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MICROBIAL GROWTH IN PERIODIC GRADOSTATS

HAL SMITH

Dedicated to the memory of Geoffrey Butler.

Introduction. A gradostat is a laboratory device in which one can study the growth of microorganisms in a nutrient gradient. As constructed by Lovitt and Wimpenny [**7, 8, 16**], a gradostat is a concatenation of several chemostats in which adjacent vessels are connected by tubes allowing pumps to exchange the material contents of each vessel. A mathematical model of the growth of a single species of microorganism in an n-vessel gradostat based on Michaelis-Menten growth response was studied by Tang in [**15**]. A model of competition between two different species in a two-vessel gradostat was studied by Jäger, So, Tang and Waltman in [**5**]. A similar model was studied by the author together with Tang in [**12**]. Models of competition between two species of microorganisms in a gradostat-like device have been studied by Stephanopoulos and Fredrickson [**13**] and Kung and Baltzis [**6**] in the bioengineering literature.

Actually, the term gradostat does not refer to a single well-defined apparatus, but to a whole family of configurations of interconnected, well-stirred vessels in which one can study the growth of microorganisms. We refer the reader to [**16**] for a general discussion of gradostat devices. See, in particular, Figures 1 and 10 describing different possible configurations.

The aim of the present paper is to study the growth of a single species of microorganism in the presence of one limiting substrate or two limiting complementary substrates in a very general gradostat where we allow essentially arbitrary connections between vessels, outside feed reservoirs of a limiting nutrient, and receiving vessels. In addition, we allow operating parameters, e.g., flow rates, input limiting nutrient concentration, to be time varying in a periodic way. Allowing periodic operating parameters is not merely a mathematical exercise. Long period variations of operating parameters can simulate noise, inevitably

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present in the system, e.g., pump operation. Periodicities can also be viewed as simulating the temporal inhomogeneities in nature such as day/night and summer/winter cycles. Thus, by periodically varying the operating parameters in a gradostat, one can simulate the full range of spatial and temporal inhomogeneities found in natural environments. Obviously, constant values of the operating parameters are a special case.

Basically, we find that single species growth in all periodic gradostats can be very simply described by a threshold criteria. If the threshold is not exceeded, then extinction of the species from the gradostat results. If the threshold is exceeded, then there is a unique positive periodically varying population level which is approached regardless of nontrivial initial nutrient and population size. This result depends essentially on the monotonicity and concavity of the microorganism's growth response function. Our result generalizes earlier work of Tang [**15**]. The present study is a prelude to the consideration of competition between two microbial species for a limiting nutrient in general gradostats.

In this section we consider only the case of a single limiting substrate. Consider a gradostat consisting of n well-stirred vessels in which microbial growth is to take place, together with as many as n separate reservoirs containing growth medium and all nutrients necessary for growth supplied in excess except for a single nutrient which may or may not be present. It is assumed that this nutrient (the same one for all reservoirs), if present in a reservoir, is present in a growth limiting concentration which may vary with time in a periodic manner. A receiving vessel which collects runoff from any subcollection of the vessels is also present. The vessels, reservoirs and receiving vessels are connected in a manner to be described such that material can be exchanged via pumps which pump the material from one vessel to another at either a constant rate or a periodically varying rate. The manner of connection and the pump rates are to be selected such that each of the n vessels in which growth is to take place maintain constant volume. Consider the i-th vessel. It can exchange material with any subcollection of the vessels, it can *receive* medium and (possibly) the essential limiting nutrient from a reservoir, and it can have a runoff to a receiving vessel. It is required that the sum of the volume flow rates into the vessel from other vessels or reservoirs at each instant be balanced by the volume flow rate out of the vessel to other vessels or receiving vessels.

Let $S_i(t)$ be the concentration of the substrate in the i-th vessel. Let $E_{ij}(t)$ be the (volumetric) flow rate at time t from vessel j to vessel i, $i \neq j$, and set $E_{ii}(t) \equiv 0$, $1 \leq i \leq n$. Let V_i be the volume of the i-th vessel, $D_i(t)$ be the flow rate at time t from a reservoir to vessel i (put $D_i = 0$ if there is no such reservoir) and $C_i(t)$ be the flow rate at time t from vessel i to a receiving vessel (put $C_i = 0$ if there is no such receiving vessel). Finally, let $\bar{S}_i^0(t)$ be the concentration of substrate at time t in the reservoir feeding into vessel i. Then the equation describing the change of $S = (S_1, S_2, \dots, S_n)$ at time t is given by

$$\operatorname{diag}[V_i]S' = \bar{A}(t)S + f(t),$$

in which $\operatorname{diag}[V_i]$ is the $n \times n$ diagonal matrix with $V_1, \dots, V_n$ down the diagonal and

$$\bar{A}(t) = E(t) - \operatorname{diag}[C_i(t)] - \operatorname{diag}\left[\sum_{l=1}^{n} E_{li}(t)\right]$$
$$f(t) = (D_1(t)\bar{S}_1^0(t), \cdots, D_n(t)\bar{S}_n^0(t)).$$

In order to insure that V_i is constant, which is implicit in the above system, we require that, for each i and all t,

$$\sum_j E_{ij}(t) + D_i(t) = \sum_l E_{li}(t) + C_i(t).$$

After multiplying through the system by $\operatorname{diag}[V_i^{-1}]$ and renaming variables in a, hopefully, obvious way, we obtain the system

$$S'(t) = A(t)S(t) + S^0(t) \tag{0.1}$$

in which $A(t)$ is a continuous, T-periodic matrix satisfying

$$A_{ij}(t) \geq 0, \quad i \neq j,$$
$$\sum_{j=1}^{n} A_{ij}(t) \leq 0,$$

for each t, and $S^0(t)$ is a continuous, T-periodic function satisfying

$$S^0(t) \geq 0, \quad S^0 \not\equiv 0.$$

We assume that, for each t, the matrix $A(t)$ is irreducible (see [**1**] for a definition) which means that, at each instant, the n vessels cannot be partitioned into two disjoint proper subsets, one of which has the property that none of it's vessels receives input from any vessel in the second subset.

