Normal and slow growth states of microbial populations in essential resource-based chemostat*

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Abstract

To mimic the striking capability of microbial culture for growth adaptation after the onset of the novel environmental conditions, a heterogeneous microbial population model in the chemostat with essential resources is proposed which considers adaptation by spontaneously phenotype-switching between normally growing cells and persister cells having reduced growth rate. A basic reproductive number R_0 is introduced so that the population dies out when $R_0 < 1$, and when $R_0 > 1$ the population will be asymptotic to a steady state of persister cells, or a steady state of normal cells, or a steady state corresponding to a heterogeneous population of both normal and persister cells. Our analysis confirms that inherent heterogeneity of bacterial populations is important in adaption to fluctuating environments and in the persistence of bacterial infections.

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1 Introduction

The monod model for a single species in the chemostat has the advantage of being easily implementable in a laboratory, hence it has been subject to extensive tests and experiments and has become a benchmark model in microbial ecology (See, e.g. [17, 21]):

$$\begin{aligned} &S' = (S^\circ - S)D - \tfrac{1}{y} f(S)x, \\ &x' = (f(S) - D)x, \\ &S(0) \geq 0, \qquad x(0) > 0. \end{aligned} \tag{1.1}$$

Here S and x are respectively the concentrations of the growth-limiting nutrient and the microorganisms. S° is the concentration of the nutrient supply into the chemostat. D is the dilution rate and y is the yield constant. The growth rate f shows saturation kinetics as

$$f(S) = \nu_{\max} \cdot S/(a + S), \tag{1.2}$$

where $\nu_{\max}$ is the maximal growth rate and a is the saturation constant.

It is however well known that the physiology of microbial population reveals a striking capability for adaptation after the onset of the novel environmental conditions ([5] and [22]). Adaptation means that cells vary their cellular composition in order to cope optimally with the fluctuate environmental conditions. For instance, bacteria have been experimentally found to protect themselves at the cost of suspending their growth against many antibiotic encounters. The slow-growing persister phenotype can save the population from extinction during times of stress. This phenomenon was first reported for staphylococcal infections treated with penicillin ([3]) and has since been observed in many bacterial species (see e.g. [2, 10, 18, 25]). Such persistence was linked to preexisting heterogeneity in bacterial populations because phenotypic switching occurred between normal growing cells and persister cells having reduced growth rates.

Standard kinetic Monod model (1.1) systematically neglects the possible adaptive variations in growth characteristics and the inherent heterogeneity of bacterial populations in the persistence of bacterial infections. As a consequence, it remains a puzzle as to why a supposed saturation constant of *E. coli* in (1.2) for glucose proved to differ by orders of magnitude under different environment (see [13, 19]). Moreover, the expected monotone kinetic dynamics in the Monod equation (1.1) was not able be observed, but showed transient oscillations before steady state is reached (see C-8 strain of *E. coli* [8] and algae *Chlamydomonas reinhardii* [4]).

Numerous experimental disproofs have led to many attempts of modification of the original kinetic Monod model (see [5, 23] and the references therein). To explicitly include an adaptive mechanism behind persistence, Malik and Smith ([15]) proposed a model of an osmotrophic bacterial population growing in a chemostat with multiple limiting resources $R = (R_1, \cdots, R_m)$:

$$\begin{aligned} &R_j' = d(R_j^\circ - R_j) - c_j^{-1} f(R)N,\ j = 1, 2, \cdots, m, \\ &N' = N[f(R) - d] + \alpha(R)D - \beta(R)N, \\ &D' = -dD + \beta(R)N - \alpha(R)D, \end{aligned} \tag{1.3}$$

where c_j's are the yield constants, R_j° is the concentration of R_j in the feed to a well-stirred continuous culture with dilution rate d. The total population of bacteria cells is divided into two subpopulations: rapidly growing normal cells $N(t)$ and nongrowing (dormant) persister cells $D(t)$. The rate of switching from normal to persister is labeled β, and the rate of switching from persister to normal is labeled α. Note that both α and β are dependent on R.

This model is able to show that, even if the nutrient input concentration remains relatively low, the bacterial organism could still not be washed out by switching between the two different phenotypes ([15]). Transient oscillatory convergence to steady states reported in [4, 8] is also observed in this model.

It should be pointed out that model (1.3) only concerns with the heterogeneous population consisting of normal cells and dormant persister cells. However, the observation in Balaban et al. [2] indicated that there are at least two types of persistent cells. The *type I persisters*, e.g., *hipA7* mutant strain of *E. coli* (Moyed and Bertrand 1983 [18]), are *nongrowing* cells that exhibit a negligible spontaneous switching rate from normal to persister. The size of the type I subpopulation depends on the number of cells that have passed through stationary phase and does not increase during growth phase. The *type II persisters*, e.g., *hipQ* mutant strain (Wolfson et al. [25]), on the other hand, constitute a subpopulation of *slow-growing* cells. The type II persisters are formed via a phenotype-switching mechanism whereby a normal cell spontaneously becomes a type II persister, and the type II persister cell can spontaneously switch back to the normal phenotype.

Although type I persisters are somehow in the category of the above equation (1.3), this model is not applicable to slow-growing (but not dormant) type II persisters any more. To understand the potential dynamics of a bacterial heterogeneous population of normally growing cells and persister cells having reduced growth rates, we propose a model (see (2.1) in Section 2) of a bacterial population where the phenotype is acquired via a spontaneous, reversible switch between normal and slow-growing persister cells. The corresponding switching rates are depending on the levels of *several essential resources*. By essential resources, it means that growth ceases in the absence of any one of them (cf. [20, 24]). Therefore, while stated in terms of bacteria, our model is equally applicable to osmotrophic organism such as phytoplankton ([11, 12]).

We are able to carry out a global analysis of the proposed model by approach of asymptotically autonomous theory. A *basic reproductive number* R_0 is introduced so that the population dies out when $R_0 < 1$, and when $R_0 > 1$ the population will be asymptotic to a steady state of only persisters, or a steady state of only normal cells, or a steady state corresponding to a heterogeneous population of both normal and persister cells. Moreover, R_0 is observed to be strictly less than that of a single phenotype without persisters. This observation suggests that clonal bacterial populations may use persister cells, whose slow division rate under growth conditions leads to lower population fitness, as an insurance policy against antibiotic encounters.

Observe that the reduced-growing persisters in our model can also be allowed to be nongrowing. Therefore our new alternative model is able to describe both the population dynamics of the mutant *hipQ* strain of *E. coli* (Wolfson et al [25]), which is of Type II phenotype, and that of the mutant *hipA7* strain of *E. coli* (Moyed and Bertrand [18]) which is of Type I phenotype. Besides, our model also exhibits the advantage of the adaptive mechanism of phenotype-switching between the normal cells and slow-growing persisters over that of switching between normal growing and dormant cells. That is, even if the concentration of nutrient input into the chemostat is very low, the bacteria cultures could still survive with persister cells in the slow-growing mode, provided that the dilution rate is low enough. For the heterogenous cultures switching between normal and dormant phenotypes in [15], the population survives only if the essential nutrients input is higher than some threshold value. We illustrate this phenomena by numerical simulation comparison in Section 4. At the end of Section 4, we also present in our model that the transient oscillations phenomena reported in [4, 8] are observed as population levels approach the survival steady state.

Mathematically, we point out that it involves much more complicated analysis in our model than that in [15] because it includes more potential steady states. In particular, a key technical lemma (Lemma 5.2) is presented, motivated by which we give the total classification of the steady states. Moreover, noticing that the switching functions α and β are usually non-smooth, one cannot utilize the linearization method in [15, 23] to analyze the local stability of the steady states. An invariant region technique is therefore introduced in this paper (see Section 5.2) to accomplish our stability analysis. Finally, in order to obtain global behavior of the system, we use a new approach by providing the sufficient and necessary conditions (see Lemmas 5.16-5.18) for determining the basins of attraction of semitrivial steady states.

Persisters have been selected to increase chances of survival of bacterial populations exposure to stress. The quantitative characterization of persistence states and the associated phenotypic transitions should find clinical application in treatment of pathogens. Single-cell microscope observation of persisters carried out in Balaban et al. [2] has further found that persisters in the *wild-type* bacterial population of *E. coli* are continuously generated during normal growth. However, passage through stationary phase also increases the number of persisters. The wild-type population can be thus described as consisting of three subpopulations: normal cells; continuously generated type II persisters; and stationary phase type I persisters. This may induce the more complex wild-type population dynamics. We leave the mathematical description and quantitative measurement of this multiple-persistence switch as our future work.

2 The model and reproductive number

The proposed model follows microbial cultures and multiple inorganic nutrients $S_1, S_2, \cdots, S_m$. The total population of microbial cells is divided into two subpopulations: normal cells and persister cells. At any time t , the number of normal cells, $n(t)$, and persister cells, $p(t)$, forms the total population

$$\tau(t) = n(t) + p(t).$$

This quantity changes in time due to the growth or decline of the two subpopulations. Nutrients are supplied as in a chemostat, with dilution rate d and input concentrations $S_1^\circ, \cdots, S_m^\circ$. Bacterial growth depends on inorganic nutrient concentrations. One usually assumes Michaelis-Menten (i.e., Monod) functional forms in (1.2). Multiple-nutrient-limited growth can be modeled by the minimum of the functions describing single-nutrient-limited growth, encoding Liebig's law of the minimum. For the most generality, we present weak assumptions on growth rates, see (P1)-(P3) in the following paragraph, of both the normal and persisters cells. The specific growth functions can be found in [1, 6, 7] including Liebig's law of the minimum. The rate of switching from normal to persister is labeled β, and the rate of switching from persister to normal is labeled α. Both α and β are dependent on the nutrients $S = (S_1, \cdots, S_n)$.

Together, these assumptions result in the following model:

$$\begin{aligned} &\dot{S}_j(t) = d(S_j^\circ - S_j) - \Theta_j g_n(S)n - \Theta_j g_p(S)p,\ j = 1, 2, \cdots, m, \\ &\dot{n}(t) = n[g_n(S) - d] + \alpha(S)p - \beta(S)n, \qquad (2.1) \\ &\dot{p}(t) = p[g_p(S) - d] + \beta(S)n - \alpha(S)p. \end{aligned}$$

Here both normal cells and slow-growing persisters, since they are of the same species, have the same yield constant $\Theta_j^{-1} > 0$. For simplicity, we rewrite model (2.1) as the following equation in vector form:

$$\begin{aligned} &\dot{S}(t) = d(S^\circ - S) - [g_n(S)n + g_p(S)p]\Theta, \\ &\dot{n}(t) = n[g_n(S) - d] + \alpha(S)p - \beta(S)n, \qquad (2.2) \\ &\dot{p}(t) = p[g_p(S) - d] + \beta(S)n - \alpha(S)p. \end{aligned}$$

Where $S^\circ = (S_1^\circ, S_2^\circ, \cdots, S_m^\circ)^T$ and $\Theta = (\Theta_1, \Theta_2, \cdots, \Theta_m)^T$. Let $\mathcal{L} = \{S \geq 0 \mid S = S^\circ - \lambda\Theta,\ 0 \leq \lambda \leq \min\limits_{1 \leq j \leq m} \frac{S_j^\circ}{\Theta_j}\}$ be the portion of the ray through S° in the direction $-\Theta$ belonging to $\mathbb{R}_+^m$. g_n and g_p are the growth rates of the normal and persister cells, respectively. We assume the following properties for both of them:

(P1) $g_n(g_p) : \mathbb{R}_+^m \to [0, \infty)$ are continuous function, which are C^1 on a neighborhood of $\mathcal{L}$ with $\nabla g_n \cdot \Theta > \nabla g_p \cdot \Theta > 0$ on $\mathcal{L}$;

(P2) $g_n(S) \geq g_p(S)$ for $S \in \mathbb{R}_+^m$, and $g_n(S) > g_p(S)$ for $S \in \mathrm{Int}\mathbb{R}_+^m$;

(P3) $g_n(S) = g_p(S) = 0$ at $S = S^\circ - \min\limits_{1 \leq j \leq m} \frac{S_j^\circ}{\Theta_j}\Theta$.

Note that persisters in this model can be either in slow-growing phase (i.e., $g_p \neq 0$) or in dormant phase (i.e., $g_p \equiv 0$). Our equations are thus able to describe both the population dynamics of Type II phenotype and that of Type I phenotype observed in Balaban et al. [2].

Motivated by [15], we assume the switching rates α and β to be continuous, and piecewise smooth as follows:

- (a) α: $\mathbb{R}_+^m \to [0, \infty)$ is continuous and $A_0 := \{S \in \mathbb{R}_+^m : \alpha(S) = 0\}$ is the closure of a compact neighborhood of $\mathbf{0}$ with a piecewise smooth boundary ∂A_0. α is C^1 except on ∂A_0 and $\nabla\alpha \geq \mathbf{0}$. In addition, we assume that $S \in A_0$ and $0 \leq R \leq S$ implies that $R \in A_0$.

