

# Theory of a microfluidic serial dilution bioreactor for growth of planktonic and biofilm populations

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## Abstract

We present the theory of a microfluidic bioreactor with a two-compartment growth chamber and periodic serial dilution. In the model, coexisting planktonic and biofilm populations exchange by adsorption and detachment. The criteria for coexistence and global extinction are determined by stability analysis of the global extinction state. Stability analysis yields the operating diagram in terms of the dilution and removal ratios, constrained by the plumbing action of the bioreactor. The special case of equal uptake function and logistic growth is analytically solved and explicit growth curves are plotted. The presented theory is applicable to generic microfluidic bioreactors with discrete growth chambers and periodic dilution at discrete time points. Therefore, the theory is expected to assist the design of microfluidic devices for investigating microbial competition and microbial biofilm growth under serial dilution conditions.

keywords: chemostat, serial transfer dilution, biofilm, microfluidics, bioreactors, and coexistence

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## 1 Introduction

Bacterial cultivation is a fundamental technique in microbiology. Traditional bacterial culture methods fall into two categories; serial dilution transfer and chemostat

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cultivation. In serial dilution transfer, the microbes are grown in a closed environment, such as a test tube with limited nutrient. After a designated period, a portion of the microbes is transferred to another container with sufficient nutrient to maintain another growth cycle. The chemostat maintains a nearly steady population for microbiology study (Novick and Szilard 1950; Smith and Waltman 1995). Since its invention by Novick and Szilard in 1950, the chemostat has become a standard microbiological laboratory technique. Meanwhile, serial dilution transfer has been considered for competition studies involving multiple species (Stewart and Levin 1973; Smith 2011). Traditional milliliter- to liter-scale bioreactors consume excessive growth medium and are very laborious to maintain and operate. However, with recent technological advances in microfluidics, nano- to micro-scale microfluidic bioreactors have become available for enzyme yield optimization, systems biology, bioenergy generation and similar investigations (Hegab et al. 2013). In particular, nanoliter microfluidics with large scale integration complexity have afforded the design of microfluidic chips with almost arbitrary complexity (Melin and Quake 2007). The growth of microorganisms in a nanoliter-scale chemostat with  $N$  compartments is governed by the low Reynolds number of the fluid flow (Balagadde et al 2005; Balagadde et al. 2008). Balagadde et al. (2005) realized a microfluidic chemostat with a nanoliter working volume and 16 compartments. Such a device approaches the traditional continuous-dilution chemostat, and has been used to characterize *Escherichia coli* engineered with a synthetic genetic circuit for population control.

Recently, we constructed a similar microfluidic bioreactor with 2 compartments and a serial dilution step that mimics macro-scale serial dilution transfer. This bioreactor sustains a long-term bacterial culture (Chiang and Yang; unpublished). Motivated by the operation of this chip design, we here develop the mechanistic theory of the device, providing a foundation for more advanced applications such as drug resistance and mutation studies. For illustrative purposes, we consider a 2-compartment bioreactor, periodically diluted at discrete times  $T_j = jT$  ( $j= 0, 1, 2, \dots$ ), but the results can be easily generalized to the  $N$ -compartment case. The operation of our device is shown in Figure 1. Figure 1(a) schematizes the 2-compartment serial dilution bioreactor ( $N = 2$ ) and the two serial dilution steps ( $M = 2$ ). Each compartment is alternately cleaned and refilled with nutrient. After the clean and refill step, the partition between the two compartments is opened, and the liquid containing the microbial culture in one compartment

mixes with that of the other. Mixing is repeated when the other compartment is cleaned. Figure 1(b) shows the plumbing design of the microfluidic device. In this configuration, the ring-shaped growth chamber is partitioned into upper and lower compartments, which are alternately cleaned and refilled with nutrient medium. The nutrients and microbes are mixed by an integrated peristaltic pump installed in the growth chamber. Following the mixing, the device enters the growth cycle. The microbial growth is monitored through optical microscopy and cell numbers are obtained by processing the images of the microbes in the sampling volume.

In another context, the high surface-to-volume ratio of nanoliter containers greatly enhances biofilm growth, thus the present device is an effective tool for biofilm study. Biofilms constitute a large fraction of natural microbial populations and are implicated in many bacterial infections (Ghannoum and O'toole 2004; Costerton et al. 1999). To understand biofilm phenomena, we must develop a robust theory for the coexistence of biofilm and planktonic cells. As a clinical example, the bacteria detached from biofilm infection sites can cause persistent symptoms even in patients treated with antibiotics. The theory can provide a framework for quantitative analysis of various scenarios, whereas the microfluidic device provides an *in vitro* model of biofilm infection. When designing biochips for bacterial detection, engineers tend to avoid the interference from biofilms. The proposed theory can provide guidelines for eliminating biofilm growth.

## **2 The simple chemostat model with wall growth: description of our model**

We now describe the theory of a simple chemostat with planktonic and biofilm growth (Pylyugin and Waltman 1999). A planktonic population  $u$  coexisting with a biofilm population  $w$  under a single nutrient source  $S$  can be modeled by the following system of differential equations:

$$\begin{aligned}
\frac{dS}{dt} &= -\frac{1}{\gamma}f_u(S)u - \frac{\delta}{\gamma}f_w(S)w \\
\frac{du}{dt} &= (f_u(S) - \alpha)u + \delta\beta w \\
\frac{dw}{dt} &= (f_w(S) - \beta)w + \frac{\alpha u}{\delta}
\end{aligned} \tag{2.1}$$

The initial conditions are constrained by  $S(0) > 0$ ,  $u(0) > 0$ ,  $w(0) > 0$ , where  $u$  is the volume density of the planktonic population and  $w$  is the surface density of the biofilm population. The surface-to-volume ratio  $\delta = A/V$  provides the proper scaling between  $u$  and  $w$  (here,  $A$  and  $V$  are the surface area and volume of the growth chamber, respectively.)  $\gamma$  is the yield constant. In Equation (2.1), the growth rates of planktonic and biofilm cells are given by  $f_u(S)$  and  $f_w(S)$  respectively, where  $f_u(0) = 0$ ,  $f'_u(S) > 0$ ,  $f_w(0) = 0$ , and  $f'_w(S) > 0$ . These two populations are exchanged by adsorption and detachment.  $\alpha$  is the adsorption rate from the planktonic population to the biofilm population and  $\beta$  is the detachment rate from the biofilm to the planktonic population.

The resetting of the initial condition at  $t = T_j = jT$  ( $j=0, 1, 2, \dots$ ) can be written as

$$\begin{aligned}
S(T_j^+) &= \eta S(T_j^-) + FS^{(0)} \\
u(T_j^+) &= \eta u(T_j^-) \\
w(T_j^+) &= \theta_w w(T_j^-) \\
0 < \theta_w < 1, \quad F &= 1 - \eta
\end{aligned} \tag{2.2}$$

$\eta$  is the dilution ratio of the existing substrate and planktonic population immediately before the dilution step. A fraction  $F$  of the existing substrate is removed and replaced with fresh input substrate  $S^{(0)}$ . This dilution step contributes a term  $FS^{(0)}$  and the  $F$  and  $\eta$  sum to one.  $\theta_w$  characterizes the fraction of the biofilm population that remains during the dilution step; that is, the fraction that adheres to the wall.  $T_j^-$  and  $T_j^+$  denote the times immediately before and after the dilution step is carried out at  $t = jT$ . Mathematically,  $T_j^-$  and  $T_j^+$  are defined as follows:

$$T_j^- = \lim_{\epsilon \rightarrow 0^-} jT + \epsilon \quad T_j^+ = \lim_{\epsilon \rightarrow 0^+} jT + \epsilon$$

For our device, two two-fold dilution steps are executed as shown in Figure 1(a) and Figure 1(b) so the substrate and the population is reset as

$$\begin{aligned} S(T_j^+) &= \frac{1}{4}S(T_j^-) + \frac{3}{4}S^{(0)} \\ u(T_j^+) &= \frac{1}{4}u(T_j^-) \\ w(T_j^+) &= \theta_w w(T_j^-) \end{aligned} \tag{2.3}$$

### 3 Stability analysis and threshold condition for extinction

In this section, we investigate the global behavior of the model presented in previous section and find out the condition such that the microbial population will be washed out. This is done by analyzing the stability around the a global extinct state defined by  $E_0 = (\tilde{S}, 0, 0)$ .

Define the map  $P : (S_0, u_0, w_0) \rightarrow (S_1, u_1, w_1)$  induced by the system of differential equations in Equation (2.1) for  $0 < t < T$  with initial condition  $S(0) = S_0 > 0$ ,  $u(0) = u_0 \geq 0$ ,  $w(0) = w_0 \geq 0$  and the resetting of initial conditions in Equation (2.2) at  $t = T^+$  :

$$\begin{cases} S_1 &= S(T^+) = \eta S(T^-) + FS^{(0)} \\ u_1 &= u(T^+) = \eta u(T^-) \\ w_1 &= w(T^+) = \theta_w w(T^-) \end{cases} \tag{3.1}$$

Consider the extinction fixed point of  $P$ ,  $E_0 = (\tilde{S}, 0, 0)$ . When  $u_0 = u(0) = 0$  and  $w_0 = w(0) = 0$ , from Equation (2.1) it follows that  $u(t) \equiv 0$ ,  $w(t) \equiv 0$ , and  $S(t) \equiv S_0$  for  $0 \leq t \leq T$ . From the resetting of the initial conditions in (2.2),we

have  $u_1 = 0$ ,  $w_1 = 0$ ,  $\tilde{S} = \eta\tilde{S} + FS^{(0)}$  or  $\tilde{S} = \frac{FS^{(0)}}{1-\eta} = S^{(0)}$ .

