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Multiple limit cycles in the chemostat with variable yield

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Abstract

The global asymptotic behavior of solutions of the variable yield model is determined. The model generalizes the classical Monod model and it assumes that the yield is an increasing function of the nutrient concentration. In contrast to the Monod model, it is demonstrated that the variable yield model exhibits sustained oscillations. Moreover, it is shown that the variable yield model may undergo a subcritical Hopf bifurcation and feature at least two distinct limit cycles. Implications for the coexistence of competing populations are discussed.

AMS: 34A34; 92D25
Keywords: Continuous culture; Variable yield model; Multiple limit cycles; Subcritical Hopf bifurcation; Competitive coexistence

1. Introduction

Modeling microbial growth is a problem of special interest in mathematical biology and theoretical ecology. One particular class of models includes deterministic models of microbial growth in the continuous culture vessel (sometimes also referred to as the bioreactor or chemostat) [1,2]. Equations of the basic model take the form

$$S' = (S^0 - S)D - \frac{x}{\gamma}p(S),$$

$$x' = x(p(S) - D),$$

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where $S(t)$ and $x(t)$ denote concentrations of the nutrient and the microbial biomass respectively; S^0 denotes the feed concentration of the nutrient and D denotes the volumetric dilution rate (flow rate/volume). The function $p(S)$ denotes the microbial growth rate and a typical choice for p is $p(S) = (mS)/(a + S)$ [3]. The stoichiometric yield coefficient γ denotes the ratio of microbial biomass produced to the mass of the nutrient consumed.

The dynamics of the basic model are simple. If γ is constant and $p(S)$ is a monotonically increasing function, then the microorganism can either become extinct or persist at an equilibrium level [4–6]. The particular outcome depends solely on the break-even concentration λ where $p(\lambda) = D$. Specifically, if $\lambda < S^0$, the organism persists, and if $\lambda \geqslant S^0$, it becomes extinct. Both monotonicity of $p(S)$ and assumption that the yield γ is constant are critical in establishing such a dichotomy.

Following the accumulation of experimental data, it became evident that the simple model requires modification. Specifically, the simple model failed to explain the observed oscillatory behavior in the chemostat [7,8]. It was suggested that the stoichiometric yield coefficient may be a function of substrate concentration. Such hypothesis was analyzed in a series of theoretical studies in chemical engineering literature [9–11]. These studies demonstrated that if the yield coefficient increases linearly with substrate concentration, then in a suitable parameter range, the stable rest state may undergo the Hopf bifurcation and a limit cycle may appear.

The fact that the yield coefficient may depend on the substrate concentration is now well established in experimental literature. The data presented by Herbert [12] show that in glycerol-limited *A. aerogenes*, the stoichiometric growth yield decreased by a factor of 2 when the specific growth rate was decreased from 0.9 to 0.05 h^{-1}. In a more recent study, Panikov [13] measured the stoichiometric growth yields for several microbial organisms such as *D. formicarius*, *P. fluorescens*, *A. globiformis*, and *B. subtilis* growing on carbon source in a chemostat. In these experiments, the yield increased as a saturating function of the specific growth rate (see Fig. 3.7 on p. 127 [13]). Despite the clear evidence for variability of the yield coefficient, its precise functional form is still largely unknown. The variability of γ can be attributed to various physiological factors such as the maintenance energy requirement [14]; 'cell-quota' [12,15,16]; mass-energy balance [17]; variation in metabolic activity [18,19]; or changes in cell morphology [20–22]. Including different combinations of these factors into a mathematical model will produce different analytic expressions for the yield.

In this paper, we modify the modeling approach developed in [9–11] and assume that the yield coefficient $\gamma(S)$ is a function of the substrate concentration S. We use the local properties of $p(S)$ and $\gamma(S)$ and the bifurcation analysis to study the Hopf bifurcation of the persistence rest point. We show that the bifurcation can be subcritical, meaning that while there is an asymptotically stable rest point there also can be multiple (at least two) limit cycles surrounding it. Specifically, we demonstrate that only supercritical bifurcations occur when the yield varies linearly with S thus correcting the previously published results [10,11]. We also show that the variability of the yield coefficient may lead to oscillatory coexistence of several microbial species in continuous culture.

This paper has the following structure. In Section 2, we formulate the variable yield model and discuss the basic properties of its solutions. We also present the main tool (a Hopf theorem) for the analysis of the Hopf bifurcation. Applications of the theorem and numerical examples are presented in Section 3, an example of oscillatory coexistence is also presented. Section 4 contains the discussion and it concludes the paper. Appendices contain the proofs of the mathematical results.

2. The model

We modify the constant yield model by simply introducing a functional dependence between the yield coefficient γ and the nutrient concentration S. In chemical engineering literature, the variable yield is traditionally modeled by a linear function [9–11,23,24],

$$\gamma(S) = c_1 + c_2 S, \quad c_1, c_2 > 0,$$

but our results hold for a more general class of functions. The equations of interest are

$$S' = (S^0 - S)D - x\frac{p(S)}{\gamma(S)}, \quad S(0) \geqslant 0,$$

$$x' = x(p(S) - D_1), \quad x(0) \geqslant 0.$$

The usual scaling is to measure concentrations in units of S^0 and time in units of $1/D$. Moreover, we further rescale x by a factor of $1/\gamma(0)$. Such rescaling results in the new system where the new $p(S)$ replaces $(1/D)p(S^0 S)$, the new $\gamma(S)$ replaces $\gamma(S^0 S)/\gamma(0)$, and the new D replaces D_1/D:

$$S' = 1 - S - x\frac{p(S)}{\gamma(S)}, \quad S(0) \geqslant 0, \tag{1}$$

$$x' = x(p(S) - D), \quad x(0) \geqslant 0. \tag{2}$$

We require that $p(S)$ be a C^1-smooth function with $p(0) = 0$ and $p'(S) > 0$ and that $\gamma(S)$ be a positive C^1-smooth function with $\gamma(0) = 1$. Note that $\gamma(0) = 1$ by the scaling.

