THE CONTINUOUS MORBIDOSTAT: A CHEMOSTAT WITH CONTROLLED DRUG APPLICATION TO SELECT FOR DRUG RESISTANCE MUTANTS

ZHENZHEN CHEN *

Department of Mathematics, Swinburne University of Technology,
Melbourne VIC 3122, Australia.

SZE-BI HSU

Department of Mathematics and National Center of Theoretical Science,
National Tsing Hua University, Hsinchu, Taiwan, R. O. C.

YA-TANG YANG

Department of Electrical Engineering, National Tsing Hua University,
Hsinchu, Taiwan, R. O. C.

(Communicated by the associate editor name)

ABSTRACT. The morbidostat is a bacteria culture device that progressively increases antibiotic drug concentration and maintains a constant challenge for study of evolutionary pathway. The operation of a morbidostat under serial transfer has been analyzed previously. In this work, the global dynamics for the operation of a morbidostat under continuous dilution is analyzed. The device switches between drug on and drug off modes according to a simple threshold algorithm. We prove the extinction and uniform persistence of all species with both forward and backward mutations. Numerical simulations for the case of logistic growth and the Hill function for drug inhibition are also presented.

1. **Introduction.** Antibiotic drug resistance is a global health problem [15]. Today, clinically important bacteria are characterized by their resistance to single or multiple drugs. Historically, penicillin-resistant Staphylococcus aureus was discovered soon after the introduction of penicillin in clinical environments [3] and still up to now the antibiotic drug resistance is still a subject of intense research [2, 7, 8, 9, 17, 23]. Although the in vivo evolution of drug resistance has been proven to be useful [2], these experiments are done retrospectively and control over the environmental factors is elusive. Recently, a more advanced chemostat [20] known as a morbidostat has been developed with the goal of imposing well controlled temporal drug profile to study the evolutionary pathway [13, 16, 18, 20]. In short, a morbidostat is a microbial selection device that progressively increase the antibiotic
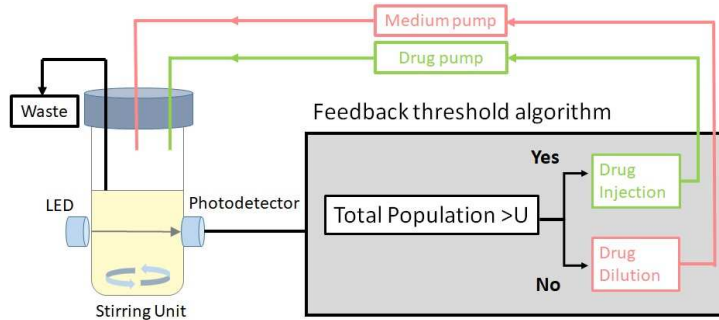
FIGURE 1. Schematic of a continuous morbidostat. There is no drug injection when the total microbes are less than threshold U. There is continuous drug injection once the total microbes reach the threshold U.

concentration to continuously challenge the microbe. During the course of evolution, drug resistant mutants prevail because of antibiotic drug resistance. In the end, daily frozen samples are analyzed retrospectively to reveal the genetic mutation events that lead to antibiotic drug resistance. In one incarnation demonstrated by Kishony's group [20], the microbial population is diluted periodically and this situation has been analyzed mathematically. In another incarnation demonstrated by Yang's group [16], the microbial population is diluted continuously and a simple threshold algorithm is used and this situation is analyzed in this work. Figure 1 shows the schematic of a continuous morbidostat device.

In [3], the cultivation of the microbes is assumed to be under serial transfer, and a simple threshold algorithm is used as feedback. In this work, we studied the case with continues dilutions and injections instead of periodical dilutions and injections. More preciously, there is no drug injection when the total microbes are less than a threshold U. It implies that without drug injection, the drug P simply decays exponentially, which is

$$\frac{dP}{dt} = -DP.$$

Thus, the drug P will die out eventually after sufficiently long time without drug injection. However, we assume that once the total microbes reach the threshold U, there is continuous drug injection in the model, which is

$$\frac{dP}{dt} = -D(P - P^0).$$

Hence the drug concentration P will converge to the input drug concentration P^0 under continuous drug injection. The operation of morbidostat can therefore be thought as switching between these two modes and the feedback threshold algorithm decide when the switching happens.

2. **Description of our models.** In the simplest scenario, we could formulate the transition from a wild type bacteria population to N mutant strains. For the drug on-drug off model with only forward mutations (see Figure 2) and both forward and backward mutations (see Figure 3), the growth dynamics with the nutrient substrate S under the influence of the drug inhibitor P are given by (1) and (2),

respectively.

$$\begin{cases} \frac{dS}{dt} = D(S^0 - S) - \frac{1}{\gamma}g_0(S)f_0(P)u - \frac{1}{\gamma}\sum_{i=1}^{N} g_i(S)f_i(P)v_i, \\ \frac{du}{dt} = (g_0(S)f_0(P)u - Du) - \sum_{k=1}^{N} q_{0k}u, \\ \frac{dv_i}{dt} = (g_i(S)f_i(P)v_i - Dv_i) + \sum_{k=0}^{i-1} q_{ki}v_k - \sum_{k=i+1}^{N} q_{ik}v_i, \\ \frac{dv_N}{dt} = (g_N(S)f_N(P)v_N - Dv_N) + \sum_{k=0}^{N-1} q_{kN}v_k, \\ \frac{dP}{dt} = \begin{cases} -DP - h_0(P)u - \sum_{i=1}^{N} h_i(P)v_i & \text{if} \quad u + \sum_{j=1}^{N} v_j < U, \\ (P^0 - P)D - h_0(P)u - \sum_{i=1}^{N} h_i(P)v_i & \text{if} \quad u + \sum_{j=1}^{N} v_j \geq U, \end{cases} \end{cases} \quad (1)$$

$$\begin{cases} \frac{dS}{dt} = D(S^0 - S) - \frac{1}{\gamma}g_0(S)f_0(P)u - \frac{1}{\gamma}\sum_{i=1}^{N} g_i(S)f_i(P)v_i, \\ \frac{du}{dt} = (g_0(S)f_0(P)u - Du) - \sum_{k=1}^{N} q_{0k}u + \sum_{k=1}^{N} \tilde{q}_{0k}v_k, \\ \frac{dv_i}{dt} = (g_i(S)f_i(P)v_i - Dv_i) + \sum_{k=0}^{i-1} q_{ki}v_k - \sum_{k=i+1}^{N} q_{ik}v_i \\ \qquad - \sum_{k=0}^{i-1} \tilde{q}_{ki}v_i + \sum_{k=i+1}^{N} \tilde{q}_{ik}v_k, \\ \frac{dv_N}{dt} = (g_N(S)f_N(P)v_N - Dv_N) + \sum_{k=0}^{N-1} q_{kN}v_k - \sum_{k=0}^{N-1} \tilde{q}_{kN}v_N, \\ \frac{dP}{dt} = \begin{cases} -DP - h_0(P)u - \sum_{i=1}^{N} h_i(P)v_i & \text{if} \quad u + \sum_{j=1}^{N} v_j < U, \\ (P^0 - P)D - h_0(P)u - \sum_{i=1}^{N} h_i(P)v_i & \text{if} \quad u + \sum_{j=1}^{N} v_j \geq U, \end{cases} \end{cases} \quad (2)$$

where $i = 1, 2, ..., N - 1$. $v_0 = u$ and v_i are the volume densities of the wild-type and mutant populations, respectively. γ denotes the yield constant, which reflects the conversion of nutrient to bacteria. In Equations (1) and (2), $g_0(S)$ and $g_i(S)$ denote the growth rates of the wild type u and mutants v_i, respectively. Furthermore, the growth rates satisfy $g_0(0) = 0$, $g'_0(S) > 0$, $g_i(0) = 0$, and $g'_i(S) > 0$ for $i = 1, 2, \cdots, N$, which implies the fact that the bacteria do not grow if there is no nutrient substrate in the bioreactor, and a higher concentration of nutrient leads to higher growth rates of bacteria. There are two important cases in the microbiology, namely, $g_i(S) = m_i(S)$ and $g_i(S) = \frac{m_i S}{a_i + S}$, $i = 0, 1, 2, ..., N$. The wild-type and mutants are assumed to consume drug while drug inhibits the growth of the bacteria. The consumption rates on drug P by the wild type bacteria u and mutants v_i are denoted by $h_0(P)$ and $h_i(P)$, respectively. The consumption functions $h_0(P)$ and $h_i(P)$ are nonnegative functions and are increasing in P. The drug inhibitions for the wild-type and mutants are described by $f_0(P)$ and $f_i(P)$, which satisfy the convention that when $P = 0$, $f_0(0) = 1$ and $f_i(0) = 1$. Meanwhile, $f'_i(P) < 0$ for $i = 1, 2, \cdots, N$ since drug with a higher-concentration leads to a stronger inhibition to both wild type and mutants. Considering the fact that mutants always have stronger resistances to the inhibitor than wild-type, and mutant v_i always have a stronger resistance to the inhibitor than mutant v_{i-1}, we have $f_{i-1}(P) \leq f_i(P)$ for $i = 1, 2, \cdots, N$. In other words, we have