Let  $x_0 = (S_0, u_0, w_0)$  and define  $\Phi_t(x_0) = (S(t, x_0), u(t, x_0), w(t, x_0))$  be the solution with initial condition  $x_0$ ,  $P$  can be written as

$$P(S_0, u_0, w_0) = L \circ \Phi_T(S_0, u_0, w_0) = (S_1, u_1, w_1) \quad (3.2)$$

where

$$L(S, u, w) = \begin{pmatrix} \eta S + FS^{(0)} \\ \eta u \\ \theta_w w \end{pmatrix} \quad (3.3)$$

Then

$$D_{x_0}P(x_0) = D_{x_0}P(S_0, u_0, w_0) = \begin{pmatrix} \eta & 0 & 0 \\ 0 & \eta & 0 \\ 0 & 0 & \theta_w \end{pmatrix} D_{x_0}\Phi_T(x_0) \quad (3.4)$$

The details of the stability analysis calculation is given in Appendix. Here we simply give the final result. The stability condition for the extinction fixed point  $E_0 = (\tilde{S}, 0, 0)$  is given by

$$\eta\theta_w e^{\lambda_1 T} e^{\lambda_2 T} < 1 \quad (3.5)$$

$$\eta V_{11}(T) + \theta_w V_{22}(T) < 1 + \eta\theta_w e^{\lambda_1 T} e^{\lambda_2 T} \quad (3.6)$$

$\lambda_1, \lambda_2$  are the roots of characteristic polynomial given by

$$g(\lambda) = \lambda^2 - [(f_u(\tilde{S}) - \alpha) + (f_w(\tilde{S}) - \beta)]\lambda + (f_u(\tilde{S}) - \alpha)(f_w(\tilde{S}) - \beta) - \alpha\beta$$

and

$$\begin{aligned} V_{11}(T) &= A_1 e^{\lambda_1 T} + A_2 e^{\lambda_2 T} \\ V_{22}(T) &= A_2 e^{\lambda_1 T} + A_1 e^{\lambda_2 T} \end{aligned}$$

Equivalently we can rewrite the condition for the global extinction in a concise form which is much easier to understand. In terms of coefficients  $A_1$  and  $A_2$ , (3.6) can be rewritten as:

$$A_1(1 - \eta e^{\lambda_1 T})(1 - \theta_w e^{\lambda_2 T}) + A_2(1 - \eta e^{\lambda_2 T})(1 - \theta_w e^{\lambda_1 T}) > 0 \quad (3.7)$$

The coefficients  $A_1$  and  $A_2$  are given by

$$A_1 = \frac{-(\lambda_1 - (f_w(\tilde{S}) - \beta))(\lambda_2 - (f_u(\tilde{S}) - \alpha))}{\alpha\beta - (\lambda_1 - (f_w(\tilde{S}) - \beta))(\lambda_2 - (f_u(\tilde{S}) - \alpha))}$$

$$A_2 = \frac{\alpha\beta}{\alpha\beta - (\lambda_1 - (f_w(\tilde{S}) - \beta))(\lambda_2 - (f_u(\tilde{S}) - \alpha))}$$

$$0 \leq A_1, A_2 \leq 1, \quad A_1 + A_2 = 1$$

From this expression, we can construct an operating diagram. If  $\alpha = 0$  and  $\beta = 0$ , the system describes exploitative competition between two populations. Smith studied two bacterial strains competing for nutrient in a serial transfer dilution culture with the same dilution rate  $\eta = \theta_w$  (Smith 2011). The system then reduces to a monotonic dynamical system with three possible outcomes, namely, competitive exclusion, stable coexistence and bistability (Hsu et al. 1996). If either  $\alpha = 0$  or  $\beta = 0$ , this formula are greatly simplified because  $A_2 = 0$ . For clarity, we give the criteria in three separate cases.

Case 1:  $\alpha > 0, \beta = 0$

From (3.5) and (3.7), the condition for extinction or total washout is given by

$$\begin{aligned} \eta e^{\lambda_1 T} < 1 \quad \text{and} \quad \theta_w e^{\lambda_2 T} < 1 \\ \lambda_1 &= f_u(S^{(0)}) - \alpha \\ \lambda_2 &= f_w(S^{(0)}) \end{aligned} \tag{3.8}$$

We can further divide this case into two subcases:

Subcase 1a:  $f_u(S^{(0)}) \leq \alpha$ .

Then  $\lambda_1 \leq 0, \lambda_2 > 0$ . In (3.8),  $\eta e^{\lambda_1 T} < 1$  always hold. Thus the stability condition becomes

$$\theta_w e^{f_w(S^{(0)})T} < 1 \quad , \quad \text{equivalently,} \quad \theta_w < e^{-f_w(S^{(0)})T} \quad \text{or} \quad T < \frac{\ln(\frac{1}{\theta_w})}{f_w(S^{(0)})} \tag{3.9}$$

Subcase 1b:  $f_u(S^{(0)}) > \alpha$

Then the stability condition

$$\eta e^{(f_u(S^{(0)}-\alpha)T} < 1, \quad \theta_w e^{f_w(S^{(0)})T} < 1$$

can be written as

$$\eta < e^{-(f_u(S^{(0)}-\alpha)T}, \quad \theta_w < e^{-f_w(S^{(0)})T} \quad \text{or} \quad T < \min\left\{\frac{\ln(\frac{1}{\theta_w})}{f_w(S^{(0)})}, \frac{\ln(\frac{1}{\eta})}{f_u(S^{(0)}) - \alpha}\right\} \quad (3.10)$$

Case 2:  $\alpha = 0, \beta > 0$

As in the Case 1,  $A_1 = 1, A_2 = 0$ . The stability condition is

$$\eta e^{\lambda_1 T} < 1 \quad \text{and} \quad \theta_w e^{\lambda_2 T} < 1 \quad \text{with} \quad \lambda_1 = f_u(S^{(0)}) \quad \lambda_2 = f_w(S^{(0)}) - \beta \quad (3.11)$$

There are two subcases:

Subcase 2a:  $f_w(S^{(0)}) \leq \beta$ .

Then  $\lambda_1 > 0, \lambda_2 \leq 0$  and the stability condition becomes

$$\eta e^{(f_u(S^{(0)})T} < 1 \quad , \quad \text{equivalently,} \quad \eta < e^{-f_u(S^{(0)})T} \quad \text{or} \quad T < \frac{\ln(\frac{1}{\eta})}{f_u(S^{(0)})} \quad (3.12)$$

Subcase 2b:  $f_w(S^{(0)}) > \beta$

The stability condition

$$\eta e^{f_u(S^{(0)})T} < 1, \quad \theta_w e^{(f_w(S^{(0)}-\beta)T} < 1$$

can be written as

$$\eta < e^{-(f_u(S^{(0)})T}, \quad \theta_w < e^{-(f_w(S^{(0)}-\beta)T} \quad \text{or} \quad T < \min\left\{\frac{\ln(\frac{1}{\eta})}{f_u(S^{(0)})}, \frac{\ln(\frac{1}{\theta_w})}{f_w(S^{(0)}) - \beta}\right\} \quad (3.13)$$

Case 3:  $\alpha > 0, \beta > 0$

For fixed parameters  $\alpha, \beta, T$  we will interpret the stability conditions (3.5), (3.7) of the extinction state  $(\tilde{S}, 0, 0)$  by the  $\eta - \theta_w$  operation diagrams. First we rewrite (3.5) as  $\theta_w < \frac{\epsilon^{-\lambda_1 T} \epsilon^{-\lambda_2 T}}{\eta}$ . (3.7) can be rewritten as

$$\theta_w < G(\eta) \quad \text{if} \quad A_1 e^{-\lambda_1 T} + A_2 e^{-\lambda_2 T} > \eta$$

where

$$G(\eta) = \frac{1 - \eta(A_1 e^{\lambda_1 T} + A_2 e^{\lambda_2 T})}{(A_1 e^{\lambda_2 T} + A_2 e^{\lambda_1 T}) - \eta e^{(\lambda_1 + \lambda_2) T}} \quad (3.14)$$

We note that  $G(\eta)$  is strictly decreasing and

$$G(0) = \frac{1}{A_1 e^{\lambda_2 T} + A_2 e^{\lambda_1 T}}, G\left(\frac{1}{A_1 e^{\lambda_1 T} + A_2 e^{\lambda_2 T}}\right) = 0$$

Furthermore, we have this property

$$\lim_{\eta \rightarrow A_1 e^{-\lambda_1 T} + A_2 e^{-\lambda_2 T}} G(\eta) = \pm\infty,$$

Of course,  $G(\eta)$  must be greater than zero to be meaningful so we need to restrict  $\eta$  to be smaller than the zero of  $G(\eta)$ , namely  $\eta < \frac{1}{A_1 e^{\lambda_1 T} + A_2 e^{\lambda_2 T}}$ . We restrict ourselves to the case of  $\lambda_1 > 0, \lambda_2 > 0$  and plot a representative operating diagram in Figure 2. The condition that  $\lambda_1 > 0, \lambda_2 > 0$  guarantees that  $G(0) < 1$  and the zero of  $G(\eta)$  is also less than one. One can see the boundary of the extinction and coexistence regions defined by the curve  $G(\eta)$ .

In the following, we state TheoremA for the threshold dynamics. In TheoremB under the assumptions (3.15), (3.16) we establish global stability for the positive fixed point  $E_c := (s^*, u^*, w^*)$ . The details of the proofs are given in the Appendix.

**Theorem A.** *For  $\alpha \geq 0, \beta \geq 0$  but not both identically zero.*

1. If (3.5) and (3.7) hold then the extinction fixed point  $E_0 = (\tilde{S}, 0, 0)$  of  $T$ -periodic map  $P$  is locally stable. Furthermore  $E_0$  attracts each positive initial condition,  $(S_0, u_0, w_0)$  such that  $P^n(S_0, u_0, w_0) \rightarrow (\tilde{S}, 0, 0)$  as  $n \rightarrow \infty$
2. If neither (3.5) or (3.7) hold then  $E_0$  becomes unstable and the map  $P$  is uniformly persistent, i.e. there exists  $\rho > 0$  such that  $S_n \geq \rho$ ,  $u_n \geq \rho$ ,  $w_n \geq \rho$ , for all  $n$ . Moreover there exists a positive fixed point  $E_c = (S^*, u^*, w^*)$ .

Next we consider a special case. Assume

$$(i) \eta = \theta_w \tag{3.15}$$

and

$$(ii) f_u(S) = f_w(S) := f(S) \text{ for all } S \geq 0. \tag{3.16}$$

**Theorem B.** For  $\alpha \geq 0, \beta \geq 0$  but not both identically zero. Let  $E_0$  be unstable and (3.15), (3.16) hold. Then the set  $W = \{(S, u, w) : \gamma S + u + w = \gamma S^{(0)}\}$  is positively invariant under the map  $P$ . Furthermore there exists a unique positive fixed point  $E_c = (S^*, u^*, w^*) \in W$  attracts each positive initial condition  $(S_0, u_0, w_0)$  such that  $P^n(S_0, u_0, w_0) \rightarrow E_c$  as  $n \rightarrow \infty$

## 4 Analytic solution for the case of equal uptake function

In this section, we will consider the special case of equal uptake function.

$$f_u(S) = f_w(S) = f(S) \tag{4.1}$$

This assumption is appropriate if the nutrient diffuses through the biofilm and planktonic cells at approximately the same rate. It may be invalidated in mature biofilm, which impedes nutrient diffusion into the inner parts. Mathematically, this assumption permits a closed-form solution, which is very useful for generating the growth curve, and for visualizing how the extinction or coexistence of two species emerges over the parameter space of interest. Assuming logistic growth, the uptake function takes the form

$$f(S) = mS \tag{4.2}$$

In the calculation below and without loss of generality, we conveniently set the starting time in a given growth cycle as  $t = 0$  and find the growth curve within such a growth cycle. Finding the exact solution of Equation (2.1) can be done by utilizing a conservation law. In addition, with the assumption of equal yield and uptake function for both populations, there is an inherent exchange symmetry between  $u$  and  $\delta w$ . Equation (2.1) is invariant with the exchange of these quantities

$$\begin{aligned}
u &\rightarrow \delta w \\
\delta w &\rightarrow u \\
\alpha &\rightarrow \beta \\
\beta &\rightarrow \alpha
\end{aligned}
\tag{4.3}$$

