ANALYSIS OF A MODEL OF THE NUTRIENT DRIVEN SELF-CYCLING FERMENTATION PROCESS

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Abstract. Self-cycling fermentation is a computer-aided process used for culturing microorganisms. Potential applications could include water purification, treatment of sewage, and the cleanup of toxic waste. We consider a model of growth of a single species in the fermenter, assuming a single limiting nutrient, with the level of this nutrient as the triggering factor. The model is formulated in terms of impulsive ordinary differential equations. The model predicts that either the system fails and the population of microorganisms essentially washes out, or more favourably, the fermenter cycles indefinitely, with one impulse per period, maintaining a positive, though oscillatory, number of cells. The predicted outcome is based on a threshold criterion that can be determined in advance in terms of biologically relevant parameters. An analytic expression for the cycle time is also derived. Using this expression, it is shown that the total yield over a specified time period depends on the choice of the optimal emptying/refilling fraction. A method for determining the optimal emptying/refilling fraction is given. The results are illustrated by means of numerical simulations.

Keywords. Self-cycling fermentation, impulsive differential equations, nutrient driven process, tolerance, moment of impulse, emptying/refilling fraction, cycle time, optimal yield.

AMS (MOS) subject classification: 34K45, 34K60, 92D25, 92D40, 62P12

1 Introduction

The development of the process of self-cycling fermentation (SCF) is described in Sheppard and Cooper [11], and a model similar to the one discussed here is given in Wincure, Cooper, and Rey [16].

Briefly, the process of self-cycling fermentation is a computer-controlled semibatch fermentation. A well-stirred tank containing fresh medium is inoculated with microorganisms that consume the nutrients in the broth. The microorganisms process the nutrient in order to grow and to reproduce. A

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probe inserted in the tank relays information to a computer that monitors the system. When certain conditions are met, the computer initiates a rapid emptying and refilling process. A set fraction of the contents of the tank is released and replaced by an equal volume of fresh medium, and then the process begins anew.

The time at which the emptying and refilling process is initiated is referred to variously as the time of impulsive effect, the harvesting time, the moment of impulse, or the end of cycle time. The time between impulsive effects is referred to as the cycle time. Under the right conditions, this cycling continues indefinitely. No a priori estimate of the natural cycle time of the microorganisms is necessary. Once the process has been initiated, there is no need for human intervention. Self-cycling fermentation has been shown to be potentially useful for a variety of applications, including sewage treatment (Sarkis and Cooper [10]), elimination of industrial pollutants (Brown and Cooper [4]), cultivation of synchronous populations for use in a second stage reactor resulting in a significant increase in the biomass production rate of a secondary metabolite (Wentworth and Cooper [15]), and production of antibiotics (Zenaitis and Cooper [18]). Since our formulation of the model is nutrient driven, applications that require decreasing contaminants to specified levels, such as water-purification, sewage treatment, and toxic waste clean up would be appropriate.

In Sheppard and Cooper [11], five different feedback mechanisms are described. The model developed in Wincure, Cooper and Rey [16] uses dissolved oxygen concentration as the feedback mechanism that triggers the emptying and refilling process. Following the depletion of the limiting nutrient, a rapid rise in the dissolved oxygen level is observed as a result of the organism's declining respiration rate. In our model, instead we take a specified concentration of the limiting nutrient as the triggering mechanism. This has several advantages. First, this model gives the experimenter more flexibility to determine a feedback mechanism to initiate the emptying and refilling process. The model applies as long as the feedback mechanism ultimately results in the cycling of the tank occurring at a consistent concentration of the limiting nutrient. This appears to be the case, for example, in the experiments and the simulations carried out in Wincure, Cooper and Rey [16], even though they used the dissolved oxygen level as the feedback mechanism. Using the concentration of the limiting nutrient directly resulted in a model for which it was possible to do a very thorough and mathematically rigorous analysis, resulting in predictions that can be tested in the laboratory. The analysis indicated that the model appears to be very robust and applies for any reasonable monotone increasing response function, not just response functions of the Monod form. This is important because models for response functions are at best approximations, and so if the predicted dynamics of a model are sensitive to the form of the response function, the model loses predictability.

In particular, the analysis predicts that the process is extremely stable. Once an acceptable level of the contaminant is set, the model predicts that

there is a threshold criterion given in terms of the biologically relevant parameters that indicates how to choose an appropriate population of microorganisms and corresponding operating parameters such as the emptying and refilling fraction, in order to obtain the desired outcome. In Wincure, Cooper and Rey [16] the species-specific death rate is ignored. In fact, if the species-specific death rate is ignored and the initial concentration of microorganisms is positive, our model predicts that the fermenter cycles indefinitely and concentrations of contaminant and microorganism rapidly approach a unique positive cycle with a fixed finite period. However, we include the species-specific death rate in our model so that we can explore how variations in this death rate might affect the outcome. Our model predicts that if the desired level of contaminant in the output, $\bar{s}$, is set sufficiently low, then if the species-specific death rate is too high relative to its growth rate for concentrations of the contaminant near $\bar{s}$, the process could break down if a threshold criterion is not satisfied. This is because the microorganism concentration does not increase fast enough to offset its death rate during the part of the cycle when the nutrient is close to $\bar{s}$, and depends upon whether the increase in the concentration of the microorganism during the rest of the cycle is sufficient to overcome this. Hence, the model predicts that selection of an appropriate population of microorganisms depends on an interplay between the species-specific death rate and the acceptable level of the contaminant that can be released, as well as selection of an appropriate emptying and refilling fraction.

In Wincure, Cooper and Rey [16], the emptying and refilling fraction r, was assumed to be half the tank ($r = 0.5$) and all of the experiments reported were for this value of this fraction. However, they did computer simulations for other values of r. Our model allowed us to explore the use of different emptying and refilling fractions. We proved that whether or not the process cycles indefinitely can depend on the choice of this fraction and that choice of this fraction can significantly affect the expected yield over time. We showed how to determine the *best* fraction predicted by the model in order to optimize the yield. Any real understanding of self-cycling fermentation will require experimental evidence of the effect of different values of r, and we hope that our analysis will stimulate such experiments.

This paper is organized as follows. In section 2 we introduce the model, define a threshold, and present our main result. We provide criteria for the existence of an asymptotically stable nontrivial periodic orbit with the property of asymptotic phase and characterize when trajectories will approach this periodic orbit. In section 3 we present numerical simulations to illustrate the various cases of the main theorem. In section 4 we find an analytic expression of the cycle time and then discuss how to select the emptying/refilling fraction in order to optimize the yield over any specified period of time. In section 5, we prove our results, and in section 6, we discuss their implications and compare the dynamics of the self-cycling fermenter with the dynamics of the chemostat.

2   A nutrient driven model for the self-cycling fermentation process

First we formulate a model that describes the nutrient driven self-cycling fermentation process. This model is an adaptation of a model described by Wincure, Cooper, and Rey [16].

Since the time taken to empty and refill the tank is negligible, compared to each cycle time, we assume that the process occurs instantaneously so that we can formulate the model as a system of impulsive differential equations. Impulsive differential equations are described in Bainov and Simeonov [1], [2], [3] and Lakshmikantham, Bainov, and Simeonov [8].

In accordance with the theory of impulsive differential equations (see Bainov and Simeonov [1], [2]), for a given function $y(t)$, and time τ,

$$\Delta y \equiv y^+ - y^-,$$

where

$$y^+ \equiv y(\tau^+) \equiv \lim_{t \to \tau^+} y(t) \quad \text{and} \quad y^- \equiv y(\tau^-) \equiv \lim_{t \to \tau^-} y(t).$$

Consider the impulsive system

$$
\begin{array}{llll}
\dfrac{ds}{dt} & = & -\dfrac{1}{Y}f(s)x, & s \neq \bar{s}, \\[2mm]
\dfrac{dx}{dt} & = & -\bar{d}x + f(s)x, & s \neq \bar{s}, \\[2mm]
\Delta s & = & -r\bar{s} + rs^i, & s = \bar{s}, \\[1mm]
\Delta x & = & -rx^-, & s = \bar{s},
\end{array}
\tag{2.1}
$$

$$s(0) > \bar{s}, \ x(0) > 0.$$

Here, t denotes time in minutes, s denotes the concentration (g/L) of the limiting nutrient in the fermentation tank, x the biomass concentration (g/L) of the population of microorganisms that consume the nutrient, Y the cell yield constant (g biomass/g limiting substrate), $\bar{d}$ the species-specific death rate (per minute), s^i the concentration (g/L) of the fresh medium added to the tank at the beginning of each new cycle, $\bar{s}$ the threshold concentration (g/L) of limiting nutrient that initiates the emptying and refilling process, and r the emptying/refilling fraction. It is assumed that $\bar{d} \geq 0$, $Y > 0$, $s^i > \bar{s} > 0$, and $0 < r < 1$.

The response function is denoted f and satisfies

$$
\begin{array}{ll}
\text{i.} & f : \mathbb{R} \to \mathbb{R}, \\
\text{ii.} & f \text{ is continuously differentiable,} \\
\text{iii.} & f(0) = 0, \\
\text{iv.} & f'(s) > 0, \ \text{if} \ s > 0.
\end{array}
\tag{2.2}
$$

Let t_k denote the time at which the kth moment of impulse occurs, i.e., the time at which the concentration of the limiting nutrient in the tank reaches the specified threshold, $\bar{s}$ for the kth time. Thus, $s(t_k^-) = \bar{s}$. From (2.1), it follows that $\Delta s(t_k) = s(t_k^+) - s(t_k^-) = -r\bar{s} + rs^i$, and so $s(t_k^+) = (1-r)\bar{s} + rs^i$. It will be convenient to define

$$\bar{s}^+ \equiv (1-r)\bar{s} + rs^i.$$

Note that, since s is decreasing during each cycle, if $s(0) < \bar{s}$ then the tolerance $\bar{s}$ is never reached and there will be no impulsive effect. In accordance with impulsive theory, we also assume that $s(0) \neq \bar{s}$, so that there is no impulsive effect initially. In fact, in most applications, $s(0) = s^i$. The applications we have in mind include water purification or waste decomposition. Here, s^i would denote the concentration of some contaminant in the environment, s the concentration of the contaminant in the fermentation tank, and $\bar{s}$ the acceptable level of the contaminant in the environment, consistent with standards set by an environmental protection agency.