$$f_0(P) \leq f_1(P) \leq \cdots \leq f_N(P). \qquad (H1)$$

It has shown [14] that in some cases, mutants remained abundant in the population or even have greater growth rate despite the absence of inhibitor, while in others the mutant has smaller growth rate than wild-type bacteria, as expected. In this work, we study the case that mutant have same or greater growth rate compared with the growth rate of wild-type bacteria in a drug free environment. Then the wild type grows more slowly than all the mutants, and mutant v_{i-1} grows more slowly than the mutant v_i in the inhibitor environment for $i = 1, 2, \cdots, N$, which is our basic assumption as follows,

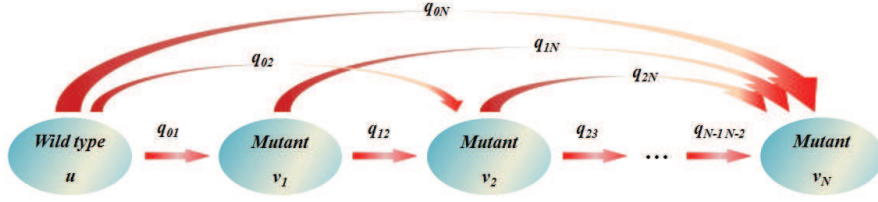$$g_0(S)f_0(P) < g_1(S)f_1(P) < \cdots < g_N(S)f_N(P) \quad \text{for} \quad S > 0, P > 0. \quad (H2)$$

FIGURE 2. Forward mutations between species. Mutant v_i mutates to mutant v_{i+1} with a forward mutation rate q_{ii+1}, and there is no backward mutations. We have $v_0 = u$ and $i = 0, 1, 2, \cdots, N-1$.
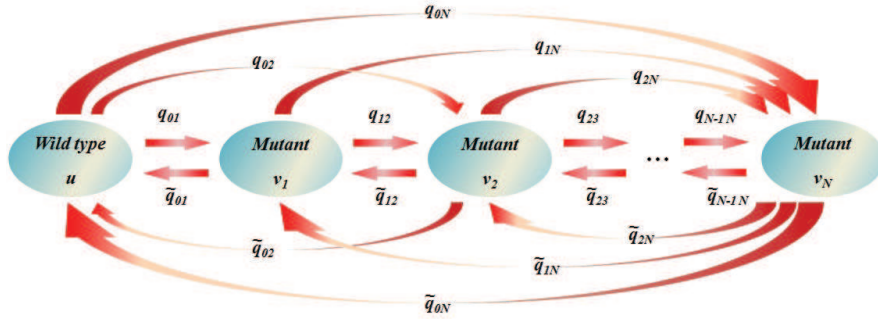

FIGURE 3. Forward-backward mutations between species. Mutant v_i mutates to mutant v_{i+1} with a forward mutation rate q_{ii+1}, while mutant v_{i+1} mutates to mutant v_i with a backward mutation rate $\tilde{q}_{ii+1}$. We have $v_0 = u$ and $i = 0, 1, 2, \cdots, N-1$.

q_{ik} denotes the forward mutation rate from mutant v_i to mutant v_k, and $\tilde{q}_{ik}$ denotes the backward mutation rate from mutant v_k to mutant v_i. We assume that the mutation rates q_{ik} and $\tilde{q}_{ki}$ are quite small compared with the difference of growth rates $g_i(S)f_i(P) - g_{i-1}(S)f_{i-1}(P)$ for all $i = 1, 2, \cdots, N$, i.e., $q_{ik}, \tilde{q}_{ki} \ll g_i(S)f_i(P) - g_{i-1}(S)f_{i-1}(P)$ for $i, k = 1, 2, \cdots, N$.

3. **Preliminary.** We first do some simplifications to make models (1) and (2) more mathematically tractable. By scaling $u \to \frac{u}{\gamma}$, $v_i \to \frac{v_i}{\gamma}$, $h_0(P) \to \gamma h_0(P)$ and $h_i(P) \to \gamma h_i(P)$ for $i = 1, 2, 3, \cdots, N$, the following scaled models of models (1) and (2) are obtained, respectively,

$$
\begin{cases}
\frac{dS}{dt} = D(S^0 - S) - g_0(S)f_0(P)u - \sum_{i=1}^{N} g_i(S)f_i(P)v_i, \\
\frac{du}{dt} = (g_0(S)f_0(P)u - Du) - \sum_{k=1}^{N} q_{0k}u, \\
\frac{dv_i}{dt} = (g_i(S)f_i(P)v_i - Dv_i) + \sum_{k=0}^{i-1} q_{ki}v_k - \sum_{k=i+1}^{N} q_{ik}v_i, \\
\frac{dv_N}{dt} = (g_N(S)f_N(P)v_N - Dv_N) + \sum_{k=0}^{N-1} q_{kN}v_k, \\
\frac{dP}{dt} = \begin{cases} -DP - h_0(P)u - \sum_{i=1}^{N} h_i(P)v_i & \text{if} \quad u + \sum_{j=1}^{N} v_j < U, \\ (P^0 - P)D - h_0(P)u - \sum_{i=1}^{N} h_i(P)v_i & \text{if} \quad u + \sum_{j=1}^{N} v_j \geq U, \end{cases} \\
S(0) \geq 0, u(0) \geq 0, P(0) \geq 0, v_i(0) \geq 0, i = 1, 2, ..., N.
\end{cases}
\tag{3}
$$

$$\begin{cases} \frac{dS}{dt} = D(S^0 - S) - g_0(S)f_0(P)u - \sum_{i=1}^{N} g_i(S)f_i(P)v_i, \\ \frac{du}{dt} = (g_0(S)f_0(P)u - Du) - \sum_{k=1}^{N} q_{0k}u + \sum_{k=1}^{N} \tilde{q}_{0k}v_k, \\ \frac{dv_i}{dt} = (g_i(S)f_i(P)v_i - Dv_i) + \sum_{k=0}^{i-1} q_{ki}v_k - \sum_{k=i+1}^{N} q_{ik}v_i \\ \qquad - \sum_{k=0}^{i-1} \tilde{q}_{ki}v_i + \sum_{k=i+1}^{N} \tilde{q}_{ik}v_k, \\ \frac{dv_N}{dt} = (g_N(S)f_N(P)v_N - Dv_N) + \sum_{k=0}^{N-1} q_{kN}v_k - \sum_{k=0}^{N-1} \tilde{q}_{kN}v_N, \\ \frac{dP}{dt} = \begin{cases} -DP - h_0(P)u - \sum_{i=1}^{N} h_i(P)v_i & \text{if} \quad u + \sum_{j=1}^{N} v_j < U, \\ (P^0 - P)D - h_0(P)u - \sum_{i=1}^{N} h_i(P)v_i & \text{if} \quad u + \sum_{j=1}^{N} v_j \geq U, \end{cases} \\ S(0) \geq 0, u(0) \geq 0, P(0) \geq 0, v_i(0) \geq 0, i = 1, 2, ..., N. \end{cases} \quad (4)$$

For scaled models (3) and (4), we have the following results.