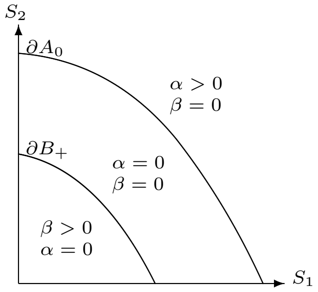


Figure 1: The positions of A_0 and B_+ when there are only two resources.

- (b) β: $\mathbb{R}_+^m \to [0, \infty)$ is continuous and $B_+ := \{S \in \mathbb{R}_+^m : \beta(S) > 0\}$ is a compact neighborhood of $\mathbf{0}$ with a piecewise smooth boundary ∂B_+. β is C^1 except on ∂B_+ and $\nabla\beta \leq \mathbf{0}$. In addition, we assume that $R \in B_+$ and $0 \leq S \leq R$ implies that $S \in B_+$.
- (c) The closure of B_+ is contained in the interior of A_0 .

Of course, in the resource phase space $\mathbb{R}_+^m$, $0 \leq S \leq R$ means that $0 \leq S_i \leq R_i$ for all $i = 1, 2, \cdots, m$. We refer to ∂A_0 as the "*normal-switching threshold*" and ∂B_+ as the "*persister-switching threshold*". See Figure 1 depicting the positions of A_0 and B_+ when there are only two resources. Moreover, we suppose that available resources in the chemostat are suitable such that ∂A_0 and ∂B_+ exist.

For system (2.2), a direct calculation yields that $S(t) + \tau(t)\Theta \to S^\circ$ as $t \to \infty$. By virtue of asymptotically autonomous theory (see [9, 16]), system (2.2) possesses an autonomous limit system given by:

$$\begin{aligned} \dot{n}(t) &= n[g_n(S^\circ - \tau\Theta) - d] + \alpha(S^\circ - \tau\Theta)p - \beta(S^\circ - \tau\Theta)n, \\ \dot{p}(t) &= p[g_p(S^\circ - \tau\Theta) - d] - \alpha(S^\circ - \tau\Theta)p + \beta(S^\circ - \tau\Theta)n, \end{aligned} \tag{2.3}$$

where $S^\circ - \tau\Theta = S$. The domain of (2.3) is the triangular region $\Gamma = \{(n, p) \in \mathbb{R}_+^2 \mid 0 \leq \tau = n + p \leq \min_{1 \leq j \leq m} \frac{S_j^\circ}{\Theta_j}\}$, which is positively invariant with respect to (2.3). Rewrite (2.3) in vector form $\begin{pmatrix} n \\ p \end{pmatrix}' = \mathcal{M} \begin{pmatrix} n \\ p \end{pmatrix}$, with

$$\mathcal{M} = \begin{pmatrix} g_n(S^\circ - \tau\Theta) - d - \beta(S^\circ - \tau\Theta) & \alpha(S^\circ - \tau\Theta) \\ \beta(S^\circ - \tau\Theta) & g_p(S^\circ - \tau\Theta) - d - \alpha(S^\circ - \tau\Theta) \end{pmatrix}.$$

Noticing that $\alpha(S^\circ - \tau\Theta)\beta(S^\circ - \tau\Theta) = 0$, one has

$$\det \mathcal{M} = H_n(S^\circ - \tau\Theta) \cdot H_p(S^\circ - \tau\Theta),$$

where

$$H_n(S^\circ - \tau\Theta) = g_n(S^\circ - \tau\Theta) - d - \beta(S^\circ - \tau\Theta) \tag{2.4}$$

and

$$H_p(S^\circ - \tau\Theta) = g_p(S^\circ - \tau\Theta) - d - \alpha(S^\circ - \tau\Theta). \tag{2.5}$$

We define

$$R_{n0} = \frac{g_n(S^\circ) - \beta(S^\circ)}{d}, \qquad R_{p0} = \frac{g_p(S^\circ)}{d},$$

and the ***Basic Reproductive Number*** for our model is given by

$$R_0 := \max\{R_{n0}, R_{p0}\}. \tag{2.6}$$

It captures the maximum of the respective numbers of descendants that both a single normal cell and a single persister cell, introduced into the sterile steady state $E_0 = (0, 0)$ of (2.3), produce in an average survival period $1/d$.

Remark 2.1. The basic reproductive number R_0 given in (2.6) naturally includes the corresponding R_0^* defined in [15], which only considered the phenotype-switching between normal and dormant cells. In this special case, $R_0 = R_{n0}$ because dormant persisters are nongrowing. A straightforward calculation then yields that $R_0 < (>)\ 1$ iff $H_n(S^\circ) \cdot H_p(S^\circ) > (<)\ 0$. Hence,

$$R_0 < (>)\ 1 \iff R_0^* \triangleq g_n(S^\circ) \cdot \frac{1}{d} \cdot \frac{\alpha(S^\circ) + d}{\alpha(S^\circ) + \beta(S^\circ) + d} < (>)\ 1.$$

Remark 2.2. It is obvious that R_0 is strictly less than that of a single phenotype without persisters. This observation suggests that clonal bacterial populations may use persister cells, whose slow division rate under growth conditions leads to lower population fitness, as an insurance policy against antibiotic encounters.

3 Main results

Hereafter, let $\mathcal{E}$ denote the set of steady states of the limit system (2.3). Then the potential elements of $\mathcal{E}$ can only be of the following forms:

$$E_0 = (0, 0),\ E_n = (\bar{n}, 0),\ E_p = (0, \bar{p}),\ \text{or}\ E_c = (n^*, p^*),$$

for which $\bar{n}, \bar{p}, n^*, p^* > 0$. We refer to E_n as the normal cell steady state, E_p the persisters steady state and E_c the coexistence of both normal and persister cells. We will show that E_n and E_c, if they exist, are locally asymptotically stable (see Lemma 5.12). However, they can never exist simultaneously (see Corollary 5.4). When neither E_n nor E_c exists, E_p will be stable (see Lemma 5.13); but E_p will lose its stability as E_n or E_c appears (see Lemma 5.14).

Our first result describes the global dynamics of (2.3):

Theorem 3.1. (i) $R_0 < 1$ *implies that* $\mathcal{E} = \{E_0\}$ *and* E_0 *is a global attractor.*

(ii) $R_0 > 1$ *implies that* $\mathcal{E} = \{E_0, E_p\}$, $\{E_0, E_c\}$, $\{E_0, E_n\}$, $\{E_0, E_c, E_p\}$ *or* $\{E_0, E_n, E_p\}$. E_n *and* E_c*, if they exist, are global attractors provided that* $n(0) > 0$. *If* $\mathcal{E} = \{E_0, E_p\}$ *then* E_p *is a global attractor provided that* $(n(0), p(0)) \neq (0, 0)$.

Theorem 3.1 will be proved in Section 5.4. We now present a simple criterion, when $R_0 > 1$, for determining which case occurs exactly in statement (ii). Roughly speaking, let subset $\Lambda_n \subset \mathbb{R}_+^m$ (resp. $\Lambda_p \subset \mathbb{R}_+^m$) be the *break-even nutrient concentration threshold* of normal (resp. persister) cells such that $g_n(\Lambda_n) = d$ (resp. $g_p(\Lambda_p) = d$). Viewing total population τ as a parameter, we determine its value such that the ray $S = S^\circ - \tau\Theta$ meets Λ_n at a resource level $S_n = S^\circ - \tau_n\Theta$ and meets Λ_p at a resource level $S_p = S^\circ - \tau_p\Theta$, respectively. Obviously, $0 \leq S_n \leq S_p \leq S^\circ$ in the resource phase space by the fundamental hypotheses of g_n and g_p. Then one can deduce which case in (ii) of Theorem 3.1 occurs by analyzing the relative positions of S_n and S_p with respect to the normal-switching threshold ∂A_0 and the persister-switching threshold ∂B_+.

More precisely, we define two functions $h_i : [0, \min_j \dfrac{S_j^\circ}{\Theta_j}] \to \mathbb{R}$ by

$$h_i(\tau) = g_i(S^\circ - \tau\Theta) - d,$$

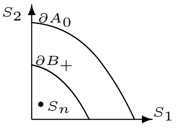
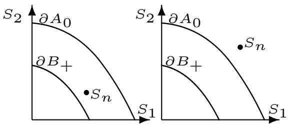
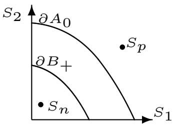
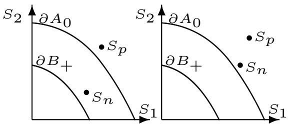
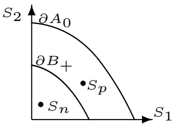
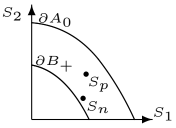
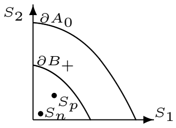
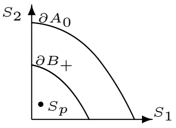
for $i = n, p$. Obviously, $h_p \leq h_n$, h_i is strictly decreasing and $h_i(\min_j \dfrac{S_j^\circ}{\Theta_j}) < 0$ for $i = n, p$. Since $R_0 = \max\{R_{n0}, R_{p0}\} > 1$, there always exists a unique $\tau_n > 0$ such that $h_n(\tau_n) = 0$. Furthermore, when $R_{p0} > 1$ there exists a unique $\tau_p \in (0, \tau_n)$ such that $h_p(\tau_p) = 0$ (τ_p does not exist when $R_{p0} \leq 1$). Let $S_n = S^\circ - \tau_n\Theta$ and $S_p = S^\circ - \tau_p\Theta$ (if S_p exists). Then $S_n \in \Lambda_n$ and $S_p \in \Lambda_p$. The exact criterion that determines all the cases in Theorem 3.1(ii) is listed in **Table 1**. The rigorous proof of statements in Table 1 will be given in next Section (see Proposition 5.1). From **Table 1**, it is worth noticing that E_n cannot exist but E_c or E_p can exist when the nutrient concentration input S° is relatively low as $\beta(S^\circ) > 0$.

Corresponding to the steady states E_0, E_n, E_c, E_p of (2.3), there are steady states e_0, e_n, e_c, e_p of (2.2). They are given by $e_0 = (E_0, S^\circ)$, $e_n = (E_n, S_n)$, $e_p = (E_p, S_p)$ and $e_c = (E_c, S^*)$ with $S^* = S^\circ - (n^* + p^*)\Theta$.

In the following two main Theorems, we will classify global dynamics of the original system (2.2) completely. The proof of these two Theorems will be provided in Section 5.5.

Theorem 3.2. *Assume that* S° *does not belong to the normal-switching threshold* ∂A_0. *Then*

Table 1: The classification of $\mathcal{E}$ when $R_0 > 1$.

	Cases			$\mathcal{E}$	Figures
$R_0 > 1$	$R_{n0} > 1$ $R_{p0} \leq 1$	$\beta(S_n) > 0$		$\{E_0, E_c\}$	
		$\beta(S_n) = 0$		$\{E_0, E_n\}$	
	$R_{p0} > 1$ $R_{n0} > 1$	$\alpha(S_p) > 0$	$\beta(S_n) > 0$	$\{E_0, E_c\}$	
			$\beta(S_n) = 0$	$\{E_0, E_n\}$	
		$\alpha(S_p) = 0$ $\beta(S_p) = 0$	$\beta(S_n) > 0$	$\{E_0, E_p, E_c\}$	
			$\beta(S_n) = 0$	$\{E_0, E_p, E_n\}$	
		$\beta(S_p) > 0$	$H_n(S_p) > 0$	$\{E_0, E_p, E_c\}$	
			$H_n(S_p) \leq 0$	$\{E_0, E_p\}$	
	$R_{p0} > 1$ $R_{n0} \leq 1$	$\beta(S_p) > 0$	$H_n(S_p) \leq 0$	$\{E_0, E_p\}$	

(i) $R_0 < 1 \Longrightarrow e = \{e_0\}$ *and* e_0 *is a global attractor;*

(ii) $R_{p0} \leq 1$ *and* $R_{n0} > 1 \Longrightarrow e = \{e_0, e_c\}$ *or* $\{e_0, e_n\}$*. If* $S^\circ \in A_0$*, then* e_0 *is an attractor for the initial value* $n(0) = 0$ *and* $p(0) > 0$*, while the nontrivial steady state is a global attractor provided that* $n(0) > 0$*. If* $S^\circ \notin A_0$*, then the nontrivial steady state is a global attractor provided that* $(n(0), p(0)) \neq (0, 0)$*.*

(iii) $R_{p0} > 1$ *and* $R_{n0} \leq 1 \Longrightarrow e = \{e_0, e_p\}$ *and* e_p *is a global attractor provided that* $(n(0), p(0)) \neq (0, 0)$*.*

Theorem 3.3. *Assume that* $R_{p0} > 1$ *(hence* $S_p := S^\circ - \tau_p \Theta$ *exists) and* $R_{n0} > 1$*. Assume also that both* S° *and* $S^\circ - \tau_p \Theta$ *do not belong to the normal-switching threshold* ∂A_0*. Then one of the following alternatives holds exactly:*

(i) $e = \{e_0, e_p\}, \{e_0, e_c\}$ *or* $\{e_0, e_n\}$*, and the nontrivial steady state is a global attractor provided that* $(n(0), p(0)) \neq (0, 0)$*;*

(ii) $e = \{e_0, e_c, e_p\}$ *or* $\{e_0, e_n, e_p\}$*, and* e_c *or* e_n *is a global attractor provided that* $n(0) > 0$*. Moreover, if* $S^\circ \in A_0$ *then* e_p *attracts all the orbit with the initial value* $n(0) = 0$ *and* $p(0) > 0$*; if* $S^\circ \notin A_0$ *then there exists a* $\hat{\tau} \in (0, \tau_p)$ *such that* e_p *attracts all the orbits with the initial value* $n(0) = 0$ *and* $p(0) \geq \hat{\tau}$*, and the other nontrivial steady state (i.e.,* e_c *or* e_n*) attracts all the orbits with the initial value* $n(0) = 0$ *and* $0 < p(0) < \hat{\tau}$*.*

Moreover, we have a simple criterion for determining which case in Theorems 3.2 and 3.3 occurs exactly from **Table 1**, because E_i in (2.3) is corresponding to e_i in (2.2) for $i = 0, c, n, p$.