Let $u(t) = (u_1(t), \ldots, u_n(t))$ be the vector whose i-th component represents the concentration of microbial species U in the i-th vessel. It is assumed that the growth rate of U at limiting nutrient concentration S (temporarily viewed as a scalar) is given by a function $f_u(S)$ satisfying

$$\begin{aligned} f_u(0) &= 0 \\ f_u(S) &\geq 0 \\ f_u'(S) &> 0 \\ f_u''(S) &< 0. \end{aligned} \qquad S \geq 0 \tag{0.2}$$

The consumption rate of nutrient by the microorganism is assumed proportional to f_u. A typical f_u is the Michaelis-Menten-Monod response function

$$f_u(S) = \frac{m_u S}{a_u + S},$$

where m_u is the maximum growth rate and a_u is the nutrient concentration at which f_u is half its maximum.

Following a familiar scaling out of the proportionality constant between growth rate and consumption rate we obtain the system of differential equations describing the rate of change of $(S(t), u(t))$

$$\begin{aligned} S' &= A(t)S - F(S)u + S^0(t) \\ u' &= A(t)u + F(S)u, \end{aligned} \tag{0.3}$$

in which $F(S)$ is the diagonal matrix with

$$F(S)_{ii} = f_u(S_i).$$

System (0.3) is the object of study in Section one.

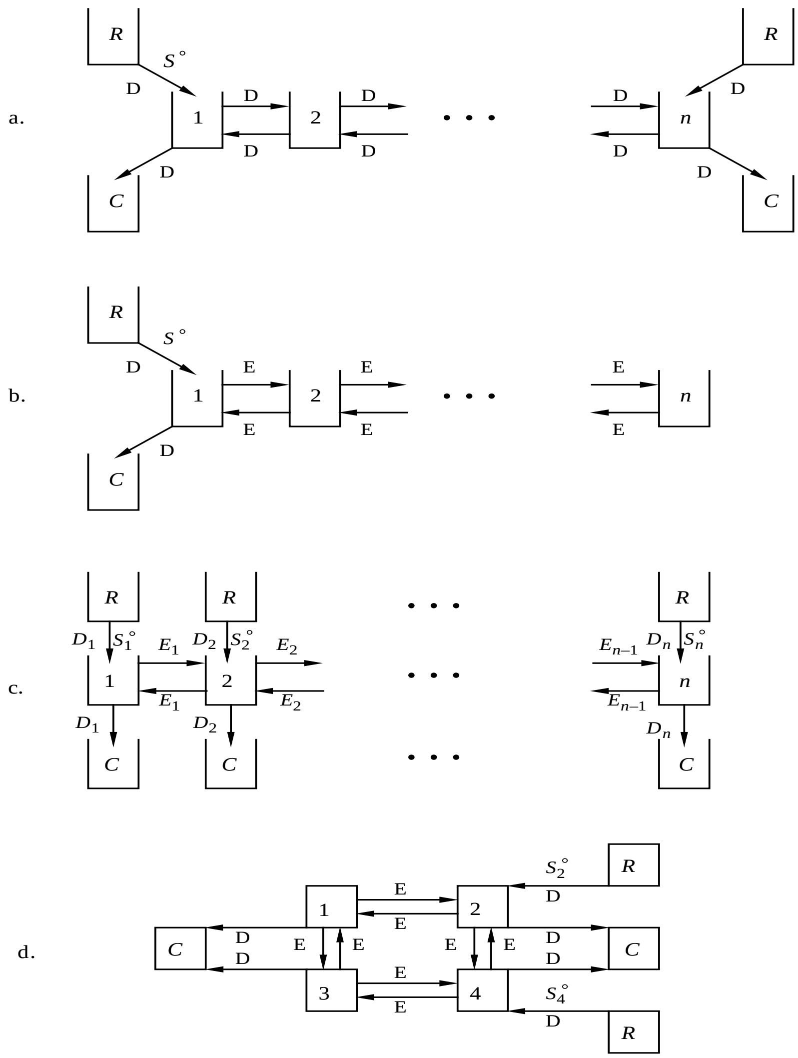

FIGURE 1. Selected gradostats. Flow rates are prescribed as are reservoir concentrations of limiting nutrient. Vessels labeled R are reservoirs and those labeled C are receiving (collecting) vessels. The gradostat in (d) is viewed from above.

Figure 1 depicts several gradostat configurations, most of which are similar to those in [**16**]. All vessels have identical volume. Clearly, there are limitless possibilities. For each of these, we give $A(t)$ and $S^0(t)$ below:

(a)

$$A = \begin{bmatrix} -2D & D & 0 & \cdot & \cdot & \cdot & 0 \\ D & -2D & D & & & & \cdot \\ 0 & D & -2D & & & & \cdot \\ \cdot & & & \cdot & & & \cdot \\ \cdot & & & & \cdot & & 0 \\ \cdot & & & & & \cdot & D \\ 0 & \cdot & \cdot & \cdot & 0 & D & -2D \end{bmatrix}$$

$S^0 = D(S^0, 0, 0, \cdots, 0)$

(b)

$$A = \begin{bmatrix} -(D+E) & E & 0 & \cdot & \cdot & \cdot & 0 \\ E & -2E & E & & & & \cdot \\ 0 & E & -2E & & & & \cdot \\ \cdot & & & \cdot & & & \cdot \\ \cdot & & & & \cdot & & 0 \\ \cdot & & & & & \cdot & E \\ 0 & \cdot & \cdot & \cdot & 0 & E & -2E \end{bmatrix}$$

$S^0 = D(S^0, 0, \cdots, 0)$

(c)

$$A = \begin{bmatrix} -(D_1+E_1) & E_1 & 0 & \cdot & \cdot & \cdot & 0 \\ E_1 & -(E_1+E_2+D_2) & E_2 & & & & \cdot \\ 0 & E_2 & -(E_2+D_3+E_3) & & & & \cdot \\ \cdot & & & \cdot & & & \cdot \\ \cdot & & & & \cdot & & 0 \\ \cdot & & & & & \cdot & E_{n-1} \\ 0 & \cdot & \cdot & \cdot & 0 & E_{n-1} & -(D_n+E_{n-1}) \end{bmatrix}$$

$S^0 = (D_1 S_1^0, D_2 S_2^0, \cdots, D_n S_n^0)$

(d)

$$A = \begin{bmatrix} -(D+2E) & E & E & 0 \\ E & -(D+2E) & 0 & E \\ E & 0 & -(D+2E) & E \\ 0 & E & E & -(D+2E) \end{bmatrix}$$

$S^0 = (0, DS_2^0, 0, DS_4^0)$

The coefficients above are allowed to vary T-periodically or can be constant. They are all nonnegative.

