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The Growth of Competing Microbial Populations in a CSTR With Periodically Varying Inputs

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The operation of a periodically forced chemostat (CSTR) in which two microbial populations compete for the same nutrient has been examined. Easily implemented criteria for the stability of the resulting cycles have been obtained, using the Floquet stability theory. After examining several possibilities it was found that stable periodic trajectories of coexistence can be achieved: (a) when the dilution rate of the chemostat is properly varied in a periodic manner between two values so chosen that the growth of one population is favored by the first and the growth of the other population is favored by the second, (b) when a certain percentage of biomass and growing medium is harvested periodically from the chemostat, and (c) when both the dilution rate and the concentration of the substrate in the feed are varied simultaneously and in a periodic manner.

SCOPE

Both experiments and theories of growth show that competition of two populations for a single limiting nutrient leads to extinction of one of the populations, if they are grown in a spatially uniform environment that is subject to time-invariant external influences. This statement, or rather a less cautiously worded version of it, is known as the "competitive exclusion principle," (Hardin 1960). Its validity and applicability have been the subjects of much discussion among researchers.

Some recent theoretical work based on models that give good description of the growth of microbial populations shows that the restricted version of the competitive exclusion principle given above must be modified if more than one nutrient is limiting. Thus, Taylor and Williams (1975) suggest that *n* competing populations can coexist with each other if a set of at least *n* nutrients is limiting. Similarly, Schuelke (1976) shows that two populations could coexist if the sets of limiting nutrients for the two populations overlapped only partially. In spite of these developments, it remains true that reduction of system diversity is the usual outcome of competition in spatially uniform systems subject to time-invariant influences.

Several schemes for sustaining microbial competitors in the same environment have appeared in the literature because of the importance of competitive interaction in practical applications involving microbial activities. One of the most common is that external influences are not time invariant but that they vary so as to provide an environment in which the competitive advantage alternates between the two populations. Unfortunately, all the above schemes have been based on non-mathematical arguments and there is no assurance that a proposed scheme of time varying external influences will, in fact, allow competitors to coexist.

This study examined the effect of periodically varying inputs to coexisting competing microbial populations which grow in a continuous, well stirred vessel, usually referred to as chemostat. Criteria for the stability of the proposed cycles and examination of the effect of some operating parameters on their stability characteristics are based on Floquet stability theory. The results can be useful in establishing patterns of operation of a CSTR which can sustain the coexistence of microbial competitors and also in providing useful information about their coexistence in natural ecosystems.

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CONCLUSIONS AND SIGNIFICANCE

Several types of periodic operations were examined. It was found that the cycling of (a) the concentration of the substrate in the feed and (b) the dilution rate between two values (one of which is very large) resulting in washout of both populations, cannot give stable cycles. On the contrary, (c) the cycling of the dilution rate between two values, each one of which favors the growth of a different population, (d) the periodic harvesting of a certain amount of biomass and growing medium and refilling of the CSTR with fresh medium and (e) the simultaneous cycling of the dilution rate and the concentration of the substrate in the feed, *can* give stable periodic trajectories of coexistence.

A parametric study, limited to cases (c) and (e), indicated that the limits of the range of variation of the operating parameters are somehow restrictive in these two cases. For case (d), however, large ranges for the values of the period and the harvesting number exist, which can secure a safe operation for the chemostat under widely varied operating conditions.

The important conclusion to be drawn from this research is that periodically varying external inputs in a chemostat can, indeed, stabilize two microbial competitors in coexistence. Thus, their activities can be utilized by growing them in the same chemostat under conditions properly varying with time.

Microbial competition is a subject that has received considerable attention, (Aris and Humphrey 1977, Yoon et al. 1977, Defreitas and Fredrickson 1978, Taylor and Williams 1975, Jost et al. 1973, Magee et al. 1972). Besides its obvious ecological importance, competition for a common nutrient occurs in important industrial operations such as waste water treatment, and it is likely to be a major feature of other processes which utilize the activities of mixed microbial populations. Clearly, a necessary condition for such utilization is the ability of competitors to coexist in the same environment.

It is generally conceded, however, that two populations which compete with one another cannot coexist in spatially homogeneous surroundings which are subject to time-invariant external influences. This nonexistence of a coexistence steady state has been amply demonstrated both on microbial and nonmicrobial competitors (Gause 1934, Vandermeer 1969, Magee et al. 1972, Jost et al. 1973, Jannasch 1967, Meers 1971, Harder and Veldkamp 1971). However, it is commonly observed that populations which compete with one another do coexist in natural ecosystems; this apparent contradiction to the competitive exclusion principle has been called the paradox of the plankton by Hutchinson (1961). The paradox may be resolved in various ways, but the most common are that 1) the environment is not spatially homogeneous or 2) external circumstances influencing it are not time-invariant. In such a case, one population here or now has the competitive advantage, whereas the other population there or then has this advantage.