Lemma 3.1. *Let $(S(t), u(t), v_1(t), ..., v_N(t), P(t))$ be the solution of the I.V.P (3) and (4). Then the solution exists and is unique for all $t \geq 0$. Furthermore,$S(t) + u(t) + \sum_{i=1}^{N} v_i(t) \to S^0$ as $t \to \infty$.*

Proof. We note that although the differential equation for P is discontinuous, the solution $(S(t), u(t), v_1(t), ..., v_N(t), P(t))$ is continuous. We claim that the maximal interval of existence is $[0, \infty)$. If not, then the solution will blow up in finite time. However, the solution has a priori bound, namely, $P(t) \leq P^{(0)}$.

$S(t) + u(t) + \sum_{i=1}^{N} v_i(t) \leq \max\{S^{(0)}, S(0) + u(0) + \sum_{i=1}^{N} v_i(0)\}$ as long as the solution exists. By a theorem of global existence [12], the maximal interval of existence of the solution $(S(t), u(t), v_1(t), ..., v_N(t), P(t))$ is $[0, \infty)$.

Adding the first $N+2$ equations of models (3) and (4) together respectively, and define

$$C(t) = S(t) + u(t) + \sum_{i=1}^{N} v_i(t),$$

then the following single equation is obtained,

$$C'(t) = S'(t) + u'(t) + \sum_{i=1}^{N} v_i'(t) = D(S^0 - S) - Du - D\sum_{i=1}^{N} v_i = S^0 - DC(t).$$

It follows at once that,

$$C(t) = S^0 + \mathcal{O}(e^{-Dt}),$$

which leads to $C(t) \to S^0$ as $t \to \infty$. That completes our proof. $\qquad \square$

Lemma 3.2. *Let $(H1)$ and $(H2)$ hold and $(S(t), u(t), v_1(t), ..., v_N(t), P(t))$ be the solution of the I.V.P (3) and (4). Then there exists $\delta > 0$ such that $S(t) \geq \delta > 0$ for all $t \geq 0$.*

Proof. For drug on-drug off models (3) and (4), we have

$$\begin{aligned} \frac{dS}{dt} &= D(S^0 - S) - g_0(S)f_0(P)u - \sum_{i=1}^{N} g_i(S)f_i(P)v_i \\ &\geq D(S^0 - S) - g_N(S)f_N(P)(u + \sum_{i=1}^{N} v_i) \\ &\geq D(S^0 - S) - g_N(S)(u + \sum_{i=1}^{N} v_i). \end{aligned}$$

From Lemma 3.1, for any $\varepsilon > 0$, there exists large enough $t_0 = t_0(\varepsilon) > 0$ such that $t \geq t_0$ implies that

$$S^0 - S(t) - \varepsilon < u(t) + \sum_{i=1}^{N} v_i(t) < S^0 - S(t) + \varepsilon. \tag{5}$$

Therefore, for $t \in [t_0, +\infty]$, we have

$$\frac{dS}{dt} > D(S^0 - S) - g_N(S)(S^0 - S(t) + \varepsilon). \tag{6}$$

Let $S^*(t)$ be the solution of the following system,

$$\frac{dS^*}{dt} = D(S^0 - S^*) - g_N(S^*)(S^0 - S^*(t) + \varepsilon). \tag{7}$$

Then we have $S(t) > S^*(t)$ for all $t \in [t_0, +\infty]$. Choose $\delta > 0$ such that $\delta < \min_{0 \leq t \leq +\infty} \{S^*(t)\}$ and $\delta < \min_{0 \leq t \leq t_0} \{S(t)\}$, we then have $S(t) > \delta$ for all $t \geq 0$. That completes our proof. $\square$

4. Dynamics of drug on-drug off model with only forward mutations.
For the forward mutations model (3), we will show that only the last mutant v_N survives in the long-term by the following result.

Lemma 4.1. *Let $(H1)$ and $(H2)$ hold. The wild type bacteria u and mutants v_i, where $1 \leq i \leq N-1$, go extinction in the long-term. More precisely, $u(t) \to 0$, $v_i(t) \to 0$ as $t \to \infty$ for $1 \leq i \leq N-1$.*

Proof. From model (3), if letting $\omega(t) = u(t) + v_1(t) + v_2(t) + \cdots + v_{N-1}(t)$, it is easy to check that

$$\frac{d\omega}{dt} = g_0(S)f_0(P)u + g_1(S)f_1(P)v_1 + \cdots + g_{N-1}(S)f_{N-1}(P)v_{N-1} - D\omega - \sum_{k=0}^{N-1} \tilde{q}_{kN} v_k.$$

Since $g_0(S)f_0(P) \leq g_1(S)f_1(P) \leq \cdots \leq g_{N-1}(S)f_{N-1}(P)$ for all $0 < S \leq S^0$ and $0 \leq P \leq P^0$, we have

$$\frac{d\omega}{dt} \leq -D\omega + g_{N-1}(S)f_{N-1}(P)\omega - \sum_{k=0}^{N-1} \tilde{q}_{kN} v_k,$$

which implies

$$\frac{1}{\omega}\frac{d\omega}{dt} \leq -D + g_{N-1}(S)f_{N-1}(P) - - \sum_{k=0}^{N-1} \tilde{q}_{kN} \frac{v_k}{\omega}.$$

Since

$$\frac{dv_N}{dt} = (g_N(S)f_N(P)v_N - Dv_N) + \sum_{k=0}^{N-1} \tilde{q}_{kN} v_k,$$

we have

$$\frac{1}{\omega}\frac{d\omega}{dt} - \frac{1}{v_N}\frac{dv_N}{dt}$$

$$\leq -D + g_{N-1}(S)f_{N-1}(P) - \sum_{k=0}^{N-1} \tilde{q}_{kN} \frac{v_k}{\omega} - g_N(S)f_N(P) + D - \sum_{k=0}^{N-1} \tilde{q}_{kN} \frac{v_k}{v_N}$$

$$\leq g_{N-1}(S)f_{N-1}(P) - g_N(S)f_N(P) \leq -\eta,$$

where $\eta = \min_{\delta \leq S \leq S^0, 0 \leq P \leq P^0}(g_N(S)f_N(P) - g_{N-1}(S)f_{N-1}(P))$. We have η is always positive from $(H2)$. Since

$$\frac{1}{\omega}\frac{d\omega}{dt} - \frac{1}{v_N}\frac{dv_N}{dt} = \frac{d\ln\frac{\omega}{v_N}}{dt},$$

straightforward calculation shows that $\omega(t) \leq \frac{\omega(0)}{v_N(0)}v_N(t)e^{-\eta t}$. It implies that $\omega(t) \to 0$ as $t \to \infty$ since $v_N(t)$ is bounded. In other words, $u(t) \to 0$, $v_i(t) \to 0$ as $t \to \infty$, $1 \leq i \leq N-1$. That completes our proof. □

From Lemmas 3.1 and 4.1, the forward mutations model (3) can be reduced to the following limiting system [19, 11].

$$\begin{cases} \frac{dS}{dt} = D(S^0 - S) - g_N(S)f_N(P)v_N, \\ \frac{dv_N}{dt} = g_N(S)f_N(P)v_N - Dv_N, \\ \frac{dP}{dt} = \begin{cases} -DP - h_N(P)v_N & \text{if } v_N < U, \\ (P^0 - P)D - h_N(P)v_N & \text{if } v_N \geq U. \end{cases} \end{cases} \quad (8)$$

We note from [19] Appendix F, [22] Theorem 1.2.2 and [10], it can be shown that the solution of original systems (3) and (4) has the same asymptotic behabiour as those in the limiting system provided the acyclic condition is satisfied and the solution of the corresponding limiting system converges to an equilibrium. We denote $(S(t), v_N(t), P(t))$ as the solution of the limiting system (8) with the initial values $S(0) > 0, v_N(0) > 0, P(0) > 0$. The long-term dynamics of the limiting system is our concern. We will analyse the global dynamics of the limiting system (8) from the following three cases.