Define λ to be the value of the nutrient that satisfies $f(\lambda) = \bar{d}$. If f is bounded below $\bar{d}$, then we define $\lambda = \infty$. Thus, λ represents the so-called *break-even* concentration of the nutrient. Provided the concentration of the nutrient is above λ, $x'(t)$ is positive, and so the concentration of the microorganism is increasing. However, if it is below λ, $x'(t)$ is negative, and so the concentration is decreasing. If f is bounded below $\bar{d}$, then $x'(t)$ is negative for all concentrations of the nutrient, and there is no hope for the process to succeed. The relative values of $\bar{s}$ and λ play an important role in the potential dynamics of the model.

Between impulses the system is modelled by a system of ordinary differential equations. This system will be called the associated system and is given by

$$\begin{aligned}
\frac{ds}{dt} &= -\frac{1}{Y}f(s)x, \\
\frac{dx}{dt} &= -\bar{d}x + f(s)x,
\end{aligned} \tag{2.3}$$

$$s(0) \geq 0, \ x(0) \geq 0.$$

For our analysis it is necessary to understand the dynamics of this associated system for a slightly expanded set of initital conditions compared to system (2.1). It is easy to show that solutions of (2.3) remain nonnegative and bounded.

If $\bar{d} = 0$, then $\lambda = 0$, by (2.2)(iii). In this case all points of the form $(s^*, 0)$ with $s^* \geq 0$ or $(0, x^*)$ with $x^* \geq 0$ are equilibrium points. See Figure 1 (a). All orbits lie along lines $Ys + x = Ys(0) + x(0)$ with $s(t)$ decreasing and $x(t)$ increasing. Any orbit with initial condition satisfying $s(0) > 0$, $x(0) > 0$, approaches $(0, x^*)$, where $x^* = Ys(0) + x(0) > 0$ and hence these equilibrium points are all stable (but not asymptotically stable). All equilibrium points of the form $(s^*, 0)$, with $s^* > 0$ are unstable.

If $\bar{d} > 0$, then only points of the form $(s^*, 0)$, with $s^* \geq 0$ are equilibrium points. See Figure 1 (b). If $s^* > \lambda$, then $(s^*, 0)$ is unstable, and if $s^* \leq \lambda$, then it is stable (but not asymptotically stable). If $s(0) > 0$ and $x(0) > 0$, then $s(t)$ is decreasing for all t. $x(t)$ increases if $s(t) > \lambda$ and then decreases to an equilibrium point of the form $(s^*, 0)$ with $0 < s^* < \lambda$, that depends on the initial conditions.

Provided $s(0) > 0$ and $x(0) > 0$, from (2.3),

$$\frac{dx}{ds} = Y\left(\frac{\bar{d}}{f(s)} - 1\right), \tag{2.4}$$

a separable differential equation. It follows that

$$x(t) = x(0) + Y \int_{s(t)}^{s(0)} \left(1 - \frac{\bar{d}}{f(u)}\right) \, du. \tag{2.5}$$

Figure 1 depicts typical phase portraits in x-s space, in the case (a) that $\bar{d} = 0$ and (b) that $\bar{d} > 0$. Note that, since x does not appear explicitly on the right hand side of (2.4), the slope of trajectories depends only on s. Therefore, if $\tilde{\gamma}(t)$ and $\hat{\gamma}(t)$ are two orbits with initial conditions, $(\tilde{s}(0), \tilde{x}(0))$ and $(\hat{s}(0), \hat{x}(0))$, respectively, with $\tilde{s}(0) = \hat{s}(0)$, but $\tilde{x}(0) - \hat{x}(0) = \eta > 0$, then for any times $\tilde{t} > 0$ and $\hat{t} > 0$, where $\tilde{s}(\tilde{t}) = \hat{s}(\hat{t})$, it follows that $\tilde{x}(\tilde{t}) - \hat{x}(\hat{t}) = \eta$. It can also be shown that $\tilde{t} < \hat{t}$. (For the proof, see Lemma 1 in section 5.)
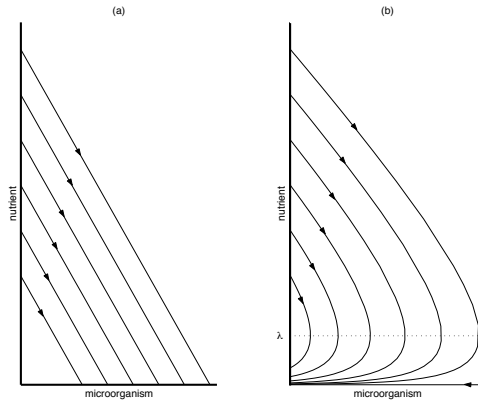

Figure 1: Phase portrait for the associated system of ordinary differential equations. Note that the bold lines on the axes indicate lines of equilibria. (a) phase portrait with $\bar{d} = 0$, and (b) phase portrait with $\bar{d} > 0$. Notice that the concentration of biomass is increasing for concentrations of nutrient above λ and decreasing for concentrations below λ.

In the analysis of (2.1), it will be helpful to define

$$s_{\text{int}} \equiv Y \int_{\bar{s}}^{\bar{s}^+} \left(1 - \frac{\bar{d}}{f(s)}\right) ds. \tag{2.6}$$

Setting $s(0) = \bar{s}^+$ and $s(\tau) = \bar{s}$ in (2.5), it follows that $x(\tau) - x(0) = s_{\text{int}}$. Therefore, whenever impulse times t_{k-1} and t_k are defined,

$$x(t_k^-) - x(t_{k-1}^+) = s_{\text{int}}. \tag{2.7}$$

Note that if $\bar{s} \geq \lambda$, then $s_{\text{int}} > 0$, whereas if $\bar{s}^+ \leq \lambda$, then $s_{\text{int}} < 0$.
The following is our main result.

Theorem 1. *Consider model (2.1). There exists a unique nontrivial positive periodic orbit if, and only if, $s_{\text{int}} > 0$. This periodic orbit has exactly one impulse per period, is asymptotically stable, and has the property of asymptotic phase.*
   At the times of impulse $\{t_n\}_{n=1}^\infty$, the periodic orbit satisfies

$$\begin{array}{llll}
s(t_n^-) & = & \bar{s}, & \qquad s(t_n^+) & = & \bar{s}^+, \\
x(t_n^-) & = & \frac{1}{r} s_{\text{int}}, & \qquad x(t_n^+) & = & \frac{1-r}{r} s_{\text{int}}.
\end{array}$$

1. *Assume that $s_{\text{int}} > 0$.*

   i) *If $\bar{s} \geq \lambda$, then the periodic orbit attracts all orbits with initial conditions satisfying $x(0) > 0$.*

   ii) *If $\bar{s} < \lambda$, then the periodic orbit attracts all orbits with initial conditions satisfying $x(0) > 0$ and $x(0) + Y \int_{\bar{s}}^{s(0)} \left(1 - \frac{\bar{d}}{f(s)}\right) ds > 0$. In particular, if $s(0) \geq \bar{s}^+$, then the periodic orbit attracts all orbits with initial conditions satisfying $x(0) > 0$.*

   iii) *If $x(0) + Y \int_{\bar{s}}^{s(0)} \left(1 - \frac{\bar{d}}{f(s)}\right) ds \leq 0$, then there are no impulses and $x(t) \to 0$, $s(t) \to s^* = s^*(s(0), x(0))$, as $t \to \infty$, where $\bar{s} \leq s^* < \lambda$.*

   Let $T > 0$ denote the period of the periodic orbit.

   In cases (i) and (ii) the fermenter cycles indefinitely, and so there exists an infinite sequence of impulse times $\{t_n\}_{n=1}^\infty$. As $n \to \infty$, $t_n \to \infty$, $t_{n+1} - t_n \to T$, $x(t_n^-) \to \frac{1}{r} s_{\text{int}}$, and $x(t_n^+) \to \frac{1-r}{r} s_{\text{int}}$.

   For all positive integers n, solutions satisfy $s(t_n^+) = \bar{s}^+$ and $s(t_n^-) = \bar{s}$, and one of the following holds:

   a) $t_{n+1} - t_n = T$, $x(t_n^-) = \frac{1}{r} s_{\text{int}}$, *and* $x(t_n^+) = \frac{1-r}{r} s_{\text{int}}$; *or*

   b) $t_{n+1} - t_n > T$, $x(t_n^-) < x(t_{n+1}^-)$, $x(t_n^+) < x(t_{n+1}^+)$, $x(t_n^-) < \frac{1}{r} s_{\text{int}}$, *and* $x(t_n^+) < \frac{1-r}{r} s_{\text{int}}$; *or*

c) $t_{n+1} - t_n < T$, $x(t_n^-) > x(t_{n+1}^-)$, $x(t_n^+) > x(t_{n+1}^+)$, $x(t_n^-) > \frac{1}{r}s_{\text{int}}$,   and $x(t_n^+) > \frac{r}{1-r}s_{\text{int}}$.