One can modify (0.3) by the addition of a periodic forcing term, $U^0(t)$, to the second equation which might represent an influx of species U into some subset of the vessels from reservoirs containing these species. Indeed, this influx might be viewed as coming from unmodeled gradostats or chemostats. In this way, one can consider systems where irreducibility is not assumed. We consider this modification in the next section. It causes only minor modification of our main result.

Several authors have considered periodic variation of operating parameters for single vessel (chemostat) competition studies. We mention the work in [**3, 4, 9, 14, 17**] which establishes that periodicity in system parameters can facilitate coexistence of competing species which could not occur for constant values of the parameters.

In the next section, we prove our main result, Theorem 1.3, which describes the global behavior of solutions of (0.3). In Section two, the special case depicted in Figure 1(a) is considered in more detail. In Section three we consider the case of a pair of complementary substrates.

It is convenient to describe, here, some notation used in the following sections. Inequalities will play a major role. If A, B are $n \times n$ matrices or n vectors, we write $A \leq B (A \ll B)$ whenever each entry of A is less than or equal to (strictly less than) the corresponding entry of B. If a and b are two vectors in $\mathbf{R}^n$ with $a \leq b$, let $[a, b] = \{x \in \mathbf{R}^n : \ a \leq x \leq b\}$.

1. Main Results. Consider the system of equations

$$\begin{aligned} S' &= A(t)S - F(S)u + S^0(t) \\ u' &= A(t)u + F(S)u \\ S(0) \geq 0\ , &\quad u(0) \geq 0, \end{aligned} \tag{1.1}$$

where

$$\begin{aligned} S^0(t+2\pi) &= S^0(t) \geq 0\ , \quad S^0(t) \not\equiv 0, \\ A(t+2\pi) &= A(t) \\ A_{ij}(t) &\geq 0\ , \quad i \neq j, \end{aligned}$$

$A(t)$ is irreducible for each t, and $F(S)$ is the diagonal matrix with

$$F(S)_{ii} = f_u(S_i),$$

with f_u as in (0.2). The time variable has been scaled to normalize the period to 2π.

The homogeneous linear system

$$x' = A(t)x \tag{1.2}$$

is assumed to be stable. More precisely, we assume all Floquet multipliers of (1.2) lie inside the unit circle in the complex plane. This hypothesis is necessary for (1.1) to be biologically reasonable as we will show. One can give a sufficient condition for stability of (1.2) which is very natural from a physical point of view. It is contained in the following lemma, the proof of which is contained in an appendix.

LEMMA 1.0. *Let $A(t)$ be as above and, in addition, suppose that, for each i, $1 \leq i \leq n$, and all t,*

$$\sum_{j=1}^{n} A_{ij}(t) \leq 0.$$

If, for each $t \in R$, there exists an i such that strict inequality holds in the above inequality, then all Floquet multipliers of (1.2) *lie inside the unit circle.*

We observed the reasonableness of the inequality in Lemma 1.0 in the previous section. The added condition can be viewed physically as saying that, at each instant t, there is flow from a reservoir into some vessel. The irreducibility assumption on $A(t)$ can be weakened (see Appendix).

Let $z = u + S$. Then (1.1) is equivalent to

$$\begin{aligned} z' &= A(t)z + S^0(t) \\ u' &= A(t)u + F(z-u)u \end{aligned} \tag{1.3}$$
$$0 \le u(0) \le z(0).$$

The advantage of (1.3) is that the first equation is independent of the second and is an inhomogeneous linear system. A second important advantage of (1.3) is that it is cooperative [**10**]. It is immediate from the form of (1.1) that solutions of (1.1) with nonnegative initial values remain nonnegative on their maximal right intervals of existence. The following lemma establishes that all solutions of

$$z' = A(t)z + S^0(t) \tag{1.4}$$

approach a unique positive 2π-periodic solution as t tends to infinity. An immediate consequence is that solutions of (1.1) with nonnegative initial values exist and are bounded on $[0, \infty)$.

LEMMA 1.1. *There is a unique 2π-periodic positive solution, $z^*(t)$, of* (1.4) *which attracts all solutions of* (1.4) *as t tends to infinity.*

PROOF. The homogeneous system (1.2) has no nontrivial 2π-periodic solutions by our stability hypothesis. A standard result (see, e.g., [**2**, p. 148]) implies that (1.4) has a unique 2π-periodic solution given by

$$z^*(t) = X(t,0)z^*(0) + \int_0^t X(t,s)S^0(s)ds$$
$$z^*(0) = [I - X(2\pi,0)]^{-1} \int_0^{2\pi} X(2\pi,s)S^0(s)ds,$$

where $X(t,\tau)$, $X(\tau,\tau) = I$, is the principal matrix solution of (1.2). Since $A(t)$ is quasi-positive and irreducible for each t, it follows [10] that $X(t,\tau) \gg 0$ for $t > \tau$. Moreover, since $X(2\pi, 0)$ has spectral radius less than one, the series

$$[I - X(2\pi,0)]^{-1} = \sum_{n=0}^{\infty} X(2\pi,0)^n = \sum_{n=0}^{\infty} X(2n\pi,0) \gg 0$$

converges absolutely to a positive matrix. It follows that $z^*(t) \gg 0$ for all t since $z^*(0) \gg 0$. Clearly, $z^*(t)$ is the limit of every solution of (1.4) as $t \to \infty$. □