Since the variables S and x are concentrations, only non-negative solutions are meaningful. The form of Eqs. (1) and (2) ensure that the positive cone in R^2 is invariant in forward time so that the model is well-posed. Since the vector field of the system is at least C^1-smooth, local existence and uniqueness of solutions follow immediately. We present the basic analysis of equilibria and their stability in the series of three lemmas whose proofs are deferred to Appendix A.

Lemma 2.1. *The positive quadrant*

$$\Omega = \{(S, x) \in R^2 | S, x > 0\}$$

is positively invariant under (1) *and* (2). *Moreover, the system* (1)–(2) *is dissipative in* $\overline{\Omega}$. *In particular, all non-negative solutions exist for all positive times.*

Lemma 2.2

(a) *The system* (1)–(2) *always admits a trivial equilibrium* $E_0 = (1, 0)$. *If* $p(1) \leqslant D$, *then all non-negative solutions of* (1) *and* (2) *converge to* E_0 *as* $t \to \infty$. *If* $p(1) > D$, *then* E_0 *is hyperbolically unstable and the system* (1)–(2) *is uniformly persistent* (*and uniformly dissipative*) *in* Ω.

(b) *The system* (1)–(2) *admits a unique equilibrium* $E_1 = (S^*, x^*) \in \Omega$ *if and only if* $p(1) > D$. *Let*

$$\rho = -x^* \left(\frac{p}{\gamma}\right)'(S^*).$$

If $\rho > 1$, *then* E_1 *is hyperbolically unstable with two-dimensional unstable manifold. If* $\rho < 1$, *then* E_1 *is hyperbolically stable. Here* S^* *is determined from* $p(S^*) = D$ *and* $x^* = (1 - S^*)\gamma(S^*)/D$.

Lemma 2.3. *Suppose that E_1 exists.*

(a) *If S^* is the only zero of*

$$R(S) = 1 - S - x^* f(S), \quad \text{where } f(S) = \frac{p(S)}{\gamma(S)}$$

on $(0,1)$, then E_1 is globally asymptotically stable in Ω.

(b) *Suppose $R(S)$ has multiple zeros on $(0,1)$. If $R'(S^*) > 1$ (equivalently if $\rho > 1$), then (1)–(2) admits a stable limit cycle in Ω.*

A necessary condition for instability of E_1 is that $\gamma'(S^*) > 0$. A necessary condition for existence of periodic solutions is that $\gamma'(S) > 0$ for some $0 < S < 1$. If $\gamma(S)$ is a non-increasing function of S, then (1)–(2) and the original Monod model have the same dynamics. The instability of the interior equilibrium E_1 inevitably leads to the existence of a stable limit cycle (sustained oscillations in practical terms). In Section 3, we show that the system (1)–(2) does undergo a subcritical Hopf bifurcation and admits at least two distinct limit cycles surrounding a stable equilibrium. The key tool is the following theorem whose proof can be found in Appendix B.

Theorem 2.1. *Consider the following system*

$$z' = F(z,c), \quad z \in R^2, \;\; c \in (-1,0]. \tag{3}$$

Assume that there exists a positively invariant bounded domain $U \subset R^2$ such that (3) is uniformly dissipative in U for $c \in (-1,0)$.

(A) *$F(z,c)$ is continuous in z, c and at least C^1 smooth in z for each fixed c;*

(B) *there exists a continuous map E: $(-1,0] \to U$ such that $F(E(c),c) = (0,0)$ for all $c \in (-1,0]$ and $E(0) = (0,0)$; moreover, $E(c)$ is the only equilibrium of (3) in U for $c \in (-1,0]$;*

(C) *the variational matrix $A(c) = F_z(E(c),c)$ has two complex conjugate eigenvalues $\lambda_{1,2} = \alpha(c) \pm i\beta(c)$ with $\beta(c) > 0$ for all $c \in (-1,0]$ and $\alpha(c) < 0$ for all $c \in (-1,0)$;*

(D) *there exists an open neighborhood V of $(0,0)$ such that $\mathrm{div}F(z,0) > 0$ for all $z \in V$ except possibly a set of Lebesgue measure zero.*

Then there exists c_0, $-1 < c_0 < 0$, such that for all $c \in (c_0,0)$ the system (3) has a limit cycle Γ_c^u surrounding $E(c)$ and another limit cycle Γ_c^s surrounding Γ_c^u. The limit cycle Γ_c^u is unstable relative to its interior and the limit cycle Γ_c^s is stable relative to its exterior. Finally, Γ_c^u converges to $(0,0)$ as $c \to 0_-$.

The important conclusion is the existence of two limit cycles. The only derivatives required are in (C) and (D) and the divergence is readily computable directly from the vector field. The continuous map E just scales the parameter to the interval $(-1,0]$ with the critical value occurring at 0. (C) implies that $E(c)$ is a local attractor for $c \in (-1,0)$ and (D) implies that O is a local repellor for $c = 0$. Without further assumptions this could be a very weak repellor. A previous result using set theoretic arguments is [25].

3. The chemostat with variable yield

In this section, we apply the main theorem to a specific class of functions $p(S)$ and $\gamma(S)$ and present a numerical example to demonstrate that the system (1)–(2) may admit multiple limit cycles. We assume that all of the parameters in $p(S)$ are fixed and that the yield function contains the bifurcation parameter c; hence we write $\gamma(S, c)$ in place of γ in (1)–(2).