2. *Assume that $s_{\text{int}} = 0$. Then $\liminf_{t\to\infty} x(t) = 0$. If $x(0) > 0$ and $x(0) + Y \int_{\bar{s}}^{s(0)} \left(1 - \frac{\bar{d}}{f(s)}\right) ds > 0$, i.e., $x(0)$ is sufficiently large, or $s(0) \geq \bar{s}^+$ and $x(0) > 0$, then there are an infinite number of impulses, but the time between impulses increases, approaching infinity. If $x(0) + Y \int_{\bar{s}}^{s(0)} \left(1 - \frac{\bar{d}}{f(s)}\right) ds \leq 0$ i.e., $\bar{s} < s(0) < \bar{s}^+$ and $x(0)$ is sufficiently small, there are no impulses and $s(t) \to s^* = s^*(s(0), x(0))$ as $t \to \infty$, where $\bar{s} \leq s^* < \lambda$.*

3. *Assume that $s_{\text{int}} < 0$. Then there are at most a finite number of impulses, the time between impulses increases, and eventually $s(t) \to s^* = s^*(s(0), x(0))$ and $x(t) \to 0$ as $t \to \infty$, where $\bar{s} \leq s^* < \lambda$.*

Thus, if $s_{\text{int}} > 0$, solutions that undergo impulsive effect once, undergo an infinite number of impulsive effects and approach the positive periodic orbit. For these orbits, the time between each impulse approaches the period of the periodic orbit monotonically. As well, the portion of the orbit between each impulse moves monotonically towards the periodic orbit. If $s_{\text{int}} = 0$, solutions that undergo impulsive effect once, also undergo impulsive effect an infinite number of times, but the time between impulses increases without bound and the population of microorganisms essentially washes out. If $s_{\text{int}} < 0$, solutions undergo impulsive effect at most a finite number of times, and eventually the microorganism washes out. (For the proof, see section 5.)

An analytic expression for the period T of the unique periodic orbit that exists when $s_{\text{int}} > 0$, will be derived in Section 4 (see (4.3)).

3   Simulations illustrating Theorem 1

We illustrate the various cases of Theorem 1 by means of numerical simulations. All simulations were run using ODE45 in MATLAB, with the appropriate events option to calculate the moment of impulsive effect. Figures 2, 4, and 5 in this section show the phase portrait in x-s space.

In the simulations shown in Figures 2–5, we select the response function of the form $f(s) = \frac{ms}{K_s + s}$, as in Wincure, Cooper and Rey [16], and as they did, choose the maximum specific growth rate $m = 0.01$, the half saturation constant $K_s = 0.007$, and the cell yield $Y = 0.73$. We set $r = 0.6$, $s^i = 1.333$, and $\bar{s} = 0.1$, Therefore, $\bar{s}^+ = 0.8398$. The species-specific death rate, $\bar{d}$ is varied in order to obtain the appropriate case of Theorem 1.   When
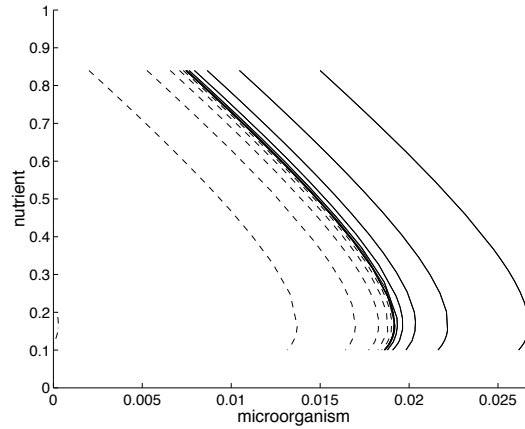
Figure 2: Orbits in phase space in the case $s_{\text{int}} = 0.011163 > 0$ and $\bar{s} < \lambda < \bar{s}^+$. There is an orbitally stable positive periodic orbit with the property of asymptotic phase in the area where the solid and dashed curves converge. Two orbits, one (solid) approaches it from the right, and the other (dashed) approaches it from the left. At each impulse, there is a discontinuity. The solid orbit moves further to the left and the dashed orbit moves further to the right. Both orbits become almost indistinguishable from the periodic orbit in approximately 5-6 cycles. A third orbit, (dot-dashed), far left, very small and very close to the ordinate axis, does not undergo any impulses, but rather approaches $(s^*, 0)$, where $s^* > \bar{s}$, in a single cycle. Initial conditions of the solid orbit are $s(0) = \bar{s}^+$ and $x(0) = 0.015$, of the dashed orbit are $s(0) = \bar{s}^+ = 0.398$ and $x(0) = 0.002$, and of the dot-dashed orbit are $s(0) = 0.2$ and $x(0) = 0.0002$. Parameters selected are $s^i = 1.333$, $\bar{s} = 0.1$, $m = 0.01$, $K_s = 0.007$, $Y = 0.73$, $r = 0.6$, and $\bar{d} = 0.0096$, and so $\lambda = 0.168$.

$f(s) = \frac{ms}{K_s + s}$, and $\bar{d} < m$, so that $\lambda < \infty$,

$$
\begin{aligned}
s_{\text{int}} &= Y\left(1 - \frac{\bar{d}}{m}\right) r(s^i - \bar{s}) - \frac{\bar{d}YK_s}{m} \ln \frac{\bar{s}^+}{\bar{s}} \\
&= \frac{\bar{d}YK_s}{m}\left(\frac{r}{\lambda}(s^i - \bar{s}) - \ln \frac{\bar{s}^+}{\bar{s}}\right). \tag{3.1}
\end{aligned}
$$

Figures 2 and 3 illustrate case 1 of Theorem 1. Figure 2 is a phase portrait, i.e., the projection of the solution onto phase space, whereas Figure 3 is a time series.

In order to illustrating case 1, we chose the death rate, $\bar{d} = 0.0096$, so that $s_{\text{int}} = 0.011163 > 0$. For this choice, $\lambda = 0.168$, and so $\bar{s} < \lambda < \bar{s}^+$. Depending on the initial conditions, either orbits cycle indefinitely and approach the unique orbitally asymptotically stable periodic orbit, or orbits do not undergo any impulses and approach $(s^*, 0)$ for some $s^* > \bar{s}$, where the
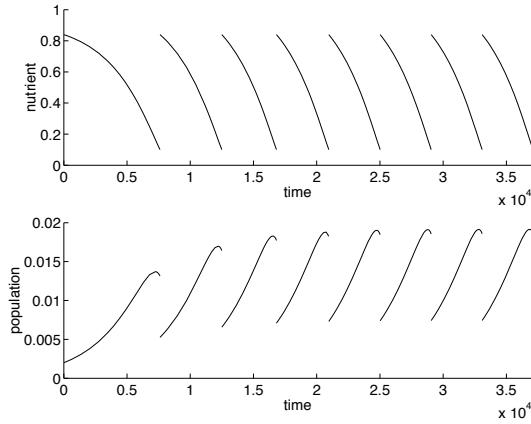
Figure 3: The corresponding time series of the dashed orbit in Figure 2, and so the initial conditions are $s(0) = \bar{s}^+$ and $x(0) = 0.002$. This orbit converges to a stable periodic cycle and is almost indistinguisable from it within approximately 5-6 cycles. The cycle time decreases and approaches the period of the attracting periodic orbit.

value of s^* also depends on the initial conditions. It is worth noting that the latter case is more a mathematical curiosity. In practise, $s(0) = s^i > \bar{s}^+$, and so as long as $x(0) > 0$ when $s_{\text{int}} > 0$, the model predicts that orbits cycle indefinitely and approach the unique orbitally asymptotically stable periodic orbit and so the eventual outcome in most applications is not really initial condition dependent. In the phase portrait, Figure 2, it was not necessary to plot the attracting periodic orbit explicitly, since the simulations show two other orbits converging to it, one from the left and the other from the right.

Figure 3 depicts the time series of the dashed orbit in Figure 2, the orbit that was initiated with a relatively small concentration of microorganism. From this Figure one can see that for this orbit the cycle time, that is the time between each impulse, also approaches the period of the periodic orbit monotonically, as predicted by the Theorem.

The case $\lambda < \bar{s}$, i.e., the species-specific death rate is relatively small, is similar, except in this case, $x(t)$ increases throughout each cycle, and so all orbits with $x(0) > 0$ cycle indefinitely and approach the unique orbitally asymptotically stable periodic cycle.

Figure 4 illustrates case 2 of Theorem 1. Here, the death rate $\bar{d} = 0.009802623$ was chosen so that $s_{\text{int}} \approx 0$. It follows that $\bar{s}^+ = 0.8398$ and $\lambda = 0.3477$, and so $\bar{s} < \lambda < \bar{s}^+$. In this case there is no periodic orbit and depending on initial conditions, either orbits undergo an infinite number of impulses and approach a singular orbit with infinite period from the right or orbits do not undergo any impulses and eventually approach $(s^*, 0)$ for some $s^* > \bar{s}$, where the value of s^* also depends on the initial conditions.
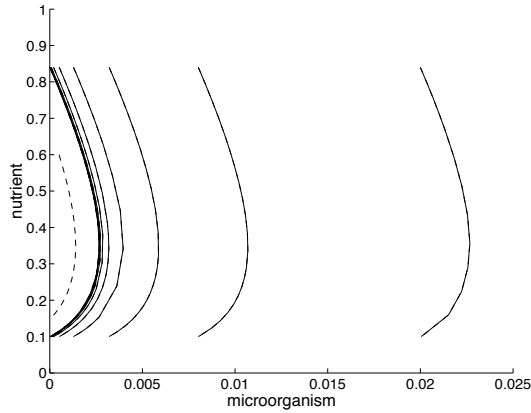
Figure 4: Orbits in phase space in the case $s_{\text{int}} = 0$. One orbit (solid) undergoes an infinite number of impulses, (with impulse points moving to the left) and approaches a heteroclinic orbit joining an unstable equilibrium of the form $(s^*, 0)$ to a stable equilibrium of the same form. Another orbit (dashed) does not undergo any impulses, but instead approaches $(s^*, 0)$, where $s^* > \bar{s}$. The initial conditions of the solid orbit are $s(0) = \bar{s}^+$ and $x(0) = 0.02$ and of the dashed orbit are $s(0) = 0.6$ and $x(0) = 0.0005$. $\bar{d} = 0.0098$. All other parameters are the same as in Figure 2, and so $\lambda = 0.343$. The corresponding time series of the solid orbit would show that each cycle takes longer and longer as the concentration of microorganism tends to zero.
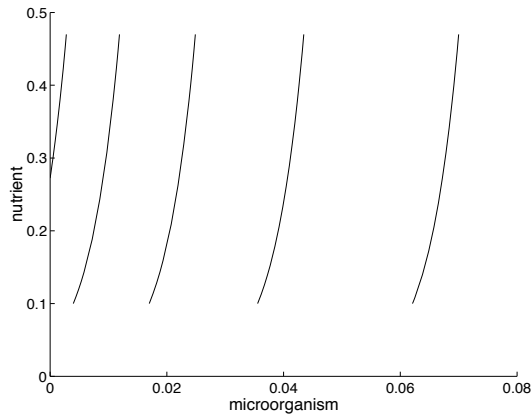

Figure 5: An orbit in phase space in the case $s_{\text{int}} = -0.01087 < 0$, illustrating a single orbit that moves to the left and undergoes a finite number of impulses before approaching $(s^*, 0)$, where $s^* > \bar{s}$. The initial conditions are $s(0) = \bar{s}^+ = 0.4699$ and $x(0) = 0.07$. $\bar{d} = 0.01$ and $r = 0.3$. All other parameters are the same as in Figure 2, and so $\lambda = \infty$.