In order to study the behavior of nonnegative solutions of (1.1), it suffices to study solutions of (1.3) with $0 \le u(0) \le z(0)$. Note that the relation $0 \le u(t) \le z(t)$ must hold for $t \ge 0$. As a prelude to the study of (1.3), consider first the system obtained by putting $z = z^*$ in the second equation of (1.3)

$$\begin{aligned} u' &= A(t)u + F(z^*(t) - u)u \\ 0 &\le u(0) \le z^*(0) \end{aligned} \tag{1.5}$$

This periodic system has the trivial solution $u = 0$ for which the variational system becomes

$$w' = [A(t) + F(z^*(t))]w \tag{1.6}$$

The following result gives the global asymptotic behavior of (1.5).

PROPOSITION 1.2. *If all Floquet multipliers of* (1.6) *lie inside or on the unit circle in the complex plane, then all solutions of* (1.5) *are asymptotic to the trivial solution. If* (1.6) *has a* (*necessarily positive*) *Floquet multiplier outside the unit circle, then* (1.5) *has a unique positive* 2π*-periodic solution* $u^*(t)$ *satisfying* $0 \ll u^*(t) \ll z^*(t)$ *for all* t, *which attracts all nontrivial solutions of* (1.5).

PROOF. The proposition essentially follows from Theorem 3.1 in [**11**]. This result does not apply immediately since the right-hand side of (1.5) is not defined for all $u \in \mathbf{R}_+^n$. Let $\hat{f}_u(S)$, $S \in \mathbf{R}$, be any twice continuously differentiable extensions of $f_u(S)$ on $[0,\infty)$ to $\mathbf{R}$ satisfying

$\hat{f}'_u(S) > 0$ and $\hat{f}''_u(S) < 0$ for all S. Extending $\hat{F}(S)_{ii} = \hat{f}_u(S)$ we obtain a system ((1.5) with $\hat{F}$ replacing F) for which Theorem 3.1 applies directly, as we now show. The hypothesis (M) and irreducibility of the Jacobian of the right-hand side in Theorem 3.1 have already been noted. The concavity assumption (C) requires that, if $0 \ll u \ll v$, then $D_uF(t,u) \geq D_uF(t,v)$ with equality *not holding* for each t in this inequality. $F(t,u)$ denotes the right-hand side of (1.5) with the modification as above. A calculation gives

$$D_uF(t,u) = A(t) + \operatorname{diag}[\hat{f}_u(z_i^* - u_i) - \hat{f}'_u(z_i^* - u_i)u_i]$$

where diag stands for the diagonal matrix with the i-th diagonal entry as above. Since

$$\frac{d}{du_i}[\hat{f}_u(z_i^* - u_i) - \hat{f}'_u(z_i^* - u_i)u_i] = -2\hat{f}'_u(z_i^* - u_i) + u_i\hat{f}'_u(z_i^* - u_i) < 0,$$

the concavity assumption (C) follows.

Theorem 3.1 of [**11**] now implies the proposition. We note that if $0 \leq u(0) \leq z^*(0)$ then $0 \ll u(t) \ll z^*(t)$ for $t > 0$, so the periodic solution $u^*(t)$, if it exists, must satisfy $0 \ll u^*(t) \ll z^*(t)$, $0 \leq t \leq 2\pi$. □

The coefficient matrix of (1.6) is a quasi-positive, irreducible matrix for each t, and so the fundamental matrix solution $Y(t,0)$, $Y(0,0) = I$, satisfies $Y(t,0) \gg 0$ for $t > 0$. Perron-Frobenius theory (see, e.g., [**1**]) implies that the spectral radius, ρ, of $Y(2\pi, 0)$ is a simple eigenvalue, strictly larger in modulus than all other eigenvalues. Of course, the eigenvalues of $Y(2\pi,0)$ are precisely the Floquet multipliers of (1.6). Hence, ρ is the largest (in modulus) Floquet multiplier of (1.6). We can then restate Proposition 1.2 as follows. If $\rho \leq 1$, then all solutions of (1.5) are asymptotic to the trivial solution; if $\rho > 1$, then (1.5) has a unique positive 2π-periodic solution $u^*(t)$ to which all nontrivial solutions approach as t tends to infinity. Henceforth, we refer to ρ as the principal Floquet multiplier of (1.6).

We can now state the main result of this paper. It asserts that all periodic gradostats behave in essentially the same way.

THEOREM 1.3. *If the principal Floquet multiplier, ρ, of* (1.6) *satisfies $\rho \leq 1$, then every solution of* (1.1) *is attracted to the 2π-periodic* *solution* $(S, u) = (z^*(t), 0)$. *If* $\rho > 1$, *then all nontrivial solutions of* (1.1) *are attracted to the unique positive* 2π*-periodic solution* $(S, u) = (S^*(t), u^*(t))$, *where* $u^*(t)$ *is as in Proposition* 1.2 *and* $S^*(t) + u^*(t) = z^*(t)$ *for all* t.

PROOF. We let T denote the Poincaré map for (1.1): $T(S(0), u(0)) = (S(2\pi), u(2\pi))$. Then T is a smooth, orientation preserving diffeomorphism of $\mathbf{R}_+^{2n}$. Given $(S(0), u(0)) \geq 0$, the orbit $\{T^j(S(0), u(0)) = (S(2j\pi), u(2j\pi))\}_{j \geq 0}$ is bounded in $\mathbf{R}_+^{2n}$, and, hence, the limit set

$$\Lambda = \{(S, u) : (S, u) = \lim_{i \to \infty} (S(2j_i\pi), u(2j_i\pi)),\ j_i \to \infty\}$$

is nonempty, compact and invariant under T.

