

Stability analysis of a self-cycling fermentation model with state-dependent impulse times

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A self-cycling fermenter is a batch fermenter subject to recurrent emptyings of liquid volume followed by the refilling with new fresh substrate. This article constructs a modified model of self-cycling fermenter, which is described by an impulsive differential equation at impulse-dependent times, which have been recently introduced. The main result is a set of conditions depending of the fraction of removed volume, the concentration of new substrate introduced, and the maximal length between two impulses, which ensure the existence and attractiveness of a periodic cycle. A second result provides alternative conditions for the biomass extinction. Copyright © 2013 John Wiley & Sons, Ltd.

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1. Introduction

A self-cycling fermenter (SCF) [1–7] consists of a controlled batch fermentation process [8, 9] containing a limiting substrate, which is degraded by a microbial population. At the same time, the device is subject to successive emptyings of a constant fraction $p \in (0, 1)$ of its total volume, which is replaced by an equivalent input containing fresh substrate. In a SCF, the time's interval between two emptying/refilling actions is determined by some measure of the fermenter's available outputs.

There are two processes in a batch fermenter: the consumption of the limiting substrate by the microorganisms and the growth of the last one. As batch fermenters have no inputs (neither substrate nor microbial biomass), the substrate concentration is always decreasing, which eventually leads to the biomass starvation in the long term. In this context, under certain conditions, the introduction of a sequence of emptying/refilling actions can promote the biomass persistence in a periodic way.

The SCF can be seen either as a complement or an alternative to the classical batch and fed-batch fermenters. Nevertheless, its theoretical and experimental literature is considerably less extended. Some well-known applications of SCF are

- *Sewage treatment*: if the limiting substrate is a contaminant that is consumed by the biomass (e.g., ethanol and phenol are respectively degraded by *Acinetobacter calcoaceticus* [1] and *Pseudomonas putida* [3]). The process is a cycle where the device is filled with a fixed contaminant concentration $S^0 > 0$ at the beginning of each refilling/emptying interval and partially released with a lower concentration at the end.
- *Production of biomass*: in general, under certain conditions, it is expected that SCF can have better productivity outcomes than batch processes. For example, in [10], the production of *Escherichia coli* in a SCF and a batch fermenter with glucose as limiting substrate are compared: by using SCF, the productivity increased by 50% and the production time decreased by 40% compared with a batch fermenter.

It is important to emphasize that a SCF has two time scales: the first one describing the continuous biological processes inside the fermenter: consumption of substrate and growth of cellular biomass. The second scale is given by the emptying/refilling actions, whose duration is considerably shorter than the global process, but leads to big changes of the substrate level.

There exists several tools for the modeling of phenomena having two time scales. A prominent one is the theory of impulsive differential equations (IDEs) [11–13], which could consider the second scale as a pulse. In spite of IDE have been extensively used to modeling

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SCF [14–19], this work employs a new type of IDE whose main property is that the value of the substrate at the k th emptying/refilling action determines (without a priori knowledge of the future continuous dynamics) the time of the next one. More details will be given later.

The main result of this paper is a condition ensuring the existence of periodic solutions of the resulting impulsive model, which is described in terms of the released fraction p of liquid volume and the maximal time between two consecutive emptying/refilling actions. The proof is carried out by studying the qualitative and asymptotic properties of the stroboscopic map associated to the impulsive system and the basic mathematical tools are the average Lyapunov functions [20, 21] (used to find conditions ensuring the nonextinction of the microbial biomass) and some results about the ω -limits of discrete dynamical systems.

The paper is organized as follows. In Section 2, we formulate an IDE describing the model and introduce some technical definitions. In Section 3, we introduce the main results of the article. Section 4 studies some stroboscopic maps related to the model and the uniform persistence problem. The main results are proved in Sections 5 and 6. Section 7 is devoted to some numerical examples.

2. The self-cycling fermenter mathematical model

To the best of our knowledge, the first model was introduced by Wincure *et al.* in [19] and subsequently generalized in a series of articles [14–17] and [18]. In general, there are three processes to be described: (i) consumption of substrate; (ii) growth of the microorganisms; and (iii) the device emptying and refilling. Given an initial instant t_0 , the first two processes take place in any time interval (t_k, t_{k+1}) , with $k \in \mathbb{Z}^+$, and the last one will be considered as a pulse taking place in the sequence $\{t_k\}$ of emptying/refilling instants.

2.1. Biological processes

We will introduce two simplifying assumptions: the consumed substrate is directly converted in new microorganisms, whose mortality will be neglected. In consequence, between two consecutive emptying/refilling instants, that is, for any $t \in (t_k, t_{k+1})$, the behavior is described by the system:

$$\begin{cases} \dot{s}(t) = -\gamma^{-1}\mu(s(t))x(t), \\ \dot{x}(t) = \mu(s(t))x(t). \end{cases} \quad (1)$$

To explain (1), let us denote by $M_s(t)$ and $M_x(t)$ the masses, at time $t \in [t_0, +\infty)$, of a limiting substrate and the microorganism, respectively. The concentrations of substrate and microorganism (at time $t \geq t_0$) in the device, with constant volume V , are denoted by $s(t)$ and $x(t)$. In consequence, $M_s(t) = Vs(t)$ and $M_x(t) = Vx(t)$.

The growth rate (by unit of mass) of the microorganisms is described by a function $\mu(s)$ of the available substrate concentration s , where $\mu : [0, +\infty) \rightarrow [0, +\infty)$ satisfies:

- (C1) $\mu(\cdot)$ is continuously differentiable.
- (C2) $\mu(\cdot)$ is strictly increasing and $\mu(0) = 0$.
- (C3) $\mu'(s) \leq \mu'(0) < +\infty$ for any $s \geq 0$.

The parameter $\gamma > 0$ denotes the conversion factor of consumption in new biomass by unit time. Hence, between two consecutive emptying/refilling instants, the variation of the substrate and microbial masses are respectively given by $M'_s(t) = -\gamma^{-1}\mu(s(t))Vx(t)$ and $M'_x(t) = \mu(s(t))Vx(t)$. Finally, we obtain (1) by dropping the volume.

2.2. Emptying/refilling instants

We will consider the concurrence of two actions at $t = t_k$: the emptying of a proportion $p \in (0, 1)$ of the whole volume and the refilling of the same volume with a substrate concentration S^0 . The process is summarized by the diagrams:



By denoting $q = 1 - p$ and using the fact that the external flow to the tank does not have microorganisms, we can deduce that the concentrations at the instant $t = t_k$ vary according to:

$$\begin{cases} s(t^+) = qs(t) + pS^0, \\ x(t^+) = qx(t), \end{cases} \quad (2)$$

where $s(t^+) = \lim_{r \rightarrow t, r > t} s(r)$ and $x(t^+) = \lim_{r \rightarrow t, r > t} x(r)$.

The coupling of (1)–(2) determines the following IDE:

$$\begin{cases} \dot{s}(t) = -\gamma^{-1}\mu(s(t))x(t), \\ \dot{x}(t) = \mu(s(t))x(t), \end{cases} \left. \vphantom{\begin{cases} \dot{s}(t) = -\gamma^{-1}\mu(s(t))x(t), \\ \dot{x}(t) = \mu(s(t))x(t), \end{cases}} \right\} t \neq t_k, \\ \begin{cases} s(t^+) = q s(t) + p S^0 \\ x(t^+) = q x(t) \end{cases} \left. \vphantom{\begin{cases} s(t^+) = q s(t) + p S^0 \\ x(t^+) = q x(t) \end{cases}} \right\} t = t_k. \\ s(t_0) \in [0, S^0] \quad \text{and} \quad x(t_0) > 0. \end{cases} \quad (3)$$

The sequence of emptying/refilling instants $\{t_k\}$ can be defined by several ways, which determine the type and the properties of the resulting IDE:

- A usual choice is to define $\{t_k\}$ as the times when the trajectories in the phase space reach the line of a prefixed substrate level $s = \bar{s}$, this approach has been followed in the related model [15, 16], which considers the mortality of the species and its generalization [14, 18], where non-monotone functions $\mu(\cdot)$ are considered.
- In [17], the system (3) has a substrate dependent yield coefficient $\gamma = \gamma(s)$ and the times t_k are defined when the microbial biomass reaches a level $x = \bar{x}$.
- The seminal model [19] adds a third equation to (3) describing the variation of oxygen and t_k is defined when this variable decreases to a critical level. Some problems with the oxygen balance equation are pointed out in [6, pp.18–20], where an alternative formulation is proposed.
- The model [22] considers several species $\{x_i\}_{i=1}^n$ in competition for the limiting substrate and the sequence is defined as $t_k = kT$ with $T > 0$.