Also note that Equation (3.1) has a conservative quantity  $C(t) = \gamma S(t) + u(t) + \delta w(t)$  that can be used to simplify the calculation. We can easily show that

$$\begin{aligned}
C'(t) &= (\gamma S + u + \delta w)' = 0 \\
C(t) &= \gamma S + u + \delta w = C(0) = K
\end{aligned}
\tag{4.4}$$

Here  $K$  is a constant and called the effective carrying capacity associated with the growth cycle. Equation (4.4) means that when substrate  $S$  is consumed, it is converted into either  $u$  or  $\delta w$ . We can define a useful quantity called the total biomass  $M$  as

$$M = u + \delta w \tag{4.5}$$

$$C(t) = \gamma S + u + \delta w = \gamma S + M \tag{4.6}$$

We can substitute this equation back to Equation (3.1) and obtain an equation for  $M(t)$  as

$$M' = f(\gamma^{-1}(K - M))M \tag{4.7}$$

For the uptake function  $f(S) = mS$  and the equation for the total biomass is a logistic equation

$$M' = m\gamma^{-1}K\left(1 - \frac{M}{K}\right)M \tag{4.8}$$

Note that in this equation, we can identify  $\mu = m\gamma^{-1}K$  as the effective growth rate and  $K$  is simply the maximal biomass within that growth cycle. The solution to this equation is

$$M(t) = \frac{K}{1 + \left(\frac{K}{M(0)} - 1\right)e^{-\mu t}} \quad (4.9)$$

The explicit form of  $M(t)$  can be used to eliminate  $w(t)$  in Equation (3.1) to obtain an equation for  $u(t)$

$$u' = f(\gamma^{-1}(K - M))u - (\alpha + \beta)u + \beta M \quad (4.10)$$

or

$$u' = m\gamma^{-1}((K - M)u - (\alpha + \beta)u) + \beta M \quad (4.11)$$

From Equation (4.8), Equation (4.11) can be written as

$$u' = \left[\frac{M'}{M} - (\alpha + \beta)\right]u + \beta M \quad (4.12)$$

Solving linear equation (4.12) we obtain

$$u(t) = u(0)e^{-(\alpha+\beta)t} \left[ \frac{M(t)}{M(0)} \right] + \frac{\beta}{\alpha + \beta} (1 - e^{-(\alpha+\beta)t}) M(t) \quad (4.13)$$

From (4.3) we have

$$\delta w(t) = \delta w(0)e^{-(\alpha+\beta)t} \left[ \frac{M(t)}{M(0)} \right] + \frac{\alpha}{\alpha + \beta} (1 - e^{-(\alpha+\beta)t}) M(t) \quad (4.14)$$

For the pure adsorption case, the solution can be found as

$$u(t) = \frac{u(0)}{M(0)} M(t) e^{-\alpha t} \quad (4.15)$$

$$\delta w(t) = M(t)(1 - e^{-\alpha t}) + \frac{\delta w(0)}{M(0)} M(t) e^{-\alpha t} \quad (4.16)$$

For the pure detachment case  $\alpha = 0, \beta > 0$  the solution is given by

$$\delta w(t) = \frac{\delta w(0)}{M(0)} M(t) e^{-\beta t} \quad (4.17)$$

$$u(t) = M(t)(1 - e^{-\beta t}) + \frac{u(0)}{M(0)} M(t) e^{-\beta t} \quad (4.18)$$

We can now shift the origin of time coordinate  $t = 0$  to  $t = T_{j-1}^+$  so that the general solution can be written for the time interval  $T_{j-1}^+ < t < T_j^-$  as

$$\begin{aligned} u(t) = & u(T_{j-1}^+) e^{-(\alpha+\beta)(t-T_{j-1}^+)} [M(t)/M(T_{j-1}^+)] \\ & + \frac{\beta}{\alpha+\beta} (1 - e^{-(\alpha+\beta)(t-T_{j-1}^+)}) M(t) \end{aligned} \quad (4.19)$$

$$\begin{aligned} \delta w(t) = & \delta w(T_{j-1}^+) e^{-(\alpha+\beta)(t-T_{j-1}^+)} [M(t)/M(T_{j-1}^+)] \\ & + \frac{\alpha}{\alpha+\beta} (1 - e^{-(\alpha+\beta)(t-T_{j-1}^+)}) M(t) \end{aligned} \quad (4.20)$$

$$M(t) = \frac{K_j}{1 + \left(\frac{K_j}{M(T_{j-1}^+) - 1}\right) e^{-\mu(t-T_{j-1}^+)}} \quad (4.21)$$

where  $K_j$  is the carrying capacity of  $j^{\text{th}}$  growth cycle. Although from (3.2), we can reset the initial conditions of  $S$ ,  $u$ , and  $w$ , more conveniently, we can reset the initial condition of  $S(t)$ ,  $M(t)$  and  $K_j$  at  $t = T_j$  for the  $(j+1)^{\text{th}}$  cycle

$$\begin{aligned} \gamma S(T_j^-) &= K_j - u(T_j^-) - \delta w(T_j^-) \\ M(T_j^+) &= u(T_j^+) + \delta w(T_j^+) = \eta u(T_j^-) + \theta_w \delta w(T_j^-) \\ K_{j+1} = C(T_j^+) &= \gamma S(T_j^+) + M(T_j^+) = \eta \gamma S(T_j^-) + F \gamma S^{(0)} + \eta u(T_j^-) + \theta_w \delta w(T_j^-) \end{aligned} \quad (4.22)$$

To generate the growth curve from the solution in Equation (4.13) and (4.14), one needs to input the initial condition. In general, this depends on the protocol we use to load the cell to begin the culture. We use the convention that the cell is mixed with the input substrate  $S^{(0)}$  and loaded into the growth chamber at  $t = -T$  to allow the microbe to grow and the first dilution step is carried out at

$t = 0$  ( $t = Tj = jT, j = 0$ ). We refer the time interval  $(-T, 0)$  as the inoculation cycle or equivalently  $0^{\text{th}}$  cycle and this convention is consistent with our definition of the label  $j$  for the growth cycle number. So the initial condition is input at  $t = -T$  with  $S(-T) = S^{(0)}$ ,  $u(-T)$ , and  $\delta w(-T)$  or more conveniently, with  $K_0 = C(-T) = \gamma S(-T) + u(-T) + \delta w(-T)$  and  $M(-T) = u(-T) + \delta w(-T)$ .

To connect between the solution given in this section and the general stability analysis presented in Section 3, we note that for the important special case  $f_u(S) = f_w(S) := f(S)$ , we can show that

$$\lambda_1 = f(\tilde{S}), \quad \lambda_2 = f(\tilde{S}) - (\alpha + \beta), \quad A_1 = \frac{\beta}{\alpha + \beta}, \quad A_2 = \frac{\alpha}{\alpha + \beta} \quad (4.23)$$

By solving Eq. (4.24), we can plot the  $\eta - \theta_w$  operating diagram. Figure 3 presents a representative operating diagram under different growth cycles  $T$ , with the other parameters fixed at  $m = 0.0018, \alpha = 0.1, \beta = 0.05, \gamma = 1, S^{(0)} = 100$ . Note that the extinction region increases with decreasing growth cycle period  $T$ , and, equivalently, with increased dilution frequency. Figures 4–7 plot the growth curves of four representative points in the operating diagram of Figure 3(b) ( $T = 10$ ) constrained by  $\eta = 1/4$ . The first two points locate near the extinction coexistence boundary, determined by  $\theta_w = G(\eta)$ . At Point 1 (above the boundary), the two populations coexist as shown in Figure 4. Conversely, at Point 2 (below the boundary), both populations die out (Figure 5). If biofilm is not removed [namely,  $\theta_w = 1$  in the dilution step, corresponding to Point 3 in Figure 3(b)], the biofilm accumulates at each cycle (Figure 6). In this scenario, the device mimics a biofilm flow reactor.

Finally, Figure 7 plots the solution at Point 4 in Figure 3(b), representing complete biofilm removal ( $\theta_w = 1$  in the dilution step). In this scenario, both populations become extinct. To verify the extinction, we must stipulate  $\eta < \frac{1}{A_1 e^{\lambda_1 T} + A_2 e^{\lambda_2 T}} = 0.34$  because the operating point lies on the horizontal axis in Figure 2. If the growth rate is increased from  $m = 0.0018$  to  $m = 0.015$ , coexistence is restored as shown in Figure 8. Also note that when both populations become extinct (Figure 5 and 7), the substrate concentration slowly recovers to its input concentration  $S^{(0)} = 100$ , which is the extinction point assumed in the stability analysis. When both populations coexist (Figure 6 and 8), the substrate concentration is nearly depleted at the end of the growth cycle. In all of these plots, the initial condition  $S(-T) = S^{(0)} = 100$  is input at  $t = -T$ . In Figure

4–7, the initial conditions  $u$  and  $\delta w$  are set to  $u(-T) = 50$  and  $\delta w(-T) = 50$ ; in Figure 8, they are set to  $u(-T) = 1$  and  $\delta w(-T) = 0$ . The parameters used in the calculation are summarized in Table 1.

## 5 Conclusion

We theoretically described the operation of a discrete serial dilution bioreactor with a 2-compartment growth chamber. The survival of a single-species microbial population depends on the balance between the growth rate and growth cycle time. In a coexisting planktonic and biofilm system, the washout threshold was decided from the stability of the global extinction state  $E_0 = (S^{(0)}, 0, 0)$ . Survival or washout in the cases of pure adsorption ( $\alpha > 0, \beta = 0$ ) or pure detachment ( $\alpha = 0, \beta > 0$ ) were determined by Eqs. (3.30) and (3.33), respectively. In the more general case,  $\alpha > 0, \beta > 0$  with fixed period  $T > 0$ , our analysis retrieves the function  $G(\eta)$  in Eq. (3.36), which defines the boundary separating the coexistence and extinction regions in the operating diagram. If the uptake of planktonic and biofilm cells can be assumed equal, we can obtain analytical solutions and explicit expressions for the growth curves of both planktonic and biofilm populations. The utility of our model was demonstrated in representative operating diagrams, varying the period of the growth cycle and imposing constraints on the dilution ratio  $\eta$ . We also investigated the cases of complete and no biofilm removal in representative operating diagrams. Our results can be readily compared with experimental results. If the unit time and unit volume in the cell density calculations are assumed as 1 h and 0.1 nl respectively, the conditions imposed in Figure 4 – 8 approximate the experimental settings in *E. coli* cultures (note that the unit volume is actually the sampling volume in the bacterial number counts.) The microbial growth rate is roughly estimated by  $f(S^0) = m\gamma^{-1}S^0$ . Setting  $m = 0.015$  and  $S^0 = 100$ , we obtain  $f(S^0) = 1.5 \text{ hr}^{-1}$ , very close to the maximal growth rate of *E. coli* cultured in rich medium (such as Luria Bertani medium) at optimal temperature. Setting  $m = 0.018$  yields  $f(S^0) = 0.18 \text{ hr}^{-1}$ , which typifies growth in poor medium or lower temperature. A planktonic cell density of 100 per 0.1 nL corresponds to the typical confluent density of *E. coli* ( $10^9 \text{ cell/ml}$ ). However, the limitations of the analytical solution should not be overlooked. The equal uptake function implies equal fitness of both populations; therefore, this function is unsuitable for investi-

gating fitness-competitive scenarios.