Actually, it is more convenient to work with the Poincaré map U of (1.3) defined for (z, u) such that $0 \leq u \leq z$. Let Λ denote the limit set of such a point (z, u) with $u \neq 0$. If $(\bar{z}, \bar{u}) \in \Lambda$, then, clearly, $\bar{z} = z^*(0)$ since all solutions of the first equation in (1.3) tend to $z^*(t)$ as $t \to \infty$. Let P be the Poincare map for (1.5) defined for u with $0 \leq u \leq z^*(0)$. Invariance of Λ under the action of U implies that $U(\Lambda) = \Lambda$. But, if $(z^*(0), \bar{u}) \in \Lambda$, then $U(z^*(0), \bar{u}) = (z^*(0), P\bar{u})$. Hence, $U^j(z^*(0), \bar{u}) = (z^*(0), P^j\bar{u}) \in \Lambda$, $j = 0, \pm 1, \pm 2, \dots$. By Proposition 1.2, $P^j\bar{u} \to 0$ or $P^j\bar{u} \to u^*(0)$ as $j \to \infty$.

Let's suppose that all Floquet multipliers of (1.6) lie inside or on the unit circle. Then $P^j\bar{u} \to 0$ as $j \to \infty$ for all u, $0 \leq u \leq z^*(0)$. Hence, $(z^*(0), 0) \in \Lambda$. We must show $\{(z^*(0), 0)\} = \Lambda$. Now $U^j\Lambda = \Lambda = \{z^*(0)\} \times \Lambda_2$ where $\Lambda_2 \subseteq [0, z^*(0)]$ satisfies $P^j\Lambda_2 = \Lambda_2$, $j = 1, 2, \dots$. But $P^j[0, z^*(0)] \to \{0\}$ as $j \to \infty$, so $\Lambda = \{(z^*(0), 0)\}$.

Now suppose (1.6) has a Floquet multiplier outside the unit circle. Hence, $P^j u \to u^*(0)$ for every u with $0 \leq u \leq z^*(0)$, $u \neq 0$, by Proposition 1.2. It follows from considerations above that $(z^*(0), u^*(0)) \in \Lambda$. We must show that $\Lambda = \{(z^*(0), u^*(0))\}$. If $(z^*(0), 0) \notin \Lambda$, then it follows that $\Lambda = \{(z^*(0), u^*(0))\}$. For then, since Λ is compact, there exists $\bar{u} \gg 0$ such that $\bar{u} \leq u \leq z^*(0)$ for all u such that $(z^*(0), u) \in \Lambda$. Hence, $\Lambda = U^j\Lambda \subset \{z^*(0)\} \times [P^j\bar{u}, P^j z^*(0)]$ and $[P^j\bar{u}, P^j z^*(0)] \to \{u^*(0)\}$ as $j \to \infty$. We are done if we show $(z^*(0), 0) \notin \Lambda$. Recall that both P and U are monotone maps [**10**].

First, suppose that $z \leq z^*(0)$, so that $z(2j\pi) \leq z^*(0)$ for $j = 1, 2, \dots$, where $(z(t), u(t))$ is the solution of (1.3) with $(z(0), u(0)) = (z, u)$.

If $(z^*(0), 0) \in \Lambda$, then we may choose j, m such that $(z^*(0), 0) \approx (z(2j\pi), u(2j\pi)) \leq (z(2m\pi), u(2m\pi)) \approx (z^*(0), u^*(0))$. But then, by Proposition K in [**10**], Λ is a periodic orbit of U, i.e., $\Lambda = \{U^j(\bar{z}, \bar{u})\}_{j=0,1,2,\cdots,l-1}$, where $U^l(\bar{z}, \bar{u}) = (\bar{z}, \bar{u})$. Since both $(z^*(0), 0)$ and $(z^*(0), u^*(0))$ belong to Λ and are fixed points of U, this is impossible. Thus, if $z \leq z^*(0)$, then $\Lambda = \{(z^*(0), u^*(0))\}$.

If (z, u) is arbitrary with $0 \leq u \leq z$, $u \neq 0$, then we can choose $(\bar{z}, \bar{u})$ with $0 \leq \bar{u} \leq \bar{z}$, $\bar{u} \neq 0$ such that $\bar{z} \leq z^*(0)$ and $\bar{u} \leq u$. By the previous case, $\Lambda_{(\bar{z}, \bar{u})} = \{(z^*(0), u^*(0))\}$, and, by monotonicity of (1.3), $(\bar{z}(t), \bar{u}(t)) \leq ((z(t), u(t))$ for $t \geq 0$. Hence, $\Lambda_{(z,u)} = \{(z^*(0), u^*(0))\}$. □

REMARK 1. The second of equations (1.1) could be modified to include an "immigration" term representing influx of species u into some subset of the vessels, perhaps from a periodically driven chemostat operating at steady state [**4, 9, 14**]. Another reason for considering an immigration term is that one can then apply our results for irreducible $A(t)$ to the reducible case by decomposing $A(t)$ into irreducible components [**1**]. These considerations lead to the second equation in (1.1), modified as follows:

$$u' = A(t)u + F(S)u + U^0(t)$$
$$U^0(t + 2\pi) = U^0(t) \geq 0.$$

System (1.3) becomes

$$z' = A(t)z + S^0(t) + U^0(t)$$
$$u' = A(t)u + F(z - u)u + U^0(t).$$

If U^0 is not identically zero, then $u = 0$ is no longer a solution of the second equation, and this fact simplifies matters. Using ideas in [**11**], one can show that there is a unique 2π-periodic positive solution of the system to which all solutions approach asymptotically.

REMARK 2. Obviously, if $A(t) \equiv A$ and $S^0(t) \equiv S^0$ are constant, then Theorem 1.3 yields steady states $(z^*, 0)$ and (S^*, u^*), $S^* + u^* = z^*$, and the inequality $\rho \leq 1$ $(\rho > 1)$ can be replaced by an inequality in terms of the stability modulus $s \leq 0$ $(s > 0)$, where $s = s(A + F(z^*)) = \max\{\text{Re } \lambda : \ \lambda \text{ is an eigenvalue of } A + F(z^*)\}$. The stability modulus is a simple eigenvalue of $A + F(z^*)$ by virtue of the quasi-positive and irreducibility assumptions.