4 New phenomena in numerical simulation

To illustrate the new phenomena of the dynamics in our model (2.2), we consider the dynamical bifurcation of model (2.2) as the environment undergoes proportional nutrient enrichment: $S^\circ = \eta v_0, \eta \geq 0$, where $v_0 > 0$ is a positive unit vector. We view η as a bifurcation parameter and explore some important bifurcations scenarios by numerical simulation. This approach was due to [15], but we shall present in our model several new bifurcation phenomena, by which one can make clear the advantage of slow-growing persister strain than dormant (nongrowing) persister strain in heterogeneous populations against stress.

Assume for definiteness that there are only two resources S_1 and S_2 and consider Michaelis-Menten Kinetics: $g_n^i(S_i) = \mu_n S_i/(K_i + S_i)$ and $g_p^i(S_i) =$

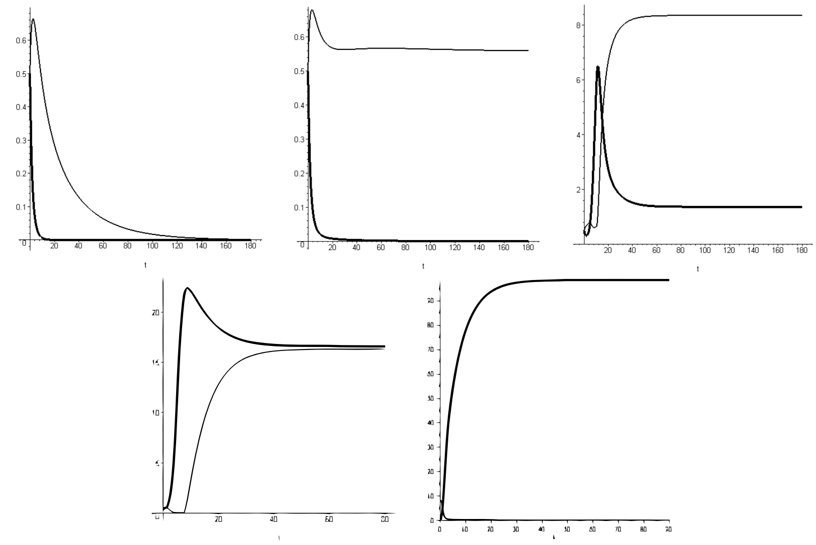


Figure 2: Time evolution of normal (**thick line**) and slow-growing persister (thin line) cells. Population tends to be washed out when the resource environment is extremely poor (top-left), to a steady state of persisters in relatively resource-poor environment (top-middle), to coexistence in resource-moderate (top-right and bottom-left) environment, and to a steady state of normal cells in resource-rich (bottom-right) environment.

$\mu_p S_i/(K_i + S_i)$, with $\mu_p < \mu_n$, for $i = 1, 2$. The growth rate of normal and persister cells, which obeys Liebig's law of minimum, takes the form of $g_i(S) = \min\{g_i^1(S_1), g_i^2(S_2)\}$, for $i = n, p$, respectively. Now let us take piecewise smooth normal-switching and persister-switching rate $\alpha(S_1, S_2) = \max\{g_n^1(S_1)g_n^2(S_2) - 0.7, 0\}$, $\beta(S_1, S_2) = \max\{0.5 - g_n^1(S_1)g_n^2(S_2), 0\}$, respectively. To incorporate proportional enrichment, we define $v_0 = (1, 2)$ and let η increase gradually to mimic the environment changing from the "resource-poor" status to the "resource-moderate" status, and to the "resource-rich" status. Other parameter values are chosen from the following Table.

μ_n	μ_p	K_1	K_2	Θ_1	Θ_2	$n(0)$	$p(0)$	$S_1(0)$	$S_2(0)$
5	2.5	20	10	0.4	0.6	0.5	0.5	0.4	0.6

By choosing the dilution rate $d = 0.15$, new bifurcation phenomenon can be observed via the numerical simulation shown in **Figure 2**. A steady state of slow-growing persisters, instead of coexistence of both normal and persister cells (observed by [15]), will bifurcate out of the washout state. More precisely, the population is washed out if the resource environment is extremely poor. But, if the resource environment is relatively poor, the population will tend to a steady state of persisters. Then in resource-moderate status the population coexistence steady state is observed, and in resource-rich status a steady state of normal cells bifurcates from the coexistence steady state. In **Figure 3**, we

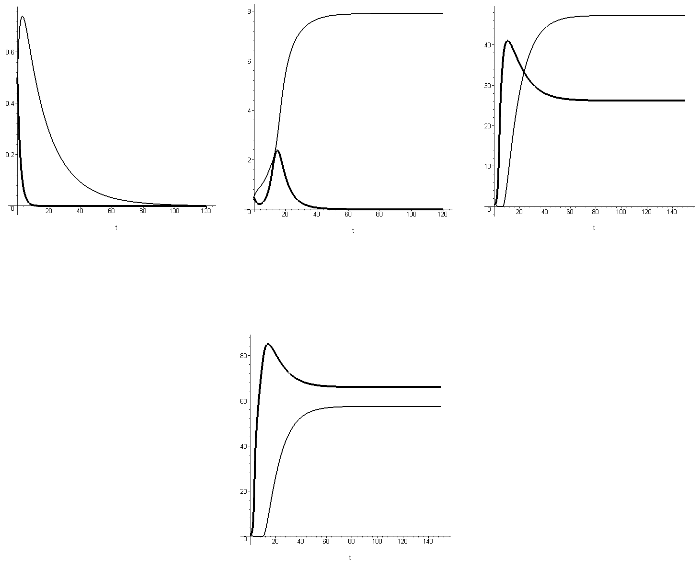


Figure 3: Population tends to be washed out when the resource environment is extremely poor (left), to a steady state of slow-growing persisters in relatively resource-poor environment (left-middle), and to coexistence in both resource-moderate and resource-rich environment (right-middle and right).

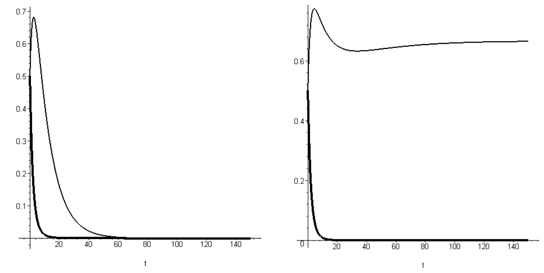


Figure 4: We take $\eta = 1.1$ and $d = 0.1$. Other parameters chosen are same as the previous Table. The population is washed out (left) as $\mu_p = 0$, i.e., the case of normal and dormant persister cells; while the population can still survive at steady state of persisters (right) as the case of normal and slow-growing persister cells.

let $d = 0.1$ and obtain the similar phenomena in Figure 2, except that the population tends to coexistence steady state in both resource-moderate status and in resource-rich status.

The evidence shown in Figures 2 and 3 implies that, even if the concentration of nutrient input into the chemostat is very low, the population could still survive with all cells in the persister mode, provided that the dilution rate d is low enough. For the heterogeneous population switching between normal and dormant cells in [15], however, the population survives only if the vector $S^\circ := (S_1^\circ, S_2^\circ)$ is higher than some critical threshold value. The simulation shown in **Figure 4** outstands this advantage of the adaptation mechanism between normal and slow-growing persister cells than that between normal and nongrowing persisters. Of course, from our main results, another advantage of our model here is that, by choosing S_1°, S_2°, d appropriately, one can obtain pure cultures of only normal cells or of only persister cells. Only pure cultures of cells of one growing mode (normal cells) can be obtained if cells switch between normal and dormant states.

Not surprisingly, when the growth rates obey Liebig's law of minimum, we take $d = 0.3$ (see Figure 5); when $g_n(S_1, S_2) = g_n^1(S_1)g_n^2(S_2)$ and $g_p(S_1, S_2) = g_p^1(S_1)g_p^2(S_2)$, we take $d = 0.2$ (see Figure 6) and $d = 0.9$ (see Figure 7). Similar three bifurcation diagrams reported in [15] can also be observed in our model.

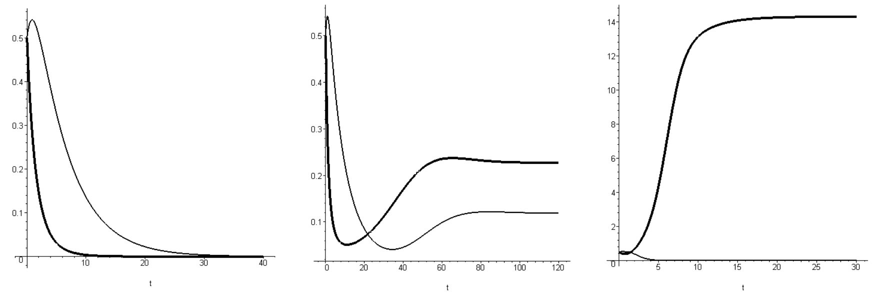


Figure 5: Population tends to be washed out in a resource-poor environment (left), to coexistence in resource-moderate (middle) environment and to normal steady state in resource-rich (right) environment.

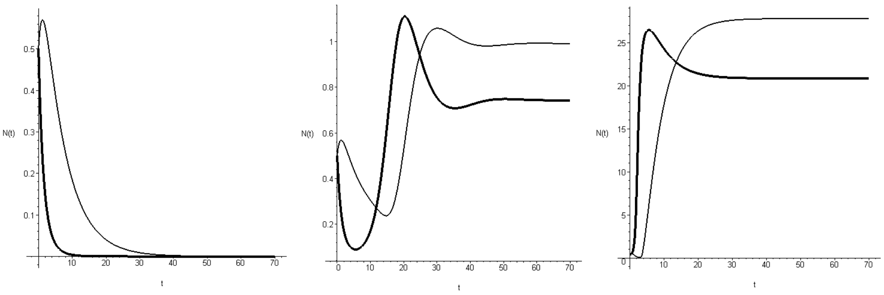


Figure 6: Population tends to extinction in a resource-poor environment (left) and to coexistence in both resource-moderate (middle) and resource-rich (right) environments.

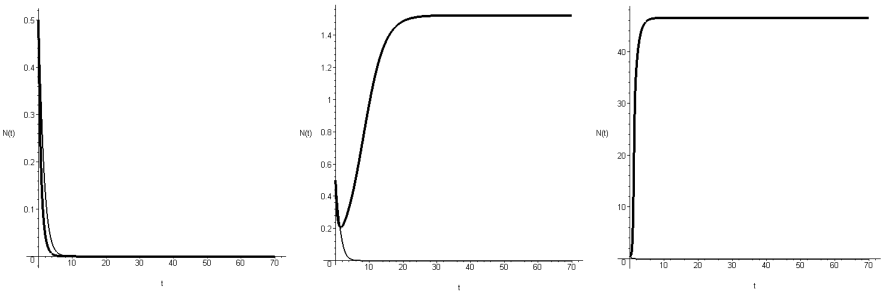


Figure 7: Population tends to extinction in a resource-poor environment (left), and to normal steady state in both resource-moderate (middle) and resource-rich (right) environments.

Transient oscillation, a phenomenon often observed in laboratory culture of bacteria [8] and algas [4], is obtained in our model (see Figure 8) when population levels approach the survival steady state.

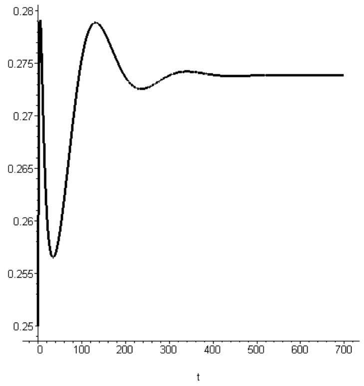


Figure 8: Transient oscillations in the change of the total concentration $\tau(t)$ of one bacterial population in the chemostat with two essential resources S_1, S_2.