Although we focused on planktonic and biofilm growth, our stability analysis is quite general, and is applicable to competition between two inter-convertible populations with different exchange rates  $\alpha$  and  $\beta$ . If both species are in the liquid phase, they are subject to the same dilution ratio; namely, we can set  $\eta = \theta_w$ . The operating diagram is unchanged in this scenario, except that the constraint becomes  $\eta = \theta_w$ . Our analysis is also generalizable to  $N$  compartments under well-mixed conditions. Our formulation subtly assumes that the biofilm populations in both compartments are cleaned to the same extent; thus, the biofilm populations in both compartments are given by  $w$ . In a general  $N$ -compartment bioreactor, both planktonic and biofilm populations would need to be assigned in each compartment. The resetting of the initial condition in the dilution step depends on the details of the dilution procedure. In our formulation, biofilm growth is described by ordinary differential equations. Our model could be rendered more sophisticated by considering other effects, such as convection of fluidic flow and diffusion of nutrient into the biofilm (Kapper and Dockery 2010).

Our theory may also be extended to drug inhibition effects and mutation. To this end, we could introduce a drug inhabitation term and single or multiple mutant population terms into Eq. (3.1). Recently, a device called the morbidostat has become available. The morbidostat maintains a constant bacterial population by monitoring the microbial growth and adjusting the drug concentration (Topak et al. 2012). The present formulation could also be extended to forward and backward mutations, and could assist the design of microbial growth experiments in compartmentalized bioreactors.

## Appendix 1: Stability Analysis

We can rewrite the initial value problem in Equation (2.1) in vector form

$$\begin{aligned} \frac{dX}{dt} &= F(X) \\ X(0) &= x_0 \end{aligned} \tag{A.1}$$

where the vector  $X$  and  $F(X)$  are defined as

$$X = \begin{pmatrix} S \\ u \\ w \end{pmatrix}, \quad F(X) = \begin{pmatrix} -\frac{1}{\gamma}f_u(S)u - \frac{\delta}{\gamma}f_w(S)w \\ (f_u(S) - \alpha)u + \delta\beta w \\ (f_w(S) - \beta)w + \frac{\alpha u}{\delta} \end{pmatrix}$$

Then we have

$$\frac{d}{dt}\Phi_t(x_0) = F(\Phi_t(x_0)), \quad \Phi_0(x_0) \equiv x_0 \quad (\text{A.2})$$

Differentiating Equation (A.2) with respect to  $x_0 \in \mathbf{R}^3$  yields

$$\begin{aligned} \frac{d}{dt}D_{x_0}\Phi_t(x_0) &= D_x F(\Phi_t(x_0))D_{x_0}\Phi_t(x_0) \\ D_{x_0}\Phi_0(x_0) &= I \end{aligned} \quad (\text{A.3})$$

Setting  $x_0 = (\tilde{S}, 0, 0)$  in Equation (A.3), then we obtain

$$\begin{aligned} \frac{d}{dt}V(t) &= AV(t) \\ V(0) &= I \end{aligned} \quad (\text{A.4})$$

where  $V(t) = D_{x_0}\Phi_t(x_0) \Big|_{x_0=(\tilde{S},0,0)}$  and

$$A = \begin{pmatrix} -\frac{u}{\gamma}f'_u(S) - \frac{\delta w}{\gamma}f'_w(S) & -\frac{1}{\gamma}f_u(S) & -\frac{\delta}{\gamma}f_w(S) \\ f'_u(S)u & f_u(S) - \alpha & \beta\delta \\ f'_w(S)w & \alpha\delta^{-1} & f_w(S) - \beta \end{pmatrix} \quad (\text{A.5})$$

Substituting  $(S, u, w) = \Phi_t(\tilde{S}, 0, 0) \equiv (\tilde{S}, 0, 0)$  we can obtain

$$A = \begin{pmatrix} 0 & -\frac{1}{\gamma}f_u(\tilde{S}) & -\frac{\delta}{\gamma}f_w(\tilde{S}) \\ 0 & f_u(\tilde{S}) - \alpha & \beta\delta \\ 0 & \alpha\delta^{-1} & f_w(\tilde{S}) - \beta \end{pmatrix} \quad (\text{A.6})$$

To prove the local stability of the extinction fixed point  $(\tilde{S}, 0, 0)$ , we need to show that the spectral radius of  $D_{x_0}P(\tilde{S}, 0, 0)$  is less than 1. From Equation(3.4), we

have

$$D_{x_0}P(\tilde{S}, 0, 0) = \begin{pmatrix} \eta & 0 & 0 \\ 0 & \eta & 0 \\ 0 & 0 & \theta_w \end{pmatrix} V(T) \quad (\text{A.7})$$

From (A.6), the eigenvalues of the matrix  $A$  are  $0, \lambda_1,$  and  $\lambda_2$ . Here  $\lambda_1, \lambda_2$  are the eigenvalues of  $2 \times 2$  matrix  $A_1$  given by

$$A_1 = \begin{pmatrix} f_u(\tilde{S}) - \alpha & \beta\delta \\ \alpha\delta^{-1} & f_w(\tilde{S}) - \beta \end{pmatrix} \quad (\text{A.8})$$

Then  $\lambda_1, \lambda_2$  are the roots of characteristic polynomial of  $A_1$

$$g(\lambda) = \lambda^2 - [(f_u(\tilde{S}) - \alpha) + (f_w(\tilde{S}) - \beta)]\lambda + (f_u(\tilde{S}) - \alpha)(f_w(\tilde{S}) - \beta) - \alpha\beta$$

The discriminant  $\vartheta$  of  $g(\lambda)$  is

$$\vartheta = [(f_u(\tilde{S}) - \alpha) - (f_w(\tilde{S}) - \beta)]^2 + 4\alpha\beta > 0 \quad (\text{A.9})$$

Thus  $\lambda_1, \lambda_2$  are real value and given by

$$\lambda_{1,2} = \frac{[(f_u(\tilde{S}) - \alpha) + (f_w(\tilde{S}) - \beta)] \pm \sqrt{\vartheta}}{2} \quad (\text{A.10})$$

To evaluate the spectral radius  $r(D_{x_0}P(\tilde{S}, 0, 0))$  from (A.7), we need to compute the matrix  $V(t) = [V_1(t) \ V_2(t) \ V_3(t)]$ . Then the dynamics of the matrix is described by

$$\begin{aligned} \frac{dV_i}{dt} &= AV_i, \quad i = 1, 2, 3 \\ V_i(0) &= e_i \end{aligned} \quad (\text{A.11})$$

where  $e_i$  are the basis vectors given by

$$e_1 = \begin{pmatrix} 1 \\ 0 \\ 0 \end{pmatrix}, \quad e_2 = \begin{pmatrix} 0 \\ 1 \\ 0 \end{pmatrix}, \quad e_3 = \begin{pmatrix} 0 \\ 0 \\ 1 \end{pmatrix}$$

It is easy to show that  $V(t)$  take the following form

$$V(t) = \begin{pmatrix} 1 & \alpha_2(t) & \alpha_3(t) \\ 0 & V_{11}(t) & V_{12}(t) \\ 0 & V_{21}(t) & V_{22}(t) \end{pmatrix} \quad \text{with} \quad \hat{V}(t) = \begin{pmatrix} V_{11}(t) & V_{12}(t) \\ V_{21}(t) & V_{22}(t) \end{pmatrix} \quad (\text{A.12})$$

Then

$$D_{x_0}P(\tilde{S}, 0, 0) = \begin{pmatrix} \eta & 0 & 0 \\ 0 & \eta & 0 \\ 0 & 0 & \theta_w \end{pmatrix} V(T) = \begin{pmatrix} \eta & \eta\alpha_2(T) & \eta\alpha_3(T) \\ 0 & \eta V_{11}(T) & \eta V_{12}(T) \\ 0 & \theta_w V_{21}(T) & \theta_w V_{22}(T) \end{pmatrix} \quad (\text{A.13})$$

Note that from Equation (A.11) and (A.12), the matrix  $\hat{V}(t)$  satisfies

$$\frac{d}{dt}\hat{V}(t) = A_1\hat{V}(t), \quad \hat{V}(0) = I \quad (\text{A.14})$$

Let

$$B = \begin{bmatrix} \eta V_{11}(T) & \eta V_{12}(T) \\ \theta_w V_{21}(T) & \theta_w V_{22}(T) \end{bmatrix} \quad (\text{A.15})$$

The matrix  $B$  have eigenvalues  $\mu_1, \mu_2$ .

Then the eigenvalues of  $D_{x_0}P(\tilde{S}, 0, 0)$  are  $\eta, \mu_1$  and  $\mu_2$ . Since  $0 < \eta < 1$ , the spectral radius  $r(D_{x_0}P(\tilde{S}, 0, 0)) < 1$  if and only if  $|\mu_i| < 1$ ,  $i = 1, 2$ .

From (Allen 2007)  $|\mu_i| < 1$ ,  $i = 1, 2$  if and only if

$$|\det B| < 1, \quad |\text{trace} B| < 1 + \det B \quad (\text{A.16})$$

From (A.10) (A.14) and (A.15) and Liouville's formula (Hsu 2013; Hale 1969), we can calculate the determinant  $\det B$  as

$$\begin{aligned} \det B &= \eta\theta_w \det \begin{bmatrix} V_{11}(T) & V_{12}(T) \\ V_{21}(T) & V_{22}(T) \end{bmatrix} \\ &= \eta\theta_w \exp\left(\int_0^T [(f_u(\tilde{S}) - \alpha) + (f_w(\tilde{S}) - \beta)] dt\right) \\ &= \eta\theta_w \exp((\lambda_1 + \lambda_2)T) \\ &= \eta\theta_w e^{\lambda_1 T} e^{\lambda_2 T} \end{aligned} \quad (\text{A.17})$$

To evaluate  $\text{trace}(B)$ , we need compute  $V_{11}(T)$  and  $V_{22}(T)$ . Let  $\tilde{V}_i$  be an eigenvector of the eigenvalue  $\lambda_i$  of matrix  $A_1$ ,  $i = 1, 2$ .

It is easy to show that

$$\tilde{V}_1 = \begin{pmatrix} \lambda_1 - (f_w(\tilde{S}) - \beta) \\ \alpha\delta^{-1} \end{pmatrix} \quad \text{and} \quad \tilde{V}_2 = \begin{pmatrix} \beta\delta \\ \lambda_2 - (f_u(\tilde{S}) - \alpha) \end{pmatrix} \quad (\text{A.18})$$

are eigenvectors of  $\lambda_1$  and  $\lambda_2$  respectively.