In another work (Stephanopoulos and Fredrickson, in press), we examined the effect of spatial inhomogeneity (modeled by two chemostats interconnected in various ways, and with different growth conditions prevailing in each), and showed that it can, indeed, stabilize the competitive coexistence state. Here we turn to the question of whether it is possible to stabilize coexistence of competitors by varying some of the inputs to a growth vessel in a periodic way. Periodicity is chosen as the pattern of time variation since it is a significant characteristic of many natural operations. An affirmative answer to the above question will offer substantial ground for the second resolution of the paradox mentioned above. At the same time, it will provide an easily implemented scheme for sustaining competing populations in the same chemostat.

DESCRIPTION OF THE SYSTEM

Consider two populations of microorganisms, X_1 and X_2, growing in a chemostat and utilizing for their growth a common substrate S for which they compete. The equations which describe this interaction have been presented elsewhere (Powell 1958, Fredrickson and Tsuchiya 1977, Aris and Humphrey 1977, Bailey and Ollis 1977). For the sake of continuity we state them here again:

$$V\dot{x}_1 = -qx_1 + Vx_1\mu_1(s) \qquad \cdot \equiv d/dt' \tag{1}$$

$$V\dot{x}_2 = -qx_2 + Vx_2\mu_2(s) \tag{2}$$

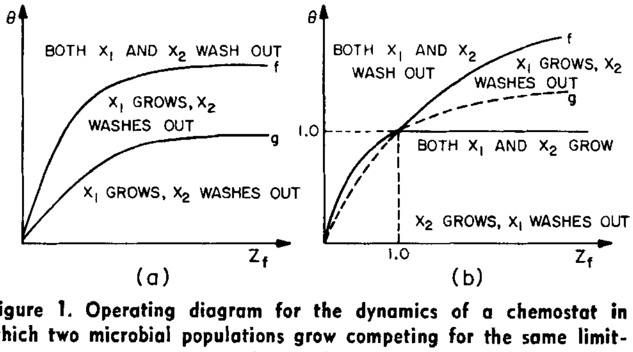
$$V\dot{s} = q(s_f - s) - V\left\{\frac{1}{Y_1}x_1\mu_1(s) + \frac{1}{Y_2}x_2\mu_2(s)\right\} \tag{3}$$

where x_1, x_2 and s are the concentrations of the two populations X_1 and X_2 and the substrate S, respectively, V the volume of the chemostat, q the flow rate, s_f the concentration of the substrate in the feed, Y_1 and Y_2 the yield coefficients, (amounts of biomass of X_1 and X_2 respectively produced per unit of substrate consumed), and μ_1, μ_2 the specific growth rates of X_1 and X_2 respectively.

The Monod model (Monod 1942) for uninhibited growth is used to describe specific growth rates as functions of s:

$$\mu_i(s) = \frac{\mu_{mi}\, s}{K_{si} + s}, \quad i = 1, 2 \tag{4}$$

with μ_{mi} the maximum specific growth rates and K_{si} the Michaelis constants.



(a) (b)

Figure 1. Operating diagram for the dynamics of a chemostat in which two microbial populations grow competing for the same limiting nutrient.

The two specific growth rates μ_1 and μ_2 can either have no common point other than the origin or they can intersect at some point (s_c, μ_c) of the positive quadrant of the (s, μ) plane. These two possibilities are shown schematically in Figure 1. The mutual disposition of the two curves, μ_1 and μ_2, is important to the final outcome of competition, as it will become clear shortly after the results of the stability analysis have been presented.

In making Equations (1) through (3) dimensionless, one needs a characteristic time and a characteristic concentration. If the two specific growth rates intersect at a point (s_c, μ_c), the inverse of the common value of the specific growth rates, $(1/\mu_c)$, can be used for the former, and the substrate concentration at which the two specific growth rates are equal (s_c), can be used for the latter. If μ_1 and μ_2 do not intersect, but are as shown in Figure 1a, the inverse of the maximum specific growth rate of the population that grows faster and the corresponding Michaelis constant can be used as scaling parameters. Since no periodic variation or spatial inhomogeneity can stabilize two competitors in coexistence if one has the competitive advantage over the other for all values of the substrate concentration, we will assume that the two specific growth rates intersect at a point different than the origin.