The last work is an example of *IDE at fixed times* (IDE-FT), whose theory is widely established and presents a development fashioned along the lines of ODE theory [13]. On the other hand, the first three groups of models are examples of *IDE at variable times* (IDE-VT), where the instants $\{t_k\}$ occur when the trajectories reach a predetermined set of hypersurfaces in the time–phase space. Its theory can be found in [11, 23–25].

From a mathematical point of view, IDE-VT have intrinsic technical difficulties (see, e.g., [13] and [25] for a detailed discussion) as the possible existence of a cluster point for $\{t_k\}$ (beating phenomenon [13]), nonuniqueness to the past, and loss of autonomy of the systems. In addition, a practical IDE-VT implementation for a batch fermenter assumes the ability of carrying out certain online measurements of the state variables; hereafter, we will relax these requirements.

2.3. Novelty of this work

In this work, we will introduce a sequence of emptying/refilling instants $\{t_k\}$ defined recursively as follows:

$$\Delta t_k = t_{k+1} - t_k = \tau \left(s \left(t_k^+ \right) \right), \quad (4)$$

where $\tau : [pS^0, S^0] \rightarrow [0, +\infty)$ will be called the *timing function*. We point out that the domain of $\tau(\cdot)$ is given by the third equation of (3).

Equation (4) points out that the unique necessary information to calculate the $(k + 1)$ th emptying/refilling instant is the substrate level at time $t = t_k$. This fact contrasts with the IDE-VT formalism, which needs to know the trajectory on an interval to the right of $t = t_k$. We emphasize that the dynamics associated to (3)–(4) leads to a new type of IDE: the *IDE at impulse dependent times* (IDE-IDT), which have been simultaneously introduced by [26] in a bioeconomic context and Karafyllis [27] in a control theory framework, with the name of *hybrid systems with sampling partition generated by the system*.

A preliminary IDE-IDT theory has been developed in [28]. On the other hand, applications to epidemiology and fisheries management are presented in [29] and [30], respectively.

In this work, we will assume that $\tau(\cdot)$ satisfies the following conditions:

(T1) $\tau(\cdot)$ is differentiable, positive, increasing, and

$$\tau(S^0) < \frac{1}{\mu(S^0)} \ln \left(1 + \frac{\mu(S^0)}{qS^0\mu'(0)} \right). \quad (5)$$

(T2) For any $y \in (pS^0, S^0)$, its derivative satisfies the inequality:

$$\tau'(y) < \frac{1}{\mu(S^0)} \left\{ \frac{1}{S^0 - y} - \frac{\mu'(0)}{\mu(S^0)} \left[e^{\mu(S^0)\tau(S^0)} - 1 \right] \right\}. \quad (6)$$

Remark 1

- Positiveness of $\tau(\cdot)$ is a technical assumption introduced to avoid the beating phenomena.
- The fact that $\tau(\cdot)$ is monotonically increasing will imply that lower substrate concentrations at $t = t_k^+$ (compared with higher ones) must lead to shorter intervals of time before the next emptying/refilling action. This property can help to promote the permanence of the biomass.
- The inequality (5) implies the positiveness of the right part of (6) and is a technical assumption imposed by the stability analysis described later.

2.4. Preliminaries

Lemma 1

For any initial condition $(s(t_0), x(t_0)) \in \Omega$, with:

$$\Omega = \left\{ (s, x) \in \mathbb{R}_+^2 : s \in [0, S^0] \text{ and } s + \gamma^{-1}x \leq 2S^0 \right\},$$

the system (3)–(4) has a unique solution $t \in [t_0, +\infty) \mapsto (s(t), x(t))$, which remains in Ω , for any $t \geq t_0$.

Proof

The existence and uniqueness follow from [28]. As the beating phenomena is excluded (Remark 1), it follows that the solution is defined in $[t_0, +\infty)$.

By (3), we have that $(s(t_0), x(t_0)) \in \Omega$ implies that $(s(t_0^+), x(t_0^+)) \in \Omega$, because

$$s(t_0^+) = qs(t_0) + pS^0 < S^0 \text{ and } x(t_0^+) = qx(t_0) > 0.$$

The continuous part of (3) implies that $t \mapsto s(t)$ and $t \mapsto x(t)$ are respectively decreasing and increasing on $(t_0, t_1]$. By evaluating at $t = t_1$, we have

$$s(t_1) < s(t_0^+) < S^0 \text{ and } x(t_1) > x(t_0^+) > 0,$$

which means that $s(t_1) < S^0$ and $x(t_1) > 0$. By following a recursive procedure, it can be proved that $s(t_k) < S^0$ and $x(t_k) > 0$ for any $k \in \mathbb{N}$.

Finally, the change of variables $w = s + \gamma^{-1}x$ leads to $\dot{w} = 0$ and

$$w(t_{k+1}) = qw(t_k) + pS^0.$$

If $w(t_0) \in (0, S^0]$, then it is easy to verify that $w(t_k)$ is a nondecreasing sequence convergent to S^0 . On the other hand, if $x(t_0) \in (S^0, 2S^0]$, it is straightforward to prove that $w(t_k)$ is strictly decreasing and the Lemma follows. \square

Remark 2

The solutions of (3)–(4) determine a unique stroboscopic map:

$$\begin{cases} s_{k+1} = \varphi_1(s_k, x_k), \\ x_{k+1} = \varphi_2(s_k, x_k), \end{cases} \tag{7}$$

where (s_k, x_k) is a solution of (3)–(4) evaluated at $t = t_k^+$. Moreover, $\varphi_1(\cdot, \cdot)$ and $\varphi_2(\cdot, \cdot)$ can be deduced by the integral equation corresponding to (3)–(4).

Definition 1

A fixed point $p^* = (s^*, x^*)$ of (7) is

- (i) Locally stable if there exists a ball centered at p^* with radius $\delta > 0$, $B_\delta(p^*)$, such that if $(s_0, x_0) \in B_\delta(p^*)$, then $(s_k, x_k) \in B_\delta(p^*)$ for any k . Locally asymptotically stable if is locally stable and $\lim_{k \rightarrow +\infty} (s_k, x_k) = p^*$.
- (ii) Globally asymptotically stable on a subset $\Omega_* \subseteq \Omega$ if is locally stable and, for any initial condition $(s_0, x_0) \in \Omega_*$, it follows that $\lim_{k \rightarrow +\infty} (s_k, x_k) = p^*$.

Remark 3

Any fixed point (s^*, x^*) of (7) leads to a $\tau(s^*)$ -periodic solution $t \mapsto (s(t), x(t))$ of (3)–(4) with $(s(t_k^+), x(t_k^+)) = (s^*, x^*)$ for any $k \geq 0$.

Definition 2

A $\tau(s^*)$ -periodic solution $t \mapsto (s(t), x(t))$ of (3)–(4), with $\Delta t_k = \tau(s^*)$, is globally asymptotically stable on Ω_* if (s^*, x^*) is a fixed point of (7) globally asymptotically stable on Ω_* .

3. Main results

Theorem 1

Assume that the timing function $\tau: [pS^0, S^0] \rightarrow [0, +\infty)$ is continuous and positive. Then, there exists a constant biomass-free solution $t \mapsto (S^0, 0)$ of (3)–(4), which is globally asymptotically stable on $\Omega_0 = \{(s, x) \in \Omega : x = 0\}$.

Proof

By following the lines of the proof of Lemma 1, it can be proved that any initial condition $(s(t_0), 0) \in \Omega_0$ leads to a unique solution $t \mapsto (s(t), 0) \in \Omega_0$, where $s(t) = s(t_k^+)$ for any $t \in (t_k, t_{k+1}]$. In addition, the stroboscopic map (7) corresponding to this initial condition is given by

$$s(t_{k+1}^+) = qs(t_k^+) + pS^0 \quad \text{and} \quad x(t_{k+1}^+) = x(t_k^+) = 0$$

Finally, it is easy to see that $s(t_k^+) \rightarrow S^0$ as $k \rightarrow +\infty$ and the result follows by Definition 2. □

The following results assume that the initial conditions have biomass, and its proof will be the consequence of a series of technical results:

Theorem 2 (Existence of a periodic cycle)

Assume that the timing function $\tau: [pS^0, S^0] \rightarrow [0, +\infty)$ satisfies the conditions (T1)–(T2). If the inequality

$$\frac{1}{\mu(S^0)} \ln\left(1 + \frac{p}{q}\right) < \tau(S^0) \tag{8}$$

is verified, then there exists a periodic positive solution $t \mapsto (\hat{s}(t), \hat{x}(t))$ of (3)–(4), which is globally asymptotically stable on $\Omega_+ = \{(s, x) \in \Omega : x > 0\}$.