Since  $\lambda_1 \neq \lambda_2$  then from theory of linear system (Hsu 2013; Hale 1969),

$$\begin{aligned} \hat{V}_2(t) &= \begin{pmatrix} V_{11}(t) \\ V_{21}(t) \end{pmatrix} = \xi_1 e^{\lambda_1 t} \tilde{V}_1 + \xi_2 e^{\lambda_2 t} \tilde{V}_2 \\ \hat{V}_3(t) &= \begin{pmatrix} V_{12}(t) \\ V_{22}(t) \end{pmatrix} = \delta_1 e^{\lambda_1 t} \tilde{V}_1 + \delta_2 e^{\lambda_2 t} \tilde{V}_2 \end{aligned} \quad (\text{A.19})$$

From (A.18) and (A.19),

$$\hat{V}_2(0) = \begin{pmatrix} 1 \\ 0 \end{pmatrix}, \quad \hat{V}_3(0) = \begin{pmatrix} 0 \\ 1 \end{pmatrix}$$

we obtain

$$\begin{aligned} \xi_1 &= \frac{\lambda_2 - (f_u(\tilde{S}) - \alpha)}{(\lambda_1 - (f_w(\tilde{S}) - \beta))(\lambda_2 - (f_u(\tilde{S}) - \alpha)) - \alpha\beta} \\ \xi_2 &= \frac{-(\alpha\delta^{-1})}{(\lambda_1 - (f_w(\tilde{S}) - \beta))(\lambda_2 - (f_u(\tilde{S}) - \alpha)) - \alpha\beta} \\ \delta_1 &= \frac{\beta\delta}{(\lambda_1 - (f_w(\tilde{S}) - \beta))(\lambda_2 - (f_u(\tilde{S}) - \alpha)) - \alpha\beta} \\ \delta_2 &= \frac{\lambda_1 - (f_w(\tilde{S}) - \beta)}{(\lambda_1 - (f_w(\tilde{S}) - \beta))(\lambda_2 - (f_u(\tilde{S}) - \alpha)) - \alpha\beta} \end{aligned} \quad (\text{A.20})$$

From (A.10), we note that

$$\lambda_1 + \lambda_2 = (f_u(\tilde{S}) - \alpha) + (f_w(\tilde{S}) - \beta)$$

or

$$(\lambda_1 - (f_w(\tilde{S}) - \beta)) = -(\lambda_2 - (f_u(\tilde{S}) - \alpha)) \quad (\text{A.21})$$

From (A.18),(A.19), (A.20), and (A.21), we can obtain

$$\begin{aligned} V_{11}(T) &= A_1 e^{\lambda_1 T} + A_2 e^{\lambda_2 T} \\ V_{22}(T) &= A_2 e^{\lambda_1 T} + A_1 e^{\lambda_2 T} \end{aligned}$$

where

$$\begin{aligned} A_1 &= \frac{-(\lambda_1 - (f_w(\tilde{S}) - \beta))(\lambda_2 - (f_u(\tilde{S}) - \alpha))}{\alpha\beta - (\lambda_1 - (f_w(\tilde{S}) - \beta))(\lambda_2 - (f_u(\tilde{S}) - \alpha))} \\ A_2 &= \frac{\alpha\beta}{\alpha\beta - (\lambda_1 - (f_w(\tilde{S}) - \beta))(\lambda_2 - (f_u(\tilde{S}) - \alpha))} \end{aligned} \quad (\text{A.22})$$

$$0 \leq A_1, A_2 \leq 1, \quad A_1 + A_2 = 1$$

Using (A.16), the stability condition for the extinction fixed point  $E_0 = (\tilde{S}, 0, 0)$  is given by

$$\eta\theta_w e^{\lambda_1 T} e^{\lambda_2 T} < 1 \quad (\text{A.23})$$

$$\eta V_{11}(T) + \theta_w V_{22}(T) < 1 + \eta\theta_w e^{\lambda_1 T} e^{\lambda_2 T} \quad (\text{A.24})$$

## Appendix 2: Proof of lemma A

**Lemma A1.** Define  $(S_n, u_n, w_n) = P^n(S_0, u_0, w_0)$ . For any  $(S_0, u_0, w_0) \in \mathbb{R}^3$ , the sequence  $(S_n, u_n, w_n)_{n=0}^{n=\infty}$  is bounded.

**Proof.** From the first equation in (3.1), we have  $S'(t) \leq 0, 0 < t < T$ , and  $S(T) \leq S_0$  since  $S_1 = \eta S(T) + FS^{(0)} \leq \eta S_0 + FS^{(0)}$ . Inductively we have  $S_{n+1} = \eta S_n + FS^{(0)}, n=0,1,2,\dots$ , then

$$\begin{aligned} S_n &\leq \eta S_{n-1} + FS^{(0)} \leq \eta(\eta S_{n-2} + FS^{(0)}) + FS^{(0)} \leq \dots \\ &\leq \eta^n S_0 + FS^{(0)}(1 + \eta + \dots + \eta^{n-1}) \end{aligned}$$

Given  $\epsilon > 0$  small for  $n$  large

$$S_n \leq \frac{FS^{(0)}}{1 - \eta} + \epsilon = S^{(0)} + \epsilon \quad \text{for } n \geq N_0$$

Let  $U(t) = \frac{1}{\gamma}(u(t) + \delta w(t))$  and  $U_n = \frac{1}{\gamma}(u_n + \delta w_n)$ ,  $n \geq 0$ .  
Then  $S(t) + U(t) = S_0 + U_0$  for  $0 \leq t \leq T$  and

$$\begin{aligned} U' &= \frac{1}{\gamma}(f_u(S)u + f_w(S)\delta w) \\ &\leq \text{Max}(f_u(S), f_w(S))U \\ &= \text{Max}(f_u(S_0 + U_0 - U(t)), f_w(S_0 + U_0 - U(t)))U \\ U(0) &= U_0 \end{aligned}$$

Hence  $U(t) \leq S_0 + U_0$ ,  $0 \leq t \leq T$ .

Let  $\theta^* = \max\{\eta, \theta_w\}$ , then

$$U_1 = \frac{1}{\gamma}(u_1(T^-) + \delta w_1(T^-)) \leq \theta^* U(T^-) \leq \theta^*(S_0 + U_0) \leq \theta^*((S^{(0)} + \epsilon) + U_0)$$

Inductively for  $n \geq 1$ , we have

$$\begin{aligned} U_n &\leq \theta^*((S^{(0)} + \epsilon) + U_{n-1}) \\ &\leq \theta^*((S^{(0)} + \epsilon) + \theta^*((S^{(0)} + \epsilon) + U_{n-2})) \\ &\dots\dots\dots \\ &\leq \theta^*((S^{(0)} + \epsilon)(1 + \theta^* + \dots\dots\dots(\theta^*)^{n-1}) + (\theta^*)^n U_0) \end{aligned}$$

For large  $n$ , we have

$$U_n \leq \frac{\theta^*(S^{(0)} + \epsilon)}{1 - \theta^*} + \epsilon_1, \quad \epsilon, \epsilon_1 \text{ are small.}$$

Hence  $(S_n, u_n, w_n)_{n=0}^{n=\infty}$  are bounded.

**Proof of Theorem A, part(i).** Consider the following auxiliary system of (3.1)

$$\begin{aligned} S' &= 0 \\ u' &= (f_u(S) - \alpha)u + \beta\delta w \\ w' &= (f_w(S) - \beta)w + \alpha\delta^{-1}u \end{aligned} \tag{A.25}$$

$S(0) = S_0$ ,  $u(0) = u_0$ ,  $w(0) = w_0$  with with the same resetting of initial condition (3.1) at  $t = T^+$ .

Define map  $\hat{P}(\hat{S}_0, \hat{u}_0, \hat{w}_0) = L \circ \psi_T(\hat{S}_0, \hat{u}_0, \hat{w}_0)$ , with  $(\hat{S}_0, \hat{u}_0, \hat{w}_0) = (S_0, u_0, w_0)$ ,  $L$

defined in (4.3) and  $\psi_t(\hat{x}_0)$  is the semi-flow defined by the system Equation (A.25),  $0 \leq t \leq T$ ,  $\hat{x}_0 = (\hat{S}_0, \hat{u}_0, \hat{w}_0)$ . We write initial value problem (A.25) in vector form

$$\begin{aligned} \frac{dX}{dt} &= \hat{F}(X) \\ X(0) &= \hat{x}_0 \end{aligned} \tag{A.26}$$

Then  $F(X) \leq \hat{F}(X)$ . We note that (A.3) is a cooperationsystem for  $0 \leq t \leq T$ . Let  $(\hat{S}_n, \hat{u}_n, \hat{w}_n) = \hat{P}^n(\hat{S}_0, \hat{u}_0, \hat{w}_0)$ .

By Kamke theorem (Smith 1995) and the resetting mechanism (3.1), it follows that

$$(S_n, u_n, w_n) \leq (\hat{S}_n, \hat{u}_n, \hat{w}_n) \text{ for all } n \geq 0.$$

We want to show that if (3.6) holds, i.e., the extinction state  $(S^{(0)}, 0, 0)$  is locally stable for the map  $P$ , then  $(\hat{u}_n, \hat{w}_n) \rightarrow (0, 0)$  as  $n \rightarrow \infty$ .

From the proof of Lemma A1, it follows that

$$\hat{S}_n = \eta^n S_0 + FS^{(0)}(1 + \eta + \dots + \eta^{n-1}) \rightarrow S^{(0)} \text{ as } n \rightarrow \infty.$$

For  $\epsilon > 0$  small,

$$S^{(0)} - \epsilon < S_n < S^{(0)} + \epsilon \text{ for } n \geq N$$

Let

$$\hat{F}^\pm(S, u, w) = \begin{pmatrix} (f_u(S^{(0)} \pm \epsilon) - \alpha)u + \beta\delta w \\ (f_w(S^{(0)} \pm \epsilon) - \beta)w + \alpha\delta^{-1}u \end{pmatrix}$$

Then

$$\hat{F}^-(S, u, w) < \hat{F}(S, u, w) < \hat{F}^+(S, u, w)$$

By Kamke's Theorem and resetting mechanism, it follows that

$$(S^{(0)} - \epsilon, u_n^-, w_n^-) < (S_n, u_n, w_n) < (S^{(0)} + \epsilon, u_n^+, w_n^+)$$

Since  $r(D_{x_0}\hat{P}(S^{(0)}, 0, 0)) = r(D_{x_0}P(S^{(0)}, 0, 0))$ , then  $r(D_{x_0}\hat{P}(S^{(0)}, 0, 0)) < 1$  implies  $r(D_{x_0}\hat{P}^\pm(S^{(0)} \pm \epsilon, 0, 0)) < 1$  for  $\epsilon > 0$ ,  $(u_n^\pm, w_n^\pm) \rightarrow (0, 0)$  as  $n \rightarrow \infty$ . Hence if the stability condition (3.6) holds then  $(u_n, w_n) \rightarrow (0, 0)$  as  $n \rightarrow \infty$ .