Using the coordinates of the common point (s_c, μ_c) as scaling constants, we make Equations (1), (2), and (3) dimensionless, using the substitution:

$$x = x_1/Y_1 s_c, \quad y = x_2/Y_2 s_c, \quad z = s/s_c, \quad z_f = s_f/s_c,$$
$$t = \mu_c t', \quad \theta = q/V\mu_c, \quad f(z) = \mu_1(z s_c)/\mu_c,$$
$$g(z) = \mu_2(z s_c)/\mu_c \qquad (5)$$

In Equation (5), x, y, and z are the dimensionless concentrations of populations X_1 and X_2 and substrate S, respectively, f and g the dimensionless specific growth rates of X_1 and X_2, respectively, and θ is the dimensionless dilution rate $(\theta = D/\mu_c, D = q/V)$.

With the previous choice of characteristic variables, f and g will intersect at $z = 1$, and their common value will be equal to 1 in dimensionless variables. Using the Monod model, f and g can be written as:

$$f(z) = a_1 z/(b_1 + z), \quad g(z) = a_2 z/(b_2 + z)$$

with $a_i = \mu_{mi}/\mu_c$ and $b_i = K_{si}/s_c$, $i = 1, 2$.

By the particular choice of μ_c and s_c, we have made $a_i = 1 + b_i$. It should be pointed out that the choice of Monod model is of no particular importance, and any other model with an increasing convex relationship between μ and s gives similar results. Nor does including inhibition for large s alter the situation.

Using Equation (5), then Equations (1), (2), and (3) become:

$$\dot{x} = x[f(z) - \theta] \qquad \cdot \equiv \frac{d}{dt} \qquad (6)$$

$$\dot{y} = y[g(z) - \theta] \qquad (7)$$

$$\dot{z} = \theta(z_f - z) - x f(z) - y g(z) \qquad (8)$$

with $0 \leq x, y, z \leq z_f$.

Arguments similar to those of Aris and Humphrey (1977) can reduce the dimensions of the system from three to two and the differential Equation (8) can be replaced by the steady state relation:

$$z = z_f - x - y. \qquad (9)$$

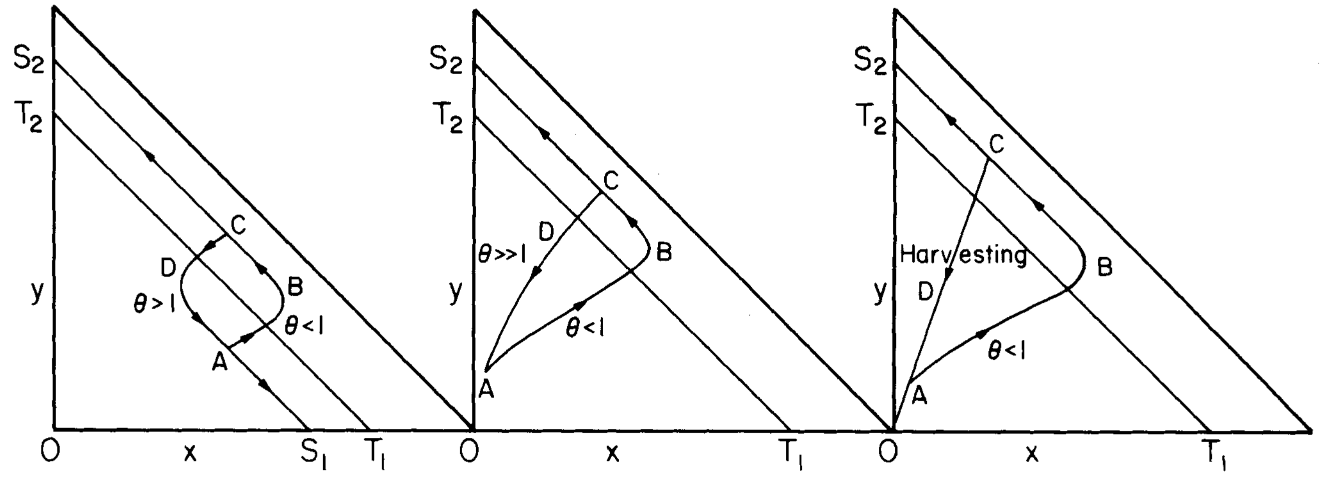
In this way, instead of trajectories in the positive octant of the (x, y, z) space, we consider their projections on the triangular domain:

$$0 \leq x, y \leq z_f \quad \text{and} \quad 0 \leq x + y \leq z_f. \qquad (9a)$$

No justification is needed for the above substitution if the state of the system lies initially on the plane defined by (9). This can be achieved by filling up the chemostat initially with fresh substrate and seeding it with the two organisms in negligible concentrations. Further arguments are provided by Aris and Humphrey (1977) and Asbjørnsen and Fjeld (1970) for the general case. These arguments derive from the fact that only the independent reactions need be considered in examining the dynamics of a CSTR. In our case there are two reactions, namely those which transform the substrate into the biomass of X_1 and X_2.