In practice, equation (1.1) will depend on several parameters, e.g., the frequency (period) of the time dependence, flow rates, etc. If we denote these parameters by $\lambda_1, \lambda_2, \dots, \lambda_k$, then it will be crucial to locate the "neutral stability surface," $\rho(\lambda_1, \lambda_2, \dots, \lambda_k) = 1$, in parameter space. This surface separates parameter space into a region ($\rho < 1$) where survival of the species is impossible and a region ($\rho > 1$) where survival occurs. In the next section, we obtain estimates for the location of such a surface.

2. An Example. As an application of Theorem 1.3, consider the periodic gradostat depicted in Figure 1(a). The corresponding system (0.3) is

$$\begin{aligned} S' &= DQS + DS^0(\omega t)e_1 \\ u' &= DQu + F(S)u, \end{aligned} \tag{2.1}$$

in which D is the (constant) dilution rate, $S^0(\omega t)$ is the periodic concentration of incoming nutrient to the first vessel:

$$S^0(r) = S^0(r + 2\pi) > 0$$

and ω is the frequency. The vector $e_1 = (1, 0, \dots, 0)^t$, and Q is given by

$$Q = \begin{bmatrix} -2 & 1 & 0 & \cdot & \cdot & \cdot & 0 \\ 1 & -2 & 1 & & & & \cdot \\ 0 & 1 & -2 & & & & \cdot \\ \cdot & & & \cdot & & & \cdot \\ \cdot & & & & \cdot & & 0 \\ \cdot & & & & & \cdot & 1 \\ 0 & \cdot & \cdot & \cdot & 0 & 1 & -2 \end{bmatrix}.$$

The change of time scale $\tau = \omega t$ will convert (2.2) to the 2π-periodic system

$$\begin{aligned} \dot{S} &= D\omega^{-1}[QS + S^0(\tau)e_1] \\ \dot{u} &= D\omega^{-1}Qu + \omega^{-1}F(S)u \end{aligned}$$

in which "$\cdot$" denotes $d/d\tau$.

The global behavior of solutions of (2.1) is determined by the principal Floquet multiplier of (1.6), according to Theorem 1.3. We will estimate the principal Floquet multiplier of (1.6) by obtaining an estimate,

$$p \le f_u(z_i^*(\tau)) \le q, \quad 1 \le i \le n, \quad \tau \in R.$$

From such an estimate, we obtain constant coefficient majorizing and minorizing comparison systems related to (1.6) by

$$\omega^{-1}DQ + \omega^{-1}pI \le \omega^{-1}DQ + \omega^{-1}F(z^*(\tau)) \le \omega^{-1}DQ + \omega^{-1}qI,$$

in which I is the identity matrix. If $Y(\tau, 0)$ denotes the principal matrix solution of (1.6), $Y(0,0) = I$, then we have the comparison

$$0 \ll e^{2\pi\omega^{-1}[DQ+pI]} \le Y(2\pi, 0) \le e^{2\pi\omega^{-1}[DQ+qI]},$$

which follows from a well-known comparison theorem [**11**]. Since $DQ + pI$ is a quasi-positive irreducible matrix, the matrix exponential is a positive matrix. Perron-Frobenius theory [**1**] implies that the spectral radii of the three matrices are ordered. It is worth noting that the spectral radius (largest Floquet multiplier) ρ of $Y(2\pi, 0)$, being a simple eigenvalue of $Y(2\pi, 0)$, is, therefore, a smooth function of system variables, e.g., D, ω, etc. We have

$$e^{2\pi\omega^{-1}[Ds(Q)+p]} \le \rho \le e^{2\pi\omega^{-1}[Ds(Q)+q]}, \tag{2.2}$$

where

$$\begin{aligned} s(Q) &= \max\{\lambda : \lambda \text{ is an eigenvalue of } Q\} \\ &= 2\left(\cos\frac{\pi}{n+1} - 1\right). \end{aligned}$$

The spectral theory of the symmetric matrix Q is well known; the formula for its stability modulus above can be found in [**15**].

It follows from (2.2) that

$$\begin{aligned} &\rho < 1 \quad \text{if } q < 2D\left(1 - \cos\frac{\pi}{n+1}\right) \\ &\rho > 1 \quad \text{if } p > 2D\left(1 - \cos\frac{\pi}{n+1}\right). \end{aligned} \tag{2.3}$$

In order to obtain explicit estimates p, q, we must estimate $z^*(t)$, the solution of

$$\dot{z} = \omega^{-1} DQz + \omega^{-1} DS^0(\tau)e_1.$$

Suppose we have bounds on S^0,

$$0 < S_m \leq S^0(\tau) \leq S_M, \quad \tau \in \mathbf{R}.$$

Application of a comparison argument then yields

$$z_m \leq z^*(\tau) \leq z_M,$$

where

$$\begin{aligned} Qz_m + S_m e_1 &= 0 \\ Qz_m + S_M e_1 &= 0. \end{aligned}$$

This system can be readily solved (see, e.g., [**15**]) to yield

$$\begin{aligned} (z_m)_i &= S_m \frac{n+1-i}{n+1} \\ (z_M)_i &= S_M \frac{n+1-i}{n+1}. \end{aligned}$$

Hence, we have

$$S_m \frac{1}{n+1} \leq z_i^*(\tau) \leq S_M \frac{n}{n+1}, \quad 1 \leq i \leq n,\ \tau \in R.$$

Since f_u is monotone, we get p and q as follows:

$$p = f_u\left(S_m \frac{1}{n+1}\right) \leq f_u(z_i^*(\tau)) \leq f_u\left(S_M \frac{n}{n+1}\right) = q.$$

To be specific, suppose f_u is the Michaelis-Menten-Monod function

$$f_u(S) = \frac{m_u S}{a_u + S}.$$

Then we conclude from the above estimates, together with (2.3), that

$$\begin{aligned} &\rho < 1 \text{ if } \frac{m_u}{D} < 2\left(1 - \cos\frac{\pi}{n+1}\right)\left(1 + \frac{(n+1)a_u}{nS_M}\right) \\ &\rho > 1 \text{ if } \frac{m_u}{D} > 2\left(1 - \cos\frac{\pi}{n+1}\right)\left(1 + \frac{(n+1)a_u}{S_m}\right) \end{aligned} \tag{2.4}$$

Recall that $\rho < 1$ implies that the solution $(z^*(t), 0)$ of (2.1) is globally attracting while $\rho > 1$ implies that it is unstable and that $(S^*(t), u^*(t))$ is globally attracting for nontrivial initial data.