4.1. **Case 1: Drug off case of the limiting system.** If $v_N(t) < U$ for all $t > 0$, then drug P follows $\frac{dP}{dt} = -DP - h_N(P)v_N$ all the time, which is the drug off case. In this case, the limiting system (8) becomes the following system,

$$\begin{cases} \frac{dS}{dt} = D(S^0 - S) - g_N(S)f_N(P)v_N, \\ \frac{dv_N}{dt} = g_N(S)f_N(P)v_N - Dv_N, \\ \frac{dP}{dt} = -DP - h_N(P)v_N. \end{cases} \quad (9)$$

It is easy to check that $P(t) \to 0$ in system (9) as $t \to \infty$. Then system (9) can be reduced to the following limiting system since $\lim_{t \to \infty} f_N(P) = 1$,

$$\begin{cases} \frac{dS}{dt} = D(S^0 - S) - g_N(S)v_N, \\ \frac{dv_N}{dt} = g_N(S)v_N - Dv_N. \end{cases} \quad (10)$$

Straightforward calculation shows that model (10) has at most two equilibria, a boundary equilibrium $E_1(S^0, 0)$ and a positive equilibrium $E^* = (S^*, v_N^*)$, where

$$S^* = g_N^{-1}(D), \quad v_N^* = S^0 - S^*.$$

E^* is physically meaningful if $g_N(S^0) > D = g_N(S^*)$, which always holds true since $S^* \in (0, S^0)$ and $g_N'(S) > 0$. From [1, 11], the following result is obtained.

Theorem 4.2. *Assume $S^{(0)} > S^* = \lambda_N$ and let $(S(t), v_N(t))$ be the solution of the limiting system (10). The the positive equilibrium $E(S^*, v_N^*)$ is globally asymptotically stable.*

Theorem 4.3. *Assume that $v_N(t) < U$ for all sufficiently large t for the system (9), then $\lim_{t \to \infty}(S(t), v_N(t), P(t)) = (S^*, v_N^*, 0)$ for all $S(0) \in (0, S^0)$, $v_N(0) \in (0, S^0)$ and $P(0) \in (0, P^0)$.*

4.2. **Case 2: Drug on case of the limiting system.** If $v_N(t) > U$ for all $t > 0$, then drug P follows $\frac{dP}{dt} = (P^0 - P)D - h_N(P)v_N$ all the time, which is the drug on case. In this case, the limiting system (8) could be reduced into the following limiting system,

$$\begin{cases} \frac{dS}{dt} = D(S^0 - S) - g_N(S)f_N(P)v_N, \\ \frac{dv_N}{dt} = g_N(S)f_N(P)v_N - Dv_N, \\ \frac{dP}{dt} = (P^0 - P)D - h_N(P)v_N. \end{cases} \tag{11}$$

Since $S + v_N \to S^0$ as $t \to \infty$ by lemmas 3.1 and 4.1, then system (11) can be reduced to the following limiting system,

$$\begin{cases} \frac{dv_N}{dt} = g_N(S^0 - v_N)f_N(P)v_N - Dv_N, \\ \frac{dP}{dt} = (P^0 - P)D - h_N(P)v_N. \end{cases} \tag{12}$$

We will first prove that every periodic solution of the limiting system (12) is orbitally stable. Let

$$F(v_N, P) = g_N(S^0 - v_N)f_N(P)v_N - Dv_N$$

and

$$G(v_N, P) = (P^0 - P)D - h_N(P)v_N,$$

if we assume $(v_N(t), P(t))$ is a periodic solution of model (12) with period T, and $(\bar{v}_N, \bar{P})$ is the equilibrium of the limiting system (12), then we have

$$\int_0^T \frac{\partial F}{\partial v_N} + \frac{\partial G}{\partial P} dt$$

$$= \int_0^T (g_N(S^0 - v_N)f_N(P) - D) - v_N(f_N(P)g_N'(S^0 - v_N)) + (-D - h_N'(P)v_N)dt$$

$$= \int_0^T -v_N(f_N(P)g_N'(S^0 - v_N)) + (-D - h_N'(P)v_N)dt.$$

It is easy to check that $\int_0^T \frac{\partial F}{\partial v_N} + \frac{\partial G}{\partial P} dt < 0$. Since $(\bar{v}_N, \bar{P})$ is locally asymptotically stable and every periodic solution (if it exists) is orbitally stable, then from [4], there is no periodic solution and thus by Poincare Bendixson Theorem, we have $(v_N(t), P(t)) \to (\bar{v}_N, \bar{P})$ as $t \to \infty$. Moreover, $(\bar{v}_N, \bar{P})$ is the unique equilibrium of the limiting system (12), which satisfies

$$\begin{cases} g_N(S^0 - \bar{v}_N)f_N(\bar{P}) - D = 0, \\ (P^0 - \bar{P})D - h_N(\bar{P})\bar{v}_N = 0. \end{cases}$$

Therefore, for limiting system (11), we have the following result.

Theorem 4.4. *Assume that $v_N(t) > U$ for all sufficiently large t for the system (8), then $\lim_{t\to\infty}(S(t), v_N(t), P(t)) = (S^0 - \bar{v}_N, \bar{v}_N, \bar{P})$ for all $S(0) \in (0, S^0)$, $v_N(0) \in (0, S^0)$ and $P(0) \in (0, P^0)$.*

From Theorems 4.3 and 4.4, we know that

$$v_N^* = S^0 - S^* = S^0 - g_N^{-1}(D).$$

And $\bar{v}_N$ satisfies

$$g_N(S^0 - \bar{v}_N)f_N(\bar{P}) = D,$$

which implies that $\bar{v}_N = S^0 - g_N^{-1}(\frac{D}{f_N(\bar{P})}) > 0$. It can be checked that $v_N^* > \bar{v}_N$ since $f_N(\bar{P}) < 1$ and $g_N'(S) > 0$.

4.3. **Case 3: oscillation of the limiting systems.** If $v_N(t)$ oscillates around U, then the drug P follows the rule $\frac{dP}{dt} = -DP - h_N(P)v_N$ if $v_N(t) < U$ at some time t, otherwise it follows the rule $\frac{dP}{dt} = (P^0 - P)D - h_N(P)v_N$. Therefore, the inhibitor concentration oscillates in this case as the system is trying to maintain constant bacteria density v_N through feedback. We also demonstrate it by numerical simulation in Section 6.

Based on the analysis in the above 3 cases, we state our main result about the drug on-drug off model with only forward mutations in the following theorem.

Theorem 4.5. *For the drug on-drug off model with only forward mutations (3), we have*

(i) *if $U > v_N^*$, then the equilibrium $(S^0 - v_N^*, 0, 0, \cdots, v_N^*, 0)$ attracts all positive initial data, in other words, $\lim_{t \to \infty} (S(t), v_1(t), v_2(t), \cdots, v_N(t), P(t)) = (S^0 - v_N^*, 0, 0, \cdots, v_N^*, 0)$. The biological interpretation is that the concentrations of the nutrient S and mutant v_N will stay at $S^0 - v_N^*$ and v_N^*, respectively after a long-term. While drug P will dilute out when time t is sufficiently large.*

(ii) *if $U < v_N^{**}$, where $v_N^{**} = S^0 - g_N^{-1}(\frac{D}{f_N(P^0)})$, then the equilibrium $(S^0 - \bar{v}_N, 0, 0, \cdots, \bar{v}_N, \bar{P})$ is globally attracting, in other words, we have $\lim_{t \to \infty} (S(t), v_1(t), v_2(t), \cdots, v_N(t), P(t)) = (S^0 - \bar{v}_N, 0, 0, \cdots, \bar{v}_N, \bar{P})$. It implies that the wild type u and mutants $v_1, v_2, \cdots, v_{N-1}$ will die out eventually, while the concentrations of the nutrient S, mutant v_N and drug P will stay at $S^0 - \bar{v}_N$, $\bar{v}_N$ and $\bar{P}$, respectively.*

(iii) *If $\bar{v}_N < U < v_N^*$, then $v_N(t)$ oscillates around U.*

It is easy to verify that $v_N^{**} < \bar{v}_N < v_N^*$.

Proof. (i) It suffices to show $v_N(t) < U$ for t large. From the equation of v_N and $f(P) \le 1$ for $P > 0$, we have that for $\varepsilon > 0$ small and t large,

$$\begin{aligned} \frac{dv_N}{dt} &= g_N(S)f_N(P) - Dv_N \\ &\le g_N(S) - Dv_N \\ &\le \left(g_N(S^{(0)} - v_N + \varepsilon) - D \right) v_N. \end{aligned}$$

It follows that

$$v_N(t) \le v_{N,\varepsilon}^* = S^0 - \lambda_{N,\varepsilon}^*,$$

where $\lambda_{N,\varepsilon}^* \to \lambda_N^*$ as $\varepsilon \to 0$. Therefore, if $U > v_N^*$, then $v_N(t) \le v_{N,\varepsilon}^* < U$ for t large.