It should be pointed out that the above reduction in dimensions is valid only when z_f is not varied during the operation. If z_f is changed Equations (6), (7), and (8) must be used.

There are four steady states for the system of Equations (6), (7), and (8) or (9): The washout of both populations, the washout of either one of them, and the coexistence steady state. Results of the stability analysis



(a) (b) (c)

Figure 2. Schematic representation of the periodic trajectories obtained by cycling the dilution rate, (a, b), and by periodic harvesting, (c).

(see Powell 1958, Fredrickson and Tsuchiya 1974, and Aris and Humphrey 1977) for these steady states are summarized in Figure 1. The effect of the dilution rate D ($D = \theta \mu_c$) on the stability characteristics of the system can be seen in Figure 1. For the case of intersecting growth rates (Figure 1b), for $\theta < \min[1.0, g(z_f)]$, population X_2 grows and X_1 washes out. For $1.0 < \theta < f(z_f)$, population X_2 washes out and population X_1 grows. Also, large dilution rates will result in washout of both populations while coexistence is possible only for one particular value of $D = \mu_c$ (corresponding to $\theta = 1$). The values of x and y at the coexistence steady state are not uniquely determined, but any point on the line

$$x + y = z_f - 1 \tag{10}$$

satisfies the steady state version of Equations (6), (7), and (8) or (9). Then, since the dilution rate is not free of disturbances, the coexistence steady state will drift randomly among the line (10), following random fluctuations of the dilution rate about the value μ_c (or θ about the value 1), until the washout of one of the populations is reached.

The periodic variation of the dilution rate, or the concentration of the substrate in the feed, or both, will now be employed to provide an environment where the competitive advantage alternates between population X_1 and X_2. Various possibilities are listed below and are schematically presented in Figure 2. They all derive, and numerous others can be constructed, from the basic features of the phase plane trajectories associated with Equations (6) to (9) and the stability characteristics presented in Figure 1.

a. Periodic variation of the dimensionless dilution rate θ between two values, one smaller and one larger than unity. Thus, if the cycle starts at point A (Figure 2a), with $\theta < 1$, washout of X_1 is the stable steady state, and the state of the system follows the trajectory ABC, directing itself toward S_2, the stable steady state for $\theta < 1$. If at point C the dilution rate is switched to a value larger than unity, the new stable steady state will be S_1, i.e. the washout of X_2. The trajectory CDA is followed then until point A is reached, at which the dilution rate is switched to the previous value and the cycle is repeated.

b. Periodic variation of the dimensionless dilution rate between a value which is smaller than unity (trajectory ABC, Figure 2b) and a value which is much larger than unity, so that washout of both populations is the stable steady state for this dilution rate, (trajectory CDA, Figure 2b). Again, a rectangular wave is applied.

c. For periodic harvesting, the dilution rate is set to a constant value which must be smaller than D_c or $\theta < 1$, Figure 2c, but a certain percentage of culture is harvested away from the chemostat at periodic intervals of time. The chemostat is filled up with fresh medium after this harvesting and allowed to operate at the previous dilution rate for another period. Harvesting is represented by the straight line, CDA0, and brings the state of the system to the same point as at the start of the cycle.

d. Periodic variation of z_f at a constant dilution rate between a small value, for which both populations wash out, and a large one, for which one population grows and the other washes out—depending on the particular value of the dilution rate used. The situation is similar to the one of Figure 2b. Notice that Equations (6), (7), and (8) must be used to describe the dynamics in this case.

e. Simultaneous periodic variation of both the dilution rate and the concentration of the substrate in the feed will be discussed later.

METHOD OF ANALYSIS

The construction of the previous cycles raises some questions regarding their stability with respect to various kinds of perturbations. Clearly, a cycle similar to the one in Figure 2a can exist, if the growth characteristics of the two populations and the operating parameters of the system are such that the two parts of the cycle exactly balance each other. This is a condition very difficult to meet, and in any case, subject to many external disturbances and random fluctuations of the operating parameters. One then would like to know what will happen if, for some reason, one part of the cycle is delayed relative to the other during one period, or what would happen if one of the operating parameters assumes a value different than the one for which the previous balancing was achieved. Is the cycle going to drift away from its position? If so, will it stabilize itself in another position, or will it drift to extinction of one of the species?