5 The proof of the main results

5.1 Classification of equilibria of limit system

The following Proposition is the main result in this subsection.

Proposition 5.1. (I) *Suppose that* $R_{p0} > 1$ *and* $R_{n0} \leq 1$. *Then* $\mathcal{E} = \{E_0, E_p\}$.

(II) *Suppose that* $R_{p0} \leq 1$ *and* $R_{n0} > 1$. *Then*

- (i) $\mathcal{E} = \{E_0, E_n\}$ *if* $\beta(S^\circ - \tau_n\Theta) = 0$, *or*
- (ii) $\mathcal{E} = \{E_0, E_c\}$ *if* $\beta(S^\circ - \tau_n\Theta) > 0$.

(III) *Suppose that* $R_{p0} > 1$ *and* $R_{n0} > 1$. *Then one of the following alternatives occurs:*

- (i) $\mathcal{E} = \{E_0, E_n\}$ *if* $\alpha(S^\circ - \tau_p\Theta) > 0$ *and* $\beta(S^\circ - \tau_n\Theta) = 0$;
- (ii) $\mathcal{E} = \{E_0, E_c\}$ *if* $\alpha(S^\circ - \tau_p\Theta) > 0$ *and* $\beta(S^\circ - \tau_n\Theta) > 0$;
- (iii) $\mathcal{E} = \{E_0, E_n, E_p\}$ *if* $\alpha(S^\circ - \tau_p\Theta) = \beta(S^\circ - \tau_n\Theta) = 0$;
- (iv) $\mathcal{E} = \{E_0, E_c, E_p\}$ *if* $\beta(S^\circ - \tau_n\Theta) > 0 = \alpha(S^\circ - \tau_p\Theta) = \beta(S^\circ - \tau_p\Theta)$;
- (v) $\mathcal{E} = \{E_0, E_c, E_p\}$ *if* $\beta(S^\circ - \tau_p\Theta) > 0$ *and* $g_n(S^\circ - \tau_p\Theta) - \beta(S^\circ - \tau_p\Theta) - d > 0$;

(vi) $\mathcal{E} = \{E_0, E_p\}$ *if* $\beta(S^\circ - \tau_p\Theta) > 0$ *and* $g_n(S^\circ - \tau_p\Theta) - \beta(S^\circ - \tau_p\Theta) - d \leq 0$.

In order to prove this proposition, we need the following key technical lemma:

Lemma 5.2. *Assume that* $R_{p0} > 1$. *Then there exist exactly two points* τ_n, $\tau_p \in (0, \min_j \frac{S_j^\circ}{\Theta_j})$ *with* $\tau_p < \tau_n$ *such that* $g_n(S^\circ - \tau_n\Theta) = d = g_p(S^\circ - \tau_p\Theta)$. *Moreover, if* E_c *exists, then* $\tau^* := n^* + p^* \in (\tau_p, \tau_n)$ *with* $g_n(S^\circ - \tau^*\Theta) - d = \beta(S^\circ - \tau^*\Theta) > 0$.

Proof. Since $R_{p0} = \dfrac{g_p(S^\circ)}{d} > 1$, one has $g_p(S^\circ) - d > 0$. Note that $g_n \geq g_p$, one can also obtain that $g_n(S^\circ) - d > 0$. By (P3) in the hypotheses of $g_n(g_p)$, one has $g_i(S^\circ - \min_j \frac{S_j^\circ}{\Theta_j}\Theta) - d = -d < 0$, for $i = n, p$. Then it follows from the monotonicity of $g_n(g_p)$ that there exist exactly two points $\tau_n, \tau_p \in (0, \min_j \frac{S_j^\circ}{\Theta_j})$ such that $g_n(S^\circ - \tau_n\Theta) - d = 0$, $g_p(S^\circ - \tau_p\Theta) - d = 0$. Recall that $g_n > g_p$ in $\mathrm{Int}\mathbb{R}^m_+$, we obtain $\tau_p < \tau_n$.

Let $E_c = (n^*, p^*) \in \mathcal{E}$ with $\tau^* := n^* + p^*$ $(n^*, p^* > 0)$. Then one has

$$[g_n(S^\circ - \tau^*\Theta) - \beta(S^\circ - \tau^*\Theta) - d]n^* + \alpha(S^\circ - \tau^*\Theta)p^* = 0, \tag{5.1}$$

$$[g_p(S^\circ - \tau^*\Theta) - \alpha(S^\circ - \tau^*\Theta) - d]p^* + \beta(S^\circ - \tau^*\Theta)n^* = 0. \tag{5.2}$$

Suppose that $\tau^* \geq \tau_n$, then

$$g_n(S^\circ - \tau^*\Theta) - d \leq g_n(S^\circ - \tau_n\Theta) - d = 0. \tag{5.3}$$

If $\beta(S^\circ - \tau^*\Theta) = 0$ then, from (5.2), $g_p(S^\circ - \tau^*\Theta) - d = \alpha(S^\circ - \tau^*\Theta) \geq 0$. But because $\tau^* \geq \tau_n > \tau_p$, $g_p(S^\circ - \tau^*\Theta) - d < g_p(S^\circ - \tau_p\Theta) - d = 0$, a contradiction. Therefore, $\beta(S^\circ - \tau^*\Theta) > 0$ which implies that $\alpha(S^\circ - \tau^*\Theta) = 0$. So $g_n(S^\circ - \tau^*\Theta) - d = \beta(S^\circ - \tau^*\Theta) > 0$ by (5.1), contradicting (5.3). Thus we have proved that $\tau^* < \tau_n$.

Now we will prove that $\tau^* > \tau_p$. Otherwise $\tau^* \leq \tau_p$, then

$$g_n(S^\circ - \tau^*\Theta) - d > g_p(S^\circ - \tau^*\Theta) - d \geq g_p(S^\circ - \tau_p\Theta) - d = 0 \tag{5.4}$$

Suppose that $\alpha(S^\circ - \tau^*\Theta) = 0$, then from (5.2), $\dfrac{p^*}{n^*} = \dfrac{\beta(S^\circ - \tau^*\Theta)}{d - g_p(S^\circ - \tau^*\Theta)} \leq 0$, a contradiction. Therefore $\alpha(S^\circ - \tau^*\Theta) > 0$ and hence $\beta(S^\circ - \tau^*\Theta) = 0$. From (5.1) it follows that $\dfrac{n^*}{p^*} = \dfrac{\alpha(S^\circ - \tau^*\Theta)}{d - g_n(S^\circ - \tau^*\Theta)}$, contradicting (5.4). Thus we have proved that $\tau^* \in (\tau_p, \tau_n)$ with $R_{p0} > 1$.

Now suppose that $\beta(S^\circ - \tau^*\Theta) = 0$, from (5.1), $\dfrac{n^*}{p^*} = \dfrac{\alpha(S^\circ - \tau^*\Theta)}{d - g_n(S^\circ - \tau^*\Theta)} \leq 0$, for $\tau^* \in (\tau_p, \tau_n)$, a contradiction. So $\beta(S^\circ - \tau^*\Theta) > 0$ and $\dfrac{p^*}{n^*} = \dfrac{\beta(S^\circ - \tau^*\Theta)}{d - g_p(S^\circ - \tau^*\Theta)}$ and $g_n(S^\circ - \tau^*\Theta) - d = \beta(S^\circ - \tau^*\Theta) > 0$. □

Lemma 5.3. (i) *E_n exists if and only if $g_n(S^\circ - \bar{n}\Theta) = d$ and $\beta(S^\circ - \bar{n}\Theta) = 0$.*

(ii) *E_p exists if and only if $g_p(S^\circ - \bar{p}\Theta) = d$ and $\alpha(S^\circ - \bar{p}\Theta) = 0$.*

Proof. The proof is obvious, we omit it here. ☐

Corollary 5.4. *E_n and E_c cannot exist simultaneously.*

Proof. By Lemma 5.3, $E_n \in \mathcal{E}$ if and only if $g_n(S^\circ - \bar{n}\Theta) = d$ and $\beta(S^\circ - \bar{n}\Theta) = 0$. So, if $E_c \in \mathcal{E}$ simultaneously, then it follows from Lemma 5.2 that $\tau^* < \bar{n}$. As a consequence, $0 < \beta(S^\circ - \tau^*\Theta) \leq \beta(S^\circ - \bar{n}\Theta) = 0$, a contradiction. ☐

Lemma 5.5. (i) *If $E_p = (0, \bar{p})$ exists, then $R_{p0} > 1$.*

(ii) *If $E_n = (\bar{n}, 0)$ exists, then $R_{n0} > 1$.*

(iii) *If $E_c = (n^*, p^*)$ exists, then $R_{n0} > 1$ and $\beta(S^\circ - \tau^*\Theta) > 0$.*

Proof. Item (i) and (ii) are direct corollary of Lemma 5.3. Now we focus on item (iii) and recall that (5.1) and (5.2) hold. Suppose that $\beta(S^\circ - \tau^*\Theta) = 0$ with $\tau^* = n^* + p^*$. Then, by (5.2), $g_p(S^\circ - \tau^*\Theta) - d = \alpha(S^\circ - \tau^*\Theta) \geq 0$. So $g_n(S^\circ - \tau^*\Theta) > d$, which contradicts (5.1). Thus we have proved $\beta(S^\circ - \tau^*\Theta) > 0$. As a consequence, $\alpha(S^\circ - \tau^*\Theta) = 0$, and hence by (5.1) $H_n(S^\circ) > H_n(S^\circ - \tau^*\Theta) = 0$, where H_n is defined in (2.4). That is to say $R_{n0} > 1$. We have completed the proof. ☐

We now break the proof of Proposition 5.1 into the following Lemmas and Corollaries.

Lemma 5.6. *Assume that $R_{p0} > 1$ and let $\tau_p < \tau_n$ as in Lemma 5.2. Suppose that $\alpha(S^\circ - \tau_p\Theta) > 0$. Then $R_{n0} > 1$ and*

(i) *$\mathcal{E} = \{E_0, E_n\}$ if $\beta(S^\circ - \tau_n\Theta) = 0$.*

(ii) *$\mathcal{E} = \{E_0, E_c\}$ if $\beta(S^\circ - \tau_n\Theta) > 0$.*

Proof. Suppose that $\alpha(S^\circ - \tau_p\Theta) > 0$. Then E_p does not exist by Lemma 5.3(ii) and

$$\beta(S^\circ - \tau_p\Theta) = 0. \tag{5.5}$$

Therefore, $g_p(S^\circ - \tau_p\Theta) - d - \beta(S^\circ - \tau_p\Theta) = 0$, which implies that

$$H_n(S^\circ - \tau_p\Theta) = g_n(S^\circ - \tau_p\Theta) - d - \beta(S^\circ - \tau_p\Theta) > 0. \tag{5.6}$$

So $g_n(S^\circ) - d - \beta(S^\circ) > 0$, i.e., $R_{n0} > 1$.

(i) If $\beta(S^\circ - \tau_n\Theta) = 0$, then it follows from Lemma 5.3(i) that $E_n = (\tau_n, 0) \in \mathcal{E}$. But, $E_c = (n^*, p^*) \notin \mathcal{E}$ by Corollary 5.4, which implies that $\mathcal{E} = \{E_0, E_n\}$.

(ii) If $\beta(S^\circ - \tau_n\Theta) > 0$ then, by Lemma 5.3(i) again, E_n does not exist. Moreover, since $H_n(S^\circ - \tau_n\Theta) = -\beta(S^\circ - \tau_n\Theta) < 0$ and $H_n(S^\circ - \tau_p\Theta) > 0$ by (5.6), there exists a unique $\tau^* \in (\tau_p, \tau_n)$ such that $H_n(S^\circ - \tau_{\tau^*}\Theta) = 0$. So $\beta(S^\circ - \tau^*\Theta) = g_n(S^\circ - \tau^*\Theta) - d > g_n(S^\circ - \tau_n\Theta) - d = 0$ and $g_p(S^\circ - \tau^*\Theta) - d < g_p(S^\circ - \tau_p\Theta) - d = 0$. Therefore, $E_c = (n^*, p^*)$ can be defined by $n^* + p^* = \tau^*$ and $\dfrac{p^*}{n^*} = \dfrac{\beta(S^\circ - \tau^*\Theta)}{d - g_p(S^\circ - \tau^*\Theta)}$, that is, E_c exists. So, $\mathcal{E} = \{E_0, E_c\}$. ☐

Lemma 5.7. *Assume that* $R_{p0} > 1$ *and let* $\tau_p < \tau_n$ *as in Lemma* 5.2. *Suppose that* $\alpha(S^\circ - \tau_p\Theta) = 0$ *and* $\beta(S^\circ - \tau_p\Theta) = 0$. *Then* $R_{n0} > 1$ *and*

(i) $\mathcal{E} = \{E_0, E_n, E_p\}$ *if* $\beta(S^\circ - \tau_n\Theta) = 0$.

(ii) $\mathcal{E} = \{E_0, E_c, E_p\}$ *if* $\beta(S^\circ - \tau_n\Theta) > 0$.