Following the approach of Hofbauer and So [26], we introduce a new vector field by multiplying the vector field of (1) and (2) by a positive function $(f(S,c))^{-1}x^{\beta-1}$ where $f(S,c) = p(S)/\gamma(S,c)$. β is to be determined below. The new system is written as

$$S' = x^{\beta-1}\frac{1-S}{f(S,c)} - x^{\beta} = x^{\beta-1}H(S,c) - x^{\beta}, \tag{4}$$

$$x' = x^{\beta}\frac{p(S)-D}{f(S,c)} = x^{\beta}G(S,c). \tag{5}$$

Clearly, the new system (4)–(5) and the system (1)–(2) have the same trajectories in Ω. The phase plane equation of trajectories is

$$\frac{dS}{dx} = \frac{x^{\beta-1}H(S,c) - x^{\beta}}{x^{\beta}G(S,c)} = \frac{1 - S - x\frac{p(S)}{\gamma(S,c)}}{x(p(S)-D)}. \tag{6}$$

Since the trajectories are the same, the point $E_1 = (S^*, x^*)$ is also a rest point of the new system (4)–(5). For a given value of c, the coordinates of $E_1(c)$ are given by the solutions of $H(S^*, c) - x^* = 0$, $G(S^*, c) = 0$. We point out that the value S^* is the solution of $p(S) = D$ and thus it is independent of c. The variational matrix of (4)–(5) at $E_1(c)$ takes the form

$$\begin{bmatrix} (x^*)^{\beta-1}H_S(S^*,c) & -(x^*)^{\beta-1} \\ (x^*)^{\beta}G_S(S^*,c) & 0 \end{bmatrix}. \tag{7}$$

Since $x^* > 0$ at $E_1(c)$, the trace will be zero at a point $\hat{c}$ where $H_S(S^*, \hat{c}) = 0$. The determinant will be positive provided $G_S(S^*, \hat{c}) > 0$. A straightforward computation shows that this is true if $p_S(S^*) > 0$ and our basic assumption is that $p(S)$ is monotone for all positive values of S. Thus $\hat{c}$ is the bifurcation point. The direction of the bifurcation is determined by the quantity $\alpha = H_{Sc}(S^*, \hat{c})$. If $\alpha > 0$, then $E_1(c)$ is hyperbolically stable for $c < \hat{c}$ and hyperbolically unstable for $c > \hat{c}$. If $\alpha < 0$, then the direction of the bifurcation is reversed. Assuming that H_{SS} exists and using the fact that $G_S(S^*, \hat{c}) > 0$, we define

$$\beta = -\frac{H_{SS}(S^*, \hat{c})}{G_S(S^*, \hat{c})}.$$

Let F denote the vector field of (4)–(5). The divergence takes the form

$$\operatorname{div} F = x^{\beta-1}H_S(S,c) + \beta x^{\beta-1}G(S,c) = x^{\beta-1}[H_S(S,c) + \beta G(S,c)].$$

To simplify the notation, we introduce $K(S,c) = H_S(S,c) + \beta G(S,c)$ and assume that $K(S,c)$ is sufficiently smooth in S for a fixed c. Although this assumption of additional smoothness is not required in Theorem 2.1, it significantly simplifies the divergence analysis and is certainly valid for particular choices of p and γ considered later in this section.

We fix c at the bifurcation value $\hat{c}$ and expand $K(S,\hat{c})$ near S^* using the Taylor polynomial of degree two:

$$K(S,\hat{c}) = K(S^*,\hat{c}) + K_S(S^*,\hat{c})(S-S^*) + \frac{K_{SS}(S^*,\hat{c})}{2}(S-S^*)^2 + o(S-S^*)^2.$$

Since $H_S(S^*,\hat{c}) = G(S^*,\hat{c}) = 0$, we have that $K(S^*,\hat{c}) = 0$. By the choice of β, we also have that

$$K_S(S^*,\hat{c}) = H_{SS}(S^*,\hat{c}) - \frac{H_{SS}(S^*,\hat{c})}{G_S(S^*,\hat{c})}G_S(S^*,\hat{c}) = 0.$$

Consequently,

$$K(S,\hat{c}) = \frac{K_{SS}(S^*,\hat{c})}{2}(S-S^*)^2 + o(S-S^*)^2,$$

and

$$\operatorname{div} F \approx \frac{\delta x^{\beta-1}}{2}(S-S^*)^2, \quad \delta = K_{SS}(S^*,\hat{c})$$

in a sufficiently small neighborhood of $E_1(\hat{c})$. If $\delta > 0$, then div$F$ is also positive in this neighborhood of $E_1(\hat{c})$ minus the set $\{S = S^*\}$ which has zero Lebesgue measure. The quantity δ defined above can be expressed as

$$\delta = H_{SSS}(S^*,\hat{c}) - \frac{H_{SS}(S^*,\hat{c})}{G_S(S^*,\hat{c})}G_{SS}(S^*,\hat{c}) = G_S(S^*,\hat{c})\left(\frac{H_{SS}}{G_S}\right)_S(S^*,\hat{c})$$

so that the signs of δ and $(H_{SS}/G_S)_S(S^*,\hat{c})$ are the same.

We summarize our bifurcation analysis as follows: if a particular choice of functions $p(S)$ and $\gamma(S,c)$ is such that the quantities α and δ are strictly positive, then all conditions (A)–(D) of the bifurcation Theorem 2.1 are satisfied. Therefore, the system (4)–(5) and consequently the system (1)–(2) must exhibit at least two distinct limit cycles in Ω for c just below the bifurcation value $\hat{c}$. If $\alpha < 0$ and $\delta > 0$, Theorem 2.1 still applies but the direction of bifurcation is reversed, that is, (1)–(2) must exhibit at least two distinct limit cycles in Ω for c just above the bifurcation value $\hat{c}$. Finally, if $\delta < 0$, then regardless of the sign of α, the Hopf bifurcation is supercritical and it does not produce a family of unstable limit cycles.

As a particular application of Theorem 2.1, we present the bifurcation analysis of (1)–(2) with $p(S) = (mS)/(a+S)$ (the usual Monod function) and $\gamma(S,c) = 1 + cS$ or $\gamma(S,c) = 1 + cS^2$ (yields that are either linear or quadratic in S). We fix the dilution rate $D = 1$.