Some of these questions can be answered for the case of simple cycles using arguments from the phase plane analysis. Our ability, however, to treat these questions qualitatively is weakened 1) if more complicated cycles are under examination, (as the simultaneous cycling of both the dilution rate and the concentration of the substrate in the feed), or 2) more complex types of periodic variations are applied (i.e., sinusoidal instead of rectangular waves). A method of analysis to answer questions pertaining to the stability of periodic cycles is clearly needed and such a method is provided by the Floquet stability theory.

A general discussion of Floquet's stability theory can be found in standard references for ordinary differential equations (see, for example, Cesari 1963, Ince 1956, or Coddington and Levinson 1955). Some useful theorems and techniques for numerical computations of nonlinear forced oscillations have been obtained by Urabe (1966) and Krasnoselskii (1970); and Sinčić and Bailey (1977) have applied the theory in analyzing the forced operation of a continuous flow, well stirred, chemical reactor.

For our purpose the theory can be summarized as follows: Suppose that the dynamics of a process can be described by the nonlinear vector equation:

$$\frac{d\mathbf{w}(t)}{dt} = \mathbf{h}(\mathbf{w}(t),\ \mathbf{u}(t)) \tag{11}$$

where $\mathbf{w}(t)$ is a vector describing the state of the system and $\mathbf{u}(t)$ a vector describing the periodic inputs to the system, (θ and z_f for the chemostat). Here, $\mathbf{u}(t)$ is a τ-periodic function:

$$\mathbf{u}(t + \tau) = \mathbf{u}(t). \tag{12}$$

Let $\mathbf{w}^*(t)$ denote a forced τ-periodic solution of Equation (11) corresponding to τ-periodic input $\mathbf{u}^*(t)$. The Jacobian matrix along the periodic solution is denoted by $\underset{\sim}{\mathrm{J}}(t)$:

$$\underset{\sim}{\mathrm{J}}(t) = \frac{\partial \mathbf{h}(\mathbf{w}^*(t),\ \mathbf{u}^*(t))}{\partial \mathbf{w}}. \tag{13}$$

Then, according to Floquet theory, the periodic solution will be stable with regard to small perturbations if all the eigenvalues of $\underset{\sim}{\mathrm{P}}(\tau)$ have moduli smaller than unity, where the matrix $\underset{\sim}{\mathrm{P}}(\tau)$ is the solution to the initial value problem:

$$\frac{d\underset{\sim}{\mathrm{P}}(t)}{dt} = \underset{\sim}{\mathrm{J}}(t)\underset{\sim}{\mathrm{P}}(t) \tag{14a}$$

$$\underset{\sim}{\mathrm{P}}(0) = \underset{\sim}{\mathrm{I}} \tag{14b}$$

The procedure for checking the stability of the previously described cycles is straightforward, if the position of the cycle is known. This, however, is difficult if the cycle is unstable, or time consuming to obtain, if it is stable. Nevertheless, some simple criteria can be obtained from the above theory for the possibility of coexistence of microbial competitors along a periodic trajectory. The procedure for deriving these criteria is described below.

It is assumed that the periodic variation of the operating parameter (inputs) of the system will make the state of the system vary also. This periodic trajectory of the state will lie either in the interior of the triangular domain (9*a*) or on one of the boundaries $x = 0$, $y > 0$ and $x > 0$, $y = 0$. Our approach then in studying the possibility of a stable periodic trajectory of coexistence consists of examining the stability of the periodic trajectories which lie on the two boundaries of the above domain. These can be determined analytically and criteria can be obtained for their stability by applying the Floquet theory. Under conditions for which the cycles on both of the above boundaries are unstable, the only remaining possibility is that the state of the system will approach a stable cycle in the interior of the domain along which both populations coexist.

Considering a periodic variation of the dilution rate of the form of a rectangular wave:

$$\theta(t) = \theta_1 \qquad 0 \leqq t \leqq \kappa\tau, \quad 0 \leqq \kappa \leqq 1 \tag{15a}$$

$$\theta(t) = \theta_2 \qquad \kappa\tau \leqq t \leqq \tau \tag{15b}$$

and an initial condition lying on the axis $x = 0$, $y > 0$, namely $x(0) = 0$ and $y(0) = y_0$, Equations (6), (7), and (9) can be integrated to give:

$$\kappa\tau = \left(\frac{1}{\beta_1} - \frac{\gamma_1}{\alpha_1}\right) \log \frac{\alpha_1 - \beta_1 y_1}{\alpha_1 - \beta_1 y_0} + \frac{\gamma_1}{\alpha_1} \log \frac{y_1}{y_0} \tag{16a}$$

$$(1 - \kappa)\tau = \left(\frac{1}{\beta_2} - \frac{\gamma_2}{\alpha_2}\right) \log \frac{\alpha_2 - \beta_2 y_2}{\alpha_2 - \beta_2 y_1} + \frac{\gamma_2}{\alpha_2} \log \frac{y_2}{y_1} \tag{16b}$$

where

$$\alpha_i = a_2 z_f - b_2\theta_i - z_f\theta_i, \quad \beta_i = a_2 - \theta_i, \quad \gamma_i = b_2 + z_f \tag{17}$$