Figure 5 illustrates case 3 of Theorem 1. Here, the death rate $\bar{d} = 0.01$ was chosen so that $s_{\text{int}} = -0.01087 < 0$. In this case $\lambda = \infty$, and so $\bar{s} < \bar{s}^+ < \lambda$. All orbits undergo impulsive effect at most a finite number of times. They eventually approach $(s^*, 0)$ for some $s^* > \bar{s}$, where the value of s^* also depends on the initial conditions. Hence, for all initial conditions the microorganism eventually washes out.

4   Selection of the emptying/refilling fraction

In the process of self-cycling fermentation, the parameter that the experimenter can control most easily is the emptying/refilling fraction r. Wincure, Cooper and Rey [16] selected $r = 0.5$. However, they did not discuss their reasons for this choice. We shall show that r can be selected in order to maximize the yield, and hence the efficiency, of the self-cycling fermentation process. By the yield, we mean the total volume of medium processed then emptied over some fixed time period, $\mathcal{T}$, assuming $\mathcal{T}$ is relatively long compared to the time between impulses.

So that the fermenter cycles indefinitely without the time between impulses becoming unbounded, it is necessary to ensure that $s_{\text{int}}(r) > 0$. Recall that a necessary condition for $s_{\text{int}}(r) > 0$ is that $\bar{s}^+ > \lambda$. Note that λ is independent of r, however, $\bar{s}^+$ increases as r increases. Thus, it is possible to choose r so that $\bar{s}^+ > \lambda$, if and only if, $s^i > \lambda$. This means that certain populations of microorganisms can only be used if the concentration of the pollutant is sufficiently high.

Next, notice that

$$\frac{d\, s_{\text{int}}}{dr} = Y(s^i - \bar{s})\left(1 - \frac{\bar{d}}{f(\bar{s}^+)}\right). \tag{4.1}$$

Thus, when $\bar{s}^+ > \lambda$, it follows that $\frac{d}{dr} s_{\text{int}} > 0$.

Therefore, once all parameters except r are fixed so that $\bar{s}^+ > \lambda$, there are three possibilities. Either $s_{\text{int}}(r)$ is negative for all r in the interval $(0, 1)$, or it is positive throughout the entire interval, or there exists a critical value, say r_0, satisfying $0 < r_0 < 1$, such that $s_{\text{int}}(r_0) = 0$, and $s_{\text{int}}(r) > 0$ for all $r_0 < r < 1$. In the case that $s_{\text{int}}(r)$ is positive throughout the entire interval $(0, 1)$, we will define $r_0 = 0$.

In order for the fermenter to be operated as desired, it is necessary to ensure that $s_{\text{int}}(r) > 0$ and that $x(0) > 0$ and $s(0)$ is sufficiently large, so that orbits rapidly approach a unique, positive, periodic orbit. Therefore, after a finite number of impulses, orbits are almost indistinguishable from the periodic orbit. Thus, for the purpose of comparing yields for different values of r, since we are assuming $\mathcal{T}$ is large compared to the cycle time, it is reasonable to choose initial conditions on the periodic orbit, that is, $s(0) = \bar{s}^+$ and $x(0) = \frac{1-r}{r} s_{\text{int}}$. However, in practice, it is more likely that

$s(0) = s^i$. Selection of $x(0)$ would depend on cost and availability. Note that the larger $x(0)$, the less time required between the first few impulses.

One of the assumptions used to develop the model was that the total time involved in emptying and refilling the tank at the end of each cycle is short compared to the time between impulses and could be ignored. However, for r sufficiently small this may not be reasonable. Therefore, in order to determine the most efficient r, the time taken to empty and refill the tank will be taken into consideration. We shall denote this time by ϵ. In experiments described in Wincure, Cooper and Rey [16], ϵ was approximately 5 minutes.

The following observations lead us to expect that there is a choice of $r \in (r_0, 1)$ that optimizes the yield. In the limiting case, $r = 1$, the process terminates, since there would no longer be any microorganisms left in the tank to process the fresh medium during the second cycle. Thus, even though the closer r is to 1, the larger the volume released at the end of each cycle, one would expect the total yield to be relatively small, because very few microrganisms are left to process the medium during each subsequent cycle, and so the cycle time would be relatively long.

In the limiting case that $r = r_0 = 0$, again the process terminates, since nothing is released. For r very close to zero, even if there were a large number of releases from the tank in a relatively short period of time, the volume released each time would be very small, resulting in a very small total yield, once the emptying and refilling time is taken into account. Also, if $r = r_0 > 0$, then $s_{\text{int}}(r_0) = 0$, and so there is no periodic orbit. Instead, there is an attracting singular orbit that takes an infinite amount of time to traverse. For r very close to r_0, the periodic orbit is very close to this singular orbit and hence the cycle time is very large and the yield is relatively small.

With the above observations in mind, in what follows we express the yield as

$$\Omega(r) \quad = \quad \frac{rV}{T(r) + \epsilon}\mathcal{T}, \qquad (4.2)$$

where r is the emptying/refilling fraction, assumed to be strictly between r_0 and 1, V is the volume of the tank, which is assumed to be constant except during the emptying and refilling time, $T(r)$ is the period of the periodic orbit and hence the time between impulses, ϵ is the time taken to empty and refill the tank, assumed to be a constant independent of r, and $\mathcal{T} \gg T(r)$ is the fixed time period over which the total yield is calculated, assumed to be long compared to the time between impulses. We would like to find the r in $(r_0, 1)$, that maximizes $\Omega(r)$.

We begin by finding an explicit formula for $T(r)$. Substituting the expression for $x(t)$ given in (2.5) into the equation for $s'(t)$ in (2.1) one obtains

$$\frac{ds}{dt} = -\frac{1}{Y}f(s)\left(x(0) + Y\int_{s(t)}^{s(0)}\left(1 - \frac{\bar{d}}{f(u)}\right)\,du\right),$$

another separable differential equation. Separating and integrating from 0 to $T(r)$, we obtain the analytic expression for $T(r)$,

$$T(r) \;=\; \int_{\bar{s}}^{\bar{s}^+} \frac{Y}{f(s)} \left(\frac{1}{\frac{(1-r)}{r} s_{\text{int}} + Y \int_s^{\bar{s}^+} \left(1 - \frac{\bar{d}}{f(u)}\right) du} \right) \, ds. \quad (4.3)$$

Unfortunately, finding an analytical expression for the optimal value of r using the expression for $T(r)$ given in (4.3) is too messy. However, after specifying the response function and all parameters except r, it is possible to use a symbol manipulation package like MAPLE to differentiate $\Omega(r)$ and then find the critical value of r where this derivative equals zero, hence obtaining the optimal emptying/refilling fraction. It is also possible to use MAPLE to graph $T(r)$ and $\Omega(r)$. See Figures 6 and 7. These graphs could also have been obtained using numerical simulations using software such as MATLAB instead of the analytical expression (4.3).

In both figures the parameter values selected were $s^i = 1.333$, $\bar{s} = 0.1$, $m = 0.01$, $K_s = 0.007$, $Y = 0.73$ and $\epsilon = 5$. r was allowed to vary throughout the range where $s_{\text{int}}(r)$ remained positive. We assumed the volume V of the medium in the fermenter was 1 litre and $\mathcal{T} = 10^5$ minutes. Initial conditions were set (for each r) so that each trajectory started on the periodic orbit with $s(0) = \bar{s}^+$. Two different values of $\bar{d}$ were selected in order to show that the optimal value of r need not be 0.5.

In Figure 6, $\bar{d} = 0.009$. For this value of $\bar{d}$, $\lambda < \bar{s}$, and so $s_{\text{int}}(r) > 0$ for $0 < r < 1$. The period $T(r)$, of the periodic orbit increases as r increases. $\Omega(r)$ attains its maximum value, 60.57 litres, for $r \approx 0.3123$. For this value of r the period is 510.578 minutes.

In Figure 7, $\bar{d} = 0.00975$. In this case $\lambda > \bar{s}$, and $s_{\text{int}}(r) < 0$ for $r < r_0$ where $r_0 \approx 0.39$. Therefore, only values of r between 0.39 and 1 are shown. In this case the period $T(r)$, of the periodic orbit is decreasing for r close to r_0 and increasing for r close to 1. $\Omega(r)$ attains its maximum value, 5.93 litres, for $r \approx 0.7791$. For this value of r the period is 13,133.07 minutes or approximately 9 days. Such a long time between impulses may not be practical and such factors may also have to be taken into consideration when selecting r.

Thus, we observe that the optimal choice for r depends on the parameters in the model. For example, a slight increase in $\bar{d}$ (and hence λ), changed the optimal choice for r from 0.3123 to 0.7791 and reduced the optimal yield, $\Omega(r)$, from 60.57 litres to 5.93 litres.