Theorem 3 (Washout of the biomass)

Assume that the timing function $\tau: [pS^0, S^0] \rightarrow [0, +\infty)$ satisfies the conditions (T1)–(T2). If the inequalities

$$\tau(S^0) \leq \frac{1}{\mu(S^0)} \ln\left(1 + \frac{p}{q}\right) < \frac{1}{\mu(S^0)} \ln\left(1 + \frac{\mu(S^0)}{qS^0\mu'(0)}\right) \tag{9}$$

are satisfied, then the constant biomass-free solution $t \mapsto (S^0, 0)$ of (3)–(4) is globally asymptotically stable on Ω_+ .

From a bioprocess point of view, of Theorems 2 and 3 prompt the existence of a trade-off between the fraction of removed volume p and the maximal cycle duration $\tau(S^0)$. Indeed,

- If the maximal time between two emptying/refilling actions is shorter than $\ln(1 + p/q)/\mu(S^0)$, the process fails and concludes in the extinction of the biomass in the long term since the resulting biomass growth at each cycle cannot compensate the average of evacuated biomass.
- If $\tau(S^0) > \ln(1 + p/q)/\mu(S^0)$, then the time between two emptying/refilling actions allows a sustainable growth of the biomass.

Remark 4

Observe that

- (i) The inequalities (5) and (8) can be written as

$$\frac{1}{1-p} < e^{\mu(S^0)\tau(S^0)} < 1 + \frac{1}{1-p} \frac{\mu(S^0)}{S^0\mu'(0)},$$

which allows to estimate an upper bound for the removed fraction p :

$$0 < p < \frac{\mu(S^0)}{S^0\mu'(0)} < 1,$$

where the last bound can be deduced from (C3).

- (ii) The left inequality of (5) is equivalent to

$$e^{\mu(S^0)\tau(S^0)} < \frac{1}{1-p}.$$

A direct byproduct of Theorem 2 is the following result:

Corollary 1

If the timing function is constant $\tau(\cdot) = T$ satisfying

$$\frac{1}{\mu(S^0)} \ln\left(1 + \frac{p}{q}\right) < T < \frac{1}{\mu(S^0)} \ln\left(1 + \frac{1}{q} \frac{\mu(S^0)}{S^0\mu'(0)}\right), \tag{10}$$

then there exists a periodic positive solution $t \mapsto (\hat{s}(t), \hat{x}(t))$ of (3)–(4), which is globally asymptotically stable on Ω_+ .

Nevertheless, we point out that this result is more restrictive than the obtained by Smith in [22, Section 3], which only requires the left inequality of (10) to ensure the stability of a periodic solution.

4. Some useful lemmata

The proof of Theorems 2 and 3 will be the consequence of a series of intermediate results about the stroboscopic map (7) and its asymptotic properties; we introduce a change of variables leading to a more tractable map. In addition, we will provide some conditions ensuring either the extinction or the permanence of the biomass.

The change of variables $w = s + \gamma^{-1}x$ and $\eta = w - s = \gamma^{-1}x$, leads to

$$\begin{cases} \dot{w}(t) = 0, \\ \dot{\eta}(t) = \mu(w(t) - \eta(t)) \eta(t), \end{cases} \quad t \neq t_k, \quad (11)$$

$$\begin{cases} w(t^+) = qw(t) + pS^0, \\ \eta(t^+) = q\eta(t). \end{cases} \quad t = t_k,$$

and

$$\Delta t_k = t_{k+1} - t_k = \tau \left(w(t_k^+) - \eta(t_k^+) \right). \quad (12)$$

It is easy to verify that (11)–(12) is well defined by using the fact that $\eta(\cdot) = \gamma^{-1}x(\cdot)$, together with (C1)–(C2) and the invariance of the set Ω under (3)–(4).

Lemma 2

The solutions of (11)–(12) determine a unique stroboscopic map:

$$\begin{cases} w_{k+1} = G_1(w_k, \eta_k) = qw_k + pS^0, \\ \eta_{k+1} = G_2(w_k, \eta_k) = q\eta_k \exp\left(\int_0^{\tau(w_k - \eta_k)} \mu[w_k - \phi(\sigma; 0, \eta_k)] d\sigma\right), \end{cases} \quad (13)$$

where $w(t_k^+) = w_k$, $\eta_k = \eta(t_k^+)$ and $\sigma \mapsto \phi(\sigma; 0, \eta_k)$ is the unique solution of

$$\begin{cases} z' = \mu(w_k - z)z \\ z(0) = \eta_k. \end{cases} \quad (14)$$

Proof

Let us consider an arbitrary solution $t \rightarrow (w(t), \eta(t))$ of (11) and its corresponding sequence $\{t_k\}$ of emptying/refilling instants determined by (12).

Notice that if $t \in (t_k, t_{k+1}]$, then $w(t) = w(t_k^+)$. By denoting $w_k = w(t_k^+)$ for any $k \geq 0$, we can verify that the associated stroboscopic map is

$$w_{k+1} = qw_k + pS^0.$$

Now, if $t \in (t_k, t_{k+1}]$, we can deduce that

$$\begin{aligned} \eta(t_{k+1}^+) &= q\eta(t_k^+) \exp\left(\int_{t_k}^{t_k + \tau(w_k - \eta_k)} \mu[w_k - \phi(r; t_k, \eta_k)] dr\right) \\ &= q\eta(t_k^+) \exp\left(\int_0^{\tau(w_k - \eta_k)} \mu[w_k - \phi(\sigma + t_k; t_k, \eta_k)] d\sigma\right) \\ &= q\eta(t_k^+) \exp\left(\int_0^{\tau(w_k - \eta_k)} \mu[w_k - \phi(\sigma; 0, \eta_k)] d\sigma\right), \end{aligned}$$

where the last equality is a consequence of the uniqueness of the solution of (14). □

An important byproduct of Lemma 2 is the boundedness of the sequence (13):

Corollary 2

The stroboscopic map (13) is bounded in the compact set:

$$\Omega_1 = \left\{ (w, \eta) \in \mathbb{R}_+^2 : 0 \leq w \leq 2S^0 \text{ and } 0 \leq \eta \leq 2S^0 \right\}.$$

Notice that (13) has a triangular structure because the first equation is not dependent of the second one. Moreover, it is easy to see that $w(\cdot)$ is a step function satisfying

$$\lim_{k \rightarrow +\infty} w_k = S^0 \quad \text{and} \quad \lim_{t \rightarrow +\infty} w(t) = S^0, \quad (15)$$

which motivates to introduce the *limit equation*:

$$\begin{cases} v'(t) = \mu[S^0 - v(t)]v(t), & t \neq \alpha_k, \\ v(t^+) = qv(t), & t = \alpha_k, \\ \Delta\alpha_k = \tau(S^0 - v(\alpha_k^+)). \end{cases} \tag{16}$$

The rest of the proof will be decomposed in several steps: firstly, we will study the stability properties of (16). Secondly, the uniform persistence problem is addressed. Finally, we will study the stroboscopic map associated to (3).

4.1. Asymptotic behavior of the limit equation (16)

By following the lines of Lemma 2, we can deduce that a stroboscopic map associated to (16) is given by

$$v(\alpha_{k+1}^+) = qv(\alpha_k^+) \exp\left(\int_0^{\tau(S^0 - v(\alpha_k^+))} \mu[S^0 - V(t; 0, v(\alpha_k^+))] dt\right),$$

where $t \mapsto V(t; 0, v)$ is the solution of the initial value problem:

$$\begin{cases} z' = \mu(S^0 - z)z \\ z(0) = v. \end{cases}$$

This map can be written as $v(\alpha_{k+1}^+) = F(v(\alpha_k^+))$, with $F : [0, qS^0] \rightarrow [0, qS^0]$ defined as follows:

$$F(v) = qve^{W(v)}, \quad \text{with} \quad W(v) = \int_0^{\tau(S^0 - v)} \mu[S^0 - V(s; 0, v)] ds. \tag{17}$$

To study the asymptotic behavior of (17), we will employ a restatement of Lemma 2 from [30]:

Proposition 1

Let us consider a one-dimensional map: $v_{k+1} = F(v_k)$ with $v_0 \in [0, K]$, where $F : [0, K] \rightarrow [0, K]$ satisfies the following properties:

- (a) $F(0) = 0$ and $F(K) < K$.
- (b) $F(\cdot)$ is differentiable and increasing.
- (c) For any $v \in (0, K]$ it follows that $(F(v)/v)' < 0$.