3.1. Linear yields

In case of a linear yield $\gamma(S,c) = 1 + cS$, the functions H and G are given by

$$H(S,c) = \frac{(1-S)(1+cS)(a+S)}{mS} = \frac{a}{mS} + \frac{1-a+ca}{m} - \frac{1+ac-c}{m}S - \frac{cS^2}{m},$$

$$G(S,c) = \frac{[(m-1)S-a](1+cS)}{mS} = -\frac{a}{mS} + \frac{m-1-ac}{m} + \frac{c(m-1)S}{m}.$$

We solve $p(S) = 1$ to find that $S^* = a/(m-1)$ so that $E_1(c)$ exists if and only if $m > 1 + a > 0$. We set $H_S(S^*, c) = 0$ and solve for c to find the bifurcation point $\hat{c}$:

$$\hat{c} = \frac{[(m-1)^2 + a](m-1)}{a(m - am - 1 - a)}.$$

The bifurcation occurs if and only if $\hat{c}$ is positive, that is, if and only if $a < ((m-1)/(m+1))$. Now we investigate whether the bifurcation is subcritical. We do so by computing H_{SS} and G_S:

$$H_{SS}(S,c) = \frac{2a}{m}\frac{1}{S^3} - \frac{2c}{m}, \quad G_S(S,c) = \frac{a}{mS^2} + \frac{c(m-1)}{m},$$

and then differentiating the ratio H_{SS}/G_S to obtain

$$\left(\frac{H_{SS}}{G_S}\right)_S = \left(\frac{2a - 2cS^3}{aS + c(m-1)S^3}\right)_S = -\frac{4caS^3 + 2a^2 + 6ac(m-1)S^2}{(aS + c(m-1)S^3)^2},$$

and since $m - 1 > 0$, δ is always negative at the bifurcation point. We conclude that linear yields do not produce subcritical bifurcations.

3.2. Quadratic yields

For quadratic yields $\gamma(S,c) = 1 + cS^2$, the value $S^* = a/(m-1)$ is unchanged but the functions H and G are now given by

$$H(S,c) = \frac{(1-S)(1+cS^2)(a+S)}{mS}, \quad G(S,c) = \frac{[(m-1)S - a](1+cS^2)}{mS}.$$

Consequently,

$$H_S = -\frac{a}{mS^2} + \frac{ac-1}{m} + \frac{2c(1-a)S}{m} - \frac{3cS^2}{m},$$

$$H_{SS} = \frac{2a}{mS^3} + \frac{2c(1-a)}{m} - \frac{6cS}{m},$$

$$G_S = \frac{a}{mS^2} - \frac{ac}{m} + \frac{2c(m-1)S}{m},$$

and

$$\left(\frac{H_{SS}}{G_S}\right)_S = \frac{(2a + 4(1 + am - m))c^2S^6 - 18cS^4a + (-16m + 20 - 4a)caS^3 + 6cS^2a^2 - 2a^2}{S^2(2mcS^3 - 2cS^3 - cS^2a + a)^2}. \tag{8}$$

Setting $H_S(S^*, c) = 0$ and solving for c, we find that the bifurcation point $\hat{c}$ is given by

$$\hat{c} = \frac{[(m-1)^2 + a](m-1)^2}{a^2(m^2 - 1 - a(1+2m))}.$$

The bifurcation occurs if and only if $\hat{c}$ is positive, that is, if and only if $a < ((m^2-1)/(1+2m))$. Now we compute

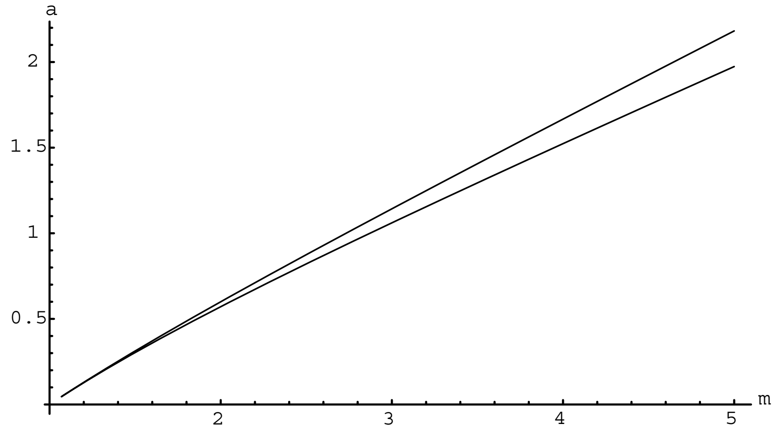

Fig. 1. Subcritical bifurcations occur inside the sector shown in this figure. The upper curve is the graph of $a = ((m^2 - 1)/(1 + 2m))$. Above this curve, no bifurcations occur. The lower curve is the set of points where the denominator of (9) equals zero. Under this curve, the bifurcations are supercritical. The sector terminates at the point $(m, a) = (1.082, 0.054)$ which is the point of intersection of these two curves.

$$\left(\frac{H_{SS}}{G_S}\right)_S (S^*, \hat{c}) = \frac{C_3(m)a^3 + C_2(m)a^2 + C_1(m)a + C_0(m)}{a^2m^2(m - 1 - a)^2}, \tag{9}$$

where $C_3(m) = (m + 4)(2m + 1)$, $C_2(m) = (m - 1)(12m^2 - 13m - 12)$, $C_1(m) = (m - 1)^2(7m^3 - 16m^2 - m + 12)$, and $C_0(m) = -(m - 1)^5(4 + 3m)$. The sign of δ is not fixed. Fig. 1 shows the region in the space of parameters a, m where the subcritical bifurcation occurs ($\delta < 0$).