In the actual experiment in Wincure, Cooper and Rey [16], $r = 0.5$ was selected, the cycle time (period of the periodic orbit) was 124 minutes, and it required 5 minutes to empty and refill the tank at the end of each cycle. When $\bar{d} = 0.004215$, $\epsilon = 5$, and $r = 0.5$ our model also predicts that the time between impulses is 124 minutes, and so this value of $\bar{d}$ might be an estimate of the species-specific death rate for the population of microorganisms that they used. If this is the case, then setting $\mathcal{T} = 10^5$ minutes, $\Omega(0.5) = 387.42$
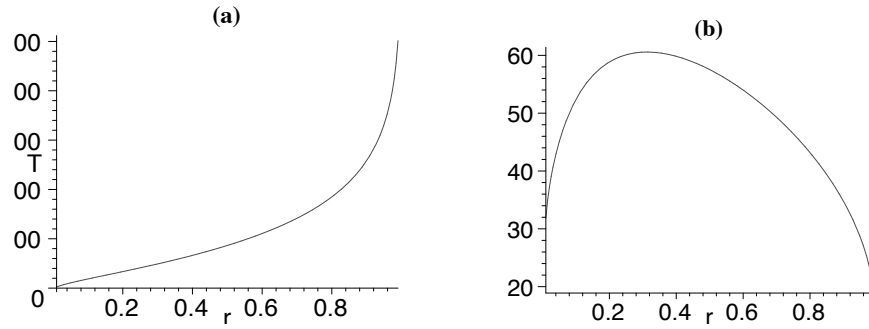
Figure 6: (a) shows how the period, $T(r)$, of the periodic orbit varies as r varies. (b) depicts how the yield, $\Omega(r)$, varies as r varies. All parameters are the same as in Figure 2 except $\bar{d} = 0.009$, $V = 1$, $\epsilon = 5$, $\mathcal{T} = 10^5$. r varies throughout $(0, 1)$, since $\lambda > \bar{s}$, and so $s_{\text{int}} > 0$ for all $r \in (0, 1)$.
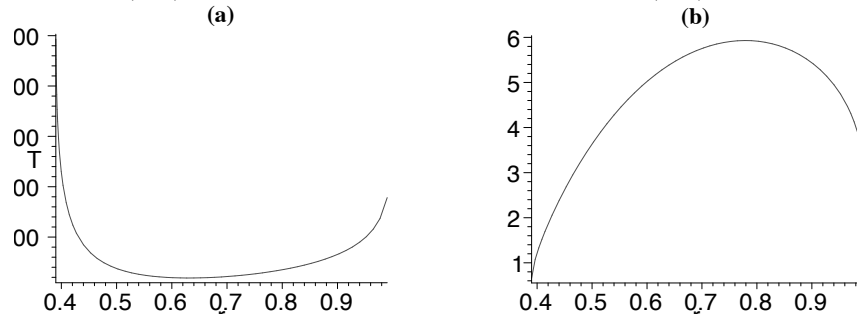

Figure 7: (a) shows how the period, $T(r)$, of the periodic orbit varies as r varies. (b) depicts how the yield, $\Omega(r)$, varies as r varies. All parameters are the same as in Figure 6, except $\bar{d} = 0.00975$. r only varies throughout $(r_0, 1)$, where $r_0 \approx 3.9$, since $s_{\text{int}} < 0$ when $0 < r < r_0$.

litres. Our model predicts that the optimal choice for r would be 0.2217, giving a time between impulses of 45.86 minutes, and an optimal yield of $\Omega(0.2217) = 436.01$ litres.

This is a significant predicted increase in the yield, demonstrating the practical advantages of understanding how the system relies on the emptying/refilling fraction and validation of this model should include experiments that include a range of values for r.

5   Proofs

Before we prove Theorem 1, we prove two Lemmas about the associated system of ordinary differential equations (2.3).

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Lemma 1. *Let $\tilde{\gamma}(t)$ and $\hat{\gamma}(t)$ be two orbits of (2.3), with initial conditions, $(\tilde{s}(0), \tilde{x}(0))$ and $(\hat{s}(0), \hat{x}(0))$, respectively, satisfying $\tilde{s}(0) = \hat{s}(0)$, but $\tilde{x}(0) - \hat{x}(0) = \eta > 0$. For any times $\tilde{t} > 0$ and $\hat{t} > 0$, where $\tilde{s}(\tilde{t}) = \hat{s}(\hat{t})$, it follows that $\tilde{x}(\tilde{t}) - \hat{x}(\hat{t}) = \eta$ and $\tilde{t} < \hat{t}$.*

Proof. Evaluating $\tilde{x}(\tilde{t}) - \hat{x}(\hat{t})$ using (2.5) yields $\tilde{x}(\tilde{t}) - \hat{x}(\hat{t}) = \eta$.

To show that $\tilde{t} < \hat{t}$, define $y(t) = \tilde{s}(t) - \hat{s}(t)$. Then $y(0) = 0$ and $y'(0) < 0$. Therefore, $y(t) < 0$ for $t > 0$, sufficiently small. Suppose there exists $\tau > 0$ such that $y(t) < 0$ for $0 < t < \tau$, and $y(\tau) = 0$. Then $y'(\tau) \geq 0$. Define $s_\tau = \tilde{s}(\tau) = \hat{s}(\tau)$. Noting that $\tilde{x}(\tau) - \hat{x}(\tau) = \eta$, using the first equation of (2.3), a simple calculations yields $y'(\tau) = -\eta \frac{f(s_\tau)}{Y} < 0$, a contradiction. Therefore, no such τ exists, and so $y(t) < 0$ for all $t \geq 0$. Since both $\tilde{s}(t)$ and $\hat{s}(t)$ are decreasing functions, $\tilde{s}(t) < \hat{s}(t)$ for all t, and so if $\tilde{s}(\tilde{t}) = \hat{s}(\hat{t})$, it follows that $\tilde{t} < \hat{t}$. $\qquad\qquad\square$

Lemma 2. *Let $\gamma(t) = (s(t), x(t))$ be an orbit of (2.3). Assume that $s_{\mathrm{int}} \geq 0$. If $x(0) > 0$ and $x(0) + Y \int_{\bar{s}}^{s(0)} \left(1 - \frac{\bar{d}}{f(s)}\right) ds > 0$, then there exists $\tau > 0$, finite, such that $s(\tau) = \bar{s}$. In particular, if $s(0) \geq \bar{s}^+$ and $x(0) > 0$, then there exists $\tau > 0$, finite, such that $s(\tau) = \bar{s}$.*

Proof. Recall that the vector field for (2.3) is C^1, all orbits are bounded, there are no equilibria with both components positive, and $s(t)$ is strictly decreasing. Therefore, either $s(t) \to 0$ as $t \to \infty$, or $s(t) \to s^* > 0$ and $x(t) \to 0$. In the first case we are done, and in the second case, if $s^* < \bar{s}$ we are also done.

Suppose $x(t) \to 0$ and $s(t) \to s^* \geq \bar{s}$ as $t \to \infty$. Since $x(t) \to 0$ as $t \to \infty$, it follows that $s^* < \lambda$. Then, letting $t \to \infty$ in (2.5), we obtain,

$$0 = x(0) + Y \int_{s^*}^{s(0)} \left(1 - \frac{\bar{d}}{f(s)}\right) ds \geq x(0) + Y \int_{\bar{s}}^{s(0)} \left(1 - \frac{\bar{d}}{f(s)}\right) ds > 0,$$

a contradiction. Therefore, $s^* < \bar{s}$, and we are done.

In particular, since $s_{\mathrm{int}} \geq 0$, $\bar{s}^+ > \lambda$. If $s(0) \geq \bar{s}^+$, then $Y \int_{\bar{s}}^{s(0)} \left(1 - \frac{\bar{d}}{f(s)}\right) ds \geq s_{\mathrm{int}} \geq 0$, and the result follows from the previous part. $\qquad\qquad\square$

The following Lemmas concern system (2.1).

Lemma 3. *Let $\gamma(t) = (s(t), x(t))$ be an orbit of (2.1). Assume that $s_{\mathrm{int}} \geq 0$. Then, if there exists a first time $t_1 > 0$, finite, such that $s(t_1^-) = \bar{s}$, then there exists an increasing sequence of distinct times $\{t_n\}_{n=1}^\infty$, such that $s(t_n^-) = \bar{s}$.*

Proof. Recall that since $s_{\text{int}} \geq 0$, it follows that $\bar{s}^+ > \lambda$. Suppose that there exists a time t_1, finite, such that $s(t_1^-) = \bar{s}$. Therefore, $x(0) > 0$ and $x(t_1^-) > 0$. It follows from (2.1) that $s(t_1^+) = \bar{s}^+$ and $x(t_1^+) = (1-r)x(t_1^-) > 0$. By Lemma 2, it follows that there is a time $t_2 > t_1$ such that $s(t_2^-) = \bar{s}$. From (2.5), $x(t_2^-) = x(t_1^+) + s_{\text{int}} > 0$, and by (2.1), $x(t_2^+) = (1-r)x(t_2^-) > 0$. This process can be repeated without end, and so there is an increasing sequence of distinct impulse times, $\{t_n\}_{n=1}^{\infty}$, and at these times $s(t_n^-) = \bar{s}$. $\qquad\square$

Lemma 4. *Consider an orbit $(s(t), x(t))$ of (2.1). If there exists an increasing sequence of distinct times $\{t_n\}_{n=1}^{\infty}$, such that $s(t_n^-) = \bar{s}$, then*

$$x(t_n^-) = (1-r)^{(n-1)}x(t_1^-) + s_{\text{int}}\left(\frac{1-(1-r)^{(n-1)}}{r}\right),$$

and so $\lim_{n\to\infty} x(t_n^-) = \frac{1}{r}s_{\text{int}}$ and $\lim_{n\to\infty} x(t_n^+) = \frac{1-r}{r}s_{\text{int}}$.