3. Two Complementary Nutrients. In this brief section, we consider the necessary modifications of the theory of Section one in order to accommodate the possibility of two complementary nutrients S and R supplied to the vessels. See the references [**7, 8, 15, 16**] for the relevant biology. Our description follows [**15**]. Substrates R and S are assumed to be essential for growth but are metabolically independent requirements for growth. At any particular time or place, one or the other of the substrates is growth limiting. Thus, species growth rate is given by

$$g(S, R) = \min(f_S(S), f_R(R))$$

where f_R and f_S are two functions satisfying (0.2).

If we assume that R and S are supplied to some subset (not necessarily the same subset) of vessels from various reservoirs, then we obtain the system of equations (where we do not scale out the proportionality constant between nutrient uptake rate and growth rate)

$$\begin{aligned} S' &= A(t)S - y_S^{-1}G(S,R)u + S^0(t) \\ R' &= A(t)R - y_R^{-1}G(S,R)u + R^0(t) \\ u' &= A(t)u + G(S,R)u \\ S(0) &\geq 0 \\ R(0) &\geq 0 \\ u(0) &\geq 0, \end{aligned} \tag{3.1}$$

where $A(t)$ is as in Section one, $S^0(t)$ and $R^0(t)$ are nonnegative, 2π-periodic functions representing inflow of S and R, respectively, to the vessels from reservoirs, y_S^{-1}, y_R^{-1} are positive constants and $G(S, R)$ is an $n \times n$ diagonal matrix with

$$G(S, R)_{ii} = g(S_i, R_i).$$

Let

$$\begin{aligned} z &= S + y_S^{-1}u \\ w &= R + y_R^{-1}u. \end{aligned}$$

Then (3.1) is equivalent to

$$\begin{aligned} z' &= A(t)z + S^0(t) \\ w' &= A(t)w + R^0(t) \\ u' &= A(t)u + G(z - y_S^{-1}u, w - y_R^{-1}u)u \end{aligned} \tag{3.2}$$

$$z(0) \geq 0, \quad w(0) \geq 0, \quad u(0) \geq 0$$
$$u(0) \leq y_S z(0), \quad u(0) \leq y_R w(0).$$

Equation (3.2) is a cooperative system. Lemma 1.1 applies to each of the first two equations of (3.2), provided $S^0 \not\equiv 0$, $R^0 \not\equiv 0$, to yield positive, 2π-periodic solutions

$$z = z^*(t) = z^*(t + 2\pi) \gg 0$$
$$w = w^*(t) = w^*(t + 2\pi) \gg 0.$$

We may then consider the third equation of (3.2) with the above positive periodic solutions z^* and w^* replacing z and w:

$$\begin{aligned} u' &= A(t)u + G(z^*(t) - y_S^{-1}u, w^*(t) - y_R^{-1}u)u \\ 0 &\leq u_i(0) \leq \min\{y_S z_i^*(0), y_R w_i^*(0)\}. \end{aligned} \tag{3.3}$$

Since (3.2) is majorized by the two systems obtained from (1.3) by replacing f_u by f_S and f_R, respectively, it follows that any 2π-periodic solutions of (3.2) must have initial conditions satisfying the inequalities above.

Associated with (3.3) is the variational equation for the trivial solution of (3.3).

$$v' = [A(t) + G(z^*(t), w^*(t))]v. \tag{3.4}$$

The following result is similar to proposition 1.2.

PROPOSITION 3.1. *If the principal Floquet multiplier, ρ, of* (3.4) *satisfies $\rho \leq 1$, then all solutions of* (3.3) *are asymptotic to the trivial solution. If $\rho > 1$, then* (3.3) *has a unique positive 2π-periodic solution $u^*(t)$, $0 < u_i^*(t) < \min\{y_S z_i^*(t), y_R w_i^*(t)\}$, $1 \leq i \leq n$, which attracts all nontrivial solutions of* (3.3).

PROOF. The proof mirrors that of Proposition 1.2 except that Theorem 2.3 of [**11**] must be used instead of Theorem 2.1 since $g(S,R)$ is not necessarily a continuously differentiable function. That is, one shows that the Poincaré map is strongly concave.

It should be remarked that (3.4) is majorized by the two systems obtained from (1.6) by replacing f_u with f_S and f_R, respectively, so that ρ of Proposition 3.1 is smaller than the ρ which would result from the majorizing systems. Biologically, U cannot survive in the gradostat with complementary nutrients unless it can survive in the gradostat where R *alone* is limiting and in the gradostat where S *alone* is limiting.

We can now state the main result of this section which is the analog of Theorem 1.3.

THEOREM 3.2. *If the principal Floquet multiplier,* ρ, *of* (3.4) *satisfies* $\rho \leq 1$, *then every solution of* (3.1) *is attracted to the* 2π-*periodic solution* $(S,R,u) = (z^*(t), w^*(t), 0)$. *If* $\rho > 1$, *then all nontrivial solutions of* (3.1) *are attracted to the unique positive* 2π-*periodic solution* $(S,R,u) = (S^*(t), R^*(t), u^*(t))$, *where* $u^*(t)$ *is as in Proposition* 3.1 *and* $z^*(t) = S^*(t) + y_S^{-1}u^*(t)$, $w^*(t) = R^*(t) + y_R^{-1}u^*(t)$ *for all* t.