Then,

- 1. If $F'(0) \leq 1$, then it follows that $v_k \rightarrow 0$ as $k \rightarrow \infty$.
- 2. If $F'(0) > 1$, then there exists a unique fixed point $v^* \in (0, K)$ which is globally asymptotically stable on $(0, K]$.

Lemma 3

Assume that (T1) and (T2) are satisfied.

- (i) If (8) is satisfied, then the stroboscopic map (17) has a globally stable (on $(0, qS^0]$) fixed point $\eta^* \in (0, qS^0)$ with $F'(\eta^*) < 1$.
- (ii) If (9) is satisfied, then 0 is the unique fixed point of the stroboscopic map (17) and is globally stable (on $[0, qS^0]$) with $F'(0) \leq 1$.

Proof

We will prove that $F(\cdot)$ satisfies the hypotheses of Proposition 1 with $K = qS^0$. Firstly, we will verify that the hypothesis (a) is satisfied: note that $F(0) = 0$ and, to check $F(qS^0) < qS^0$, we proceed by contradiction by assuming that there exists α_k^+ such that

$$0 < v(\alpha_j^+) < qS^0 \quad \text{for any } j \in \{0, 1, \dots, k-1\} \quad \text{and} \quad F(v(\alpha_k^+)) = v(\alpha_{k+1}^+) = qS^0,$$

then it follows that $v(\alpha_{k+1}) = S^0$. In addition, by (16) we can observe that $v'(t) = 0$ and $v(t) = S^0$ for any $t \in (\alpha_k^+, \alpha_{k+1}]$, which implies $v(\alpha_k^+) = qv(\alpha_k) = S^0$, obtaining a contradiction with the inequality stated earlier.

The hypothesis (b) is satisfied because $F'(v) = qe^{W(v)}\{1 + vW'(v)\}$, with

$$\begin{aligned} W'(v) &= -\mu[S^0 - V(\tau(S^0 - v); 0, v)]\tau'(S^0 - v) \\ &\quad - \int_0^{\tau(S^0 - v)} \mu'[S^0 - V(s; 0, v)] \frac{\partial V}{\partial v}(s; 0, v) ds. \end{aligned}$$

By defining $u(s; 0, v) = S^0 - V(s; 0, v)$ for any $v \in [0, qS^0]$, we have that $F'(\cdot) > 0$ if and only if

$$v \left\{ \mu[u(\tau(S^0 - v); 0, v)]\tau'(S^0 - v) + \int_0^{\tau(S^0 - v)} \mu'[u(s; 0, v)] \frac{\partial V}{\partial v}(s; 0, v) ds \right\} < 1, \tag{18}$$

for any $v \in [0, qS^0]$.

To determine if (18) is satisfied, observe that $s \mapsto (\partial V/\partial v)(s; 0, v)$ is solution of the initial value problem:

$$\begin{cases} z'(s) = \{\mu[u(s; 0, v)] - \mu'[u(s; 0, v)]V(s; 0, v)\}z(s), \\ z(0) = 1, \end{cases}$$

and by integral representation, we obtain that for any $s \in [0, \tau(y)]$:

$$\begin{aligned} \frac{\partial V}{\partial v}(s; 0, v) &= \exp\left(\int_0^s \{\mu(u(r; 0, v)) - \mu'(u(r; 0, v))V(r; 0, v)\}dr\right) \\ &\leq e^{\mu(S^0)s}. \end{aligned}$$

Now, by using (C1)–(C2) and (T1) combined with $0 < u(s; 0, v) < S^0$, the change of variables $y = S^0 - v \in (pS^0, S^0)$ and the last inequality, it follows that

$$\begin{aligned} \mu[u(\tau(y); 0, v)]\tau'(y) + \int_0^{\tau(y)} \mu'[u(s; 0, v)]\frac{\partial V}{\partial v}(s; 0, v)ds &< \mu(S^0)\tau'(y) + \mu'(0) \int_0^{\tau(y)} e^{\mu(S^0)s} ds \\ &< \mu(S^0)\tau'(y) + \frac{\mu'(0)}{\mu(S^0)} [e^{\mu(S^0)\tau(y)} - 1] \end{aligned}$$

for any $y \in (pS^0, S^0)$. Now, by using this fact and noticing that the inequality (6) from (T2) is equivalent to

$$(S^0 - y) \left\{ \mu(S^0)\tau'(y) + \frac{\mu'(0)}{\mu(S^0)} [e^{\mu(S^0)\tau(y)} - 1] \right\} < 1,$$

which implies that (18) is satisfied.

The hypothesis (c) is satisfied because $(F(v)/v)' = qe^{W(v)}W'(v)/v$ and by using the fact that $W'(v)$ is negative.

If (8) is verified, we have $F'(0) = qe^{W(0)} = qe^{\mu(S^0)\tau(S^0)} > 1$ and Proposition 1 imply that (17) has a fixed point $\eta^* \in (0, qS^0)$, which is globally asymptotically stable for any initial condition in $(0, qS^0]$. Finally, by following the proof of Proposition 1, it can be deduced that $0 < F'(\eta^*) < 1$ and the statement (i) follows. Otherwise, $F'(0) < 1$ is equivalent to (9) and (ii) can be proved in a similar way. \square

4.2. Uniform persistence

The microbial species described by (3)–(4) is said to be *uniformly persistent* (see, e.g., [31] for details) if there exists $\delta > 0$ independent of the initial condition such that

$$\liminf_{t \rightarrow +\infty} x(t) > \delta.$$

Uniform persistence is a necessary condition for the self-cycling fermentation.

Lemma 4

Assume that (T1)–(T2) are verified.

(i) If (8) is satisfied, then there exists $\beta > 0$ independent of η_0 , such that

$$\liminf_{k \rightarrow +\infty} \eta_k > \beta, \tag{19}$$

for any initial condition of (13).

(ii) If (9) is satisfied, then $\lim_{k \rightarrow +\infty} \eta_k = 0$, for any initial condition of (13).

Proof

To prove (i), we will use theory of average Lyapunov functions for discrete dynamical systems (see, e.g., [20, 21] for details). Let us note that the solutions of (13) define a discrete semiflow over the compact metric space Ω_1 .

Let us define the subset $\Gamma_1 = \{(w, \eta) \in \Omega_1 : \eta = 0\} \subset \partial\Omega_1$. In addition, let us introduce the function $P : \Omega_1 \rightarrow \mathbb{R}^+$ defined by $P(w, \eta) = \eta$ and notice that

- (a) $P(w, \eta) = 0$ if and only if $(w, \eta) \in \Gamma_1$.
- (b) For any $(w_0, \eta_0) \in \Gamma_1$, it follows that

$$\begin{aligned} P(w_{k+1}, \eta_{k+1}) &= \eta_{k+1} = q \exp\left(\int_0^{\tau(w_k)} \mu[w_k - \phi(\sigma; 0, 0)]d\sigma\right) \eta_k \\ &= qe^{\tau(w_k)\mu(w_k)} \eta_k = \Psi(w_k)\eta_k. \end{aligned}$$

Notice that (8) implies that

$$\Psi(S^0) = qe^{\tau(S^0)\mu(S^0)} > 1.$$

By using this fact, together with (15) and continuity properties, it can be proved that

$$\sup \left\{ \prod_{j=0}^{k-1} \Psi(w_j) : j \geq 1 \right\} > 1.$$

Now, by following Corollary 2.3 from [20] (see also [21]), we have that Γ_1 is a repellor, which is equivalent to (19) and the statement (i) follows. To prove (ii), notice that Lemma 1 implies that

$$w_k - \eta_k \leq S^0 \quad \text{and} \quad w_k - \phi(\sigma; 0, \eta_k) \leq S^0,$$

this fact combined with (C2), (T1), and (9) implies the inequalities:

$$\frac{\eta_{k+1}}{\eta_k} = q \exp \left(\int_0^{\tau(w_k - \eta_k)} \mu[w_k - \phi(\sigma; 0, \eta_k)] d\sigma \right) \leq qe^{\tau(S^0)\mu(S^0)} < 1$$

and the result follows straightforwardly. □

An important consequence of Lemma 4 is the following corollary:

Corollary 3

Assume that (T1)–(T2) are verified.