Proof. From (2.5),

$$x(t_{n+1}^-) = x(t_n^+) + s_{\text{int}} = (1-r)x(t_n^-) + s_{\text{int}}, \quad n = 1, 2, 3, \dots \quad (5.1)$$

This has the general solution

$$\begin{aligned} x(t_n^-) &= (1-r)^{(n-1)}x(t_1^-) + s_{\text{int}}\left(1 + (1-r) + \cdots + (1-r)^{(n-2)}\right) \\ &= (1-r)^{(n-1)}x(t_1^-) + s_{\text{int}}\left(\frac{1-(1-r)^{(n-1)}}{r}\right). \quad (5.2) \end{aligned}$$

Letting $n \to \infty$, it follows that $\lim_{n\to\infty} x(t_n^-) = \frac{1}{r}s_{\text{int}}$. Noting that $x(t_n^+) = (1-r)x(t_n^-)$, we obtain $\lim_{n\to\infty} x(t_n^+) = \frac{1-r}{r}s_{\text{int}}$. $\qquad\square$

Proof of Theorem 1. Suppose $(t, s(t), x(t))$ is a T-periodic solution of (2.1). Since the associated system of ordinary differential equations (2.3), does not admit any periodic solutions, there must be at least one impulse per period. Without loss of generality, assume that $t_1 = T$, and so $s(T^-) = \bar{s}$. Then, from the impulsive conditions in (2.1), $s(T^+) = \bar{s}^+$ and $x(T^+) = (1-r)x(T^-)$. Since the solution is T-periodic, $s(0^+) = s(T^+) = \bar{s}^+$ and $x(0^+) = x(T^+)$. Therefore, $x(T^-) = \frac{1}{1-r}x(0^+)$. From (2.5),

$$x(T^-) = x(0^+) + Y\int_{s(T^-)}^{s(0^+)}\left(1 - \frac{\bar{d}}{f(s)}\right)ds.$$

Therefore,

$$\frac{1}{1-r}x(0^+) = x(0^+) + s_{\text{int}}.$$

After rearranging,

$$\frac{r}{1-r}x(0^+) = s_{\text{int}}.$$

Thus a T-periodic orbit exists, only if $s_{\text{int}} > 0$. It follows also, that if $s(T^-) = \bar{s}$, then $s(0^+) = \bar{s}^+$, $x(0^+) = \frac{1-r}{r}s_{\text{int}}$, and $x(T^-) = \frac{1}{r}s_{\text{int}}$.

Also, if $s_{\text{int}} > 0$, $s(0^+) = \bar{s}^+$, and $x(0^+) = \frac{1-r}{r}s_{\text{int}}$, then by Lemma 2, there exists a first time t_1 such that $s(t_1^-) = \bar{s}$. As above, by (2.5), $x(t_1^-) = \frac{1}{r}s_{\text{int}}$. Therefore, $(t, s(t), x(t))$ is a T-periodic solution with exactly one impulse each period and this is the only periodic solution possible (up to translation in time). Hence there is a unique periodic orbit.

Clearly, at the impulse points, the periodic orbit satisfies $s(t_n^-) = s(T^-) = \bar{s}$, $s(t_n^+) = s(0^+) = \bar{s}^+$, $x(t_n^-) = x(T^-) = \frac{1}{r}s_{\text{int}}$, and $x(t_n^+) = x(0^+) = \frac{1-r}{r}s_{\text{int}}$.

Next we apply impulsive Floquet theory to system (2.1) to establish orbital asymptotic stability of the periodic orbit and asymptotic phase. We calculate the nontrivial impulsive Floquet multiplier (see Bainov and Simeonov [2]).

For convenience of notation, let

$$\zeta_0 = \bar{s}^+, \ \zeta_1 = \bar{s}, \ \xi_0 = \frac{1-r}{r}s_{\text{int}}, \ \xi_1 = \frac{s_{\text{int}}}{r},$$

and define

$$
\begin{aligned}
P &= -\frac{f(s)x}{Y}, & Q &= -\bar{d}x + f(s)x, \\
a &= -rs + rs^i, & b &= -rx, \\
\phi &= s - \bar{s}.
\end{aligned}
$$

Therefore, the nontrivial Floquet multiplier is

$$\Delta_1 \exp\left[\int_0^T \left(\frac{\partial P}{\partial s} + \frac{\partial Q}{\partial x}\right) dt\right],$$

where

$$\Delta_1 = \frac{P(\zeta_0,\xi_0)\left(\frac{\partial b}{\partial x}\frac{\partial \phi}{\partial s} - \frac{\partial b}{\partial s}\frac{\partial \phi}{\partial x} + \frac{\partial \phi}{\partial s}\right) + Q(\zeta_0,\xi_0)\left(\frac{\partial a}{\partial s}\frac{\partial \phi}{\partial x} - \frac{\partial a}{\partial x}\frac{\partial \phi}{\partial s} + \frac{\partial \phi}{\partial x}\right)}{P(\zeta_1,\xi_1)\frac{\partial \phi}{\partial s} + Q(\zeta_1,\xi_1)\frac{\partial \phi}{\partial x}},$$

and $\frac{\partial a}{\partial s}$, $\frac{\partial a}{\partial x}$, $\frac{\partial b}{\partial s}$, $\frac{\partial b}{\partial x}$, $\frac{\partial \phi}{\partial s}$, $\frac{\partial \phi}{\partial x}$ are evaluated at the point (ζ_1,ξ_1).

Therefore,

$$\Delta_1 = (1-r)^2\frac{f(\zeta_0)}{f(\zeta_1)}.$$

Furthermore,

$$
\begin{aligned}
\int_0^T \left(\frac{\partial P}{\partial s} + \frac{\partial Q}{\partial x} \right) dt &= \int_0^T \left(-\frac{f'(s)x}{Y} - \bar{d} + f(s) \right) dt \\
&= \int_0^T \left(\frac{f'(s)s'}{f(s)} + \frac{x'}{x} \right) dt \\
&= \ln f(s) \Big|_{\zeta_0}^{\zeta_1} + \ln \xi \Big|_{\xi_0}^{\xi_1} \\
&= \ln \frac{f(\zeta_1)}{f(\zeta_0)} + \ln \frac{1}{1-r}.
\end{aligned}
$$

Thus it follows that the nontrivial multiplier is

$$
(1-r)^2 \frac{f(\zeta_0)}{f(\zeta_1)} \cdot \frac{f(\zeta_1)}{f(\zeta_0)} \cdot \frac{1}{1-r} = 1 - r.
$$

It lies inside the unit circle, and so the T-periodic orbit is orbitally asymptotically stable and has the property of asymptotic phase.

1 . Assume that $s_{\text{int}} > 0$.

1. i) If $\bar{s} > \lambda$, then if $x(0) > 0$, there exists a time t_1 such that $s(t_1^-) = \bar{s}$, and so by Lemma 3, there exists an infinite sequence of times $\{t_n\}_{n=1}^\infty$, such that $s(t_n^-) = \bar{s}$ and $s(t_n^+) = \bar{s}^+$. By Lemma 4, $\lim_{n \to \infty} x(t_n^-) = \frac{1}{r} s_{\text{int}}$ and $\lim_{n \to \infty} x(t_n^+) = \frac{1-r}{r} s_{\text{int}}$, and so the periodic orbit attracts all orbits with initial conditions satisfying $x(0) > 0$.

1. ii) Assume that $\bar{s} < \lambda$. By Lemma 2, under the given conditions, there exists a time $t_1 > 0$ such that $s(t_1^-) = \bar{s}$. The argument is now similar to that given for 1. i) .

1. iii) Assume that $x(0) + Y \int_{\bar{s}}^{s(0)} \left(1 - \frac{\bar{d}}{f(s)} \right) ds \leq 0$. Suppose that there exists a first time $t_1 > 0$, finite, such that $s(t_1^-) = \bar{s}$. By (2.5),

$$
x(t_1^-) = x(0) + Y \int_{\bar{s}}^{s(0)} \left(1 - \frac{\bar{d}}{f(u)} \right) du \leq 0,
$$

a contradiction. Hence, there are no impulses and iii) holds.

In cases 1. (i) and (ii), we have shown that for appropriate initial conditions, the fermenter cycles indefinitely and all orbits with the appropriate initial conditions approach the unique periodic orbit asymptotically. Hence, there exists a sequence of times $\{t_n\}_{n=1}^\infty$ such that $s(t_n^-) = \bar{s}$ and by Lemma 4, $\lim_{n \to \infty} x(t_n^-) = \frac{1}{r} s_{\text{int}}$ and $\lim_{n \to \infty} x(t_n^+) = \frac{1-r}{r} s_{\text{int}}$. Since the periodic orbit is asymptotically stable and has the property of asymptotic phase, it follows that $t_{n+1} - t_n \to T$ as $n \to \infty$.

By the impulse condition in (2.1), $s(t_n^+) = \bar{s}^+$ and $s(t_n^-) = \bar{s}$. Next we show that one of a), b), or c), in the statement of the theorem must hold.

a) If $x(t_1^-) = \frac{1}{r}s_{\text{int}}$, then by the impulse condition in system (2.1), $x(t_n^+) = \frac{1-r}{r}s_{\text{int}}$ and we are on the periodic orbit so a) is satisfied.

b) If $x(t_1^-) < \frac{1}{r}s_{\text{int}}$, by Lemma 4,

$$
\begin{aligned}
x(t_n^-) &= (1-r)^{(n-1)}x(t_1^-) + s_{\text{int}}\left(\frac{1-(1-r)^{(n-1)}}{r}\right) \\
&< \frac{(1-r)^{(n-1)}s_{\text{int}}}{r} + s_{\text{int}}\left(\frac{1-(1-r)^{(n-1)}}{r}\right) \\
&= \frac{s_{\text{int}}}{r}
\end{aligned}
$$

for all positive integers n. Therefore, $x(t_n^+) = (1-r)x(t_n^-) < \frac{(1-r)}{r}s_{\text{int}}$, and so by Lemma 1, $t_{n+1} - t_n > T$. Hence, b) is satisfied.

c) If $x(t_1^-) > \frac{1}{r}s_{\text{int}}$, the argument is similar to that given for case b).

2. Assume that $s_{\text{int}} = 0$. If $x(0) > 0$ and $x(0) + Y\int_{\bar{s}}^{s(0)}\left(1 - \frac{\bar{d}}{f(s)}\right)ds > 0$, by Lemma 2, $\bar{s}$ is reached in finite time. By Lemma 3, there are an infinite number of impulses.