To illustrate the result of this subsection numerically, we fix the parameters $m = 2.0$ and $a = 0.58$ and present computer simulations of the system (1)–(2) with the quadratic yield $\gamma(S, c) = 1 + cS^2$. The critical quantities S^*, $\hat{c}$, α, β, δ are as follows:

$$S^* = 0.58, \quad \hat{c} = 46.98, \quad \alpha = 0.029, \quad \beta = 4.076, \quad \delta = 23.02.$$

Since $\alpha > 0$, the direction of the bifurcation is the same as in Theorem 2.1 so that for c slightly less than $\hat{c}$ there exist two families of stable and unstable limit cycles of (1)–(2). Fig. 2 shows two limit cycles of (1)–(2) computed with $c = 46.0$. Fig. 3 shows the continuation diagram of the subcritical bifurcation at $\hat{c} = 46.98$.

3.3. Implications for coexistence

The fact that continuous cultures with variable yields exhibit sustained oscillations has an important implication for coexistence. The principle of competitive exclusion states that at most one microbial species can survive the competition for a single substrate in the continuous culture. The rigorous proof of this assertion was first presented in [27] for microbial cultures with Monod uptake rates and it was later extended to a much broader class of uptake functions in [28]. In both cases, the yield coefficients were treated as constants. Competitive exclusion generally holds for microbial populations which exhibit only steady-state behavior in the chemostat with a single resident population. It has been argued that sustained oscillations in the single population cultures

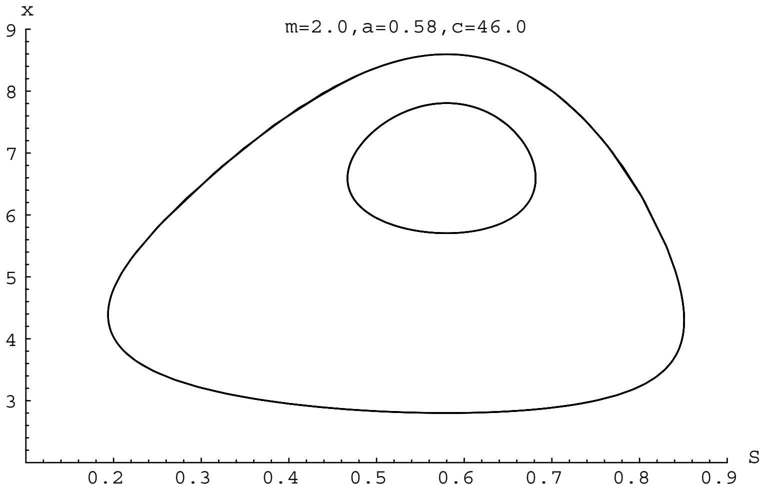

Fig. 2. This figure presents two limit cycles of (1) and (2) computed with $m = 2.0$, $a = 0.58$, and $c = 46.0$. Of the two periodic trajectories shown here, the outer is asymptotically stable and the inner is unstable. The asymptotically stable equilibrium E_1 (not shown) is located inside the inner cycle. The limit cycles were computed as the forward trajectory of the point (0.58,10) for $0 \leqslant t \leqslant 5000$ and the backward trajectory of the point (0.58,8.3) for $-3000 \leqslant t \leqslant 0$. To ensure that there are no additional limit cycles, we computed the trajectory of (0.58,8.3) in forward time for $0 \leqslant t \leqslant 5000$ and it successfully converged to the outer limit cycle.

may ultimately lead to coexistence of more than one microbial populations competing for a single substrate [29–31]. In [32], a two predator–one prey ecosystem was studied using the chemostat setting. It was shown that such system may exhibit a stable periodic solution with both competing predators present at all times. Specifically, it was demonstrated that the stable limit cycle corresponding to sustained oscillations of a single predator population can bifurcate into the region of coexistence and preserve its stability. The rest of this section presents a numerical example of periodic competitive coexistence of two microorganisms in the chemostat with one of them exhibiting the variable yield coefficient. The bifurcation described below is formally similar to the bifurcation studied in [32].

We consider the chemostat model of two populations x and y competing for a single substrate S. We set the dilution rate $D = 1$. Population x has a specific growth rate $p_1(S) = (2S/(0.7 + S))$ and the variable yield coefficient $\gamma_1(S) = 1 + 50S^3$. These parameters are chosen so that x exhibits sustained oscillations when $y = 0$. Population y has a specific growth rate $p_2(S) = (m_2 S)/(6.5 + S)$ and the constant yield coefficient $\gamma_2 = 120$. We treat m_2 as a bifurcation parameter. Let $\Gamma = (S(t), x(t))$ be the stable periodic solution of period $T > 0$ when $y = 0$. The bifurcation value m_2^* is determined from the equation

$$1 = \frac{m_2^*}{T} \int_0^T \frac{S(t)}{6.5 + S(t)}\, \mathrm{d}t,$$

that is, x and y coexist for m_2 slightly higher than m_2^*. We performed the integration numerically and found that $m_2^* \approx 9.87$. Fig. 4 shows the family of stable limit cycles for the system