**Proof of Theorem A, part(ii).** We shall prove uniformly persistence of the map  $P$  if  $r(D_{x_0}P(S^{(0)}, 0, 0)) > 1$  i.e., Equation (3.6) does not hold with strict inequality. Since  $E_0 = (S^{(0)}, 0, 0)$  is the only fixed point on the boundary of  $\text{Int}(\mathbb{R}_+^3)$ , from

Theorem 1.3.1 (Zhao 2003; Freedman and So 1989) and Lemma A1, it suffices to show that

$$W^s(E_0) \cap \text{Int}(\mathbb{R}_+^3) = \emptyset,$$

where  $W^s(E_0) = \{x_0 \in \mathbb{R}_+^3 : P^n(x_0) \rightarrow E_0 \text{ as } n \rightarrow \infty\}$  is the stable set of fixed point  $E_0$ .

We prove by contradiction. If this is not the case, there exists  $x_0 = (S_0, u_0, w_0) \in \text{Int}(\mathbb{R}_+^3)$  such that  $P^n(x_0) \rightarrow E_0$  as  $n \rightarrow \infty$ .

Then we have  $S_n \rightarrow \tilde{S}$ ,  $u_n \rightarrow 0$ ,  $w_n \rightarrow 0$  as  $n \rightarrow \infty$ . From Equation (5.5) and (5.6) and stability condition  $r(D_{x_0}P(S^{(0)}, 0, 0)) > 1$ , it follows that  $(u_n^-, w_n^-) \rightarrow \infty$  as  $n \rightarrow \infty$ , a contradiction. From Theorem 1.3.7 (Zhao 2003), there exists a positive fixed point  $E = (S^*, u^*, w^*)$  of the map  $P$ .

**Proof of Theorem B.** First we claim that under the assumptions (3.15) and (3.16) we have

$$\gamma S_n + u_n + \delta w_n \rightarrow \gamma S^{(0)} \text{ as } n \rightarrow \infty. \quad (\text{A.27})$$

From (2.1) it follows that

$$\gamma S(t) + u(t) + \delta w(t) = K_n = \gamma S_n + u_n + \delta w_n \text{ for } nT \leq t \leq (n+1)T$$

By the assumption (3.15) we have

$$\begin{aligned} \gamma S_{n+1} + u_{n+1} + \delta w_{n+1} &= \gamma S(T_{n+1}^+) + u(T_{n+1}^+) + \delta w(T_{n+1}^+) \\ &= \gamma(\eta S_n + (1-\eta)S^{(0)}) + \eta u_n + \theta_w \delta w_n \\ &= \eta(\gamma S_n + u_n + \delta w_n) + \gamma(1-\eta)S^{(0)} \end{aligned} \quad (\text{A.28})$$

From above the set  $W = \{(S, u, w) : \gamma S + u + \delta w = \gamma S^{(0)}\}$  is positively invariant under the map  $P$ . Furthermore, from (A.28) inductively we have

$$\gamma S_n + u_n + \delta w_n = \eta^n(\gamma S_0 + u_0 + \delta w_0) + \gamma(1-\eta)S^{(0)}(1 + \eta + \dots + \eta^{n-1}) \rightarrow \gamma S^{(0)} \text{ as } n \rightarrow \infty.$$

Let  $U = u + \delta w$ . Under the assumption (3.16) we have

$$\begin{aligned} \frac{dU}{dt} &= f(S)U \\ U(T_n^+) &= \eta U(T_n^-). \end{aligned}$$

From (A.27) we consider the following limiting system

$$\begin{aligned}\frac{dU}{dt} &= f(S^{(0)} - \frac{1}{\gamma}U)U \\ U(T_n^+) &= \eta U(T_n^-).\end{aligned}\tag{A.29}$$

from [4] let  $R_1 = \frac{f(S^{(0)})T}{\ln(\frac{1}{\eta})}$ , it follows that if  $R_1 < 1$ , then  $U(T_n^+) \rightarrow 0$ ; if  $R_1 > 1$ , then there exists a unique fixed point  $\hat{U}$  of the system (A.29) such that

$$U(T_n^+) \rightarrow \hat{U} \text{ as } n \rightarrow \infty \text{ for any initial condition } U_0 > 0.$$

For the case  $R_1 > 1$  equivalently  $\eta > \eta^* = e^{-f(S^{(0)})T}$ , we consider the limiting equation of the second equation of (2.1)

$$\begin{aligned}\frac{du}{dt} &= (f(S^{(0)} - \frac{1}{\gamma}\hat{U}) - \alpha)u + \beta(\hat{U} - u) \\ u(T_n^+) &= \eta u(T_n^-).\end{aligned}$$

The above equation can be written as

$$\begin{aligned}\frac{du}{dt} &= (f(S^{(0)} - \frac{1}{\gamma}\hat{U}) - (\alpha + \beta))u + \beta\hat{U} \\ &:= Au + B.\end{aligned}$$

It is easy to show that

$$\begin{aligned}\frac{du}{dt} &= (f(S^{(0)} - \frac{1}{\gamma}\hat{U}) - (\alpha + \beta))u + \beta\hat{U} \\ &:= Au + B.\end{aligned}$$

Since  $\eta > e^{-f(S^{(0)})T}$ , it is easy to check that  $\eta e^{AT} < 1$  holds. Hence

$$u_n = u(T_n^+) \rightarrow \hat{u} = \frac{B}{A}\eta(e^{AT} - 1)\frac{1}{1 - \eta e^{AT}}$$

and

$$(S_n, u_n, \delta w_n) \rightarrow (S^{(0)} - \hat{U}, \hat{u}, \hat{U} - \hat{u}) \text{ as } n \rightarrow \infty.$$

We note that from chapter1 [zhao, 2003] it is easy to lift the limiting systems (A.29) and (A.30) to the original system (2.1). Thus we complete the proof.

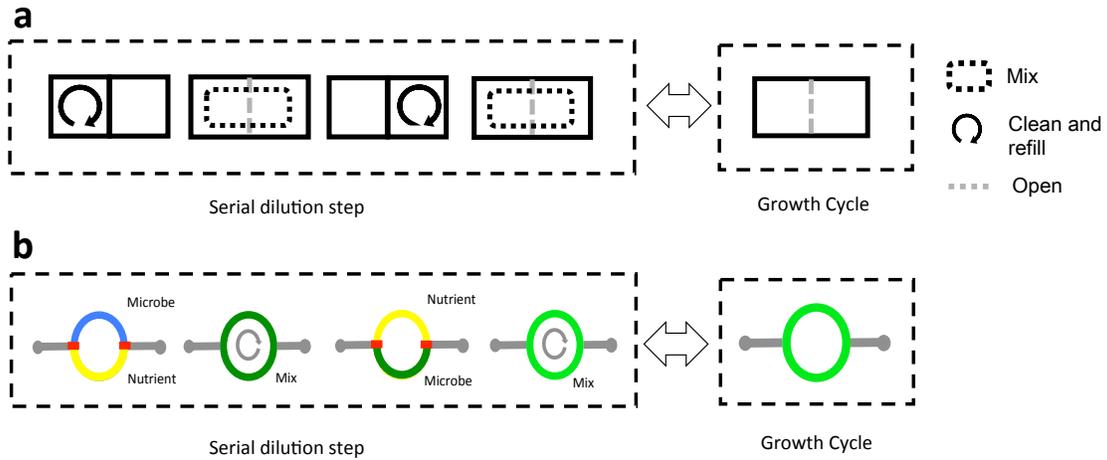


Figure 1 Operation of a serial dilution bioreactor. a. A serial dilution bioreactor with two compartments ( $N = 2$ ). Two compartments are alternatively cleaned and refilled with fresh medium. As a result of two serial dilutions, the microbial population is diluted  $2^2$ -fold. The device runs between the serial dilution step and the growth cycle. After the device stays in the growth cycle for a period  $T$ , it runs a serial dilution step and the whole process repeats itself. In our analysis, the duration of the serial dilution step are assumed to be instantaneous. The cleaning and mixing are represented in an arrowed circle symbol and a closed dash curve, respectively, as indicated in the legend. b. Microfluidic embodiment of serial dilution bioreactor. The bioreactor has ring shape growth chamber, which is divided into upper and lower compartments. The upper and lower compartments can be alternatively cleaned and refilled with medium and such dilution step results in  $2^2$ -fold dilution in platonic population.

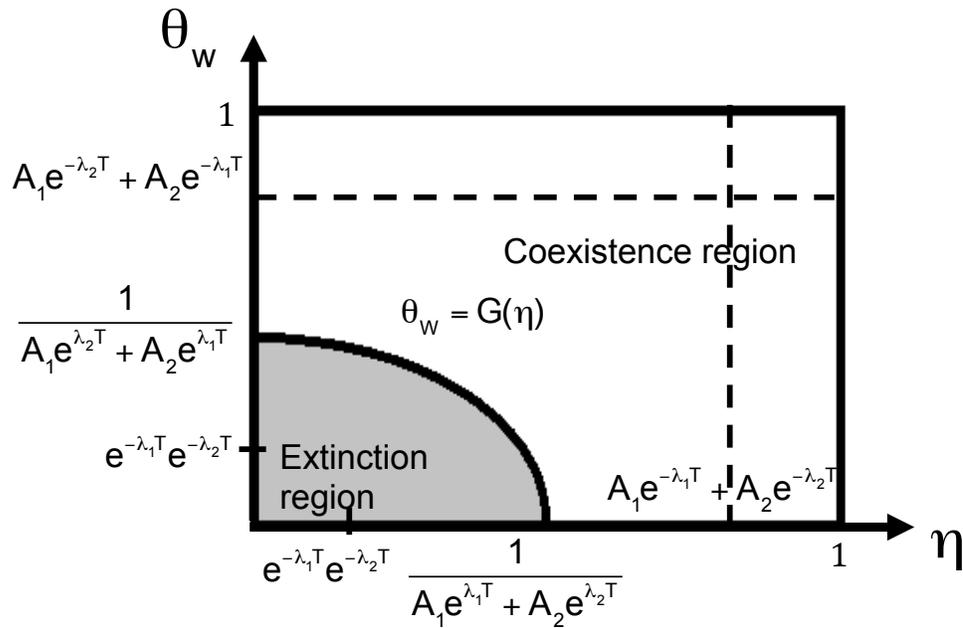


Figure 2 Operating diagram for  $\alpha > 0$  and  $\beta > 0$  and  $\lambda_1 > 0$  and  $\lambda_2 > 0$  as a result of the stability analysis. The extinction and coexistence regions in this diagram are separated by the curve  $\theta_w = G(\eta)$ .