But then, by (2.5), noting that $s_{\text{int}} = 0$, it follows that $x(t_n^-) = x(t_{n-1}^+) = (1-r)x(t_{n-1}^-) = (1-r)^{(n-1)}x(t_1^-)$. Hence $x(t_n^-) \to 0$, monotonically, as $n \to \infty$, and so $\liminf_{t\to\infty} x(t) = 0$.

It remains to prove that the time between impulses approaches infinity. Note also that $x(t_n^+)$ decreases monotonically to zero as $n \to \infty$. Therefore by Lemma 1, the time between impulses increases. The segment of the orbit of (2.1) between time t_n and time t_{n+1} approaches the heteroclinic orbit of (2.3) joining the equilibrium points $(\bar{s}^+, 0)$ and $(\bar{s}, 0)$, and hence the time between impulses approaches infinity.

If $x(0) + Y\int_{\bar{s}}^{s(0)}\left(1 - \frac{\bar{d}}{f(s)}\right)ds \leq 0$ the proof is similar to 1. iii).

3. Assume that $s_{\text{int}} < 0$. Suppose that there exists an infinite sequence of distinct impulse times $\{t_n\}_{n=1}^{\infty}$ with $s(t_n^-) = \bar{s}$ and $x(t_n^-) > 0$. Then there exists a positive integer k, such that $x(t_1^-) + ks_{\text{int}} < 0$. But then $x(t_k^+) = (1-r)x(t_k^-) < x(t_k^-)$, and so by (2.7) $x(t_{k+1}^-) = x(t_k^+) + s_{\text{int}} < x(t_k^-) + s_{\text{int}} < \cdots < x(t_1^-) + ks_{\text{int}} < 0$, a contradiction. Therefore, there are at most a finite number of impulses. By Lemma 1, the time between impulses increases and eventually there exists at time $\tau > 0$, finite so that $s(t) > \bar{s}$ for all $t > \tau$, i.e., there are no impulses for $t > \tau$. Therefore, for $t > \tau$ the orbit behaves precisely the same as an orbit of the associated system of ODEs (2.3) for which $s(t) > \bar{s}$ for all $t > 0$. The result follows.

$\square$

6   Discussion

Self-cycling fermentation has a variety of applications, as described in the introduction. The model developed here uses nutrient concentration as the triggering factor, in order to describe nutrient minimizing processes such as water purification, sewage treatment, or toxic waste cleanup. The relevant environmental protection agency sets a maximum acceptable concentration of contaminants in the environment. This concentration is chosen to be $\bar{s}$, the value of the parameter that triggers the cycling of the fermenter. None of the contents of the bioreactor are released into the environment until the concentration of nutrient (pollutant) is below this acceptable standard. In practice, there is a small delay between the detection of the threshold concentration of the pollutant and the initiation of the cycling process. Thus, the fraction of the contents in the tank released will always be strictly below $\bar{s}$. At worst, the process terminates without releasing anything.

As explained in the introduction, the model also applies to any feedback mechanism that is independent of the actual consumption dynamics, and results in the cycling process being initiated at a consistent concentration of the limiting nutrient. From experiments, (see Wincure, Cooper and Rey [16]), this appears to be the case, for example, for the dissolved oxygen level criterion.

Analysis of our model indicates that knowledge of the sign of the quantity s_{int} (defined in (2.6), and given in the special case when the response function is of the Monod form by (3.1)), allows us to determine and manipulate the dynamics of the fermenter. This quantity depends on factors inherent to the system, such as the concentration of pollutant in the fresh medium at the beginning of each cycle, the maximum acceptable concentration of contaminants, and the emptying and refilling fraction, as well as the properties of the particular microorganism used to consume the nutrient, including its species-specific death rate, its functional response, and the yield constant.

Our analysis of the model gives criteria that predict whether or not a stable periodic cycle will eventually be achieved or whether the fermenter will eventually fail to cycle. Our model predicts that only when s_{int} is positive is it possible to select initial conditions so that the fermenter cycles indefinitely as desired. For appropriate initial conditions, the fermenter asymptotically approaches a cycle with a fixed period (see (4.3)) in which the nutrient concentration, just before and after the emptying and refilling process, is $\bar{s}$ and $\bar{s}^+ = (1 - r)\bar{s} + rs^i$, respectively, and the concentration of microorganisms is $\frac{1}{r}s_{\text{int}}$ and $\frac{(1-r)}{r}s_{\text{int}}$, respectively. Otherwise, if s_{int} is not positive, either the fermenter reaches the threshold infinitely often, but the time between cycles increases without bound, or the fermenter reaches the threshold concentration at most a finite number of times. In either of these cases, the fermenter eventually fails to reach the threshold value of the pollutant (within a reasonable length of time), the process terminates, and the population of microorganisms eventually dies out.

Furthermore, our results indicate that even if s_{int} is positive, successful operation also depends on the initial concentrations of the population of microorganisms and the pollutant when the species-specific death rate is taken into account. However, if s_{int} is positive, and the initial concentration of pollutant in the fermenter is greater than or equal to $\bar{s}^+$, then as long as the initial concentration of microorganism is positive, the reactor will function as desired. Normally, this is the case since one would expect that the initial concentration of pollutant in the fresh medium of the reactor would be s^i, the concentration of pollutant in the environment and $s^i > \bar{s}^+$. It is interesting to note that the model also predicts that the larger the initial concentration of microorganisms the faster the threshold of pollutant required for cycling is reached during the initial cycles before the period of the limiting cycle is reached.

Assuming that the initial concentration of pollutant is sufficiently large, i.e., $s(0) = s^i$, and that the initial biomass concentration is very small, as would be the case in most practical applications, the model predicts that if $s_{\text{int}} < 0$, the fermenter fails to cycle within 1 cycle, and if $s_{\text{int}} = 0$, although in theory, the fermenter cycles indefinitely, the concentration of micoorganisms tends to zero and the cycle time tends to infinity, so for all practical purposes, the fermenter fails. However, if $s_{\text{int}} > 0$, a stable periodic cycle is achieved with a finite cycle time. In the example simulated in Figures 2 and 3, where $s(0) = \bar{s}^+$, it took approximately 5-6 cycles for the fermenter to reach the stable periodic orbit.

In order that $s_{\text{int}} > 0$, it is necessary that $\bar{s}^+ > \lambda$, the break-even concentration, and hence $s^i > \lambda$. Note also that,

$$\frac{d}{ds^i} \, s_{\text{int}} = Y r \left(1 - \frac{\bar{d}}{f(s^i)}\right), \qquad (6.1)$$

and so s_{int} increases as s^i increases when $s^i > \lambda$. It follows that in order for the process to work properly, there must be a high enough concentration of the pollutant in the fresh medium. Thus, the model predicts that a process that was functioning well to clean up pollution in the environment might be made to fail, if measures were taken independently that resulted in a reduction of the concentration of the pollutant in the environment!

In practice, if we measure s_{int} in advance and if it is less than or equal to zero, we can either try to adjust any operating parameters over which we have control, or we can judiciously choose a different population of microorganisms. (Note that if the species-specific death rate is assumed to be zero, then s_{int} is always greater than zero. Our model therefore predicts that taking the species-specific death rate into account might prevent the failure of the process.) The parameter that can be controlled most easily is the emptying/refilling fraction, r. Once the population of microorganisms has been selected and all other parameters except r fixed, it was shown that in order for $s_{\text{int}} > 0$, r must be selected so that $\bar{s}^+ > \lambda$. If the threshold concentration $\bar{s} \geq \lambda$, then $\bar{s}^+ > \lambda$, and $s_{\text{int}} > 0$ for any choice of $r \in (0, 1)$.

Otherwise, $\bar{s}^+ > \lambda$, if and only if the concentration of the pollutant $s^i > \lambda$. In this case, $s_{\text{int}} > 0$ is an increasing function of r, and so either $s_{\text{int}} > 0$ for all $r \in (0,1)$, or there exists a critical value $r_0 \in (0,1)$ such that for $r = r_0$, $s_{\text{int}} = 0$ and for $r > r_0$, $s_{\text{int}} > 0$, or $s_{\text{int}} < 0$ for all $0 < r < 1$.

Because the time required to empty a prescribed fraction of the tank and refill it is usually insignificant compared to the time required for the microorganisms to consume the pollutant so that the concentration of pollutant is below the threshold concentration, we chose to model the system using a system of impulsive, ordinary differential equations. We thus made the simplifying assumption that the emptying and refilling process is instantaneous. However, when we were concerned with finding the optimal emptying and refilling fraction in order to process as much pollutant as possible in as short a time as possible, we did take this emptying and refilling time into consideration. See ϵ in (4.2). Although, in practice, dependence of ϵ on r is likely to be negligible, in an actual application the function $\epsilon(r)$ could easily be determined and ϵ could then be replaced by $\epsilon(r)$ in (4.2). One refinement of the model that might warrant further analysis is to include the emptying and refilling process in the model, especially if very small values of r are predicted to be optimal.

We were able to show that when the process works for a particular population of microorganisms, there is an optimal value of the emptying/refilling fraction r that maximizes the efficiency of the self-cycling fermentation process, i.e., resulting in the maximum yield over any specified period of time and that this optimal value of r need not be $\frac{1}{2}$. We showed how to determine this value of r and we determined this value in the case of the experimental data used in Wincure, Cooper and Rey [16] to suggest an improved emptying/refilling fraction for the particular system they studied. It was interesting to note that in the examples we considered, this optimal value of r was very sensitive to the species-specific death rate $\bar{d}$, (compare Figure 6 with 7) and suggested that this might be useful for determining $\bar{d}$ for a given species. Experiments to verify if the species-specific death rate actually plays a role in determining the transient behaviour before the stable cycle is reached, as our analysis predicts, would also be useful to help validate the model.