Proof. Since $\alpha(S^\circ - \tau_p\Theta) = 0$, it follows from Lemma 5.3(ii) that $E_p = (0, \tau_p)$ exists. A careful examination in the proof of Lemma 5.6 yields that (5.5), i.e., $\beta(S^\circ - \tau_p\Theta) = 0$, is a critical condition. Note also that (5.5) is an exact hypothesis in the statement of this Lemma. Then, similarly as in the proof of Lemma 5.6, one can obtain that $\mathcal{E} = \{E_0, E_n, E_p\}$ if $\beta(S^\circ - \tau_n\Theta) = 0$, and $\mathcal{E} = \{E_0, E_c, E_p\}$ provided that $\beta(S^\circ - \tau_n\Theta) > 0$. ☐

Lemma 5.8. *Assume that* $R_{p0} > 1$ *and let* $\tau_p < \tau_n$ *as in Lemma* 5.2. *Suppose that* $\beta(S^\circ - \tau_p\Theta) > 0$. *Then*

(i) $\mathcal{E} = \{E_0, E_c, E_p\}$ *if* $g_n(S^\circ - \tau_p\Theta) - \beta(S^\circ - \tau_p\Theta) - d > 0$.

(ii) $\mathcal{E} = \{E_0, E_p\}$ *if* $g_n(S^\circ - \tau_p\Theta) - \beta(S^\circ - \tau_p\Theta) - d \leq 0$.

Proof. Obviously $\beta(S^\circ - \tau_p\Theta) > 0$ implies $\alpha(S^\circ - \tau_p\Theta) = 0$. Then $E_p = (0, \tau_p)$ exists by Lemma 5.3(ii) and Lemma 5.2. Moreover, $\beta(S^\circ - \tau_p\Theta) > 0$ also implies that $\beta(S^\circ - \tau_n\Theta) > 0$, then E_n does not exist by Lemma 5.3(i).

(i) If $g_n(S^\circ - \tau_p\Theta) - \beta(S^\circ - \tau_p\Theta) - d > 0$, then (5.6) holds. By the similar arguments as in the proof of Lemma 5.6 (ii), one obtains that E_c exists. Consequently, $\mathcal{E} = \{E_0, E_c, E_p\}$ in this case.

(ii) If $g_n(S^\circ - \tau_p\Theta) - \beta(S^\circ - \tau_p\Theta) - d \leq 0$, we claim that $E_c = (n^*, p^*)$ does not exist. Otherwise, it follows from Lemma 5.2 that $n^* + p^* = \tau^* \in (\tau_p, \tau_n)$ such that $H_n(S^\circ - \tau^*\Theta) = g_n(S^\circ - \tau^*\Theta) - d - \beta(S^\circ - \tau^*\Theta) = 0$. On the other hand, since $H_n(S^\circ - \tau\Theta)$ is strictly decreasing for τ, $H_n(S^\circ - \tau\Theta) < H_n(S^\circ - \tau_p\Theta) \leq 0$ for all $\tau \in (\tau_p, \tau_n)$. This contradicts that $H_n(S^\circ - \tau^*\Theta) = 0$. Thus we have proved our claim, and hence $\mathcal{E} = \{E_0, E_p\}$ in this case. ☐

Corollary 5.9. *Assume that* $R_{p0} > 1$ *and* $R_{n0} \le 1$*, then* $\mathcal{E} = \{E_0, E_p\}$.

Proof. By our assumption, it follows from Lemma 5.6 - Lemma 5.8 that only (ii) of Lemma 5.8 holds. So, $\mathcal{E} = \{E_0, E_p\}$. ☐

Corollary 5.10. *Assume that* $R_{p0} \le 1$ *and* $R_{n0} > 1$*, then*

(i) $\mathcal{E} = \{E_0, E_n\}$ *if* $\beta(S^\circ - \tau_n \Theta) = 0$,

(ii) $\mathcal{E} = \{E_0, E_c\}$ *if* $\beta(S^\circ - \tau_n \Theta) > 0$.

Proof. We first claim that $E_p = (0, \bar{p})$ does not exist. Otherwise, $g_p(S^\circ - \bar{p}\Theta) = d$ by Lemma 5.3, which implies that $g_p(S^\circ) > d$, contradicting that $R_{p0} \le 1$. Thus, we proved our claim. Since $R_{n0} > 1$, there exists a unique $\tau_n \in (0, \min_j \frac{S_j^\circ}{\Theta_j})$ such that $g_n(S^\circ - \tau_n\Theta) - d = 0$. So $E_n \in \mathcal{E}$ if $\beta(S^\circ - \tau_n\Theta) = 0$ according to Lemma 5.3. For the case that $\beta(S^\circ - \tau_n\Theta) > 0$, one can obtain that $\mathcal{E} = \{E_0, E_c\}$ by repeating the proof of Lemma 5.6 (ii) sentence by sentence with τ_p replaced by 0. ☐

5.2 Stability of the equilibria of limit system

Lemma 5.11. (i) *If* $R_0 < 1$*, then* E_0 *is locally asymptotically stable.*

(ii) *If* $R_{n0} > 1$ *and* $R_{p0} < 1$*, then* E_0 *is a saddle, the stable manifold of* E_0 *lies outside* Γ *if* $S^\circ \notin A_0$*, it is the portion of p-axis in* Γ *if* $S^\circ \in A_0$.

(iii) *If* $R_{n0} < 1$ *and* $R_{p0} > 1$*, then* $S^\circ \in B_+$ *and* E_0 *is a saddle, the stable manifold of* E_0 *lies outside* Γ.

(iv) *If* $R_{n0} > 1$ *and* $R_{p0} > 1$*, then*

  (1) *If* $g_p(S^\circ) - \alpha(S^\circ) - d > 0$*, then* E_0 *is an unstable node.*

  (2) *If* $g_p(S^\circ) - \alpha(S^\circ) - d \le 0$*(which implies that* $S^\circ \notin A_0$*), then* E_0 *is a saddle, but its stable manifold is outside* Γ.

Proof. Noticing $\alpha(S^\circ)\beta(S^\circ) = 0$, a straightforward calculation yields (i)-(iv). We just omit it here. ☐

Next we will discuss the local stability of each nontrivial equilibrium point provided that $R_0 > 1$. However, before proceeding to our further stability analysis of the nontrivial equilibria, we have to be more careful about the piecewise smooth hypotheses for the transition functions α and β. This implies that system (2.3) may not be smooth in Γ, because $S = S^\circ - (n+p)\Theta$ may belong to ∂A_0 where $\alpha(S)$ is not smooth or to ∂B_+ where $\beta(S)$ is not smooth.

Consequently, one cannot utilize the linearization method in [15, 23] to analyze the local stability of the steady states. Fortunately, we introduce an invariant region technique in this section to accomplish our stability analysis.

Lemma 5.12. *E_c and E_n are locally asymptotically stable when they exist.*

Proof. We first consider the coexistence equilibrium point E_c with $n^*+p^*=\tau^*$. By Lemma 5.5(iii), one has $S^\circ - \tau^*\Theta \in \mathrm{Int}B_+$, which implies that both $\alpha(S)$ and $\beta(S)$ are C^1 near $S^\circ - \tau^*\Theta$. So the Jacobian matrix of (2.3) at E_c exists and is given by

$$J_{E_c} = \begin{pmatrix} n^*(-\nabla g_n \cdot \Theta + \nabla\beta \cdot \Theta) & n^*(-\nabla g_n \cdot \Theta + \nabla\beta \cdot \Theta) \\ & \\ -p^*(\nabla g_p \cdot \Theta) - n^*(\nabla\beta \cdot \Theta) + \beta & -p^*(\nabla g_p \cdot \Theta) - n^*(\nabla\beta \cdot \Theta) + g_p - d \end{pmatrix}.$$

A direct computation yields that $\mathrm{det}J_{E_c} > 0$ and $\mathrm{Trace}J_{E_c} < 0$, which implies that E_c is locally asymptotically stable if it exists.

At E_n, $g_n(S^\circ - \bar{n}\Theta) = d$ and $\beta(S^\circ - \bar{n}\Theta) = 0$ by Lemma 5.3. So $S^\circ - \bar{n}\Theta \notin B_+$. Since α is not C^1 on ∂A_0 and β is not C^1 on ∂B_+, we cannot use the similar arguments of linearization in the proof of the stability of E_c as above. Therefore, we have to consider two cases, that is, (i) $S^\circ - \bar{n}\Theta \notin \bar{B}_+$, where $\bar{B}_+$ is the closure of set B_+; and (ii) $S^\circ - \bar{n}\Theta \in \partial B_+$, respectively.

Case (i): $S^\circ - \bar{n}\Theta \notin \bar{B}_+$. Then there exists a neighborhood V of E_n in Γ such that $\beta(S^\circ - (n+p)\Theta) = 0$ for all $(n,p) \in V$. So system (2.3) in V becomes

$$\begin{aligned} n' &= [g_n(S^\circ - \tau\Theta) - d]n + \alpha(S^\circ - \tau\Theta)p, \\ p' &= [g_p(S^\circ - \tau\Theta) - d - \alpha(S^\circ - \tau\Theta)]p, \end{aligned} \tag{5.7}$$

with $\tau = n+p$. Observe that $g_p(S^\circ - \bar{n}\Theta) - d - \alpha(S^\circ - \bar{n}\Theta) < g_n(S^\circ - \bar{n}\Theta) - d - \alpha(S^\circ - \bar{n}\Theta) = -\alpha(S^\circ - \bar{n}\Theta) \le 0$. Considering a smaller V if necessary, one can find a $\delta > 0$ such that $g_p(S^\circ - \tau\Theta) - d - \alpha(S^\circ - \tau\Theta) < -\delta$ for all $(n,p) \in V$. As a consequence, $p' < -\delta p$ in V, and hence $p(t) \to 0$ as $t \to \infty$. Now consider the limit equation of (5.7), $n' = [g_n(S^\circ - n\Theta) - d]n$. Noticing that $g_n(S^\circ - n\Theta) < g_n(S^\circ - \bar{n}\Theta) = d$ when $n > \bar{n}$, one has $n' < 0$ as $n > \bar{n}$. Similarly, $n' > 0$ when $n < \bar{n}$. Therefore E_n is locally asymptotically stable in this case.

Case (ii): $S^\circ - \bar{n}\Theta \in \partial B_+$. Then $\alpha(S^\circ - \bar{n}\Theta) = 0$ and $\beta(S^\circ - \bar{n}\Theta) = 0$. By hypothesis (c) concerning α and β, one can choose a neighborhood V of $\bar{n}$ such that $\alpha(S^\circ - \tau\Theta) = 0$ for all $\tau \in V$. Let $n+p = \tau$ in V. Then system (2.3) turns into

$$\begin{aligned} n' &= [g_n(S^\circ - \tau\Theta) - d - \beta(S^\circ - \tau\Theta)]n, \\ p' &= [g_p(S^\circ - \tau\Theta) - d]p + \beta(S^\circ - \tau\Theta)n. \end{aligned} \tag{5.8}$$

In the following, we will find a subdomain $\Gamma_1 \subset \Gamma$, with piecewise smooth boundary $\partial\Gamma_1$, such that $E_n \in \Gamma_1$ and Γ_1 is positively invariant with respect to the vector field of (2.3). To this end, we draw in Γ a straight dashed line $\mathcal{L}_1 : n = p$, which meets another straight dashed line $\mathcal{L}_2 : n + p = \bar{n}$ at point A (see Figure 9). Choose a point $G \in \mathcal{L}_1, G > A$, sufficiently close to A, we obtain a domain $\Gamma_1 \subset \Gamma$, whose boundary $\partial\Gamma_1 = CA \cup AG \cup GB \cup BC$, where the segment CA is parallel to the p-axis, and GB parallel to $\mathcal{L}_2$. Obviously, $E_n \in \Gamma_1$. We will analyze in the following the vector field of (2.3) along different parts of $\partial\Gamma_1$ one by one. Firstly, on segment CA, it is easy to see that $n + p \leq \bar{n}$. So $S^\circ - (n+p)\Theta \geq S^\circ - \bar{n}\Theta$, and hence $\beta(S^\circ - (n+p)\Theta) = 0$, which implies that the first equation of (2.3)

$$n' = [g_n(S^\circ - \tau\Theta) - d]n + \alpha(S^\circ - \tau\Theta)p \geq [g_n(S^\circ - \tau\Theta) - d]n.$$

Note also that $g_n(S^\circ - \bar{n}\Theta) = d$. Then $n' \geq 0$ on CA. Secondly, on segment AG, (2.3) becomes into

$$\begin{aligned} n' &= [g_n(S^\circ - \tau\Theta) - d - \beta(S^\circ - \tau\Theta)]n, \\ p' &= [g_p(S^\circ - \tau\Theta) - d + \beta(S^\circ - \tau\Theta)]n. \end{aligned}$$

because $n = p$, (5.8) and G is sufficiently close to A. Thus, by taking G closer to A if necessary, it follows from Lemma 5.3 (i) that $n' \leq 0$, $p' < 0$ and $|p'|/|n'| > 1$. So the vector field of (2.3) along AG points into Γ_1. Thirdly, on GB, a direct calculation from (5.8) yields that $(n+p)' = n(g_n(S^\circ - \tau\Theta) - d) + p(g_p(S^\circ - \tau\Theta) - d) \leq 0$. Overall, we have proved that Γ_1 is positively invariant w.r.t (2.3).