(ii) It suffices to show $v_N(t) > U$ for t large. Since $0 < P < P^0$ for t large,

$$\begin{aligned} \frac{dv_N}{dt} &= g_N(S)f_N(P) - Dv_N \\ &\ge g_N(S)f_N(P^0) - Dv_N \\ &\ge \left(g_N(S^0 - v_N - \varepsilon)f_N(P^0) - D \right) v_N. \end{aligned}$$

Then we have

$$v_N(t) \ge v_{N,\varepsilon}^{**} = S^0 - \varepsilon - g_N^{-1}(\frac{D}{f_N(P^0)}),$$

where $\lim_{\varepsilon \to 0} v_{N,\varepsilon}^{**} = v_N^{**}$. Hence, if $U < v_N^{**}$, then $v_N(t) \ge v_{N,\varepsilon}^{**} > v_N^{**} - \varepsilon > U$ for t large.

(iii) Suppose it is not true, then we have $v_N(t) < U$ or $v_N(t) > U$ for t large. If

$v_N(t) < U$, then from Theorem 4.3, we have $v_N(t) \to v_N^*$ as $t \to \infty$. This leads to $v_N^* \leq U$, which is a contradiction. Similarly, if $v_N(t) > U$ for t large, then from Theorem 4.4, $v_N(t) \to \bar{v}_N$ as $t \to \infty$. This leads to $\bar{v}_N \geq U$, which is also a contradiction. Hence, $v_N(t)$ oscillates around U. □

Remark 1. We conjecture that if $v_N^{**} < U < \bar{v}_N$, the conclusion of (ii) holds. We will verify this conjecture in Section 6.

5. **Dynamics of drug on-drug off model with forward-backward mutations.**

Lemma 5.1. *For model (4), if $v_j(t) \to 0$ as $t \to \infty$ for some j, $0 \leq j \leq N$, then $v_k(t) \to 0$ as $t \to \infty$ for all $k \neq j$, and $0 \leq k \leq N$. This result indicates that wild type and all the mutants will go extinction if any species of them goes extinction in the long-term.*

Proof. We may assume $0 < j < N$, the proofs for the cases with $j = 0$ or $j = N$ are the same as that for $0 < j < N$. Since $v_j(t) \to 0$ as $t \to \infty$, then for any $\epsilon > 0$, there exists $N_j > 0$ such that $0 < v_j(t) < \epsilon$ for $t \geq N_j$.

From model (4), we have

$$\frac{dv_j}{dt} = \left(g_j(S)f_j(P) - D - \sum_{k=i+1}^{N} q_{jk} - \sum_{k=0}^{j-1} \tilde{q}_{kj} \right) v_j + \sum_{k=0}^{j-1} q_{kj}v_k + \sum_{k=j+1}^{N} \tilde{q}_{jk}v_k. \quad (13)$$

It follows that

$$\begin{aligned}
\frac{d^2v_j}{dt^2} &= \left(g_j(S)f_j(P) - D - \left(\sum_{k=i+1}^{N} q_{jk} + \sum_{k=0}^{j-1} \tilde{q}_{kj} \right) \right) v_j' \\
&\quad + (g_j'(S)S'f_j(P) + g_j(S)f_j'(P)P')v_j + \sum_{k=0}^{i-1} q_{ki}v_k' + \sum_{k=i+1}^{N} \tilde{q}_{ik}v_k'.
\end{aligned}$$

From Lemma 3.2, we have $S(t) \geq \delta > 0$, $0 < P(t) < P^0$ for all $t > 0$.

It is easy to verify that from the equations of (3) and Lemma 3.1, we have

$$|S'| \leq \left(D + \max_{\delta \leq S \leq S^0, 0 \leq P < P^0} \{f_N(P)g_N(S)\} \right) S^0,$$

and $|v_j'| \leq CS^0$, where C is a constant satisfies

$$C = D + \max_{\delta \leq S \leq S^0, 0 \leq P < P^0} \{f_N(P)g_N(S)\} + \sum_{k=i+1}^{N} q_{jk} + \sum_{k=0}^{j-1} \tilde{q}_{kj} + \sum_{k=0}^{j-1} q_{kj} + \sum_{k=j+1}^{N} \tilde{q}_{jk}.$$

Similarly, $|v_k'|$ is bounded for $k = 0, 1, 2, ..., N$. Since $f_j'(P) < 0$, and $|g_j(S)f_j(P)| \leq \max_{\delta \leq S \leq S^0, 0 \leq P < P^0} \{f_N(P)g_N(S)\}$, we have $|\frac{d^2v_j}{dt^2}| \leq M_j$ for some $M_j > 0$, which implies that $|v_j''|$ is also bounded.

Therefore, from [5], we have $v_j' \to 0$ as $t \to \infty$, since $v_j \to 0$ as $t \to \infty$, and v_j'' is bounded. It implies $\sum_{k=0}^{j-1} q_{kj}v_k + \sum_{k=j+1}^{N} \tilde{q}_{jk}v_k \to 0$ as $t \to \infty$ from (13).

Therefore, $v_k \to 0$ as $t \to \infty$ for $k \neq j$. That completes our proof. □

For the drug on drug off model with both forward and backward mutations, we have the following result.

Theorem 5.2. *Consider the drug on drug off model (4), we have two cases for the long-term dynamics of the model,*

(i) *When $g_0(S^0) \leq D$, the wild type bacteria u and mutants v_i, $i = 1, 2, 3, \cdots, N$ go extinction, there is no inhibitor P exist in the device. The nutrient S will persist in a concentration of S^0 in the long-term.*

(ii) *When $g_0(S^0) > D$, the wild type bacteria u and mutants v_i, $i = 1, 2, 3, \cdots, N$ coexist, and the most resistant microbe v_N dominates the rest of the other species provided the mutation rates $\tilde{q}_i$ are sufficiently small.*

Proof. From Lemma 5.1, either all species go extinction or all species coexist.

(i) If all species go extinction, then the system (4) can be reduced to the following limiting system,

$$\begin{cases} \frac{dS}{dt} = D(S^0 - S), \\ \frac{dP}{dt} = -DP. \end{cases} \tag{14}$$

Hence we have $S \to S^0$, and $P \to 0$ as $t \to \infty$. From the second equation in the drug on and off model (4), it follows that $g_0(S^0) \leq D$. Therefore, for the drug on drug off model (4), when $g_0(S^0) \leq D$, we have the wild type bacteria u and mutants v_i, $i = 1, 2, 3, \cdots, N$ go extinction. Furthermore, there is no inhibitor P exist while the nutrient S will persist at the concentration of S^0 in the long-term.

(ii) When $g_0(S^0) > D$, then all species coexist. By continuous dependence on parameter $\tilde{q}_{ij}$, $i = 0, 1, ..., N - 1$, $j = 1, 2, ..., N$ and Lemma 4.1, we have the most resistant microbe dominates the rest of the other species provided the mutation rates $\tilde{q}_{ij}$, $i = 0, 1, ..., N - 1$, $j = 1, 2, ..., N$ are sufficiently small. □

6. **Numerical simulations.** In this section, we carry out numerical simulations with realistic parameters to verify our theoritical results.