- (i) If (8) is verified, then the microbial species described by (3)–(4) is uniformly persistent.
- (ii) If (9) is verified, then the microbial species satisfies $\lim_{t \rightarrow +\infty} x(t) = 0$.

Proof

Notice that $x(t) = \gamma \eta(t)$ is strictly increasing in $(t_k, t_{k+1}]$ and satisfies

$$x(t_k^+) < x(t) \leq x(t_{k+1}) = (1 - p)^{-1} x(t_{k+1}^+), \quad \text{for any } t \in (t_k, t_{k+1}]. \tag{20}$$

If (8) is verified, Lemma 4 implies the existence of $\delta = \gamma\beta > 0$ (independent of the initial conditions) such that $\liminf_{t \rightarrow +\infty} x(t) > \delta$ and (i) follows.

On the other hand, if (9) is verified, Lemma 4 combined with (20) implies statement (ii). □

5. Proof of Theorem 2

Firstly, we will show the following discrete stability results:

Lemma 5

If (T1)–(T2) and (8) are satisfied, the unique fixed points of (13) are $E_0 = (S^0, 0)$ and $E^* = (S^0, \eta^*)$, where η^* is the non-trivial fixed point (17). In addition, E^* is locally asymptotically stable.

Proof

If $(\bar{w}, \bar{\eta})$ is a fixed point of (13), then it is easy to verify that $G_1(\bar{w}, \bar{\eta}) = q\bar{w} + pS^0 = \bar{w}$ if and only if $\bar{w} = S^0$. Now, we can observe that the map

$$G_2(S^0, \eta) = q\eta \exp \left(\int_0^{\tau(S^0 - \eta)} \mu[S^0 - \phi(\sigma; 0, \eta)] d\sigma \right) = F(\eta)$$

has two fixed points, a trivial one $\bar{\eta} = 0$ and $\bar{\eta} = \eta^* \in (0, qS^0)$, whose existence was proved by using Lemma 3.

The linearization of (13) around E^* leads to a triangular matrix whose diagonal terms are $q \in (0, 1)$ and

$$\frac{\partial G_1}{\partial w}(w, z) \Big|_{E^*} = q, \quad \text{and} \quad \frac{\partial G_2}{\partial \eta}(w, \eta) \Big|_{E^*} = qe^{Z(S^0, \eta^*)} \left\{ 1 + \eta^* \frac{\partial Z}{\partial \eta}(S^0, \eta^*) \right\}$$

where $Z(w, \eta) = \int_0^{\tau(w - \eta)} \mu[w - \phi(s; 0, \eta)] ds$ and $\phi(s; 0, \eta)$ is solution of the initial value problem

$$\begin{cases} z' = z\mu(w - z) \\ z(0) = \eta. \end{cases} \tag{21}$$

As $(\partial G_1/\partial w)(E^*) = q \in (0, 1)$, the local asymptotic stability of E^* follows if and only if $|\partial G_2/\partial \eta)(E^*)| < 1$.
By following the lines of the proof of Lemma 3, it can be proved that

$$\left. \frac{\partial G_2}{\partial \eta}(w, \eta) \right|_{E^*} = qe^{Z(S^0, \eta^*)} \left\{ 1 + \eta^* \frac{\partial Z}{\partial \eta}(S^0, \eta^*) \right\} = F'(\eta^*) < 1,$$

where the last inequality follows from Lemma 3 and (8). □

Lemma 6

If (T1)–(T2) and (8) are satisfied, then E^* is globally asymptotically stable.

Proof

Let $\bar{u}_0 = (w_0, \eta_0)$ be any initial condition of (13) and let us define its orbit and ω -limit respectively by

$$\theta(\bar{u}_0) = \{G^n(\bar{u}_0) : n \geq 0\} \quad \text{where} \quad G^n = \underbrace{G \circ \dots \circ G}_{n \text{ times}} \quad \text{and} \quad G(\cdot) = (G_1(\cdot), G_2(\cdot)),$$

with G_1 and G_2 defined in (13), and

$$\omega(\bar{u}_0) = \left\{ (\tilde{w}, \tilde{\eta}) : \exists n_k \rightarrow +\infty \text{ such that } \lim_{k \rightarrow +\infty} G^{n_k}(\bar{u}_0) = (\tilde{w}, \tilde{\eta}) \right\}.$$

It is straightforward to verify that if $(\tilde{w}, \tilde{\eta}) \in \omega(\bar{u}_0)$, then it follows that $\tilde{w} = S^0$. In addition, Lemma 4 implies that $\tilde{\eta} > 0$.
As $\omega(\bar{u}_0)$ is invariant and $(\tilde{w}, \tilde{\eta}) = (S^0, \tilde{\eta}) \in \omega(\bar{u}_0)$, we can deduce that

$$\theta(S^0, \tilde{\eta}) = \{G^n(S^0, \tilde{\eta}) = (S^0, G_2^n(S^0, \tilde{\eta})) : n \geq 0\} \in \omega(\bar{u}_0).$$

On the other hand, notice that $n \mapsto G_2^n(S^0, \tilde{\eta})$ is a solution of the map (17) with $v_0 = \tilde{\eta}$ and by using (T1)–(T2) together with Lemma 3, we obtain that

$$\lim_{n \rightarrow +\infty} G^n(S^0, \tilde{\eta}) = (S^0, \eta^*),$$

which implies that $(S^0, \eta^*) \in \omega(\bar{u}_0)$. Hence, there exists a subsequence $\{n_k\}_k$ such that

$$\lim_{k \rightarrow +\infty} G^{n_k}(\bar{u}_0) = (S^0, \eta^*) \in \omega(\bar{u}_0). \tag{22}$$

Finally, by using (22) combined with the local asymptotic stability of E^* , we can conclude that $G^n(\bar{u}_0)$ enters the basin of attraction of E^* in a finite time and the Lemma follows. □

Now, by using $w_k = s_k + \gamma^{-1}x_k$ and $\eta_k = w_k - s_k$ together with Lemma 6, we can deduce that the stroboscopic map (7) has a fixed point $(s^*, x^*) = (S^0 - \eta^*, \gamma \eta^*)$, which is globally asymptotically stable on Ω_+ , and the Theorem follows by using Definition 2 and Remark 3.

6. Proof of Theorem 3

The structure of the proof is similar to the previous one. Firstly, we have the stability results.

Lemma 7

If (T1)–(T2) and (9) are satisfied, then $E^0 = (S^0, 0)$ is the unique fixed point of (13) and is locally asymptotically stable.

Proof

By following the lines of the proof of Lemma 5, we know that if $(\bar{w}, \bar{\eta})$ is a fixed point of (13), then $G_1(\bar{w}, \bar{\eta}) = q\bar{w} + pS^0 = \bar{w}$ if and only if $\bar{w} = S^0$, and by using Lemma 3 combined with (5), it follows that the map

$$G_2(S^0, \eta) = q\eta \exp \left(\int_0^{\tau(S^0 - \eta)} \mu[S^0 - \phi(\sigma; 0, \eta)] d\sigma \right) = F(\eta)$$

has a unique fixed point $\bar{\eta} = 0$.

As before, the linearization of (13) around E^0 leads to a triangular matrix whose diagonal terms are $q \in (0, 1)$ and

$$\left. \frac{\partial G_1}{\partial w}(w, z) \right|_{E^0} = q \quad \text{and} \quad \left. \frac{\partial G_2}{\partial \eta}(w, \eta) \right|_{E^0} = qe^{\tau(S^0)\mu(S^0)} < 1,$$

which implies local asymptotic stability. □

Lemma 8

If (T1)–(T2) and (9) are satisfied, then the fixed point E^0 of (13) is globally asymptotically stable on Ω_+ .

Proof

Let $\vec{u}_0 \in \Omega_+$ be any initial condition of (13) and $\omega(\vec{u}_0)$ its ω -limit. By using statement (ii) of Lemma 4, it can be proved that for any $(\vec{w}, \tilde{\eta}) \in \omega(\vec{u}_0)$, it follows that $\tilde{\eta} = 0$.

On the other hand, the invariance of $\omega(\vec{u}_0)$ combined with $(\vec{w}, \tilde{\eta}) = (\vec{w}, 0) \in \omega(\vec{u}_0)$ implies that

$$\theta(\vec{w}, 0) = \{G^n(\vec{w}, 0) = (G_1^n(\vec{w}), 0): n \geq 0\} \in \omega(\vec{u}_0).$$

Now, observe that $k \mapsto G_1^k(\vec{w}, 0)$ is a solution of the discrete map:

$$\begin{cases} w_{k+1} = qw_k + pS^0 \\ w_0 = \vec{w}, \end{cases}$$

and it is easy to verify that $w_k \rightarrow S^0$, which implies that $E_0 \in \omega(\vec{u}_0)$.

