2}{\partial R} + \frac{\hat{x}_{2s}}{y_s} \frac{\partial f_2}{\partial S} \right) \right).
 \end{aligned}$$

Hence the eigenvalues of $M(E_{2s})$ are

$$-D, \quad -D, \quad (1 - q)f_1(\lambda_{s2}, \hat{R}) - D, \quad -\left(\frac{\hat{x}_{2s}}{y_r} \frac{\partial f_2}{\partial R} + \frac{\hat{x}_{2s}}{y_s} \frac{\partial f_2}{\partial S} \right).$$

Since $\partial f_2/\partial R > 0$, and $\partial f_2/\partial S > 0$, the equilibrium E_{2s} is locally asymptotically stable if

$$(1 - q)f_1(\lambda_{s2}, \hat{R}) - D < 0, \tag{A.7}$$

or equivalently,

$$\lambda_{s2} < \lambda_{s1} \text{ or } \hat{R} < \lambda_{r1}.$$

Similarly, the equilibrium $E_{2s} = (\hat{S}, \lambda_{r2}, 0, \hat{x}_{2r})$, where $\hat{S} > \lambda_{s2}$, is locally asymptotically stable if

$$(1 - q)f_1(\hat{S}, \lambda_{r2}) - D < 0, \tag{A.8}$$

or equivalently

$$\hat{S} < \lambda_{s1} \text{ or } \lambda_{r2} < \lambda_{r1}.$$

From (2.4) we have $(1 - q)f_1 - D = 0$. The variational matrix of (2.4) at (E_c) is

$$M(E_c) = \begin{bmatrix} -D - \frac{x_{1c}}{y_s} \frac{\partial f_1}{\partial S} - \frac{x_{2c}}{y_s} \frac{\partial f_2}{\partial S} & -\frac{x_{1c}}{y_s} \frac{\partial f_1}{\partial R} - \frac{x_{2c}}{y_s} \frac{\partial f_2}{\partial R} & -\frac{f_1}{y_s} & -\frac{f_2}{y_s} \\ -\frac{x_{1c}}{y_r} \frac{\partial f_1}{\partial S} - \frac{x_{2c}}{y_r} \frac{\partial f_2}{\partial S} & -D - \frac{x_{1c}}{y_r} \frac{\partial f_1}{\partial R} - \frac{x_{2c}}{y_r} \frac{\partial f_2}{\partial R} & -\frac{f_1}{y_r} & -\frac{f_2}{y_r} \\ (1 - q)x_{1c} \frac{\partial f_1}{\partial S} & (1 - q)x_{1c} \frac{\partial f_1}{\partial R} & 0 & 0 \\ x_{2c} \frac{\partial f_2}{\partial S} + qx_{1c} \frac{\partial f_1}{\partial S} & x_{2c} \frac{\partial f_2}{\partial R} + qx_{1c} \frac{\partial f_1}{\partial R} & qf_1 & f_2 - D \end{bmatrix}.$$

The characteristic polynomial of the matrix $M(E_c)$ is

$$F(\lambda) = \det \begin{bmatrix} -D - \frac{x_{1c}}{y_s} \frac{\partial f_1}{\partial S} - \frac{x_{2c}}{y_s} \frac{\partial f_2}{\partial S} - \lambda & -\frac{x_{1c}}{y_s} \frac{\partial f_1}{\partial R} - \frac{x_{2c}}{y_s} \frac{\partial f_2}{\partial R} & -\frac{f_1}{y_s} & -\frac{f_2}{y_s} \\ -\frac{x_{1c}}{y_r} \frac{\partial f_1}{\partial S} - \frac{x_{2c}}{y_r} \frac{\partial f_2}{\partial S} & -D - \frac{x_{1c}}{y_r} \frac{\partial f_1}{\partial R} - \frac{x_{2c}}{y_r} \frac{\partial f_2}{\partial R} - \lambda & -\frac{f_1}{y_r} & -\frac{f_2}{y_r} \\ (1-q)x_{1c} \frac{\partial f_1}{\partial S} & (1-q)x_{1c} \frac{\partial f_1}{\partial R} & -\lambda & 0 \\ x_{2c} \frac{\partial f_2}{\partial S} + qx_{1c} \frac{\partial f_1}{\partial S} & x_{2c} \frac{\partial f_2}{\partial R} + qx_{1c} \frac{\partial f_1}{\partial R} & qf_1 & f_2 - D - \lambda \end{bmatrix}.$$