For simplicity, we assume that both the wild type and the mutants have equal uptake function and the growth takes the logistic form, namely,

$$g_0(S) = g_i(S) = mS, \quad i = 1, 2, 3, \cdots, N.$$

The consumption functions $h_0(P)$ and $h_i(P)$ are assumed to take the Holling Type II function,

$$h_0(P) = h_i(P) = \frac{rP}{a + P}, \quad i = 1, 2, 3, \cdots, N.$$

We assume the functions $f_0(P)$ and $f_i(P)$ take the Hill function form of order L, which are

$$\begin{cases} f_0(P) & = \frac{1}{1 + \left(\frac{P}{K_0}\right)^L}, \\ f_i(P) & = \frac{1}{1 + \left(\frac{P}{K_i}\right)^L}, \end{cases} \tag{15}$$

where $i = 1, 2, 3, \cdots, N$. Note that the order L stems from the allosteric cooperativity of the drug inhibition [21]. The drug inhibition effects depend on the detailed mechanism. For example, they can result from the binding of the antibiotic drug to the metabolic enzyme, which synthesizes the key precursor of biomass production of the bacteria. Taking trimethoprim (TMP) as a specific example, this antibiotic binds to dihydrofolate reductase (DHFR), an enzyme that controls the biosynthesis of folic acid. The mutation of the gene encoding DHFR will modify the binding affinity of TMP [20]. The parameters K_0 and K_i in (15) can actually

be extracted from the experimental values of IC_{50}, defined as the drug inhibitor concentration at which the growth rate is 50% of the maximal growth rate. The sample volume in the culture vessel of the morbidostat is 10 ml, and the confluent density of $EColi$ is typically 10^9 cell/ml. For simplicity, we assume that the morbidostat operates at around 10% of the confluent density, which is set by the threshold population U. We can conveniently set the yield constant γ to be 1, so that one unit of substrate density will transform to one unit of bacteria. With the volume units set to 0.1 nl, the input substrate $S^0 = 10$ corresponds to 10^8 cell/ml. The constants K_0 and K_i in the drug inhibition function are set to 1 and 10^i, and the order in the Hill function $L = 1, 2, 3$. We first consider the forward mutation rates $q = 10^{-2}\text{hr}^{-1}$. Typically, the dilution ratio $D = 0.9$. The growth rate is set by the constant m to be 0.3hr^{-1} in the logistic growth function. The initial conditions are $S(0) = 10, u(0) = 0.01, v(0) = 0$, and $P(0) = 0$. We demonstrate our theoretical results by conducting the following simulations.

6.1. **Three mutants with only forward mutations.** We first assume that there is wild type u and three mutants v_1, v_2 and v_3 in the bioreactor and there is no backward mutation. Then we have the drug on-drug off model in the following form,

$$
\begin{cases}
\frac{dS}{dt} = D(S^0 - S) - g_0(S)f_0(P)u - \sum_{i=1}^3 g_i(S)f_i(P)v_i, \\
\frac{du}{dt} = (g_0(S)f_0(P)u - Du) - q_{01}u - q_{02}u - q_{03}u, \\
\frac{dv_1}{dt} = (g_1(S)f_1(P)v_1 - Dv_1) + q_{01}u - q_{12}v_1 - q_{13}v_1, \\
\frac{dv_2}{dt} = (g_2(S)f_2(P)v_2 - Dv_2) + q_{02}u + q_{12}v_1 - q_{23}v_2, \\
\frac{dv_3}{dt} = (g_3(S)f_3(P)v_3 - Dv_3) + q_{03}u + q_{13}v_1 + q_{23}v_2, \\
\frac{dP}{dt} = \begin{cases} -DP - h_0(P)u - \sum_{i=1}^3 h_i(P)v_i & \text{if } u + v_1 + v_2 + v_3 < U, \\ (P^0 - P)D - h_0(P)u - \sum_{i=1}^3 h_i(P)v_i & \text{if } u + v_1 + v_2 + v_3 \geq U, \end{cases}
\end{cases}
\tag{16}
$$

Straightforward calculation shows that $v_3^* = 7.000$ and $\bar{v}_3 = 6.327$ by the parameters given above and $P^0 = 10$, $r = 0.5$ and $a = 0.5$. We verified the three cases of our theoretical analysis in Section 4 using numerical simulations as follows.

(i) If $U = 8$, then it satisfies $U > v_3^*$ as $v_3^* = 7.000$, which is Case 1 in Section 4, we have $v_3(t) \to v_3^* = 7.000$ and $P(t) \to 0$ as $t \to \infty$. More precisely, the fixed point $(S^*, 0, 0, 0, v_3^*, 0) = (3.000, 0, 0, 0, 7.000, 0)$ attracts all positive initial data, which agrees well with the theoretical analysis, please see Figure 4.

(ii) If $U = 5$, then it satisfies $U < v_3^{**}$ as $v_3^{**} = 6.000$, which is Case 2 in Section 4. In this case, we have $v_3(t) \to \bar{v}_3 = 6.327$ and $P(t) \to \bar{P} = 6.728$ as $t \to \infty$. More precisely, the unique fixed point $(\bar{S}, 0, 0, 0, \bar{v}_3, \bar{P}) = (3.673, 0, 0, 0, 6.327, 6.728)$ is globally attracting in this case, please see Figure 5.

(iii) If $U = 6.5$, then we have $\bar{v}_3 < U < v_3^*$, which is Case 3 according to Section 4. From the simulation results, we can see the inhibitor concentration P oscillates between 0 and $\bar{P}$, which is $P \in (0, 6.728)$, while the bacteria density v_3 will be maintained at nearly constant around U, please see Figure 6.

Remark 2. If $U = 6.1$, we have $v_3^{**} < U < \bar{v}_3$, since $v_3^{**} = 6.000$ $\bar{v}_3 = 6.327$. In this case, Figure 7 shows that the conclusion of (ii) holds in Section 4, which verifies our conjecture in Remark1.
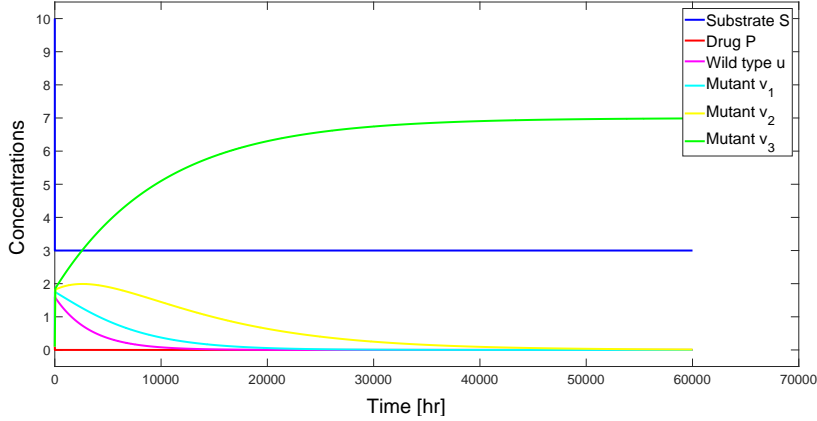
FIGURE 4. Cell, substrate, and inhibitor densities of system (3) when $U = 8$. The wild type u, mutants v_1, v_2 and inhibitor P go extinction in the drug on drug off model, while mutant v_3 and substrate S persist at fixed values in the long-term. In this figure, we take $S^0 = 10$, $D = 0.9$, $P^0 = 10$, $q_{01} = q_{02} = q_{03} = q_{12} = q_{13} = q_{23} = 10^{-4}$, $m = 0.3$, $r = 0.5$, $a = 0.5$, $L = 1$, $K_1 = 1$, $K_2 = 3$, $K_3 = 10$ and $K_4 = 30$.
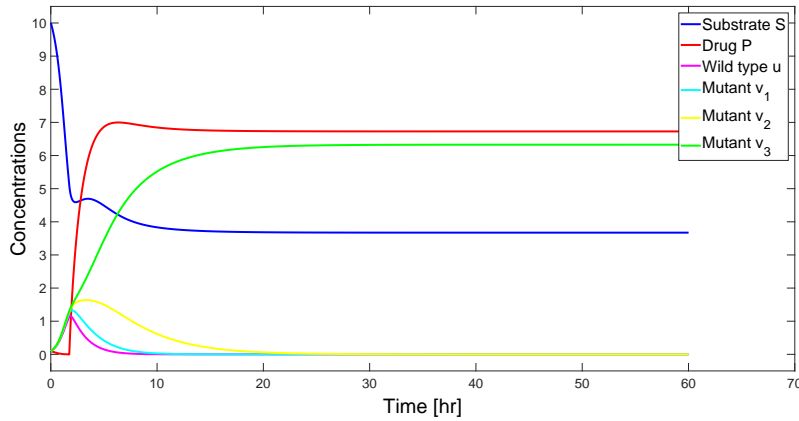

FIGURE 5. Cell, substrate, and inhibitor densities of system (3) when $U = 2$. The wild type u and mutants v_1, v_2 go extinction in the drug on drug off model, while mutant v_3, substrate S, and inhibitor P persist at fixed values in the long-term. In this figure, we take $S^0 = 10$, $D = 0.9$, $P^0 = 10$, $q_{01} = q_{02} = q_{03} = q_{12} = q_{13} = q_{23} = 10^{-4}$, $m = 0.3$, $r = 0.5$, $a = 0.5$, $L = 1$, $K_1 = 1$, $K_2 = 3$, $K_3 = 10$ and $K_4 = 30$.