Finally, the global stability follows by using $E_0 \in \omega(\vec{u}_0)$ combined with its local asymptotic stability. □

As before, we can verify that (7) has a fixed point $(S^0 - \eta^*, \gamma\eta^*)$, which is globally asymptotically stable on Ω_+ , and the Theorem follows by using Definition 2 and Remark 3.

7. Numerical examples

When considering ethanol as limiting substrate and *Acinetobacter calcoaceticus* as the microbial species, the growth is usually described by the Michaelis–Menten function:

$$\mu(s) = \mu_{\max} \frac{s}{k_s + s} \quad \text{with} \quad \mu_{\max} > 0 \quad \text{and} \quad k_s > 0, \tag{23}$$

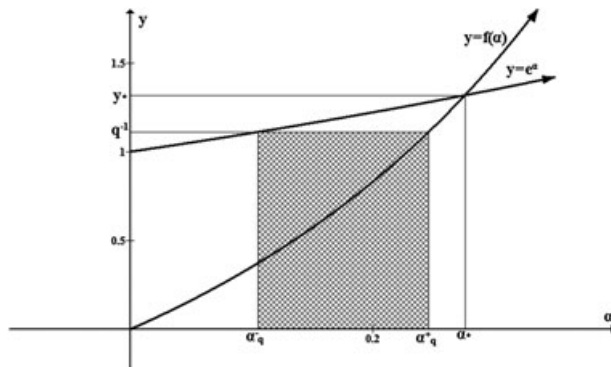


Figure 1. Graph of $f(\alpha)$ and e^α . The fraction $q \in (0, 1)$ satisfying (27) must be chosen such that if $q^{-1} \in (1, y^*) = (1, e^{\alpha^*})$, then $\alpha \in (\alpha_q^-, \alpha_q^+)$.

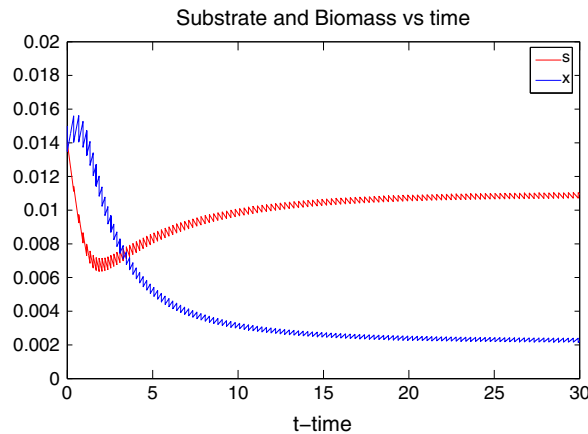


Figure 2. Convergence to the periodic solution of (3) obtained with timing function (24), the parameters (26), $\tau_{\max} = 3/8$ and $q = 0.9$. The substrate is convergent to a periodic function centered around 0.011 while the biomass converges to a function lowerly bounded by 0.002, which ensures uniform persistence.

which satisfies (C1)–(C3).

In addition, we will consider a timing function $\tau: [pS^0, S^0] \rightarrow \mathbb{R}_+$ defined as follows:

$$\tau(y) = \frac{\tau_{\max}}{qS^0}(y - pS^0). \tag{24}$$

By defining $\Theta = \frac{\mu(S^0)}{S^0\mu'(0)}$ and $\alpha = \mu(S^0)\tau(S^0)$ (where $\tau(S^0) = \tau_{\max}$), it is easy to verify that (T1)–(T2) are implied by the inequality $q(e^\alpha - 1) < \Theta(1 - \alpha)$, which is equivalent to

$$\frac{1}{\Theta} \frac{e^\alpha - 1}{1 - \alpha} = f(\alpha) < \frac{1}{q}, \text{ for any } \alpha \in (0, 1). \tag{25}$$

Let us consider the kinetic parameters from [19]:

$$\gamma = 0.73, \quad \mu_{\max} = 0.6 [1/h], \quad \text{and} \quad k_s = 0.007 [gr/L], \tag{26}$$

where h , gr , and L denote hours, grams and liters respectively. So that, if $S^0 = 0.014 [gr/L]$, then $\mu'(0) = 600/7 [L/(gr h)]$ and $\mu(S^0) = 2/5 [1/h]$. Finally, we obtain the additional parameters $\Theta = 1/3$ and $\alpha = (2/5)\tau_{\max}$.

7.1. Conditions for Theorem 2

Considering (8) in terms of Θ , α , and the inequality (25), the pair (q, α) must satisfy

$$f(\alpha) < \frac{1}{q} < e^\alpha, \text{ for any } \alpha \in (0, 1). \tag{27}$$

Notice that $f(\alpha) < e^\alpha$ if and only if $\alpha \in (0, \alpha_*)$ with $\alpha_* \approx 0.27613$. Because $f(\alpha_*) = e^{\alpha_*} \approx 1.31802$, we conclude that q^{-1} has to be chosen in the interval $(1, e^{\alpha_*})$, that is, $q \in I_q = (0.7587 \dots, 1)$. According to Figure 1, given some $q \in I_q$, the parameter α has to be in (α_q^-, α_q^+) , with $f(\alpha_q^+) = e^{\alpha_q^-} = 1/q$.

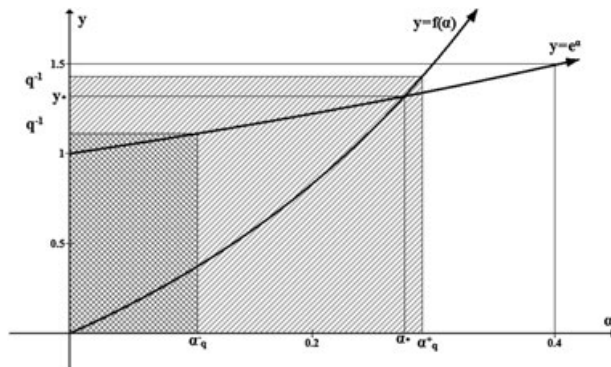


Figure 3. Graphs of $f(\alpha)$ and e^α . The fraction $q \in (0, 1)$ satisfying (28) could be chosen of two ways: (a) If $q^{-1} \in (1, y^*)$, then $\alpha \in (0, \alpha_q^-)$. (b) If $q^{-1} \in (y^*, 3/2)$, then $\alpha \in (0, \alpha_q^+)$.

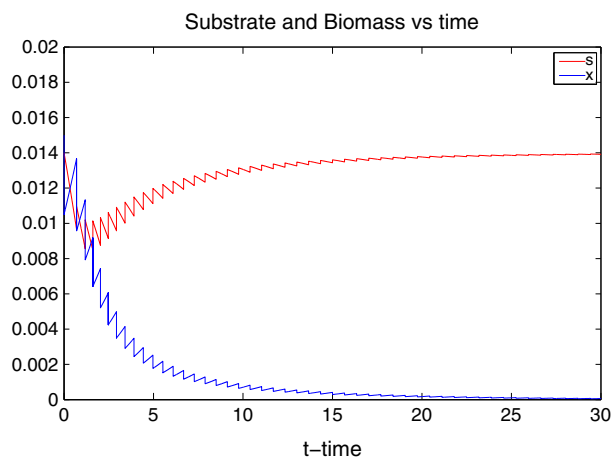


Figure 4. Washout of the biomass in (3) verified with timing function (24), the parameters (26), $\tau_{\max} = 7/10$ and $q = 7/10$.