Since E_n is the unique steady state in Γ_1 and there is no periodic orbit in Γ_1 (see Proposition 5.15), E_n is locally asymptotically stable, which completes our proof. ☐

Lemma 5.13. *Suppose that E_p is the only nontrivial equilibrium of* (2.3)*, then E_p is locally asymptotically stable.*

Proof. Suppose that $\mathcal{E} = \{E_0, E_p\}$. Then it follows from Lemmas 5.6-5.8 that $E_p = (0, \bar{p})$ with $\beta(S^\circ - \bar{p}\Theta) > 0$ and $g_n(S^\circ - \bar{p}\Theta) - \beta(S^\circ - \bar{p}\Theta) - d \leq 0$. So $S^\circ - \bar{p}\Theta \in \mathrm{Int}B_+$, which implies that system (2.3) is C^1 in the neighborhood of $S^\circ - \bar{p}\Theta$. As a consequence, we consider the linearization of (2.3) at E_p and get the Jacobian Matrix

$$J_{E_p} = \begin{pmatrix} g_n(S^\circ - \bar{p}\Theta) - d - \beta(S^\circ - \bar{p}\Theta) & 0 \\ -\bar{p}(\nabla g_p \cdot \Theta) + \beta(S^\circ - \bar{p}\Theta) & -\bar{p}(\nabla g_p \cdot \Theta) \end{pmatrix}. \tag{5.9}$$

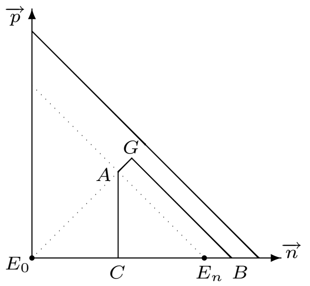


Figure 9: $\partial\Gamma_1 : CA \cup AG \cup GB \cup BC$ where Γ_1 is attractive region of E_n as $S^\circ - \bar{n}\Theta \in \partial B_+$.

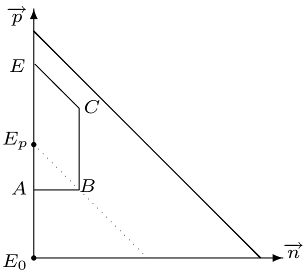


Figure 10: $\partial\Gamma_2$: $AB \cup BC \cup CE \cup EA$ where Γ_2 is attractive region of E_p as $\mathcal{E} = \{E_0, E_p\}$.

Obviously, E_p is a locally asymptotically stable node if $g_n(S^\circ - \bar{p}\Theta) - d - \beta(S^\circ - \bar{p}\Theta) < 0$. However, when $g_n(S^\circ - \bar{p}\Theta) - d - \beta(S^\circ - \bar{p}\Theta) = 0$, J_{E_p} is degenerate and one can not deduce any information just from J_{E_p}. Consequently, we return back to the original vector field (2.3) near E_p when $g_n(S^\circ - \bar{p}\Theta) - d - \beta(S^\circ - \bar{p}\Theta) = 0$. It is easy to see that, in a neighborhood U of E_p in Γ, (2.3) turns into (5.8) with $\beta(S^\circ - \tau\Theta) > 0$ for all $(n, p) \in U$, $\tau = n + p$.

We choose a subdomain $\Gamma_2 \subset U$, as in Figure 10, whose boundary $\partial\Gamma_2 = AB \cup BC \cup CE \cup EA$, where B is on the dashed line $\ell : n + p = \bar{p}$, AB is the horizontal segment, BC is the vertical segment and CE is a segment which is parallel to ℓ. Similarly as in the proof of Lemma 5.12, an easy calculation directly yields that Γ_2 is positively invariant. Since one can choose A, E arbitrarily close to E_p, E_p is asymptotically stable. Thus we have completed the proof. $\Box$

Lemma 5.14. *Suppose that E_p is not the unique nontrivial steady state. Then there exists a neighborhood U of E_p in Γ such that the orbit $(n(t), p(t))$ will leave U provided that $n(0) > 0$. Moreover, E_p just attracts a portion of p-axis.*

Proof. Let $E_p = (0, \bar{p})$. Then by Lemma 5.3, $\alpha(S^\circ - \bar{p}\Theta) = 0$ and $g_p(S^\circ - \bar{p}\Theta) = d$. Hence $S^\circ - \bar{p}\Theta \in A_0$. Moreover, since E_p is not the unique nontrivial steady state, $\mathcal{E} = \{E_0, E_p, E_n\}$ or $\{E_0, E_p, E_c\}$ by Lemmas 5.6-5.8. We will discuss the following three cases, respectively.

(i) $S^\circ - \bar{p}\Theta \notin \partial A_0 \cup \partial B_+$. Then system (2.3) is C^1 at $S^\circ - \bar{p}\Theta$, and hence one can utilize the linearization of (2.3) at E_p and obtain the Jacobian J_{E_p} as in (5.9). By virtue of Lemmas 5.7 and 5.8(i), it is easy to see that $g_n(S^\circ - \bar{p}\Theta) - d - \beta(S^\circ - \bar{p}\Theta) > 0$ if $\mathcal{E} = \{E_0, E_p, E_n\}$ or $\{E_0, E_p, E_c\}$. So from (5.9), E_p is a saddle point, whose stable manifold is a portion of p-axis.

(ii) $S^\circ - \bar{p}\Theta \in \partial B_+$. Then $\alpha(S^\circ - \bar{p}\Theta) = 0$ and $\beta(S^\circ - \bar{p}\Theta) = 0$. In this case, the set $\mathcal{E}=\{E_0, E_p, E_c\}$ by Lemma 5.7(ii), where $E_c = (n^*, p^*)$ with $\tau^* = n^*+p^*$. Moreover, there exists a neighborhood U of E_p in Γ such that $\alpha(S^\circ - \tau\Theta) = 0$ for all $\tau = n+p$ with $(n, p) \in U$. Therefore, (2.3) can be written as (5.8) again. Recall that $\tau^* > \bar{p}$ from Lemma 5.2. Then $H_n(S^\circ - \bar{p}\Theta) > H_n(S^\circ - \tau^*\Theta) = 0$. So one can choose U so small that $H_n(S^\circ - \tau\Theta) > \delta > 0$ for all $\tau = n+p$ with $(n, p) \in U$. It then follows from the first equation of (5.8) that $n(t)$ will leave U provided that $n(0) > 0$. On p-axis, it is easy to see from the second equation of (5.8) that E_p attracts all the points in U.

(iii)$S^\circ - \bar{p}\Theta \in \partial A_0$. In this case, the set $\mathcal{E}=\{E_0, E_p, E_n\}$ or $\{E_0, E_p, E_c\}$ by Lemma 5.7. Take a neighborhood U of E_p in Γ such that $\beta(S^\circ - \tau\Theta) = 0$ for all $\tau = n+p$ with $(n, p) \in U$. Then system (2.3) turns into (5.7). By virtue of the first equation of (5.7), $n' \geq [g_n(S^\circ - \tau\Theta) - d]n$. Noticing that $g_n(S^\circ - \bar{p}\Theta) - d > g_p(S^\circ - \bar{p}\Theta) - d = 0$, one can make U so small that $g_n(S^\circ - \tau\Theta) - d > \delta > 0$ for all $\tau = n+p$ with $(n, p) \in U$. So $n(t)$ will leave the neighborhood U provided that $n(0) > 0$. While on p-axis, consider the second equation of (5.7), i.e., $p' = [g_p(S^\circ - p\Theta) - d - \alpha(S^\circ - p\Theta)]p$. When $p > \bar{p}$, one has $p' < 0$ because $0 \leq \alpha(S^\circ - p\Theta) \leq \alpha(S^\circ - \bar{p}\Theta) = 0$. So each orbit with initial value $n(0) = 0$ and $p(0) > \bar{p}$ is asymptotic to $E_p = (0, \bar{p})$. ☐

5.3 Nonexistence of the closed orbits

Proposition 5.15. *System* (2.3) *has no periodic orbit.*

Proof. Suppose that α and β are C^1 in Γ. Then, by taking Dulac's function $\frac{1}{np}$ and the fact that

$$\nabla \cdot \tfrac{1}{np}(n', p') = -\tfrac{\nabla g_n \cdot \Theta}{p} - \tfrac{\alpha(S^\circ - \tau\Theta)}{n^2} - \tfrac{\nabla g_p \cdot \Theta}{n} - \tfrac{\beta(S^\circ - \tau\Theta)}{p^2} < 0, \tag{5.10}$$

we have verified that system (2.3) has no periodic orbits in Γ.

We are now focusing on the case that α and β are piecewise smooth in Γ. Our assumptions on A_0 and B_+ guarantee that the segment $S = S^\circ - \tau\Theta,\ \tau \in [0, \min_j S_j^\circ/\Theta_j]$, may meet ∂A_0 at most one point (or value of τ) which, if it exists, we label τ_0. Similarly, this line may meet ∂B_+ at most once and we label τ_+ the corresponding value of τ if it exists. So the worst case is that both τ_0 and τ_+ exist, and hence system (2.3) fails to be C^1 in Γ only along two lines $n + p = \tau_0$ and $n + p = \tau_+$. The argument is similar and simpler in neither or only one line meets Γ. Hereafter we only need to consider this case, i.e., τ_0 and τ_+ exist simultaneously.

Suppose that system (2.3) has a periodic orbit, which we label O. Then it must enclose the equilibrium point E_c. By virtue of Lemma 5.5(iii), one has

$S^\circ - \tau^*\Theta \in \mathrm{Int}B_+$. Compared with the Hypotheses (c) concerning α and β, it is easy to see that $\tau_0 < \tau_+ < \tau^*$.

Now we divide the triangular region Γ into three regions I, II and III, where

$$\mathrm{I} = \{(n,p) \in \Gamma : \alpha(S^\circ - \tau\Theta) > 0, \beta(S^\circ - \tau\Theta) = 0\},$$

$$\mathrm{II} = \{(n,p) \in \Gamma : \alpha(S^\circ - \tau\Theta) = 0, \beta(S^\circ - \tau\Theta) = 0\},$$

$$\mathrm{III} = \{(n,p) \in \Gamma : \alpha(S^\circ - \tau\Theta) = 0, \beta(S^\circ - \tau\Theta) > 0\}.$$

Motivated by [15], we introduce new variables $u = p/n$ and $\tau = n + p$, whose inverse transformations are $n = \dfrac{\tau}{1+u}$, $p = \dfrac{u\tau}{1+u}$. Then the three regions I, II and III are transformed into regions

$$\begin{aligned}\omega_1 &= \{(u,\tau) : u \geq 0, 0 \leq \tau < \tau_0\},\\ \omega_2 &= \{(u,\tau) : u \geq 0, \tau_0 \leq \tau \leq \tau_+\},\\ \omega_3 &= \{(u,\tau) : u \geq 0, \tau_+ < \tau \leq \tau_{\max}\}\end{aligned}$$

in the $u\tau-$plane, respectively. Of course, $\tau_{\max} = \min_j \frac{S_j^\circ}{\Theta_j}$. Moreover, system (2.3) is then transformed into the following

$$\begin{aligned} u' &= u\{[g_p(S^\circ - \tau\Theta) - d - \alpha(S^\circ - \tau\Theta)] - [g_n(S^\circ - \tau\Theta)\\ &\quad -d - \beta(S^\circ - \tau\Theta)] - u\alpha(S^\circ - \tau\Theta)\} + \beta(S^\circ - \tau\Theta), \\ \tau' &= \tfrac{\tau}{1+u}\{[g_n(S^\circ - \tau\Theta) - d] + u[g_p(S^\circ - \tau\Theta) - d]\}. \end{aligned} \tag{5.11}$$

Obviously, $E_c \in \omega_3$. Moreover, the $u' = 0$ nullcline for (5.11) consists of $u = 0$ in regions $\omega_1 \cup \omega_2$ and the graph G_1 of the function

$$u = \frac{\beta(S^\circ - \tau\Theta)}{g_n(S^\circ - \tau\Theta) - g_p(S^\circ - \tau\Theta) - \beta(S^\circ - \tau\Theta)}\ , \qquad \tau_+ < \tau \leq \tau_{\max}, \tag{5.12}$$

in ω_3. A straightforward calculation yields that

$$u'_\tau = \frac{-(g_n - g_p)\nabla\beta \cdot \Theta + \beta(\nabla g_n - \nabla g_p) \cdot \Theta}{(g_n - g_p - \beta)^2} > 0$$

by the fact that $\nabla g_n \cdot \Theta > \nabla g_p \cdot \Theta$, $g_n > g_p$ and $\nabla\beta \cdot \Theta < 0$. So, along G_1, u is a strictly increasing, continuous function of τ, vanishing at $\tau = \tau_+$ and has a horizontal asymptote $\tilde{\tau}$, where $g_n(S^\circ - \tilde{\tau}\Theta) - g_p(S^\circ - \tilde{\tau}\Theta) - \beta(S^\circ - \tilde{\tau}\Theta) = 0$. Recall that $g_n(S^\circ - \tau^*\Theta) - g_p(S^\circ - \tau^*\Theta) - \beta(S^\circ - \tau^*\Theta) = d - g_p(S^\circ - \tau^*\Theta) > 0$ from Lemma 5.2, and $g_n(S^\circ - \tau_{\max}\Theta) - g_p(S^\circ - \tau_{\max}\Theta) - \beta(S^\circ - \tau_{\max}\Theta) = -\beta(S^\circ - \tau_{\max}\Theta) < 0$, one has $\tau_+ < \tau^* < \tilde{\tau} < \tau_{\max}$ (see Figure 11).