6.2. **Three mutants with forward-backward mutations.** We then assume there is wild type u and two mutants v_1 and v_2 in the bioreactor, and assume
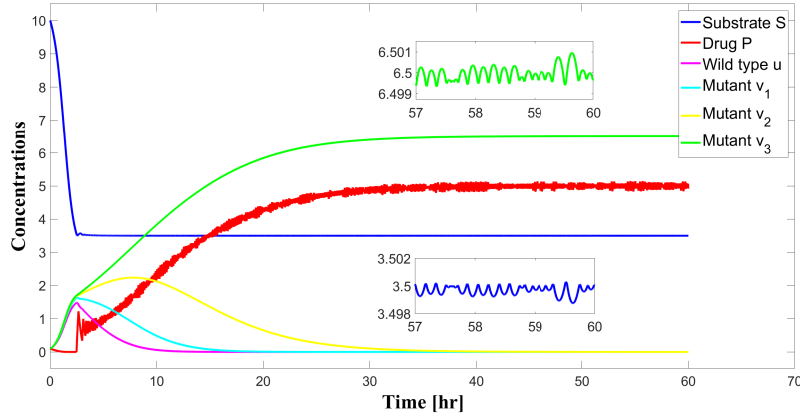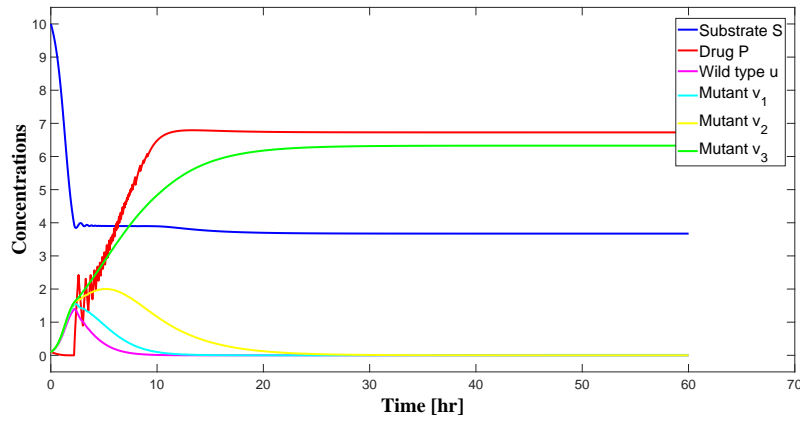
FIGURE 6. Cell, substrate, and inhibitor densities of system (3) when $U = 6.5$. The wild type u and mutants v_1, v_2 go extinction in the drug on drug off model, while mutant v_3, substrate S, and inhibitor P oscillate in the long-term. The inset figure shows the density of mutant v_3 (green) and concentration of the Substrate S (blue) in the long-term. In this figure, we take $S^0 = 10$, $D = 0.9$, $P^0 = 10$, $q_{01} = q_{02} = q_{03} = q_{12} = q_{13} = q_{23} = 10^{-4}$, $m = 0.3$, $r = 0.5$, $a = 0.5$, $L = 1$, $K_1 = 1$, $K_2 = 3$, $K_3 = 10$ and $K_4 = 30$.

FIGURE 7. Cell, substrate, and inhibitor densities of system (3) when $U = 6.1$. The wild type u and mutants v_1, v_2 go extinction in the drug on drug off model, while mutant v_3, substrate S, and inhibitor P persist at fixed values in the long-term. In this figure, we take $S^0 = 10$, $D = 0.9$, $P^0 = 10$, $q_{01} = q_{02} = q_{03} = q_{12} = q_{13} = q_{23} = 10^{-4}$, $m = 0.3$, $r = 0.5$, $a = 0.5$, $L = 1$, $K_1 = 1$, $K_2 = 3$, $K_3 = 10$ and $K_4 = 30$.
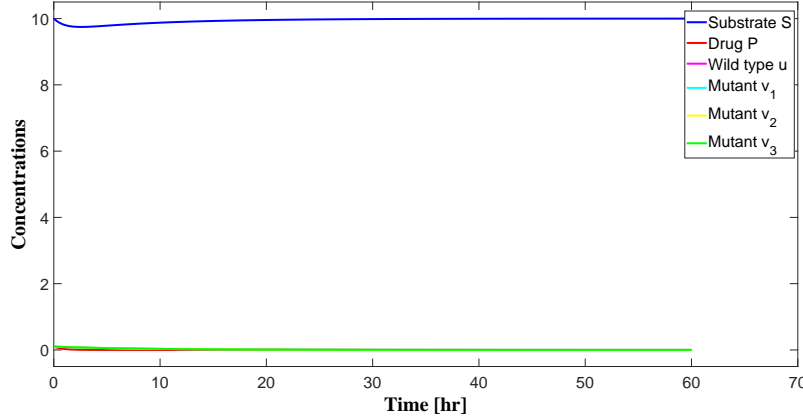
FIGURE 8. Extinction of all the microbes of system (4). In this case, all the cells and inhibitor go to extinction in the drug on drug off model, while the substrate persists at a fixed level. In this figure, we take $S^0 = 10$, $D = 0.9$, $P^0 = 10$, $U = 5$, $q_{01} = q_{02} = q_{03} = q_{12} = q_{13} = q_{23} = 10^{-4}$, $\tilde{q}_{01} = \tilde{q}_{02} = \tilde{q}_{03} = \tilde{q}_{12} = \tilde{q}_{13} = \tilde{q}_{23} = 10^{-4}$, $m = 0.08$, $r = 0.5$, $a = 0.5$, $L = 1$, $K_1 = 1$, $K_2 = 3$, $K_3 = 10$ and $K_4 = 30$.

that the mutants are with forward-backward mutations. Then we have the drug on drug off model in the following form,

$$
\begin{cases}
\frac{dS}{dt} = D(S^0 - S) - g_0(S)f_0(P)u - \sum_{i=1}^{3} g_i(S)f_i(P)v_i, \\
\frac{du}{dt} = (g_0(S)f_0(P)u - Du) - \sum_{i=1}^{3} q_{0i}u + \sum_{i=1}^{3} \tilde{q}_{0i}v_i, \\
\frac{dv_1}{dt} = (g_1(S)f_1(P)v_1 - Dv_1) + q_{01}u - q_{12}v_1 - q_{13}v_1 + \tilde{q}_{12}v_2 - \tilde{q}_{01}v_1 + \tilde{q}_{13}v_3, \\
\frac{dv_2}{dt} = (g_2(S)f_2(P)v_2 - Dv_2) + q_{02}u + q_{12}v_1 - q_{23}v_2 - \tilde{q}_{12}v_2 - \tilde{q}_{02}v_2 + \tilde{q}_{23}v_3, \quad (17) \\
\frac{dv_3}{dt} = (g_3(S)f_3(P)v_3 - Dv_3) + q_{03}u + q_{13}v_1 + q_{23}v_2 - \tilde{q}_{03}v_3 - \tilde{q}_{13}v_3 - \tilde{q}_{23}v_3, \\
\frac{dP}{dt} = \begin{cases} -DP - h_0(P)u - \sum_{i=1}^{3} h_i(P)v_i & \text{if} \quad u + v_1 + v_2 + v_3 < U, \\ (P^0 - P)D - h_0(P)u - \sum_{i=1}^{3} h_i(P)v_i & \text{if} \quad u + v_1 + v_2 + v_3 \geq U, \end{cases}
\end{cases}
$$

The simulation results of system (17) show that either all the cells go to extinction (see Figure 8) or persist (see Figure 9 and Figure 10) in the drug on drug off model, which verifies our theoretical results in Theorem 5.2. In Figure 9, the most resistant mutant dominates the final population with the coexistence of a small fraction of other species (wild type u, mutants v_1 and v_2) due to the assumption of small mutation rates and big dilution rate D as compared to the growth rates $g_i(S^0)$ ($i = 0, 1, 2, 3$). In Figure 10, the coexistence of all the species is obvious when $\tilde{q}_{01} = \tilde{q}_{02} = \tilde{q}_{03} = \tilde{q}_{12} = \tilde{q}_{13} = \tilde{q}_{23} = 0.05$.