The proof is similar to that of Theorem 1.3 and is omitted.

Finally, we note that immigration terms, as in Remark 1 of Section one, can be included.

APPENDIX

In this appendix we prove Lemma 1.0. We first observe

PROPOSITION . *If* $A(t)$ *satisfies the hypotheses of Lemma* 1.0, *then* (1.2) *cannot have a* 2π-*periodic solution* $x(t)$ *satisfying* $x(t) \gg 0$ *for all* t.

PROOF. If the result were false, then we may suppose that (1.2) has a positive, 2π-periodic solution $x(t)$ satisfying $\max_i \max_t x_i(t) = 1$ and $x_{i_0}(t_0) = 1$ for some i_0 and t_0. Let $I = \{i : x_i(t_0) = 1\}$. If $i \in I$ then ${x_i}'(t_0) = 0$ so that

$$0 = \sum_{j \in I} A_{ij}(t_0) + \sum_{j \in N-I} A_{ij}(t_0) x_j(t_0)$$

where $N = \{1, 2, \dots, n\}$ and $N - I$ is the complement of I in N. If $I = N$, then we have

$$0 = \sum_{j=1}^{n} A_{ij}(t_0), \quad 1 \leq i \leq n,$$

which contradicts the hypotheses of Lemma 1.0. Since $0 < x_j(t_0) < 1$ for all $j \in N - I$, we must have $A_{ij}(t_0) = 0$ for all $j \in N - I$, or else

$$0 = \sum_{j \in I} A_{ij}(t_0) + \sum_{j \in N-I} A_{ij}(t_0) x_j(t_0) < \sum_{j=1}^{n} A_{ij}(t_0) \leq 0,$$

which is a contradiction. Hence, $A_{ij}(t_0) = 0$ for all $i \in I$ and $j \in N - I$. Since I is a proper subset of N, this contradicts the irreducibility of $A(t_0)$. The proof is complete. □

Observe that Lemma 1.0 holds for constant matrices A satisfying the hypotheses. For, $s(A) = \max\{\text{Re } \lambda : \lambda \text{ an eigenvalue of } A\}$ satisfies $s(A) \leq 0$ by the well known Gerschgorin's circle theorem. Since $s(A)$ is a simple eigenvalue of A with a corresponding positive eigenvector [**1**], $s(A) = 0$ gives a contradiction to the above proposition.

Now consider the general case of the lemma. Define $\bar{A} = (1/2\pi) \int_0^{2\pi} A(s)\, ds$, and observe that $\bar{A}$ satisfies the hypotheses of the lemma, as does $A(t, s) \equiv s\bar{A} + (1 - s)A(t)$, $0 \leq s \leq 1$, $t \in R$. Let $\rho(s)$, $0 \leq s \leq 1$, be the principal Floquet multiplier of $x' = A(t, s)x$. Then $\rho(1) = e^{2\pi s(\bar{A})} < 1$. Since $A(t, s)$ is smooth in s and $\rho(s)$ is a simple eigenvalue of the Poincaré map associated with $x' = A(t, s)x$, $\rho(s)$ is smooth in s. If $\rho(s) < 1$ does not hold for all $s \in [0, 1]$, then $\rho(s_0) = 1$ for some $s_0 \in [0, 1)$. But, then $x' = A(t, s_0)x$ has a positive 2π-periodic solution, in violation of the proposition. □

The example

$$x' = \begin{pmatrix} -1 & 1 \\ 1 & -1 \end{pmatrix} x$$

shows the necessity of the added hypothesis in Lemma 1.0.

The irreducibility assumption of Lemma 1.0 can be weakened at the expense of a slight strengthening of the strict inequality condition of the lemma. We indicate very briefly this extension of Lemma 1.0. Suppose that there exists a permutation of the standard basis vectors so that the matrix $A(t)$ takes the following triangular form *for every* $t \in R$:

$$\bar{A}(t) = \begin{bmatrix} B^{11}(t) & 0 & 0 & \cdot & \cdot & \cdot & 0 \\ B^{21}(t) & B^{22}(t) & 0 & & & & \cdot \\ \cdot & & & \cdot & & & \cdot \\ \cdot & & & & \cdot & & \cdot \\ \cdot & & & & & \cdot & 0 \\ B^{p1}(t) & & \cdot & \cdot & \cdot & & B^{pp}(t) \end{bmatrix},$$

where the $B^{ll}(t)$, $1 \le l \le p$, are square matrices of size $n_l \times n_l$ and, if $n_l > 1$, are irreducible for each $t \in \mathbf{R}$. Observe that this assumption is stronger than simply dropping the irreducibility assumption since it requires that the permutation which puts $A(t)$ in canonical reducible form $\bar{A}(t)$, also puts $A(s)$ in canonical reducible form, $\bar{A}(s)$ (see [**1**]). As before, we assume that $A_{ij}(t) \ge 0$, $i \ne j$, and that each system $y_l' = B^{ll}(t)y_l$, $1 \le l \le p$, satisfies the hypotheses of Lemma 1.0. Then the conclusion of Lemma 1.0 holds.

The proof follows almost immediately from Lemma 1.0 and the fact that the principal matrix solution $\Phi(t)$ of $x' = \bar{A}(t)x$ at $t = 0$ is a nonnegative matrix which can be expressed in canonical reducible form with the same structure as $\bar{A}(t)$ with diagonal matrices $\Phi^{ll}(t)$ which are the principal matrix solutions of $y_l' = B^{ll}(t)y_l$ at $t = 0$. By Lemma 1.0, ρ_l, the principal Floquet multiplier of this last equation satisfies $\rho_l < 1$. But it is known (see [**1**]) that $\rho = \rho_l$ for some l where ρ is the spectral radius of $\Phi(2\pi)$, the largest Floquet multiplier. Hence, $\rho < 1$ as asserted.

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Department of Mathematics, Arizona State University, Tempe, AZ 85287