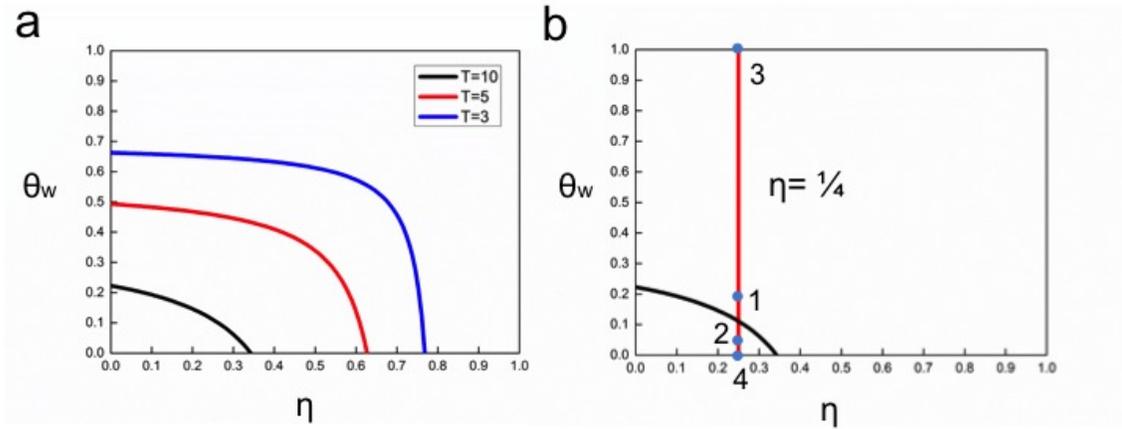


Figure 3 Representative operating diagram for equal uptake function. a. Operating diagram with different growth cycle period  $T$ . The extinction and coexistence regions in this diagram are separated by the function  $\theta_w = G(\eta)$ . Three growth cycle periods, namely  $T = 10, 5$ , and  $3$ , are used to illustrate the effect of  $T$ . In general, shorter  $T$  or more frequent dilution leads to larger extinction region. b. Operating diagram with the constraint  $\eta = 1/4$ . Four operating points are marked out. In both figures, we use  $T = 10$ ,  $\alpha = 0.1$ ,  $\beta = 0.05$ ,  $\gamma = 1$ ,  $S^{(0)} = 100$ ,  $m = 0.0018$ , and  $f(S^{(0)}) = 0.18$ .

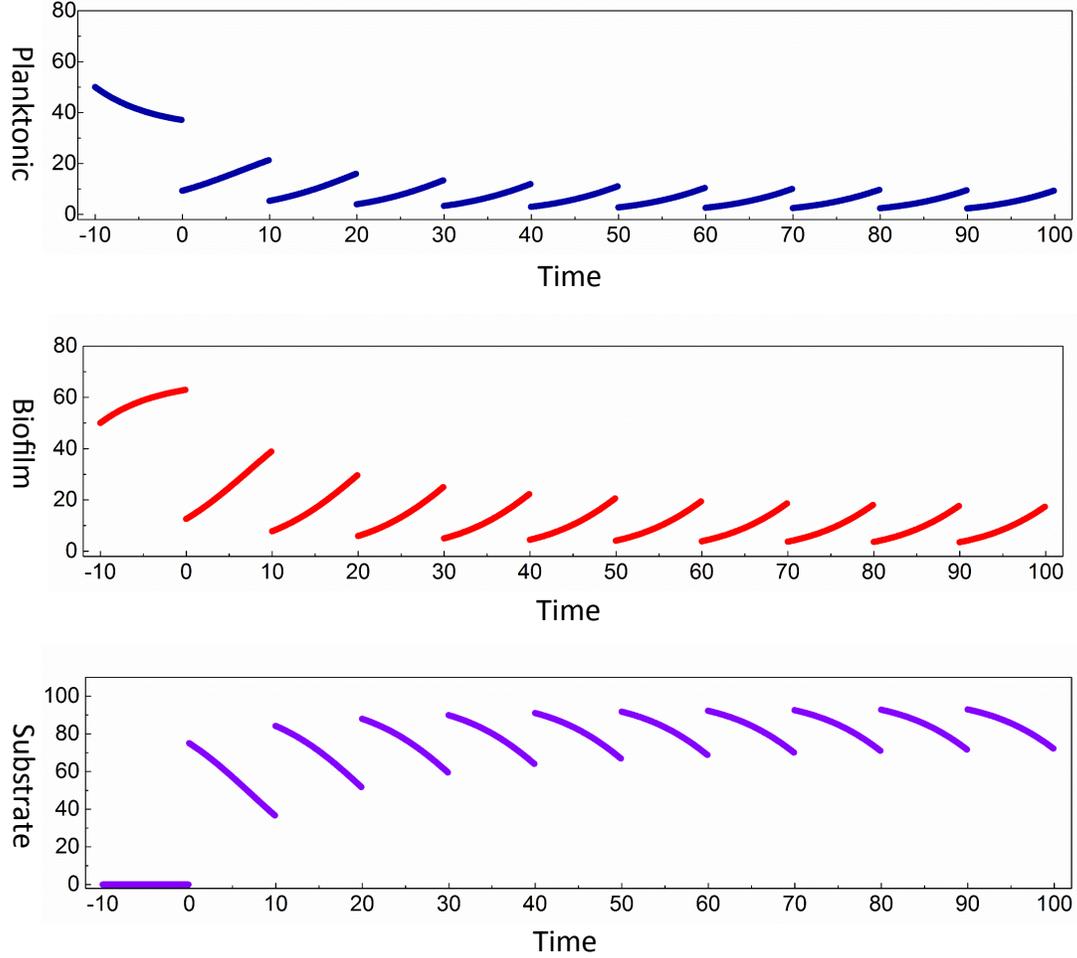


Figure 4 Growth curve near the boundary of coexistence and extinction regions. The growth curve corresponding to Point 1 ( $\theta_w = 0.05, \eta = 0.25$ ) of Figure 3b is given here and shows the coexistence of two species. In this figure, we use  $T = 10$ ,  $\alpha = 0.1$ ,  $\beta = 0.05$ ,  $\gamma = 1$ ,  $S^{(0)} = 100$ , and  $m = 0.0018$ . Initial conditions are input at  $t = -T$  with  $S(-T) = 0$ ,  $u(-T) = 50$  and  $\delta w(-T) = 50$ .

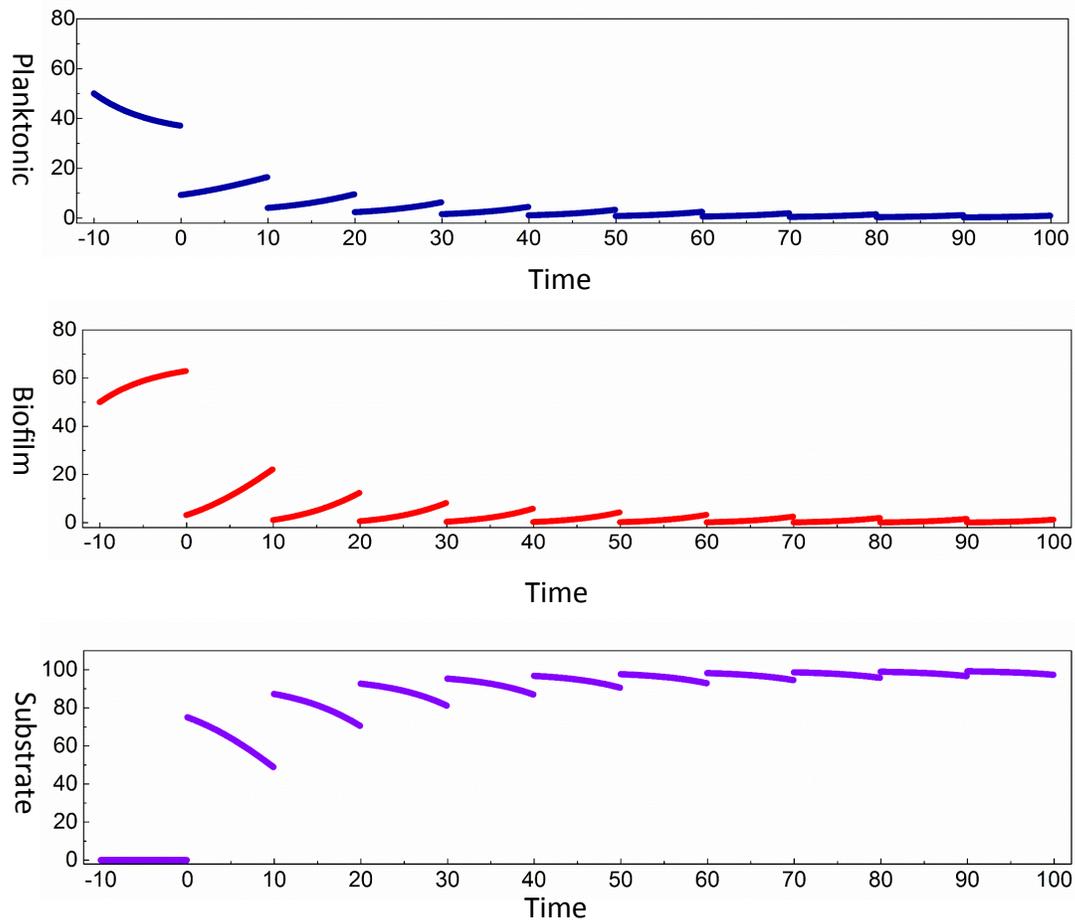


Figure 5 Growth curve near the boundary of coexistence and extinction regions. The growth curve corresponding to Point 2 ( $\theta_w = 0.2, \eta = 0.25$ ) of Figure 3b is given here and shows the extinction of two species. In this figure, we use  $T = 10$ ,  $\alpha = 0.1$ ,  $\beta = 0.05$ ,  $\gamma = 1$ ,  $S^{(0)} = 100$ , and  $m = 0.0018$ . Initial conditions are input at  $t = -T$  with  $S(-T) = 0$ ,  $u(-T) = 50$  and  $\delta w(-T) = 50$ .