The $\tau' = 0$ nullcline for (5.11) are given by $\tau = 0$, and the graph G_2 of the function

$$u = -\frac{g_n(S^\circ - \tau\Theta) - d}{g_p(S^\circ - \tau\Theta) - d}. \tag{5.13}$$

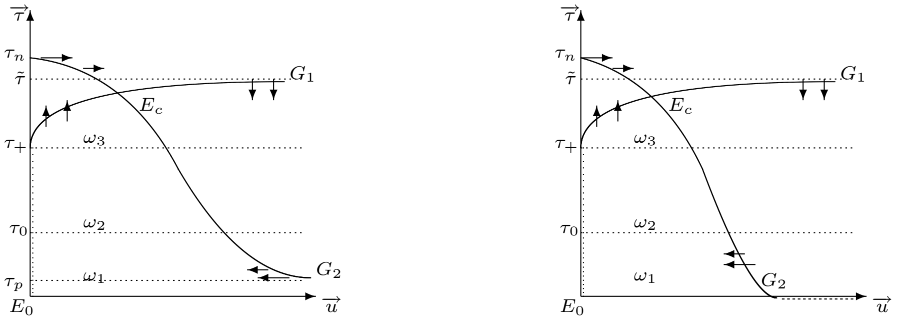


Figure 11: Graph (5.12) and graph (5.13) in the $u\tau-$plane when $R_{p0} > 1$ (left), and when $R_{p0} \leq 1$ (right).

of τ, which is defined on $[0, \tau_n]$ if $R_{p0} \leq 1$, or on $(\tau_p, \tau_n]$ if $R_{p0} > 1$. Here τ_p, τ_n are as in Lemma 5.2 (Note that τ_n always exists because $R_{n0} > 1$ which is implied by the existence of E_c). Function (5.13) vanishes at $\tau = \tau_n$. An easy computation shows that

$$u'_\tau = \frac{(g_p - d)\nabla g_n \cdot \Theta + (d - g_n)\nabla g_p \cdot \Theta}{(g_p - d)^2}.$$

Then, along G_2, u is strictly decreasing in $[0, \tau_n]$ if $R_{p0} \leq 1$ (resp. strictly decreasing in $(\tau_p, \tau_n]$ if $R_{p0} > 1$, and has an asymptote $\tau = \tau_p$ in this case)(see Figure 11). The rest of the proof is then almost the same as its counterpart in [15, Lemma 4] with a minor change, so we omit it here. $\square$

5.4 Proof of Theorem 3.1

Proof. If $R_0 < 1$ then both $R_{p0} < 1$ and $R_{p0} < 1$, and hence $\mathcal{E} = \{E_0\}$ by Lemma 5.5. By virtue of Lemma 5.11, $R_0 < 1$ also implies that E_0 is locally asymptotically stable. So it is globally attractive by Proposition 5.15 and Poincaré-Bendixson Theory.

If $R_0 > 1$ then nontrivial equilibria will appear. By virtue of Proposition 5.1, one can obtain that $\mathcal{E}$ has one of the following forms: $\{E_0, E_p\}$, $\{E_0,\ E_n\}$, $\{E_0,\ E_c\}$, $\{E_0, E_p, E_n\}$, $\{E_0, E_p, E_c\}$. Since $R_0 > 1$, it follows from Lemma 5.11 (ii)-(iv) that E_0 is an unstable node, or a saddle whose stable manifold lies on p-axis or outside Γ. By Lemma 5.12, E_c and E_n are locally asymptotically stable when they exist. It then follows from Lemma 5.14, Proposition 5.15 and Poincaré-Bendixson Theory that E_c (resp. E_p) attracts all the orbits with $n(0) > 0$, provided $\mathcal{E} = \{E_0, E_n\}, \{E_0, E_c\}$, $\{E_0, E_p, E_n\}$ or $\{E_0, E_p, E_c\}$.

If $\mathcal{E} = \{E_0, E_p\}$, then $R_{p0} > 1$ by Lemma 5.5(ii). So Lemma 5.11(iii)-(iv) implies that E_0 is an unstable node, or a saddle whose stable manifold lies outside Γ. Note also that E_p is locally asymptotically stable by Lemma 5.13. So E_p is globally asymptotically stable for all $(n(0),p(0)) \neq (0,0)$ in Γ. $\square$

5.5 Proofs of Theorems 3.2 and 3.3

Define $\Sigma := S^\circ - [S + (n+p)\Theta]$. Then system (2.2) can be transformed into the following system of equations

$$\begin{aligned} n' &= n[g_n(S) - \beta(S) - d] + \alpha(S)p, \\ p' &= p[g_p(S) - \alpha(S) - d] + \beta(S)n, \\ \Sigma' &= -d\Sigma, \end{aligned} \tag{5.14}$$

where $S = S^\circ - \Sigma - (n+p)\Theta$. System (5.14) is restricted to the positively invariant set $\tilde{\Gamma} = \{(n,p,\Sigma) | n,p \geq 0, \Sigma + (n+p)\Theta \leq S^\circ\}$. Moreover, let $\tilde{\Gamma}_{n+} = \tilde{\Gamma} \cap \{(n,p,\Sigma)|n > 0\}$ and $\tilde{\Gamma}_{p+} = \tilde{\Gamma} \cap \{(n,p,\Sigma)|p > 0\}$. Then $\tilde{\Gamma}_{n+}$ and $\tilde{\Gamma}_{p+}$ are also positively invariant. The corresponding trivial, normal-growing only, slow-growing only and coexistence steady states of (5.14) are $\sigma_0 = (E_0, 0)$, $\sigma_n = (E_n, 0)$, $\sigma_p = (E_p, 0)$ and $\sigma_c = (E_c, 0)$, respectively. It also follows easily from the third equation of (5.14) that $\Sigma(t) \to 0$ as $t \to \infty$. Hereafter denote by $\phi(t) := (n(t), p(t), \Sigma(t))$ the nontrivial solution of (5.14).

Being different from the methods in [23, 15], we here use a new approach by first providing sufficient and necessary conditions (see the following three Lemmas) for determining the basins of attraction of semitrivial steady states.

Lemma 5.16. *Let $R_{p0} > 1$. Assume that S° does not belong to the normal-switching threshold ∂A_0. Then, for* (5.14)*, $\phi(t) \to 0$ as $t \to \infty$ if and only if $n(t) = p(t) = 0$ for all $t \geq 0$.*

Proof. Since $\Sigma(t) \to 0$ as $t \to \infty$, we only need to consider the necessariness.

Case (i): Suppose that $S^\circ \in A_0$. Then $S^\circ \in \text{Int} A_0$ by our assumption. We also obtain that $S(t) = S^\circ - \Sigma(t) - (n(t) + p(t))\Theta \to S^\circ$ as $t \to \infty$, because $\phi(t) \to 0$ as $t \to \infty$. So there exists a neighborhood U_0 of S° such that $S(t) \in U_0 \subset A_0$ for all t sufficiently large, and hence $\alpha(S) = 0$, which implies that (5.14) along $\phi(t)$, for t sufficiently large, becomes

$$\begin{aligned} n' &= n[g_n(S) - \beta(S) - d], \\ p' &= p[g_p(S) - d] + \beta(S)n, \\ \Sigma' &= -d\Sigma. \end{aligned} \tag{5.15}$$

Moreover, one can choose U_0 sufficiently small, if necessary, such that $g_p(S) > g_p(S^\circ - \tau_p\Theta)$ for all $S \in U_0$, where τ_p as in Lemma 5.2 since $R_{p0} > 1$. Consequently, $g_p(S) - d > g_p(S^\circ - \tau_p\Theta) - d = 0$ for all $S \in U_0$. It then follows from the second equation of (5.15) that $p(t) = 0$ for all $t > 0$ sufficiently large. So the second equation of (5.15) becomes

$$p' = \beta(S)n, \tag{5.16}$$

for all t sufficiently large. Thus we can also claim that $n(t) = 0$ for all $t > 0$ sufficiently large. Otherwise, by the positive invariance of $\tilde{\Gamma}_{n+}$, one has $n(t) > 0$ for all $t > 0$ sufficiently large. Thus $\beta(S(t)) = 0$ for all t sufficiently large, which implies that $n'(t) = n(t)[g_n(S(t)) - d] > n(t)[f_p(S(t)) - d] > 0$, contradicting $n(t) \to 0$ as $t \to \infty$. Thus we have proved that $n(t) = p(t) = 0$ for all t sufficiently large. Note also that $\tilde{\Gamma}_{n+}$ and $\tilde{\Gamma}_{p+}$ are positively invariant with respect to (5.14). This implies that $n(t) = p(t) = 0$ for all $t \geq 0$.

Case (ii): Suppose that $S^\circ \notin A_0$. Then (5.14) along $\phi(t)$, for t sufficiently large, becomes

$$\begin{aligned} n' &= n[g_n(S) - d] + \alpha(S)p, \\ p' &= p[g_p(S) - \alpha(S) - d], \\ \Sigma' &= -d\Sigma. \end{aligned} \tag{5.17}$$

Similarly as the proof in case (i), one can easily obtain from the first equation of (5.17) that $n(t) = 0$ for all t sufficiently large. So the first equation of (5.17) becomes

$$n' = \alpha(S)p, \tag{5.18}$$

for all t sufficiently large. We claim that $p(t) = 0$ for all t sufficiently large. Otherwise, it follows from (5.18) that $\alpha(S(t_j)) = 0$ for some sequence $t_j \to \infty$. By letting $j \to \infty$, one has $\alpha(S^\circ) = 0$, which contradicts $S^\circ \notin A_0$. Thus, we have proved the claim, i.e., $p(t) = 0$ for all t sufficiently large. The remaining is the same as the similar arguments in the proof in case (i). □

Lemma 5.17. *Let $R_{p0} \leq 1$ and $R_{n0} > 1$. Assume that S° does not belong to the normal-switching threshold ∂A_0. Then, for* (5.14)*,*

(i) *If $S^\circ \in A_0$, then $\phi(t) \to 0$ as $t \to \infty$ if and only if $n(t) = 0$ for all $t \geq 0$.*

(ii) *If $S^\circ \notin A_0$, then $\phi(t) \to 0$ as $t \to \infty$ if and only if $n(t) = p(0) = 0$ for all $t \geq 0$.*

Proof. (i) $S^\circ \in A_0$. Then $S(t) \in A_0$ because $\alpha(S(t)) \leq \alpha(S^\circ)$ for all $t \geq 0$ and A_0 is lower-closed. And hence, the system (5.14) becomes the system (5.15).

If $n(t) = 0$ for all $t \geq 0$, then it follows from $\frac{g_p(S(t))}{d} \leq \frac{g_p(S^\circ)}{d} = R_{p0} \leq 1$ and the second equation of (5.15) that $p(t)$ converges decreasingly to some $a \geq 0$ as $t \to \infty$. Suppose that $a > 0$, then $S(t) \to S^\circ - a\Theta$ as $t \to \infty$, and hence $(0, a, 0)$

is a steady state of (5.14), which implies that $a(g_p(S^\circ - a\Theta) - d) = 0$ from the second equation of (5.15). But $\frac{g_p(S^\circ - a\Theta)}{d} < \frac{g_p(S^\circ)}{d} = R_{p0} \leq 1$, a contradiction. Thus we obtain that $p(t) \to 0$ as $t \to \infty$, which implies that $\phi(t) \to 0$ as $t \to \infty$.

On the other hand, suppose that $\phi(t) \to 0$ as $t \to \infty$. Then $S(t) \to S^\circ$ as $t \to \infty$. So there exists a neighborhood U of S such that $g_n(S(t)) - \beta(S(t)) - d \geq \frac{d(R_{n0}-1)}{2} > 0$ for all t sufficiently large. Combined with the first equation of (5.15), $\phi(t) \to 0$ yields that $n(t) = 0$ for all t sufficiently large. Note also that $\tilde{\Gamma}_{n+}$ is positively invariant with respect to (5.14). This implies that $n(t) = 0$ for all $t \geq 0$.