In Equations (16*a*, 16*b*), y_1 is the value of y at $t = \kappa\tau$ and y_2 the value of y at the end of a period. For a periodic solution we have $y_0 = y_2$ and Equations (16*a*, 16*b*) can be combined to give:

$$\tau = \left(\frac{1}{\beta_1} - \frac{\gamma_1}{\alpha_1}\right) \log \frac{\alpha_1 - \beta_1 y_1}{\alpha_1 - \beta_1 y_0} + \left(\frac{1}{\beta_2} - \frac{\gamma_2}{\alpha_2}\right) \cdot \log \frac{\alpha_2 - \beta_2 y_0}{\alpha_2 - \alpha_2 y_1} + \left(\frac{\gamma_1}{\alpha_1} - \frac{\gamma_2}{\alpha_2}\right) \log \frac{y_1}{y_0} \tag{18a}$$

or, after introducing the expressions for α_i, β_i and γ_i given by (17):

$$\tau = \left[\frac{1}{a_2 - \theta_1} - \frac{1}{g(z_f) - \theta_1}\right] \log \frac{y_{s1} - y_1}{y_{s1} - y_0} + \left[\frac{1}{a_2 - \theta_2} - \frac{1}{g(z_f) - \theta_2}\right] \log \frac{y_{s2} - y_0}{y_{s2} - y_0} + \left[\frac{1}{g(z_f) - \theta_1} - \frac{1}{g(z_f) - \theta_2}\right] \log \frac{y_1}{y_0} \tag{18b}$$

where y_{s1} and y_{s2} are the steady state biomass concentrations of population X_2 growing in a chemostat under dilution rates equal to θ_1 and θ_2, respectively.

We can simplify Equation (18*b*) by using the following arguments based on the order of magnitude of the variables involved in the above equation. The Michaelis constants of the growth rates of most microbial populations are of the order of magnitude of a few mg/L. The characteristic substrate concentration, s_c, then will also be of the same order of magnitude and, for a typical feed concentration of a few g/L, z_f will be of the order of thousands. Therefore, since the value of the substrate concentration stays close to the value of unity during a period it can be seen from Equation (9) that the values of the biomass concentration y will stay close to the value of z_f during this period so that the third term in Equation (18*b*) can be neglected. Also, since $g(z) \rightarrow a_2$ as $z \rightarrow \infty$, for a large value of z_f, the terms in the first two brackets of Equation (18*b*) are very small. Consequently, the value of biomass concentration at the end of the first phase of the period, during which $\theta = \theta_1$, must be very close to the steady state of y, y_{s1}, that corresponds to θ_1. Similarly, y_0 must be very close to y_{s2} if Equation (18*b*) is to be satisfied for a value of τ applicable in usual chemostat operation. The above arguments are useful in determining the periodic trajectory that corresponds to a cycling of the dilution rate described by Equations (15*a*), (15*b*). Similar results can be obtained for the cycling of the feed concentration alone or together with the cycling of the dilution rate.

For a given period τ, initial condition y_0 and θ_1, θ_2 values between which the dilution rate oscillates, Equation (18*b*) can be employed to give the value of the biomass concentration, y_1, at the end of the first part of the period during which $\theta = \theta_1$. From Equation (16*a*), then, the duration of this part can be determined. For a given pattern of periodic oscillation of the dilution rate, (15*a*, 15*b*), Equations (16*a*, 16*b*) can be solved simultaneously for y_0 and y_1, ($y_2 = y_0$), to determine the end points of the cycle that corresponds to the periodic input of Equations (15*a*, 15*b*). A solution always exists and the previously described arguments can be used to facilitate a successive approximation scheme for solving for y_0 and y_1. The exact location of the part of the cycle obtained for $\theta = \theta_1$ is determined from

$$t = \left(\frac{1}{\beta_1} - \frac{\gamma_1}{\alpha_1}\right) \log \frac{\alpha_1 - \beta_1 y}{\alpha_1 - \beta_1 y_0} + \frac{\gamma_1}{\alpha_1} \log \frac{y}{y_0} \tag{19}$$

An analogous equation describes the part of the cycle obtained for $\theta = \theta_2$. The initial assumption, then, regarding the existence of periodic trajectories when the system is cycled periodically, can be relaxed to exclude the case of periodic trajectories lying on the boundaries. This is the case because the mere construction of the latter according to the previously outlined procedure can be considered as proof of their existence.