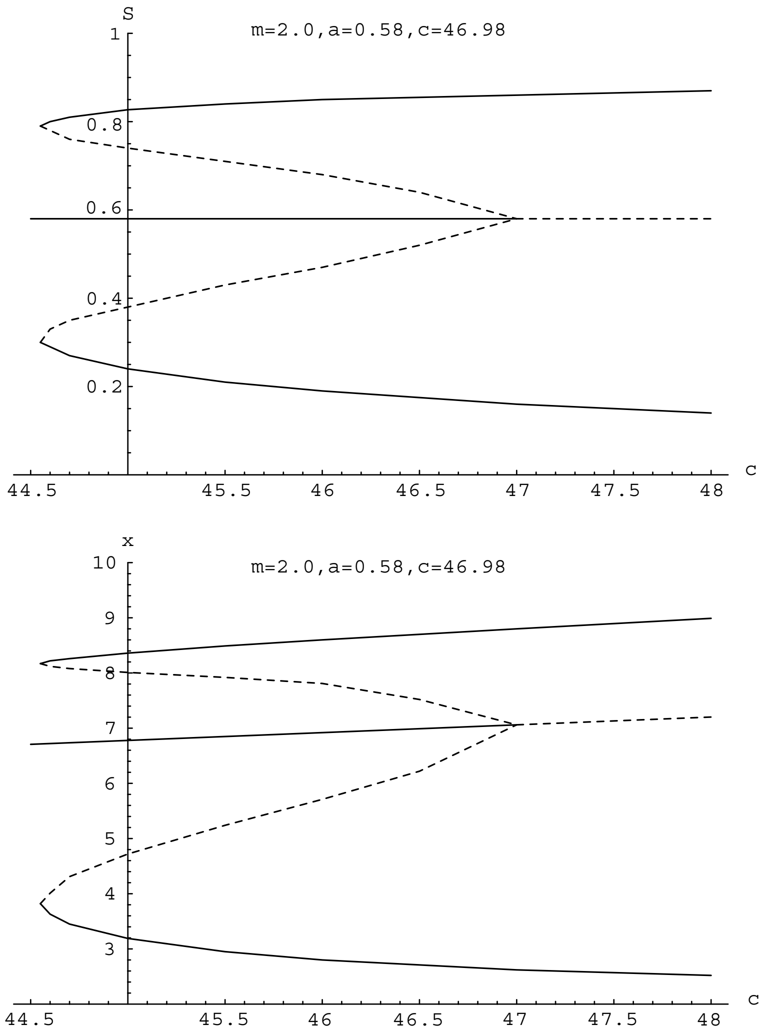

Fig. 3. This figure presents the continuation diagram of the subcritical bifurcation at $\hat{c} = 46.98$. The upper and lower solid lines are given by the maxima and minima of the corresponding phase variable on the stable limit cycle. The upper and lower dashed lines correspond to the unstable limit cycle. The line in the middle corresponds to E_1 which is stable for $c < \hat{c}$ and unstable for $c > \hat{c}$. Additional bifurcation occurs at $c = 44.55$ when the stable and unstable branches converge to form a semistable limit cycle. No periodic solutions of (1) and (2) exist and E_1 is a global attractor for $c < 44.55$.

$$
\begin{aligned}
S' &= 1 - S - \frac{x}{1 + 50S^3}\frac{2S}{0.7 + S} - \frac{y}{120}\frac{m_2 S}{6.5 + S}, \\
x' &= x\left(\frac{2S}{0.7 + S} - 1\right), \\
y' &= y\left(\frac{m_2 S}{6.5 + S} - 1\right),
\end{aligned}
\tag{10}
$$

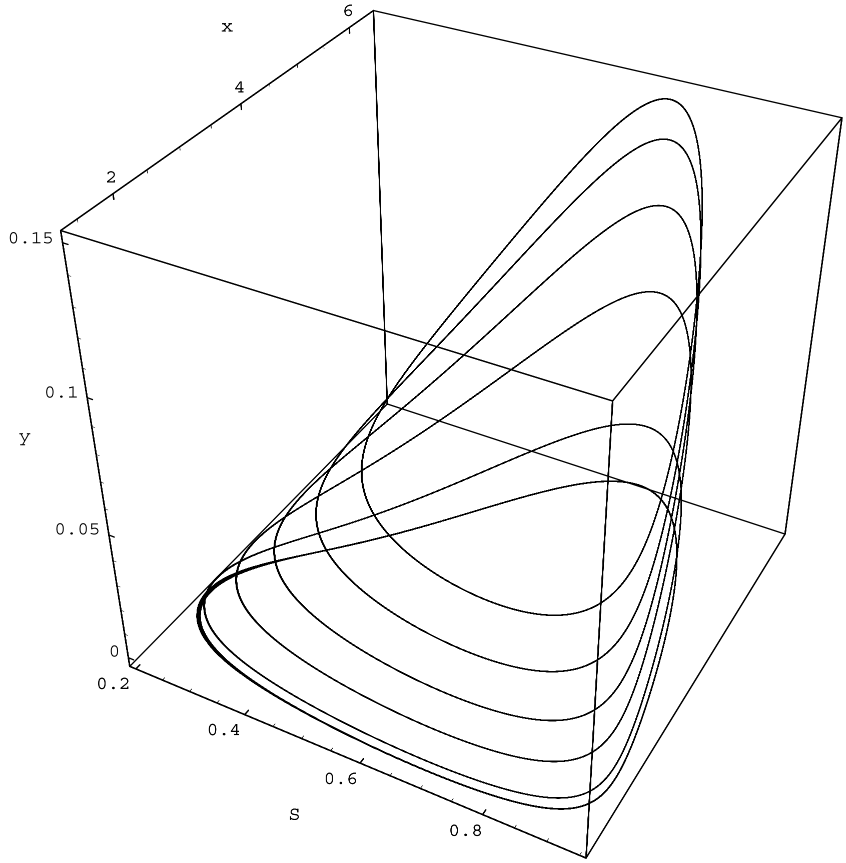

Fig. 4. The five stable limit cycles in the positive orthant correspond to $m_2 = 9.85 + 0.05k$ with $k = 1, \ldots, 5$. They were computed as numerical simulations of (10) with initial conditions $S(0) = 0.4$, $x(0) = 2.0$, $y(0) = 0.01$ for $0 \leqslant t \leqslant t_{\max} = 5000$. The figure shows the parametric plots of these solutions for $4500 \leqslant t \leqslant 5000$. The limit cycle in the $S - x$ plane is the trajectory of Γ. It was computed by setting $S(0) = 0.4$, $x(0) = 2.0$, $y(0) = 0$.

which bifurcate from Γ for $m_2 > m_2^*$. The five stable limit cycles in the positive orthant correspond to $m_2 = 9.85 + 0.05k$ with $k = 1, \ldots, 5$. The limit cycle in the $S - x$ plane is the trajectory of Γ. The fact that x and y coexist must be attributed solely to the variable yield coefficient exhibited by x. Indeed, even when $m_2 = 10.1$, the break-even concentration $\lambda_2 = (6.5)/(10.1 - 1) = 0.714$ of y is still greater than the break-even concentration $\lambda_1 = (0.7)/(2 - 1) = 0.7$ of x. If γ_1 were constant, then x would drive y to extinction for all $m_2 \leqslant 10.1$ [27].