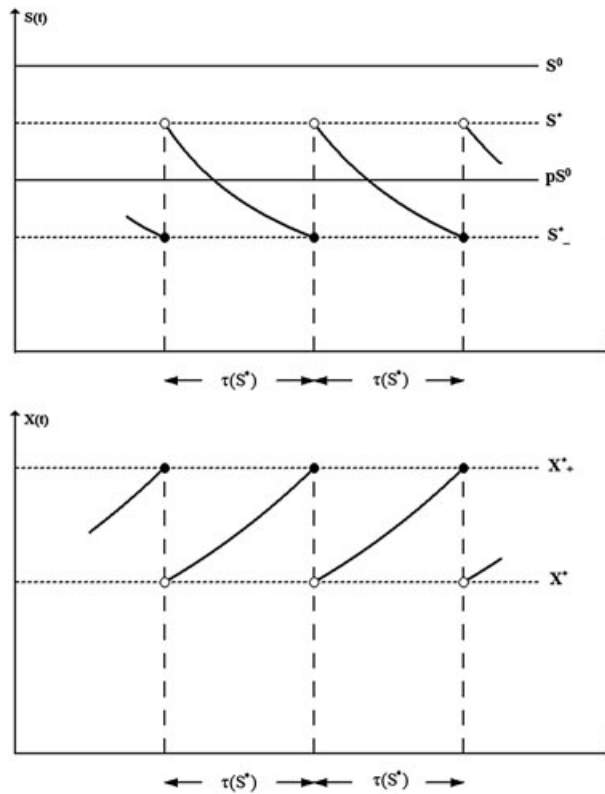


Figure 5. Idealized image of the periodic solution $t \mapsto (\hat{s}(t), \hat{x}(t))$.

For instance, if $q = 0.9$, then $\alpha_q^- \approx 0.1054$ and $\alpha_q^+ \approx 0.21216$. We can take $\alpha = 15/100$, which leads to $\tau_{\max} = 3/8$. The numerical simulation of the trajectories $t \mapsto s(t)$ and $t \mapsto x(t)$, with the initial condition $(s(0), x(0)) = (0.014, 0.015)$, are presented in Figure 2 and confirms our result.

7.2. Conditions for Theorem 3

Let us consider (9) in terms of θ, α , and the inequality (25). These conditions can be summarized as

$$\max\{f(\alpha), e^\alpha\} < \frac{1}{q} < \frac{3}{2}, \quad \text{for any } \alpha \in (0, 1). \tag{28}$$

If $q^{-1} < f(\alpha_*) = e^{\alpha_*}$, then from Figure 3, it can be deduced that α has to be restricted to the interval $(0, \alpha_q^-)$. On the other hand, if $q^{-1} \in (e^{\alpha_*}, 3/2) = (1.31802\dots, 3/2)$, then $\alpha \in (0, \alpha_q^+)$.

For example, if $q = 7/10$ (i.e., $q^{-1} \approx 1.4285$), then α has to be in $(0, \alpha_q^+)$ with $\alpha_q^+ \approx 0.29092$. By choosing $\alpha = 28/100$, we obtain $\tau_{\max} = 7/10$, and Figure 4 shows the numerical simulation (carried out with the previous initial conditions), and the washout of the biomass is illustrated.

8. Discussion

Provided that the assumptions (T1)–(T2) are satisfied, we have deduced sufficient conditions ensuring either the existence and attractiveness of a periodic cycle ($\mathcal{R}_0 > 1$) or the washout of biomass ($\mathcal{R}_0 < 1$), where

$$\mathcal{R}_0 = \frac{\mu(S^0)\tau(S^0)}{\ln(1 + p/q)}.$$

A key step in the proof of our main results is the existence and stability of a fixed point of the stroboscopic map $x_{k+1} = F(x_k)$, with $F(\cdot)$ defined by (17). In this paper, the assumptions (T1)–(T2) were introduced to ensure the increasing monotonicity of $F(\cdot)$ and to use Proposition 1. This choice provides a simple study of the one-dimensional stroboscopic map but gives us conservative results. Less restrictive conditions could be obtained by relaxing assumptions (T1)–(T2) but, it is still a pending task.

Theorem 2 proves the existence of a globally asymptotically stable periodic solution $t \mapsto (\hat{s}(t), \hat{x}(t))$ of (3)–(4). A careful lecture of the proof shows the following properties described in Figure 5: (i) the function $t \mapsto \hat{s}(t)$ is strictly decreasing on (t_k, t_{k+1}) ; and (ii) the function $t \mapsto \hat{x}(t)$ is strictly increasing on (t_k, t_{k+1}) . The values at the boundary are

$$\begin{aligned} \hat{s}(t_k^+) &= s^* = S^0 - \eta^* \in (pS^0, S^0) & \text{and} & \quad \hat{s}(t_{k+1}) = s_-^* = \frac{s^* - pS^0}{1-p}, \\ \hat{x}(t_k^+) &= x^* = \gamma\eta^* & \text{and} & \quad \hat{x}(t_{k+1}) = x_+^* = \frac{x^*}{1-p}, \end{aligned}$$

where η^* is the positive fixed point of the map (17).

Our result has some shortcomings and advantages from an applied point of view. Indeed, note that Theorem 2 only ensures that at each emptying/refilling instant, the state variables can be asymptotically stabilized at a level (s^*, x^*) , which can be numerically estimated but not analytically known. In spite that this result is weak compared with those presented in [16] and [18], where the levels of substrate [16] and biomass [18] are measured online, which allows a stabilization in the required level, our model needs to measure the substrate only at the recursive sequence of the emptying/refilling instants.

To obtain lower (resp. bigger) asymptotic levels of substrate (resp. biomass), we propose some possible strategies:

8.1. Optimization issues

As the substrate concentration of the positive periodic solution $t \rightarrow (\hat{s}(t), \hat{x}(t))$ at the emptying/refilling instants, namely $\hat{s}(t_k) = s_-^*$, with $t_k = t_0 + k\tau(s^*)$, $k \in \mathbb{N}$, is dependent of the fraction p , we have that

$$\frac{ds_-^*}{dp}(p) = -\frac{1}{(1-p)^2} \left\{ \eta^*(p) + (1-p) \frac{d\eta^*}{dp}(p) \right\}. \tag{29}$$

To determine the sign of (29), we use the identities

$$\eta^*(p) = F(\eta^*(p)) = (1-p)\eta^*(p)e^{W(\eta^*(p))},$$

where $W(\cdot)$ is defined in (17) and satisfies $F'(v) = ve^{W(v)} + W'(v)$. Now, it is easy to deduce

$$\frac{d\eta^*}{dp}(p) = -\frac{F(\eta^*(p))}{(1-p)} + F'(\eta^*(p)) \frac{d\eta^*}{dp}(p),$$

which implies that

$$\frac{d\eta^*}{dp}(p) = -\frac{1}{(1-p)} \frac{\eta^*(p)}{1 - F'(\eta^*(p))} < 0$$

because $0 < F'(\eta^*(p)) < 1$ (see Lemma 3 for details). Upon inserting in (29), we have that the periodic solution has a level of evacuated substrate at $t = t_k$, which is increasing with respect to the emptying/refilling fraction p , because

$$\frac{ds_-^*}{dp}(p) = \frac{\eta^*(p)}{(1-p)^2} \frac{F'(\eta^*(p))}{1 - F'(\eta^*(p))} > 0.$$

From a sewage treatment point of view, lower levels $s_-^*(p)$ of contaminant at the emptying/refilling instant can be obtained by reducing the released liquid volume pV . Nevertheless, this volume is removed with a bigger frequency because

$$\frac{d}{dp} \left(\frac{1}{\tau(s^*(p))} \right) = \frac{\tau'(s^*(p))}{\tau^2(s^*(p))} \frac{d\eta^*}{dp}(p) < 0.$$

By the reasons stated earlier, it is natural to state the problem of static optimization of the released liquid volume per unit of time:

$$\max_{p \in (0,1)} \Phi(p), \quad \text{where} \quad \Phi(p) = \frac{p}{\tau(s^*(p))} V,$$

subject to the existence of the periodic solution.

Note that (if the conditions of Theorem 2 are satisfied) a fraction \hat{p} satisfying the first order condition is defined by the expression:

$$\frac{\tau'(s^*(\hat{p}))s^*(\hat{p})}{\tau(s^*(\hat{p}))} = \left(\frac{s^*(\hat{p})\hat{p}}{s^*(\hat{p})} \right)^{-1},$$

that is, the product of the elasticities of $\tau(\cdot)$ and $s^*(\cdot)$ at \hat{p} is equal to 1.