(ii) $S^\circ \notin A_0$. The sufficiency is obvious. The necessariness is the same as in the proof of case (ii) of Lemma 5.16. We omit it here. ☐

Lemma 5.18. *Let $R_{p0} > 1$ (and hence τ_p exists). Assume that $S^\circ - \tau_p\Theta$ does not belong to the normal-switching threshold ∂A_0 and E_p is not the only nontrivial equilibrium of* (2.3). *Then, for* (5.14), *$\phi(t) \to \sigma_p$ as $t \to \infty$ if and only if $p(0) > 0$ and $n(t) = 0$ for all $t \geq 0$.*

Proof. The sufficiency is obvious by Lemma 5.16. We now focus on the necessariness and suppose that $\phi(t) \to \sigma_p$ as $t \to \infty$.

Since E_p is not the only nontrivial equilibrium of (2.3), it then follows from Lemmas 5.7 and 5.8(i) that $g_n(S^\circ - \tau_p\Theta) - \beta(S^\circ - \tau_p\Theta) - d > 0$ and $\alpha(S^\circ - \tau_p\Theta) = 0$. As a consequence, $S^\circ - \tau_p\Theta \in \mathrm{Int}A_0$ by our assumption. We also obtain that $S(t) = S^\circ - \Sigma(t) - (n(t) + p(t))\Theta \to S^\circ - \tau_p\Theta$ as $t \to \infty$, because $E_p = (0, \tau_p)$ and $\phi(t) \to \sigma_p$ as $t \to \infty$. So there exists a neighborhood V of $S^\circ - \tau_p\Theta$ such that $S(t) \in V \subset A_0$ for all $t > 0$ sufficiently large, and hence $\alpha(S) = 0$, which implies that (5.14) along $\phi(t)$, for t sufficiently large, becomes (5.15) again. Moreover, one can choose V sufficiently small, if necessary, such that

$$g_n(S(t)) - \beta(S(t)) - d \geq \delta > 0 \tag{5.19}$$

for some $\delta > 0$ and all t sufficiently large. Note that $n(t) \to 0$ as $t \to \infty$. Then, by virtue of the first equation of system (5.15), (5.19) implies that $n(t) = 0$ for all t sufficiently large. Moreover, one can also obtain that $n(t) = 0$ for all $t \geq 0$, because $\tilde{\Gamma}_{n+}$ is positively invariant with respect to (5.14). Thus, $p(0) > 0$ (otherwise $\phi(t) \equiv 0$, a contradiction), which completes our proof. ☐

Proof of Theorem 3.3. Define $\Sigma := S^\circ - [S + (n+p)\Theta]$ and transform (2.2) into (5.14). Then the equilibria e_0, e_n, e_c, e_p of system (2.2) are corresponding to the equilibria σ_0, σ_n, σ_c, σ_p of system (5.14), respectively. Therefore, we hereafter only consider the set $\sigma = \{\sigma_0, \sigma_n, \sigma_c, \sigma_p\}$ of the equilibria of system (5.14). Let

$\phi(t) := (n(t), p(t), \Sigma(t))$ be the nontrivial solution of (5.14). Then it is easy to see that $\Sigma(t) \to 0$ as $t \to \infty$. So (5.14) is asymptotically autonomous with limit system (2.3). Let $\Omega(\phi)$ be the ω-limit set of $\phi(t)$. Then $\Omega(\phi)$ can be written as $\Omega(\phi) := (\Omega_0, 0)$, where $\Omega_0 \subset \mathbb{R}^2_+$. Then, by the theory of asymptotically autonomous systems (see e.g., [16, Theorem 1.8]), Ω_0 is a chain-recurrent set of the flow generated by (2.3). Note also that system (2.3) has no periodic orbit, homoclinic cycle or heteroclinic cycle. Consequently, $\Omega_0 = E_i$, where $i = 0, p, n$ or c, and hence, $\Omega(\phi) = \sigma_i$ for $i = 0, p, n$ or c.

(i) If $\mathcal{E} = \{E_0, E_p\}, \{E_0, E_c\}$ or $\{E_0, E_n\}$, then $\sigma = \{\sigma_0, \sigma_p\}, \{\sigma_0, \sigma_c\}$ or $\{\sigma_0, \sigma_n\}$, respectively. It follows from Lemma 5.16 that $\Omega(\phi) = \sigma_0$ if and only if $n(t) = p(t) = 0$ for all $t \geq 0$. Therefore, $\Omega(\phi) = \sigma_p, \sigma_c$ or σ_n, respectively, if $(n(0), p(0)) \neq (0, 0)$.

(ii) If $\mathcal{E} = \{E_0, E_p, E_c\}$ (resp. $\{E_0, E_p, E_n\}$), then $\sigma = \{\sigma_0, \sigma_p, \sigma_c\}$ (resp. $\{\sigma_0, \sigma_p, \sigma_n\}$). By Lemmas 5.16 and 5.18, we obtain that $\Omega(\phi) = \sigma_c$ (resp. $\Omega(\phi) = \sigma_n$) provided that $n(0) > 0$.

We now consider the solution $\phi(t)$ with the initial value $n(0) = 0$ and $p(0) > 0$. *Case (a)*: $S^\circ \in A_0$. Then $S(t) \in A_0$ for all $t \geq 0$ because $0 \leq S(t) \leq S^\circ$ and A_0 is lower-closed. As a consequence, $\alpha(S(t)) = 0$ for all $t \geq 0$, and hence, the system (5.14) becomes the system (5.15). Thus $n(0) = 0$ implies that $n(t) \equiv 0$ for all $t \geq 0$. Noticing $p(0) > 0$, it then follows from Lemma 5.18 that $\phi(t) \to \sigma_p$ as $t \to \infty$. *Case (b)*: $S^\circ \notin A_0$. Since E_p is not the only nontrivial equilibrium of (2.3), $\alpha(S^\circ - \tau_p \Theta) = 0$ by Lemmas 5.7 and 5.8(i). So one has $S^\circ - \tau_p \Theta \in \mathrm{Int} A_0$. Then there exists a unique $\hat{\tau} \in (0, \tau_p)$ such that $S^\circ - \hat{\tau}\Theta \in \partial A_0$. By virtue of the first equation of the system (5.14), it is easy to see that $\phi(t) \to \sigma_p$ if $p(0) \geq \hat{\tau}$, and $\phi(t) \to \sigma_c$ or σ_n if $0 < p(0) < \hat{\tau}$. Thus we have completed the proof of Theorem 3.3. □

Proof of Theorem 3.2. We keep using the notations in the proof of Theorem 3.3 and the fact that $\Omega(\phi) = \sigma_i$ for $i = 0, p, n$ or c.

(i) If $R_0 < 1$, then $\sigma = \{\sigma_0\}$ and all the trajectories of (5.14) are attracted to σ_0. Therefore $e = \{e_0\}$ and all the trajectories of (2.1) are attracted to e_0.

(ii) Let $R_{p0} \leq 1$ and $R_{n0} > 1$. Then by Corollary 5.10, $\mathcal{E} = \{E_0, E_n\}$ if $\beta(S^\circ - \tau_n \Theta) = 0$, or $\mathcal{E} = \{E_0, E_c\}$ if $\beta(S^\circ - \tau_n \Theta) > 0$. Hence $\sigma = \{\sigma_0, \sigma_n\}$ if $\beta(S^\circ - \tau_n \Theta) = 0$, or $\sigma = \{\sigma_0, \sigma_c\}$ if $\beta(S^\circ - \tau_n \Theta) > 0$.

If $S^\circ \in A_0$, then it follows from Lemma 5.17 (i) that $\Omega(\phi) = \sigma_0$ if and only if $n(t) = 0$ for all $t \geq 0$. Therefore, $\Omega(\phi) = \sigma_c$ or σ_n, respectively, if $n(0) > 0$. If $S^\circ \notin A_0$, then it follows from Lemma 5.17 (ii) that $\Omega(\phi) = \sigma_0$ if and only if $n(t) = p(t) = 0$ for all $t \geq 0$. Therefore, $\Omega(\phi) = \sigma_c$ or σ_n, respectively, if

$(n(0), p(0)) \neq (0, 0)$.

(iii) Let $R_{p0} > 1$ and $R_{n0} \leq 1$. Then by Corollary 5.9, $\mathcal{E} = \{E_0, E_p\}$. Hence $\sigma = \{\sigma_0, \sigma_p\}$. It follows from Lemma 5.16 that $\Omega(\phi) = \sigma_0$ if and only if $n(t) = p(t) = 0$ for all $t \geq 0$. Therefore, $\Omega(\phi) = \sigma_p$ provided that $(n(0), p(0)) \neq (0, 0)$. We have completed the proof of Theorem 3.2. □

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References

[1] J. E. Bailey and D. Ollis, "*Biochemical engineering fundamentals*", 2n ed. NY: McGraw Hill, 1986.

[2] N. Q. Balaban, J. Merrin, R. Chait and S. Leibler, Bacterial persistence as a phenotypic switch. *Science* **305** (2004) 1622-1625.

[3] J. W. Bigger, Treatment of staphylococcal infections with penicillin. Lancet ii, (1944), 497-500.

[4] A. Cunningham and P. Maas, Time lag and nutrient storage effects in the transient growth response of Chlamydomonas reinhardii in nitrogen-limited batch and continuous culture. *J. Gen. Microbiol.* **104** (1978), 227-231.

[5] T. Ferenci, 'Growth of bacterial cultures' 50 years on: towards an uncertainty principle instead of constants in bacterial growth kinetics. *Res. Microbiol.* **150** (1999), 431-438.

[6] W. T. De Groot, Modelling the multiple nutrient limitation of algal growth, *Ecol. Model* (1983), 99-119.

[7] J. P. Grover, "*Resource competition. Population and community biology*" series 19. New York: Chapman and Hall, 1997.

[8] S. R. Hansen and S. P. Hubbell, Single nutrient microbial competition: agreement between experimental and theoretical forecast outcomes. *Science* **207** (1980), 1491-1493.

[9] M. W. Hirsch, H. L. Smith and X. Zhao, Chain Transitivity, Attractivity, and Strong Repellers for Semidynamical Systems, *Journal of Dynam. and Diff. Eqns,* **13** (2001), 107-131.

[10] I. Keren, N. Kaldalu, A. Spoering, Y. P. Wang and K. Lewis, Persister cells and tolerance to antimicrobials. *FEMS Microbiol. Lett.* **230** (2004), 13-18.

[11] C. A. Klausmeier, E. Litchman and S. A. Levin, Phytoplankton growth and stoichiometry under multiple nutrient limitation, *Limnol. Oceanogr.* **49** (2004), 1463-1470.

[12] C. A. Klausmeier, E. Litchman and S. A. Levin, A model of flexible uptake of two essential resources, Journal of Theoretical Biology **246**, (2007), 278-289.

[13] A. L. Koch and C. Wang, How close to the theoretical diffusion limit do bacterial uptake systems function? *Arch. Microbiol.* **131** (1982), 36-42.

[14] S. S. Lee et al, A two state microbial growth kinetics model, *Water Res.* **9** (1975), 491-498.

[15] T. Malik and H. Smith, A Resource-based Model of Microbial Quiescence, *J. Math. Biol.* **53** (2006), 231-252.

[16] K. Mischaikow, H. Smith and H. R. Thieme, Asymptotically autonomous semiflows: Chain recurrence and Lyapounov functions, *Trans. A.M.S.* **347** (1995), 1669-1685.

[17] J. Monod, La technique de culture continué: theorie et applications, *Ann. Inst. Pasteur* **79** (1950), 390-410.

[18] H. Moyed, and K. P. Bertrand, Hipa, a newly recognized gene of Escherichia-coli K-12 that affects frequency of persistence after inhibition of murein synthesis. *J. Bacteriol.* **155** (1983), 768-775.

[19] H. Senn, U. Lendenmann, M. Snozzi, G. Hamer and T. Egli, The growth of Escherichia coli in glucose-limited chemostat cultures: a reexamination of the kinetics, *Biochim. Biophys. Acta* **1201** (1994), 424-436.

[20] H. L. Smith and B. Li, Competition for essential resources: A brief review, in "*Dynamical Systems and Their Applications in Biology*", ed. by S. Ruan et al., Fields Institute Communication, 2003.

[21] H. Smith and P. Waltman, "*The Theory of Chemostat*", Cambridge University Press, Cambridge, UK, 1995.

[22] B. Sonnleitner, Dynamic adaptation of microbes, *J. Biotechnol.* **65** (1997), 47-60.

[23] B. Tang, A. Sitomer and T. Jackson, Population dynamics and competition in chemostat models with adaptive nutrient uptake. *J Math. Biol.* **35** (1997), 453-479.

[24] D. Tilman, Resource Competition and Community Structure. Princeton University Press, Princeton, NJ, 1982.

[25] J.S. Wolfson, D. C. Hooper, G. L. McHugh, M. A. Bozza and M. N. Swartz, Mutants of Escherichia coli K-12 exhibiting reduced killing by both quinolone and beta-lactam antimicrobial agents, *Antimicrob. Agents Chemother.* **34** (1990), 1938-1943.