4. Discussion

We have analyzed a mathematical model describing the microbial growth dynamics in the continuous culture when the yield coefficient may depend on the limiting nutrient concentration. Depending on a particular choice of parameters, the variable yield model may exhibit sustained oscillations and at least two distinct limit cycles. The main tool for the existence of multiple limit cycles is a theorem showing subcritical bifurcation in the plane and producing two distinct limit cycles (a practical Hopf theorem). Specifically, we demonstrated that only supercritical bifurcations occur in chemostats with linear yields. This finding is a correction of the previously published results [10,11]. In addition, we want to point out that use of the divergence criterion in a bifurcation theorem was first introduced in the work of Hofbauer and So [26].

The variable yield model conforms with the experimental data on microbial growth in continuous cultures that exhibit sustained oscillations. Although we have only considered the case of a single microorganism in the culture vessel, the findings that stable limit cycles do occur in the model naturally have theoretical implications for the case of several competing microorganisms. In Section 4, we presented a numerical example of periodic coexistence of two competing microorganisms with one of them exhibiting the variable yield coefficient. The open problems include obtaining analytic criteria for different outcomes of competition and investigating the possible dynamic types of solutions corresponding to competitive coexistence.

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Appendix A. Basic lemmas

Proof of Lemma 2.1. On the part of $\partial\Omega$ where $S = 0$ and $x > 0$ the vector field is pointing strictly inside Ω since $S' \equiv 1 > 0$ there. The line $l = \{x = 0, S \geqslant 0\}$ is invariant under (1)–(2); thus it consists of full trajectories. This proves the positive invariance of Ω.

Any solution $u(t) = (S(t), x(t))$ of (1)–(2) in Ω satisfies the differential inequality $S' \leqslant 1 - S$. Thus for every solution u in Ω, $\limsup S(t) \leqslant 1$. In particular, there exists $T \geqslant 0$ such that $S(t) \leqslant 2$ for all $t \geqslant T$. Let $q = \max_{[0,2]} \gamma(S)$ and let $z(t) = S(t) + x(t)/q$, then

$$z' = 1 - S - x\frac{p(S)}{\gamma(S)} + \frac{x}{q}(p(S) - D), \quad t \geqslant T.$$

By definition of q, $\gamma(S) \leqslant q$, and thus

$$z' \leqslant 1 - S - \frac{D}{q}x \leqslant 1 - \min\left(1, \frac{D}{q}\right)z, \quad t \geqslant T.$$

Consequently,

$$\limsup x(t) \leqslant \limsup z(t) \leqslant \frac{1}{\min\left(1, \frac{D}{q}\right)}.$$

The dissipativity of (1)–(2) in $\overline{\Omega}$ follows. □

Proof of Lemma 2.2. The point $E_0 = (1, 0)$ is clearly an equilibrium of (1)–(2). The eigenvalues of the variational matrix $J(E_0)$ are $\lambda_1 = -1$ and $\lambda_2 = p(1) - D$. If $p(1) > D$, then E_0 is hyperbolically unstable. Moreover, the last condition is both necessary and sufficient for the existence of E_1. The non-trivial equilibrium E_1 is unique because $p(S)$ is monotone for $S \geqslant 0$ and the equation $p(S) = D$ admits at most one solution $S^* > 0$. The trace and determinant of the variational matrix $J(E_1)$ are

$$\mathrm{Tr}(J(E_1)) = -1 - x^* \left(\frac{p}{\gamma}\right)'(S^*), \quad \mathrm{Det}(J(E_1)) = x^* p'(S^*) \frac{p(S^*)}{\gamma(S^*)} > 0.$$

Consequently, if $\rho > 1$, then E_1 is hyperbolically unstable with two-dimensional unstable manifold, and if $\rho < 1$, then E_1 is hyperbolically stable.

Suppose that $p(1) \leqslant D$ and E_1 does not exist. No solution $u(t) = (S(t), x(t))$ of (1)–(2) in Ω can have its ω-limit set different from E_0 since otherwise, the Poincaré–Bendixson theorem would imply the existence of a non-trivial equilibrium.

Suppose that $p(1) > D$, then E_0 is hyperbolically unstable and its unstable manifold intersects with Ω. It is clear that no cyclic orbits occur on the boundary $\partial\Omega$. The standard argument in the general theory of persistence shows that (1)–(2) is uniformly persistent (and uniformly dissipative) in Ω under these conditions. □

Proof of Lemma 2.3. To prove (a), define

$$V(S,x) = \int_{S^*}^{S} \frac{p(z) - D}{f(z)} \, \mathrm{d}z + \int_{x^*}^{x} \left(1 - \frac{x^*}{z}\right) \mathrm{d}z, \tag{A.1}$$

where $f(S)$ is as defined above. Substituting (A.1) into (1) and (2), we obtain

$$\frac{\mathrm{d}V}{\mathrm{d}t} = \frac{p(S) - D}{f(S)} (1 - S - x^* f(S)).$$

The assumption that S^* is the only zero of $R(S)$ together with $R(0) = 1$ and $R(1) < 0$ imply that $\mathrm{d}V/\mathrm{d}t < 0$ for all $S \in [0,1] \setminus S^*$. Consequently, $V(S,x)$ is a Liapunov function for (1)–(2) in Ω and E_1 attracts all solutions in Ω.

If $\rho > 1$, then by Lemma 2.2 E_1 has two-dimensional unstable manifold. Thus E_1 cannot belong to the ω-limit set of any solution in Ω but itself. Since (1)–(2) is dissipative in Ω by Lemma 2.2, we apply the Poincaré–Bendixson theorem to conclude that (1)–(2) admits a stable limit cycle in Ω. □

Appendix B. Proof of Theorem 2.1

The key to the proof rests with the following general technical lemma whose proof makes use of the work of [33]. It is the construction of a particular neighborhood in this proof that guarantees the existence of two distinct limit cycles.