Next we examine the stability of a cycle lying on the axis $x = 0$, $y > 0$. The Jacobian matrix is:

$$\underset{\sim}{J}(t) = \begin{bmatrix} f(z^*) - \theta^* & 0 \\ -y^* g'(z^*) & g(z^*) - \theta^* - y^* g'(z^*) \end{bmatrix} \tag{20}$$

where superscript * indicates that the corresponding quantities are evaluated along the periodic trajectory. If $p_{ij}(t)$

are the elements of the matrix $\underset{\sim}{\mathbf{P}}(t)$ of Equation (14*a*, 14*b*), we can write for this case:

$$\frac{dp_{11}(t)}{dt} = [f(z^*) - \theta^*]\,p_{11}(t); \quad p_{11}(0) = 1 \quad (21a)$$

$$\frac{dp_{12}(t)}{dt} = [f(z^*) - \theta^*]\,p_{12}(t); \quad p_{12}(0) = 0 \quad (21b)$$

$$\frac{dp_{21}(t)}{dt} = -\,y^*g'(z^*)\,p_{11}(t) + [g(z^*) - \theta^* - y^*g'(z^*)]\,p_{21}(t); \quad p_{21}(0) = 0 \quad (21c)$$

$$\frac{dp_{22}(t)}{dt} = -\,y^*g'(z^*)\,p_{12}(t) + [g(z^*) - \theta^* - y^*g'(z^*)]\,p_{22}(t); \quad p_{22}(0) = 1 \quad (21d)$$

Equation (21*b*) gives $p_{12}(t) = 0$ for $t \geq 0$. The eigenvalues then of matrix $\underset{\sim}{\mathbf{P}}(\tau)$ are its two diagonal elements, $p_{11}(\tau)$ and $p_{22}(\tau)$. From Equations (21*a*) and (21*d*) we obtain:

$$p_{11}(\tau) = \exp\left[\int_0^\tau [f(z^*) - \theta^*]dt\right] \quad (22a)$$

$$p_{22}(\tau) = \exp\left[\int_0^\tau [g(z^*) - \theta^* - y^*g'(z^*)]dt\right] \quad (22b)$$

and such a cycle will be unstable if at least one of the $p_{11}(\tau)$, $p_{22}(\tau)$ is larger than one.

For a periodic trajectory lying on the axis $x = 0$, $y > 0$:

$$\int_0^\tau [g(z^*) - \theta^*]dt = 0 \quad (23)$$

so that $p_{22}(\tau) < 0$. The condition, then, that the above periodic trajectory be unstable is:

$$\int_0^\tau [f(z^*) - \theta^*]dt > 0. \quad (24)$$

The procedure for checking the stability of the above cycle would be to determine the location of the cycle by using Equation (19) and then to examine the stability criterion (24). A similar criterion

$$\int_0^\tau [g(z^*) - \theta^*]dt > 0 \quad (25)$$

can be employed for the stability of a periodic trajectory on the axis $x > 0$, $y = 0$.

Although simple and quite straightforward, the procedure suggested by Equation (19) and inequalities (24) and (25) requires rather involved programming and considerable amount of time. Another possibility would be to examine Equations (6), (7), and (9) for small values of x. Let $y^*(t)$ be the periodic trajectory obtained for a periodic variation of the dilution rate described by Equations (15*a*, 15*b*) and initial conditions $x^*(0) = 0$ and $y^*(0) = y_0^*$ and $[x(t), y(t)]$ the coordinates of a trajectory obtained with the same variation of the dilution rate and initial conditions $x(0) = x_0$, $y(0) = y_0^*$ with x_0 small. By linearizing Equations (6) and (7) about the steady states $(0, y_{s1})$ and $(0, y_{s2})$ for $\theta = \theta_1$ and $\theta = \theta_2$ respectively, the transients for $x(t)$ and $y(t)$ can be obtained and comparing them to $y^*(t)$ it is easy to show that

$$x(t) + y(t) > y^*(t) \quad (26)$$

for $0 \leq t \leq \tau$. From Equation (9) we see that (26) implies $z(t) < z^*(t)$ for $0 \leq t \leq \tau$ so that $f(z) < f(z^*)$ during the entire period. Inequality (24) then will certainly be satisfied if