On the other hand, from a biomass production point of view, we have that the production function of biomass by unit of time is

$$\Psi(p) = \frac{pVx_+^*(p)}{\tau(s^*(p))} = \frac{\gamma pV}{1-p} \frac{\eta^*(p)}{\tau(S^0 - \eta^*(p))}.$$

Provided that the conditions of Theorem 2 are satisfied, it can be proved that the production function is decreasing with respect to p because

$$\Psi'(p) = -\frac{\gamma V \eta^*(p)}{(1-p)^2 \tau^2(s^*(p))} \frac{\tau(s^*)F'(\eta^*(p)) + \eta^*(p)\tau'(s^*(p))}{1-F'(\eta^*(p))} < 0.$$

8.2. Chain of fermenters

We can construct a chain of two batch fermenter interconnected in series, where the output of the first device becomes the input of the second one. The resulting IDE-IDT is given by the coupling of (3) with

$$\begin{cases} \dot{s}_2(t) = -\gamma^{-1} \mu(s_2(t))x_2(t), \\ \dot{x}_2(t) = \mu(s_2(t))x_2(t), \end{cases} \left. \vphantom{\begin{cases} \dot{s}_2(t) = -\gamma^{-1} \mu(s_2(t))x_2(t), \\ \dot{x}_2(t) = \mu(s_2(t))x_2(t), \end{cases}} \right\} t \neq t_k, \\ \begin{cases} s_2(t^+) = q s_2(t) + p s_1(t), \\ x_2(t^+) = q x_2(t), \end{cases} \left. \vphantom{\begin{cases} s_2(t^+) = q s_2(t) + p s_1(t), \\ x_2(t^+) = q x_2(t), \end{cases}} \right\} t = t_k,$$

where $s_2(\cdot)$ and $x_2(\cdot)$ denote the state variables of the second device and $\{t_k\}$ is still defined by (4) in the first fermenter. We conjecture that (provided some additional conditions) the concentration of the limiting substrate and biomass can be stabilized in a level (s_2^*, x_2^*) , where $s_2^* < s^*$ and $x_2^* > x^*$. Similarly, larger chains of interconnected fermenters can also be constructed.

This chain and its generalization have direct applications in sewage treatment and biomass production because at each step, we can either reduce the contaminant level or increase the biomass concentration. Nevertheless, to give mathematical proofs is an ongoing work.

Another extension of our results is the following one:

8.3. Variable fraction of removed liquid volume

We can consider a sequence $\{p_k\} \subset (0, 1)$ instead of a fixed emptying/refilling fraction. This idea is motivated by the possible perturbations and uncertainties at each emptying/refilling action. We have in mind a sequence of type $p_k = p + \delta_k$, where $p \in (0, 1)$ is a fixed value and δ_k is a perturbation with properties

$$\lim_{k \rightarrow +\infty} \delta_k = \delta \approx 0 \quad \text{or} \quad \lim_{K \rightarrow +\infty} \frac{1}{K} \sum_{k=1}^K \delta_k = \delta_\infty \approx 0,$$

the first case is an asymptotically constant perturbation and the second one is called an *ergodic perturbation*. We point out that the case of periodic and almost periodic sequences are well known examples of ergodic perturbations [13].

In this framework, the resulting stroboscopic map (7) becomes a non autonomous system, where the classical theory of discrete dynamical systems cannot be used. We expect to carry out an asymptotic analysis by using skew-product semi flows techniques [32].

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References

1. Brown WA, Cooper DG. Self-cycling fermentation applied to antibacterial oxaloacetic RAG-1. *Applied and Environment Microbiology* 1991; **57**:2901–2906.
2. Brown WA. The self-cycling fermentor: development, applications, and future opportunities. *Recent Research Developments in Biotechnology & Bioengineering* 2001; **4**:61–90.
3. Hughes SM, Cooper DG. Biodegradability of phenol using the self-cycling fermentation (SF) process. *Biotechnology and Bioengineering* 1996; **51**:112–119.
4. Sauvageau D, Storms Z, Cooper DG. Synchronized populations of Escherichia coli using simplified self-cycling fermentation. *Journal of Biotechnology* 2010; **149**:67–73.
5. Sheppard JD, Cooper DG. Development of computerized feedback control for the phasing of Bacillus subtitles. *Biotechnology and Bioengineering* 1990; **36**:539–545.
6. Smith RJ. Impulsive differential equations with applications to self-cycling fermentation. *Ph.D Thesis*, MC Master's University, 2001.
7. Van Walsum GP, Cooper DG. Self-cycling fermentation in a stirred tank reactor. *Biotechnology and Bioengineering* 1993; **42**:1175–1180.
8. Bastin G, Dochain D. *On-Line Estimation and Adaptive Estimation of Bioreactors*. Elsevier: Amsterdam, 1990.
9. Dinar A, Parkas SJ, Denney C, Biro G. *Batch Fermentation: Modeling, Monitoring, Control*. CRC Press: New York, 2003.
10. Storms ZJ, Brown T, Sauvageau D, Cooper DG. Self-Cycling operation increases productivity of recombinant protein in Escherichia coli. *Biotechnology and Bioengineering* 2012; **109**:2262–2270.
11. Bainov DD, Simeonov PS. *Systems with Impulse Effect: Stability, Theory and Applications*. Ellis Horwood Limited: Chichester, 1989.
12. Haddad WM, Chellaboina V, Nersisov SG. *Impulsive and Hybrid Dynamical Systems: Stability, Dissipation and Control*. Princeton University Press: Princeton NJ, 2006.

13. Samoilenko AM, Perestyuk NA. *Impulsive Differential Equations*. World Scientific: River Edge NJ, 1995.
14. Fan G, Wolkowicz GSK. Analysis of a model of nutrient driven self-cycling fermentation allowing unimodal response functions. *Discrete and Continuous Dynamical Systems Series B* 2007; **8**:801–831.
15. Smith RJ, Wolkowicz GSK. Growth and competition in the nutrient driven self-cycling fermentation process. *Canadian Applied Mathematics Quarterly* 2003; **10**:171–177.
16. Smith RJ, Wolkowicz GSK. Analysis of a model of the nutrient driven self-cycling fermentation process. *Dynamics of Continuous, Discrete & Impulsive Systems. Series B. Applications & Algorithms* 2003; **11**:239–265.
17. Sun K, Tian Y, Chen L, Kasperski A. Universal modeling and qualitative analysis of an impulsive process. *Computers and Chemical Engineering* 2011; **35**:492–501.
18. Tian Y, Kasperski A, Sun K, Chen L. Theoretical approach to modeling and analysis of the procession with product inhibition and impulse effect. *BioSystems* 2001; **104**:77–86.
19. Wincure BM, Cooper DG, Rey A. Mathematical model of self-cycling fermentation. *Biotechnology and Bioengineering* 1995; **46**:180–183.
20. Hofbauer J, So JWS. Uniform persistence and repellors for maps. *Proceedings of the American Mathematical Society* 1989; **107**:1137–1142.
21. Hutson V, Moran W. Persistence obeing difference equations. *Journal of Mathematical Biology* 1982; **15**:203–213.
22. Smith HL. Bacterial competition in serial transfer culture. *Mathematical Biosciences* 2011; **229**:149–159.
23. Bainov DD, Simeonov PS. *Impulsive Differential Equations: Periodic Solutions and Applications*. Longman scientific & technical: New York NY, 1989.
24. Lakshmikantham V, Liu XZ. *Stability Analysis in Two Measures*. World Scientific: River Edge NJ, 1993.
25. Yang T. *Impulsive Systems and Control: Theory and Applications*. Nova Scientific Publishers: Huntington NY, 2001.
26. Cordova-Lepe F. Advances in a theory of impulsive differential equations at impulsive-dependent times. In *BIOMAT 2006, International Symposium on Mathematical and Computational Biology*, Mondaini RP, Dilao R (eds). World Scientific: Hackensack NJ, 2007; 343–357.
27. Karafyllis I. A system theoretic framework for a wide class of systems I: applications to numerical analysis. *Journal of Mathematical Analysis & Applications* 2007; **328**:876–899.
28. Córdoba-Lepe F, Pinto M, González-Olivares E. A new class of differential equations with impulses at instants-dependent of preceding pulses. Applications to management of renewable resources. *Nonlinear Analysis: Real World Applications* 2012; **13**:2313–2322.
29. Córdoba-Lepe F, Del-Valle R, Robledo G. A pulse vaccination strategy at variable times depending on incidence. *Journal of Biological Systems* 2011; **19**:329–344.
30. Córdoba-Lepe F, Del-Valle R, Robledo G. A pulse fishery model with closures as function of the catch: conditions for sustainability. *Mathematical Biosciences* 2012; **239**:169–177.
31. Butler GJ, Waltman P. Uniformly persistent systems. *Proceedings of the American Mathematical Society* 1986; **96**:425–430.
32. Elaydi S N. Non autonomous difference equations: open problems and conjectures. In *Difference and Differential Equations. Proceedings of the 7th International Conference on Difference Equations and Applications (IDEA) 2002*, Vol. 42, Elaydi S, Ladas G, Wu J, Zou X (eds). Fields Institute Communications: Providence RI, 2004; 423–428.