Lemma B.1. *Consider an R^n-vector field $F(z,c)$, $z \in R^n$, $c \in (-1,0]$. Suppose the following conditions hold*:

(*i*) *$F(z,c)$ is C^1-smooth in z and continuous in c*;
(*ii*) *there exists a continuous map E: $(-1,0] \to R^n$ such that $F(E(c),c) = 0$ for all $c \in (-1,0]$ and $E(0) = O$*;

(*iii*) *$E(c)$ is a local attractor of the system $z' = F(z,c)$ for $c < 0$, and $E(0) = O$ is a local repellor for $c = 0$.*

If $A(c)$ denotes the basin of attraction of $E(c)$ for $c < 0$, then for any $\epsilon > 0$ there exists $c_0 < 0$ such that $\overline{A(c)} \subset B_\epsilon(O)$ for all $c \in (c_0, 0)$.

Proof of Lemma B.1. The proof will be based on an argument using a converse Liapunov function. The original result in this directions seems to be Massera, [34]; it was improved by Barbashin [35] and further improved again by Massera [36]. For more details on the theory of Liapunov functions, we refer the reader to the books [37–40]. For our purposes the statement in [33] suffices.

Let A be the basin of attraction as $t \to -\infty$ of 0 for the system $z' = F(z, 0)$. Then there exists a Liapunov function $V(z)$ in A such that [33]

(V1) $V \in C^1(A \setminus \{O\})$, $V \in C_{lip}(A)$;
(V2) $V(z) > 0$ in $A \setminus \{O\}$, $V(O) = 0$;
(V3) grad $V(z) \cdot F(z, 0) > 0$ for all $z \in A \setminus \{O\}$.

Consider a bounded neighborhood W of O such that $\overline{W} \subset A$, let $Z = \partial W$ be the boundary of W, and let

$$v_0 = \min_{z \in Z} V(z).$$

Obviously, $v_0 > 0$, and for any $v \in (0, v_0)$, the set

$$M_v = \{z \in W : V(z) = v\}$$

is a smooth $(n-1)$-dimensional manifold transversal to the vector field $F(z, 0)$ [33]. The property (V3) implies that the inequality,

$$\text{grad } V(z) F(z, 0) > 2k, \tag{B.1}$$

holds for all $z \in M_v$ for some $k > 0$ (where k depends on v). It is also clear that M_v is the boundary of the neighborhood $N_v = \{z : V(z) < v\}$ of O. Since $V(z_k) \to 0$ if and only if $z_k \to O$ for $z_k \in W$, the relation

$$\sup_{y \in N_v} \text{dist}(y, O) \to 0 \text{ as } v \to 0$$

holds. Now, given $\epsilon > 0$, one can find a $v \in (0, v_0)$ such that $N_v \subset B_\epsilon(O)$. Choose a sufficiently small $k > 0$ for which (B.1) holds. Since $F(z, c)$ is continuous in c, there exists $c_1 < 0$ such that

$$\text{grad } V(z) F(z, c) > k \tag{B.2}$$

for all $z \in M_v$ and all $c \in (c_1, 0)$. It follows from (B.2) that trajectories of $z' = F(z, c)$, $c \in (c_1, 0)$ intersect M_v, leaving the neighborhood N_v as t increases. Finally, choose $c_0 \in (c_1, 0)$ so that $E(c) \in B_{\epsilon/2}(O)$ for all $c \in (c_0, 0)$. It follows immediately that

$$\overline{A(c)} \subset N_v \subset B_\epsilon(O)$$

for all $c \in (c_0, 0)$. □

That trajectories leave the neighborhood N_v will be important in the proof of Theorem 2.1 to demonstrate that there are two distinct limit cycles. Since the boundary of $A(c)$ is itself an invariant set, N_v contains at least one limit set distinct from $E(c)$. Thus, given a neighborhood V of the origin, for c sufficiently close to 0, V contains the rest point $E(c)$ and at least one other invariant set. Lemma B.1 contains the essentials for subcritical bifurcation.

Proof of Theorem 2.1. Condition (C) implies that $E(c)$ is a local attractor of (3) for all $c \in (-1, 0)$. Conditions (C) and (D) imply that O is an unstable spiral point and thus a local repellor of (3) for $c = 0$. Conditions (A) and (B) imply that the boundary of the basin of attraction of $E(c)$ is a compact invariant set with no equilibria, so it must consist of a single periodic trajectory Γ_c^u by Poincarè–Bendixson theorem. It is now evident that Γ_c^u is a limit cycle which is unstable relative to its interior domain. Lemma B.1 implies that Γ_c^u tends to O as $c \to 0_-$.

In the proof of Lemma B.1, it as shown that there exists $c_0 < 0$ such that (B.2) holds for all $c \in (c_0, 0)$, and in particular the set $U \setminus \overline{N_v}$ is bounded and positively invariant with respect to (3) for $c \in (c_0, 0)$. By (B), the set $U \setminus \overline{N_v}$ contains no equilibria of (3), thus it must contain a limit cycle Γ_c^s. Since (3) is uniformly dissipative in U, we can always choose Γ_c^s to be the largest (in terms of geometric inclusion) limit cycle in $U \setminus \overline{N_v}$; then it follows immediately that Γ_c^s is stable relative to its exterior domain. Finally, since $\Gamma_c^u \subset N_v$ and $\Gamma_c^s \subset U \setminus \overline{N_v}$, the stable limit cycle Γ_c^s surrounds the unstable limit cycle Γ_c^u. □

For emphasis we repeat that for the relevant numbers c, one limit cycle is inside the set N_v, defined in Lemma B.1, and one is in $U \setminus \overline{N_v}$. It is not the case that a single, semi-stable limit cycle has bifurcated. In the region between the two limit cycles claimed in the theorem, one has no information about limit sets. To our knowledge, the first connection of the divergence condition to the Hopf bifurcation is found in Hofbauer and So [26].

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