The experiments by Wincure, Cooper and Rey [16] with $r = 0.5$ identified that self-cycling fermentation produces a synchronous cell population and that the stable cycle time matched the population's doubling time, i.e. the time required to double the number of cells. This contrasts with batch fermentations in which the cell population is not in synchrony. Scientists are still not sure what aspect of the technique is responsible for the synchrony, i.e., whether it is due to the value of r, or the possibility that cells at certain stages in their development die in the face of low levels of nutrient (starvation), or some combination of both. As in the case of the model of Wincure, Cooper, and Rey [16], our model suggests that knowledge of the cell population's state of synchrony is unnecessary to predict the stable periodicity for all values of r for which $s_{\text{int}} > 0$. In fact, our prediction of convergence to

a stable periodic cycle when $s_{\text{int}} > 0$, is also independent of the form of the response function and does not require knowledge of the maximum growth rate, as long as the response function is monotone increasing for all concentrations of the pollutant less than s^i. Our model predicts that on the stable periodic attracting cycle, the biomass concentration increases from $\frac{(1-r)}{r}s_{\text{int}}$ to $\frac{1}{r}s_{\text{int}}$. It thus doubles during a cycle if $r = 0.5$, but increases by a factor of $\frac{1}{1-r}$ for any feasible r between 0 and 1. Of course, this estimate might need revising if the time for emptying and refilling the tank is significant and again live data would be required to validate this claim. Our model makes no predictions about synchrony, since it uses macroscopic equations. However, in applications such as water purification, treatment of sewage, or cleanup of toxic waste, synchrony of the population need not be an issue.

Now that we understand the the dynamics predicted by the global mathematical analysis of our model for the nutrient driven self-cycling fermentation process in the case of any monotone response function, it is interesting to make a comparison with the dynamics predicted by an analogous analysis of a well established model for the chemostat. The chemostat represents another apparatus that can be used for similar applications. It is also referred to as a continuous stirred tank reactor. See Novick and Szilard [9] for a description of the apparatus and Butler and Wolkowicz [5] and Wolkowicz and Lu [17] for a complete global analysis of the model of the chemostat in the case of general monotone (as well as nonmonotone) response functions.

The chemostat differs from the self-cycling fermenter because for the chemostat, the volume in the tank always remains the same. Instead of emptying and refilling a portion of the tank, there is a continuous inflow and outflow of fresh medium at some rate D. The model for the chemostat where the species-specific death rate is not assumed to be negligible compared to the dilution rate is given by

$$s'(t) \;=\; (s^i - s(t))D - \frac{1}{Y}f(s)x, \tag{6.2}$$

$$x'(t) \;=\; x(-\bar{d} - D + f(s)), \tag{6.3}$$

where except for D defined above, the variables and parameters all have the same meaning as in (2.1). To describe the dynamics of the chemostat, it is useful to define a parameter λ_c, called the break-even concentration. This is the unique extended real number so that $f(\lambda_c) = \bar{d} + D$. ($\lambda_c = \infty$ if $f(s) < \bar{d} + D$, for all $s > 0$.) This is the analogue of λ for the self-cycling fermenter. Asssuming $D > 0$, global analysis of the model predicts that the outcome does not depend on the initial concentrations of nutrient and microorganisms, as long as $s(0) \geq 0$ and $x(0) > 0$. If $\lambda_c \geq s^i$, then the population of microorganisms dies out and $s(t)$ approaches s^i as t tends to ∞. However, if $\lambda_c < s^i$ then $x(t)$ and $s(t)$ approach the positive equilibrium concentrations $\frac{Y(s^i - \lambda_c)D}{D + \bar{d}}$ and λ_c, respectively, as t tends to ∞.

The mathematical analysis of the models predicts that the nutrient driven self-cycling fermenter has several advantages over the chemostat when used

for processes such as water purification. Note that, since we are assuming that the response function, $f(s)$ is monotone increasing, λ_c is always larger than λ. (They would be equal if $D = 0$, but the smaller D the less output from the chemostat and when $D = 0$ the process ceases to operate as a chemostat and becomes batch fermentation.) Given an $\bar{s}$ set by some environmental protection agency, it is much easier to find an appropriate population of microorganisms in the case of self-cycling fermentation. The chemostat can only be used to reduce the concentration of pollutant below $\bar{s}$, if $D > 0$ can be chosen so that $\bar{s} > \lambda_c$, since at best, $s(t)$ tends to λ_c as t tends to ∞. On the other hand, the nutrient driven self-cycling fermenter only requires $s_{\text{int}} > 0$. This always holds if $\bar{s} \geq \lambda$, and can even hold when $\bar{s} < \lambda$, as we have shown. (See for example, Figure 2). Thus the operator has much more flexibility in their choice of $\bar{s}$. For the sake of comparison, for the data used in Figure 2, to reduce the level of the pollutant to $\bar{s}$ using the chemostat, one would have to choose the dilution rate to be $D \leq 0.00935$. This is less than the assumed species-specific death rate!

If dissolved oxygen concentration is used as the triggering mechanism in the self-cycling fermentation process, the operator has less flexibility than if nutrient concentration is used, but still more flexibility than with the chemostat. As well, microorganisms have a tendency to mutate. In the case of the chemostat, the output concentration λ_c could thus change, resulting in a release of water that does not meet the required standard before the problem is detected. However, in the case of nutrient driven self-cycling fermentation, in the worst possible case the process would terminate and nothing would be released. Further study is still required when dissolved oxygen concentration is used as the triggering mechanism instead of nutrient concentration and the species-specific death rate is taken into consideration.

As pointed out above, if the dilution rate is selected to be $D = 0$ in the chemostat, the process is no longer a chemostat, but rather becomes batch fermentation. If the emptying and refilling fraction r in the self-cycling fermenter is selected to be $r = 1$ or $r = 0$, then the process is no longer a self-cycling fermenter, but also becomes a batch culture. This is because there is a discontinuity in how the process works at the extremes $r = 1$ and $r = 0$. In the first case, the entire tank is emptied once $\bar{s}$ is reached, and so microorganisms would have to be re-inoculated each cycle. In the second case, nothing is ever released.

Analysis of the model of the chemostat generalized in the obvious way to allow competition between more than one population of microorganisms for the limiting nutrient indicates that coexistence is not possible. Rather, the population with the lowest break-even concentration, λ_c, wins and drives all other populations to extinction. On the other hand, coexistence of more than one species competing for a single limiting nutrient in the nutrient driven self-cycling fermenter has been proved to be possible (see [12] and [14]). The question of whether it can be more effective to use more than one population of microorganisms in processes like water purification is explored

in [14]. Refinement of the model of self-cycling fermentation to include size-structured populations is discussed in [12] and [13] and a mass population balance model is studied in Godin, Cooper, and Rey [6] and [7].

7   Acknowledgements

References

[1] D.D. Bainov and P.S. Simeonov, *Systems with Impulsive Effect,* Ellis Horwood Ltd, Chichester [1989].

[2] D.D. Bainov and P.S. Simeonov, *Impulsive Differential Equations: Periodic Solutions and Applications,* Longman Scientific and Technical, Burnt Mill [1993].

[3] D.D. Bainov and P.S. Simeonov, *Impulsive Differential Equations: Asymptotic Properties of the Solutions,* World Scientific, Singapore [1995].

[4] W.A. Brown and D.G. Cooper, *Hydrocarbon degradation by Acinetobacter calcoaceticus RAG-1 using the self-cycling fermentation technique,* Journal of Biotechnology and Bioengineering, Vol. 40, 797-805 [1992].

[5] G.J. Butler and G.S.K. Wolkowicz, *A mathematical model of the chemostat with a general class of response functions describing nutrient uptake,* SIAM Journal on Applied Mathematics, Vol. 45, 138-151 [1985].

[6] F.B. Godin, D.G. Cooper, and A.D. Rey, *Numerical methods for a population-balance model of a periodic fermentation process,* Bioengineering, Food, and Natural Products, AIChE Journal, Vol. 45, 1359-1364 [1999].

[7] F.B. Godin, D.G. Cooper, and A.D. Rey, *Development and solution of a cell mass population balance model applied to the SCF process,* Chemical Engineering Science, Vol. 54, 565-578 [1999].

[8] V. Lakshmikantham, D.D. Bainov and P.S. Simeonov, *Theory of Impulsive Differential Equations,* World Scientific, Singapore [1989].

[9] A. Novick and L. Szilard, *Description of the chemostat,* Science, Vol. 112, 715-716 [1950].

[10] B.E. Sarkis and D.G. Cooper, *Biodegradation of aromatic compound in a self-cycling fermenter (SCF),* The Canadian Journal of Chemical Engineering, Vol. 72, 874-880 [1994].

[11] J.D. Sheppard and D.G. Cooper, *Development of computerized feedback control for the continuous phasing of Bacillus subtilis,* Journal of Biotechnology and Bioengineering, Vol. 36, 539-545 [1990].

[12] R.J. Smith, *Impulsive differential equations with applications to self-cycling fermentation,* PhD thesis, McMaster University [2001].

[13] R.J. Smith and G.S.K. Wolkowicz, *A size-structured model for the nutrient-driven self-cycling fermentation process,* Dynamics of Continuous, Discrete and Impulsive Systems, Vol. 10, 207-220 [2003].

[14] R.J. Smith and G.S.K. Wolkowicz, *Coexistence of competing species in nutrient driven self-cycling fermentation,* preprint.

[15] S.D. Wentworth and D.G. Cooper, *Self-cycling fermentation of a citric acid producing strain of Candida lipolytica,* Journal of Fermentation and Bioengineering, Vol. 81, No. 5, 400-405 [1996].

[16] B.M. Wincure, D.G. Cooper and A. Rey, *Mathematical model of self-cycling fermentation,* Journal of Biotechnology and Bioengineering, Vol. 46, 180-183 [1995].

[17] G.S.K. Wolkowicz and Z. Lu, *Global Dynamics of a Mathematical Model of Competition in the Chemostat: General Response Functions and Differential Death Rates* SIAM Journal on Applied Mathematics, Vol. 52, 222-233 [1992].

[18] M.G. Zenaitis and D.G. Cooper, *Antiobiotic production by Streptomyces aureofaciens using self-cycling fermentation,* Journal of Biotechnology and Bioengineering, Vol. 44, 1331-1336 [1994].