REFERENCES

[1] R. A. Armstrong and R. McGehee, Competitive exclusion, *Am. Nat.*, **115** (1980), 151–170.

[2] M. Barber, Infection by penicillin resistant Staphylococci, *Lancet*, **2** (1948), 641–644.

[3] Z. Chen, S.B. Hsu and Y.T. Yang, The Morbidostat: a bio-reactor that promotes selection for drug resistance in bacteria, *SIAM J. Appl. Math.*, **77** (2017), 470–499.

[4] K.S. Cheng, S.B. Hsu and S.S. Lin, Some results on global stability of a predator-prey system, *J. Math. Biology.*, **12** (1981), 115–126.
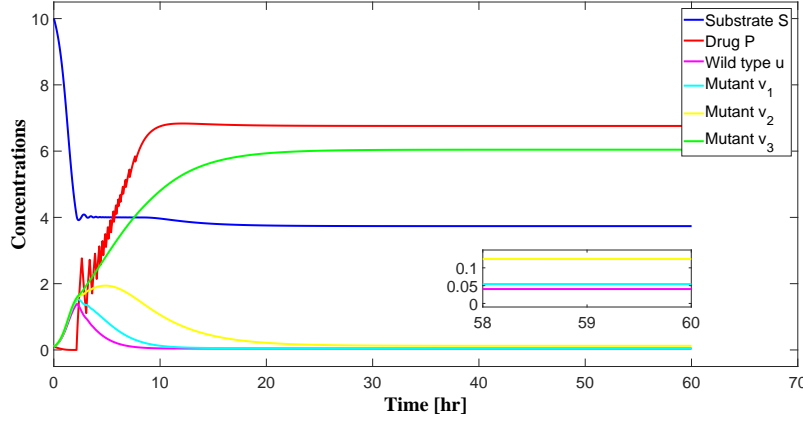
FIGURE 9. Persistence of the all the microbes of system (4). In this case, all the microbes persist in the drug on drug off model. However, the most resistant microbe dominates all the species. In this figure, we take $S^0 = 10$, $D = 0.9$, $P^0 = 10$, $U = 6$, $q_{01} = q_{02} = q_{03} = q_{12} = q_{13} = q_{23} = 0.005$, $\tilde{q}_{01} = \tilde{q}_{02} = \tilde{q}_{03} = \tilde{q}_{12} = \tilde{q}_{13} = \tilde{q}_{23} = 0.005$, $m = 0.3$, $r = 0.5$, $a = 0.5$, $L = 1$, $K_1 = 1$, $K_2 = 3$, $K_3 = 10$ and $K_4 = 30$.
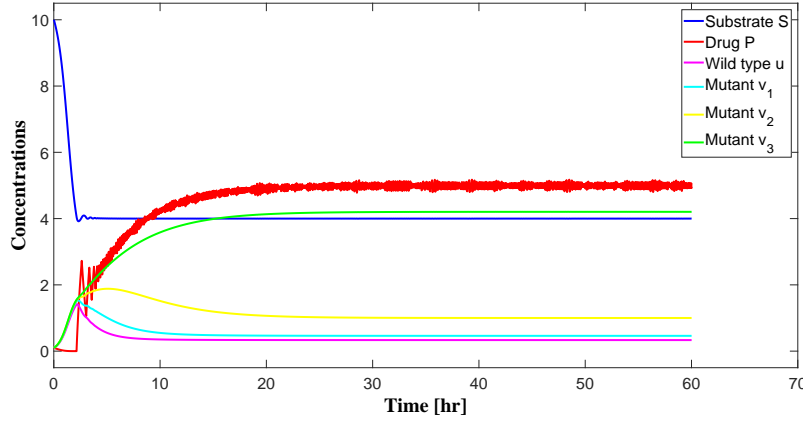

FIGURE 10. Persistence of the all the microbes of system (4). In this case, all the microbes persist in the drug on drug off model. In this figure, we $S^0 = 10$, $D = 0.9$, $P^0 = 10$, $U = 6$, $q_{01} = q_{02} = q_{03} = q_{12} = q_{13} = q_{23} = 0.05$, $\tilde{q}_{01} = \tilde{q}_{02} = \tilde{q}_{03} = \tilde{q}_{12} = \tilde{q}_{13} = \tilde{q}_{23} = 0.05$, $m = 0.3$, $r = 0.5$, $a = 0.5$, $L = 1$, $K_1 = 1$, $K_2 = 3$, $K_3 = 10$ and $K_4 = 30$

[5] W. A. Coppel, Stability and asymptotic behavior of differential equations, Health. Math. Monograph, 1965.
[6] J. B. Deris, M. Kim, Z. Zhang, H. Okano, R. Hermsen, A. Groisman, and T. Hwa, The innate growth bistability and fitness landscapes of antibiotic resistant bacteria, *Science*, **342** (2013), 1237435.

[7] M. Dragosits and D. Mattanovich, Adaptive laboratory evolution - principles and applications for biotechnology, *Microb. Cell Fact.*, **12** (2013), 64.

[8] R. Hermsen, J. B. Deris, and T. Hwa, On the rapidity of antibiotic resistance evolution facilitated by a concentration gradient, *Proc. Natl. Acad. Sci.*, **109** (2012), 10775–10780.

[9] R. Hermsen and T. Hwa, Sources and Sinks: A stochastic model of evolution in heterogeneous environments, *Phys. Rev. Lett.*, **105** (2015), 248104.

[10] W. M. Hirsch, H. L. Smith and X. Q. Zhao, Chain transitivity, attractivity and strong repellers for semidynamical systems, *J. Dynam. Differential. Equations*, **13** (2001), 107–131.

[11] S.B. Hsu, Limiting behavior for competing species system, *SIAM J. Applied Math.*, **34** (1978), 760–763.

[12] S.B. Hsu, Ordinary differential equations with applications, World Scientific Press, 2013, 2nd Edition.

[13] S. B. Hsu and P. E. Waltman, Analysis of a model of two competitors in a chemostat with an external inhibitor, *SIAM J. Applied Math.*, **52** (1992), 528–540.

[14] A. H. Melnyk, A. Wong and R. Kassen, The fitness costs of antibiotic resistance mutations, *Evol. Appl.*, **8** (2015), 273–283.

[15] S. B. Levy and B. Marshall, Antibiotic resistance worldwide: causes, challenges, and responses, *Nat. Med.*, **10** (2004), s122–s129.

[16] P. Liu, Y. T. Lee, C. Y. Wang, and Y.-T. Yang, Design and use of a low cost, automated Morbidostat for adaptive evolution of bacteria under antibiotic drug selection, *J. Vis. Exp.*, **115** (2016), e54426, DOI:10.3791/54426.

[17] M. Mwangi, S. W. Wu, Y. Zhou, et al., Tracking the in vivo evolution of jultidrug resistance in staphylococus aureus by whole genome sequencing, *Pro. Natl. Acad. Sci.*, **104** (2007), 9451–9456.

[18] H. L. Smith, Bacterial competition in serial transfer culture, *Math. Biosci.*, **229** (2011), 149–159.

[19] H. L. Smith and P. E. Waltman,The theory of the chemostat, Cambridge University Press, Cambridge, 1995.

[20] E. Toprak, A. Veres, J.B. Mitchel, D. L. Hartl, and R.Kishony, Evolutionary paths to antibiotic resistance under dynamically sustained drug selection, *Nat. Genet.*, **44** (2012), 101–106.

[21] A. Uri, An introduction to system biology design principles of biological Circuits, Chapman and Hall Taylor and Francis Group, London, 2007.

[22] X. Q. Zhao, Dynamical systems in population biology, Springer, New York, 2015, 2nd Edition.

[23] Q. Zhang, G. Lambert, D. Liao, H. Kim, K. Robin, C. Tung, N. Pourmand,and R. H. Austin, Acceleration of emergence of bacterial antibiotic resistance in connected microenvironment, *Science*, **333** (2011), 1764–1767.

E-mail address: zhenzhenchen1221@yahoo.com
E-mail address: sbhsu@math.nthu.edu.tw
E-mail address: ytyang@ee.nthu.edu.tw