Multiplying fourth row by $1/y_s$, $1/y_r$ and adding to the first row, second row respectively yields

$$F(\lambda) = \det \begin{bmatrix} -D - (1-q) \frac{x_{1c}}{y_s} \frac{\partial f_1}{\partial S} - \lambda & -(1-q) \frac{x_{1c}}{y_s} \frac{\partial f_1}{\partial R} & -\frac{1}{y_s}(1-q)f_1 & -\frac{1}{y_s}(D + \lambda) \\ -(1-q) \frac{x_{1c}}{y_r} \frac{\partial f_1}{\partial S} & -D - (1-q) \frac{x_{1c}}{y_r} \frac{\partial f_1}{\partial R} - \frac{x_{2c}}{\partial R} - \lambda & -\frac{1}{y_r}(1-q)f_1 & -\frac{1}{y_r}(D + \lambda) \\ (1-q)x_{1c} \frac{\partial f_1}{\partial S} & (1-q)x_{1c} \frac{\partial f_1}{\partial R} & -\lambda & 0 \\ x_{2c} \frac{\partial f_2}{\partial S} + qx_{1c} \frac{\partial f_1}{\partial S} & x_{2c} \frac{\partial f_2}{\partial R} + qx_{1c} \frac{\partial f_1}{\partial R} & qf_1 & f_2 - D - \lambda \end{bmatrix}.$$

Multiplying the third row by $1/y_s$, $1/y_r$ and adding to the first row, second row respectively yields

$$F(\lambda) = \det \begin{bmatrix} -(D + \lambda) & 0 & -\frac{1}{y_s}(D + \lambda) & -\frac{1}{y_s}(D + \lambda) \\ 0 & -(D + \lambda) & -\frac{1}{y_r}(D + \lambda) & -\frac{1}{y_r}(D + \lambda) \\ (1-q)x_{1c} \frac{\partial f_1}{\partial S} & (1-q)x_{1c} \frac{\partial f_1}{\partial R} & -\lambda & 0 \\ x_{2c} \frac{\partial f_2}{\partial S} + qx_{1c} \frac{\partial f_1}{\partial S} & x_{2c} \frac{\partial f_2}{\partial R} + qx_{1c} \frac{\partial f_1}{\partial R} & f_1 - D & f_2 - D - \lambda \end{bmatrix}.$$

Adding fourth row to the third row yields

$$\begin{aligned}
 1_{pt}F(\lambda) &= \det \begin{bmatrix} -(D + \lambda) & 0 & -\frac{1}{y_s}(D + \lambda) & -\frac{1}{y_s}(D + \lambda) \\ 0 & -(D + \lambda) & -\frac{1}{y_r}(D + \lambda) & -\frac{1}{y_r}(D + \lambda) \\ x_{1c} \frac{\partial f_1}{\partial S} + x_{2c} \frac{\partial f_2}{\partial S} & x_{1c} \frac{\partial f_1}{\partial R} + x_{2c} \frac{\partial f_2}{\partial R} & f_1 - D - \lambda & f_2 - D - \lambda \\ x_{2c} \frac{\partial f_2}{\partial S} + qx_{1c} \frac{\partial f_2}{\partial S} & x_{2c} \frac{\partial f_2}{\partial R} + qx_{1c} \frac{\partial f_1}{\partial R} & f_1 - D & f_2 - D - \lambda \end{bmatrix} \\
 &= \frac{(D + \lambda)^2}{y_s y_r} \det \begin{bmatrix} y_s & 0 & 1 & 1 \\ 0 & y_r & 1 & 1 \\ x_{1c} \frac{\partial f_1}{\partial S} + x_{2c} \frac{\partial f_2}{\partial S} & x_{1c} \frac{\partial f_1}{\partial R} + x_{2c} \frac{\partial f_2}{\partial R} & f_1 - D & f_2 - D - \lambda \\ x_{2c} \frac{\partial f_2}{\partial S} + qx_{1c} \frac{\partial f_1}{\partial S} & x_{2c} \frac{\partial f_2}{\partial R} + qx_{1c} \frac{\partial f_1}{\partial R} & f_1 - D - \lambda & f_2 - D - \lambda \end{bmatrix} \\
 &= \frac{(D + \lambda)^2}{y_s y_r} \left(y_s y_r \lambda^2 + \lambda \left[y_s \left(x_{1c} \frac{\partial f_1}{\partial R} + x_{2c} \frac{\partial f_2}{\partial R} \right) + y_r \left(x_{1c} \frac{\partial f_1}{\partial S} + x_{2c} \frac{\partial f_2}{\partial S} \right) \right] - y_s y_r (f_2 - D) \right).
 \end{aligned}$$

Since $\partial f_i / \partial S > 0$, $\partial f_i / \partial R > 0$, $i = 1, 2$, from Roth–Hurwicz criterion if

$$f_2(S_c, R_c) - D < 0, \tag{A.9}$$

i.e.,

$$S_c < \lambda_{s2} \text{ or } R_c < \lambda_{r2},$$

then (E_c) is asymptotically stable. Since (A.9) is precisely (4.9), we conclude that if (E_c) exists then (E_c) is asymptotically stable.