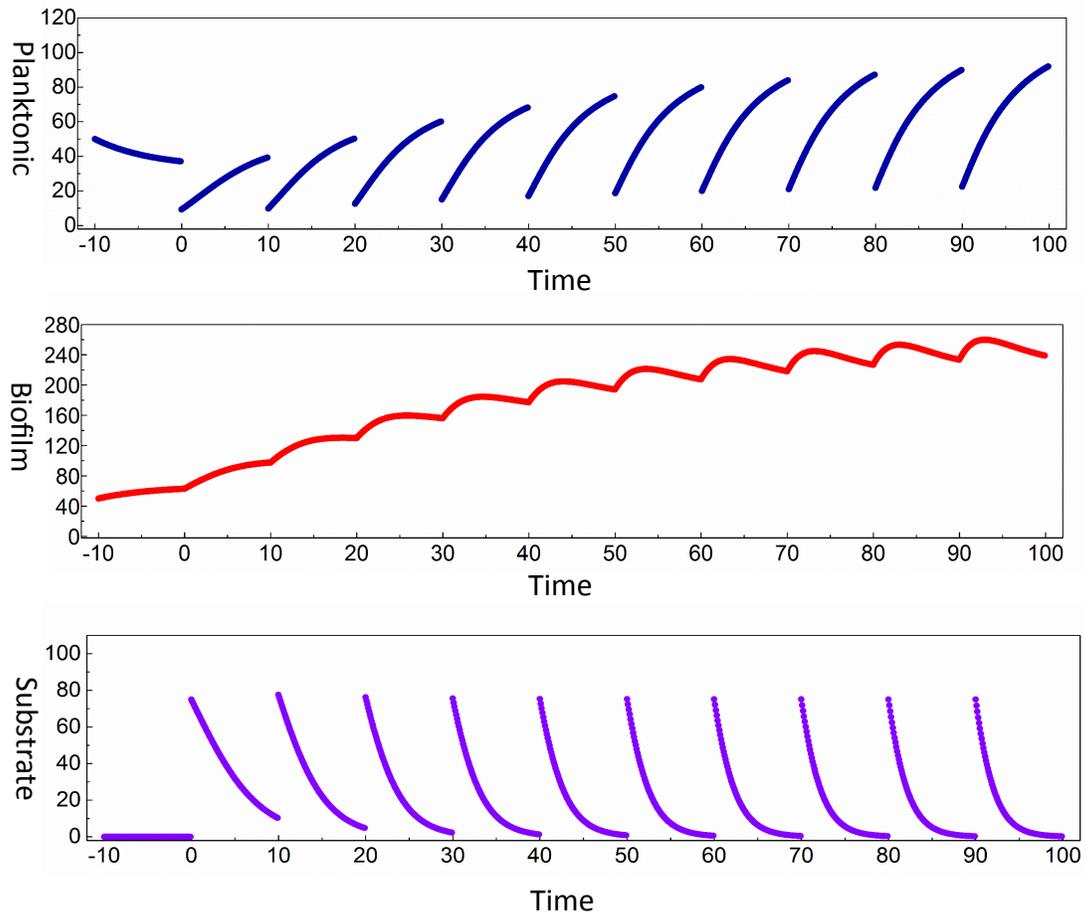


Figure 6 Growth curve with no biofilm removal in the dilution step. The growth curve corresponding to Point 3 ( $\theta_w = 1, \eta = 0.25$ ) of Figure 3b or equivalently no biofilm removal during dilution step is given here and shows pronounced growth of biofilm. In this figure, we use  $T = 10$ ,  $\alpha = 0.1$ ,  $\beta = 0.05$ ,  $\gamma = 1$ ,  $S^{(0)} = 100$ , and  $m = 0.0018$ . Initial conditions are input at  $t = -T$  with  $S(-T) = 0$ ,  $u(-T) = 50$  and  $\delta w(-T) = 50$ .

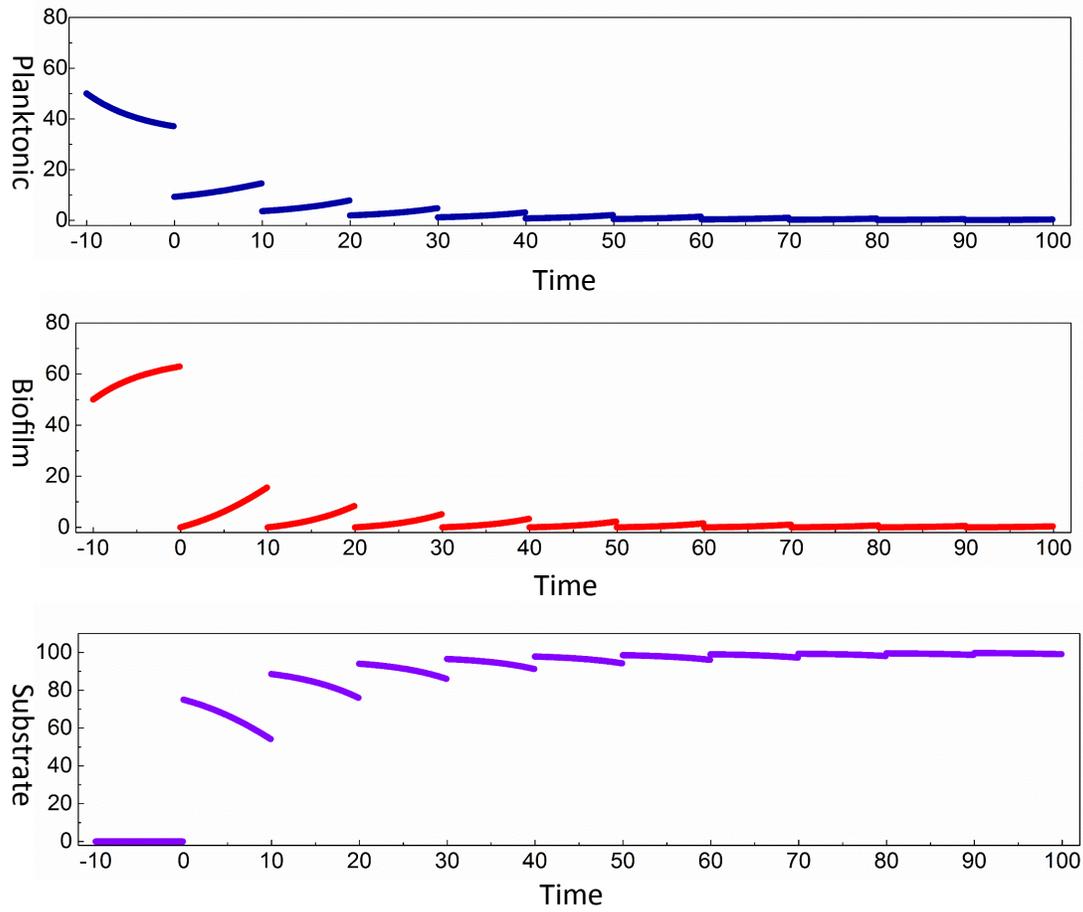


Figure 7 Growth curve with complete removal in the dilution step. The growth curve corresponding to Point 4 ( $\theta_w = 0, \eta = 0.25$ ) of Figure 3b or equivalently no biofilm removal during dilution step is given here and shows pronounced growth of biofilm. In this figure, we use  $T = 10$ ,  $\alpha = 0.1$ ,  $\beta = 0.05$ ,  $\gamma = 1$ ,  $S^{(0)} = 100$ , and  $m = 0.0018$ . Initial conditions are input at  $t = -T$  with  $S(-T) = 0$ ,  $u(-T) = 50$ , and  $\delta w(-T) = 50$ .

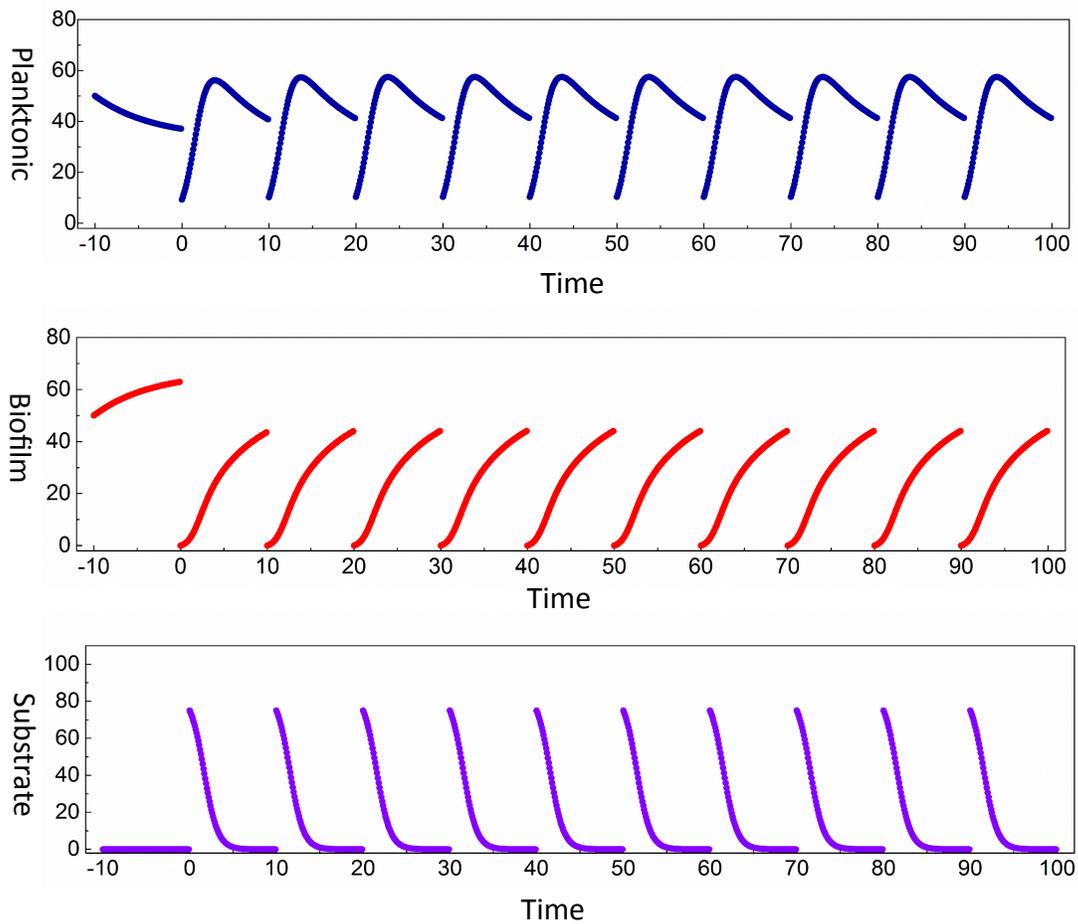


Figure 8 Growth curve for complete removal in the dilution step and larger growth rate. The growth curve with condition similar to Point 4 ( $\theta_w = 0, \eta = 0.25$ ) of Figure 3b but with larger  $m$  shows coexistence of two species. In this figure, we use  $T = 10$ ,  $\alpha = 0.1$ ,  $\beta = 0.05$ ,  $\gamma = 1$ ,  $S^{(0)} = 100$ , and  $m = 0.015$ . Initial conditions are input at  $t = -T$  with  $S(-T) = 0$ ,  $u(-T) = 50$ , and  $\delta w(-T) = 50$ .

Condition	$\theta_w$	$\eta$	m	$\lambda_1$	$\lambda_2$	Comment	Fig. No.
1	0.2	0.25	0.0018	0.18	0.03	Near boundary Coexistence	4
2	0.05	0.25	0.0018	0.18	0.03	Near boundary Extinction	5
3	1	0.25	0.0018	0.18	0.03	No biofilm removal Coexistence	6
4	0	0.25	0.0018	0.18	0.03	Complete biofilm removal Extinction	7
5	0	0.25	0.015	1.5	1.35	Complete biofilm removal Coexistence	8

Table 1 Summary of all conditions used for the growth curves. For all conditions, we use  $T = 10$ ,  $\alpha = 0.1$ ,  $\beta = 0.05$ ,  $A_1 = 1/3$ ,  $A_2 = 2/3$ ,  $\gamma = 1$ , and  $S^{(0)} = 100$ . Initial conditions are input at  $t = -T$  with  $S(-T) = 0$ ,  $u(-T) = 50$ , and  $\delta w(-T) = 50$ .

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