From (4.1) and (4.2), $E_c = (S_c, R_c, x_{1c}, x_{2c})$ and $E_2 = (\widehat{S}, \widehat{R}, 0, \widehat{x}_2)$ satisfy

$$\begin{aligned}
 \widehat{R} &= \frac{y_s}{y_r} (\widehat{S} - S^{(0)}) + R^{(0)}, \\
 R_c &= \frac{y_s}{y_r} (S_c - S^{(0)}) + R^{(0)}, \\
 \widehat{S} &= \frac{y_r}{y_s} (\widehat{R} - R^{(0)}) + S^{(0)}, \\
 S_c &= \frac{y_r}{y_s} (R_c - R^{(0)}) + S^{(0)}.
 \end{aligned} \tag{A.10}$$

Obviously from (A.10) it follows that

$$\text{if } \widehat{S} > S_c, \text{ then } \widehat{R} > R_c, \tag{A.11}$$

and

$$\text{if } \widehat{R} > R_c, \text{ then } \widehat{S} > S_c. \tag{A.12}$$

Proof of Lemma 4.2. Assume E_c exists from (A.9) we have $S_c < \lambda_{s2}$ or $R_c < \lambda_{r2}$. Since $\widehat{S} \geq \lambda_{s2}$ and $\widehat{R} \geq \lambda_{r2}$, it follows that $\widehat{S} > S_c$ or $\widehat{R} > R_c$. Then from $\widehat{R} > R_c$ if $S_c = \lambda_{s1}$, $R_c > \lambda_{r1}$, then we have $\widehat{S} > \lambda_{s1}$ and $\widehat{R} > \lambda_{r1}$. Similarly we have $\widehat{S} > \lambda_{s1}$ and $\widehat{R} > \lambda_{r1}$ if $S_c > \lambda_{s1}$, $R_c = \lambda_{r1}$. If $E_2 = E_{2s} = (\lambda_{s2}, \widehat{R}, 0, \widehat{x}_{2s})$, then we have $\lambda_{s2} > \lambda_{s1}$ and $\widehat{R} > \lambda_{r1}$ and hence from (A.7) E_{2s} is unstable. Similarly if $E_2 = E_{2r} = (\widehat{S}, \lambda_{r2}, 0, \widehat{x}_{2r})$ then we have $\widehat{S} > \lambda_{s1}$ and $\lambda_{r2} > \lambda_{r1}$ and hence from (A.8) E_{2r} is unstable.

Assume E_2 is unstable. Then from (A.7) we have $\lambda_{s2} > \lambda_{s1}$ and $\widehat{R} > \lambda_{r1}$ if $E_2 = E_{2s} = (\lambda_{s2}, \widehat{R}, 0, \widehat{x}_{2s})$ and from (A.8) we have $\widehat{S} > \lambda_{s1}$ and $\lambda_{r2} > \lambda_{r1}$ if $E_2 = E_{2r} = (\widehat{S}, \lambda_{r2}, 0, \widehat{x}_{2r})$. For simplicity we only consider the case $E_2 = E_{2s}$. The proof for the case $E_2 = E_{2r}$ follows by similar arguments. To show the existence of positive rest point $E_c = (S_c, R_c, x_{1c}, x_{2c})$ we need to prove $S_c < \lambda_{s2}$ or $R_c > \lambda_{r2}$. There are two possible cases. *Case 1:* $S_c = \lambda_{s1}$ and $R_c < \lambda_{r1}$. Then obviously we have $\lambda_{s2} > \lambda_{s1} = S_c$ and hence E_c exists. *Case 2:* $S_c > \lambda_{s1}$ and $R_c = \lambda_{r1}$. In this case we have $\widehat{R} > \lambda_{r1} = R_c$ and by (A.12) it follows that $\lambda_{s2} = \widehat{S} > S_c$ and hence E_c exists.

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