$$\int_0^\tau [f(z) - \theta^*]dt > 0 \quad (27)$$

which is equivalent to

$$x(\tau) > x_0. \quad (28)$$

Similarly, inequality (25) will be satisfied if, for small y:

$$y(\tau) > y_0. \quad (29)$$

Inequalities (28) and (29) suggest another procedure for establishing the instability of the periodic trajectories which lie on the two axes of the triangular domain (9*a*). For a given periodic variation of the dilution rate, Equations (16*a*, 16*b*) are used to determine the end points y_0^* and y_1^* of the periodic trajectory $[0, y^*(t)]$. With y_0^* and a small value for x as initial conditions, Equations (6), (7), and (9) are integrated for one period using one of the standard Runge-Kutta routines and the value of x at the end of the period is compared to the initial value of x. If inequality (28) is satisfied the cycle lying on the axis $x = 0$, $y > 0$ will be unstable.

Because of the introduction of inequality (26), the above is a sufficiency criterion only. However, the two sides of inequality (26) differ by a factor which tends to zero as $\exp(-\,y_{si}\,t)$, so that $z(t) \doteq z^*(t)$ during most of the period. Therefore whenever inequality (28) is not satisfied, this is a strong indication that the cycle on the axis $x = 0$, $y > 0$ is stable. In fact, this was observed in the numerical computations described in the following section.

Our discussion so far has centered around cycling patterns of the dilution rate of the type described by Equations (15*a*, 15*b*). Analogous procedure can be followed in examining the periodic harvesting or the periodic variation of the feed concentration. For more complex types of periodic variation, analytical solutions similar to Equations (16), (18), or (19) may not be available and numerical integration may be needed to determine the periodic trajectories $[0, y^*(t)]$ and $[x^*(t), 0]$. Also, if the feed concentration z_f is varied during the cycle, Equations (6), (7), and (8) must be used and the system now is three dimensional. Inequalities (24) and (25) or (28) and (29) still provide sufficiency conditions for instability, although now the determination of the periodic trajectory is a more difficult task.

Although the theory of nonlinear forced oscillations is sufficiently developed to provide conditions for the existence of τ-periodic forced oscillatory states for cycling at very low or very high frequencies, it is much less developed for cycling at intermediate frequency. Since a proof for the existence of τ-periodic trajectories in the interior of the triangular domain is not provided, it should be pointed out that satisfaction of the inequalities (24) and (25) or (28) and (29) only assures that none of the populations becomes extinct. It does not necessarily imply that a stable periodic trajectory of coexistence will be approached by the state of the system, although the latter was verified by all the numerical simulations performed for this system of microbial competitors.

RESULTS AND DISCUSSION

Using the method presented in the previous section, various cycles were analyzed for the competition interaction between *E. coli* and *Spirillum sp.* The growth parameters reported by Jannasch (1968) were used: $\mu_{m1} = 0.80$ h^{-1} and $K_{s1} = 0.009$ g/L, $\mu_{m2} = 0.45$ h^{-1} and $K_{s2} = 0.003$

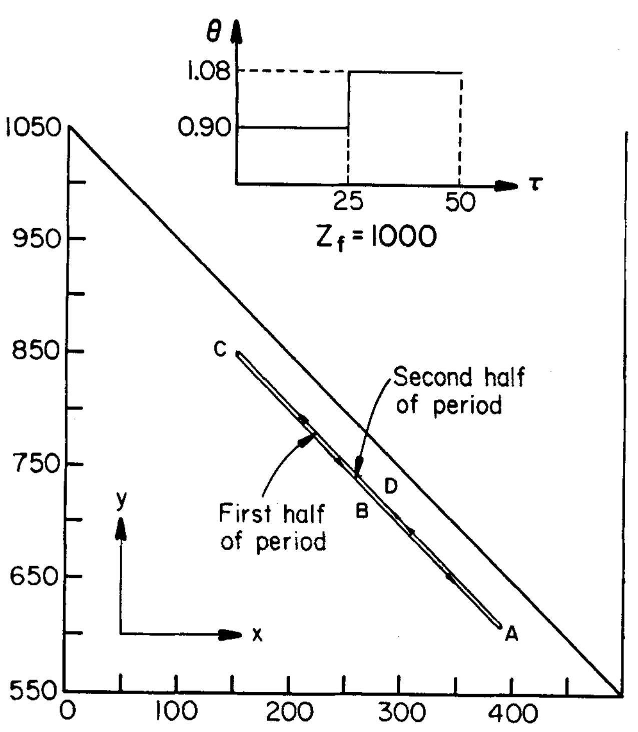


Figure 3(a). Stable periodic trajectory obtained by cycling the dilution rate.

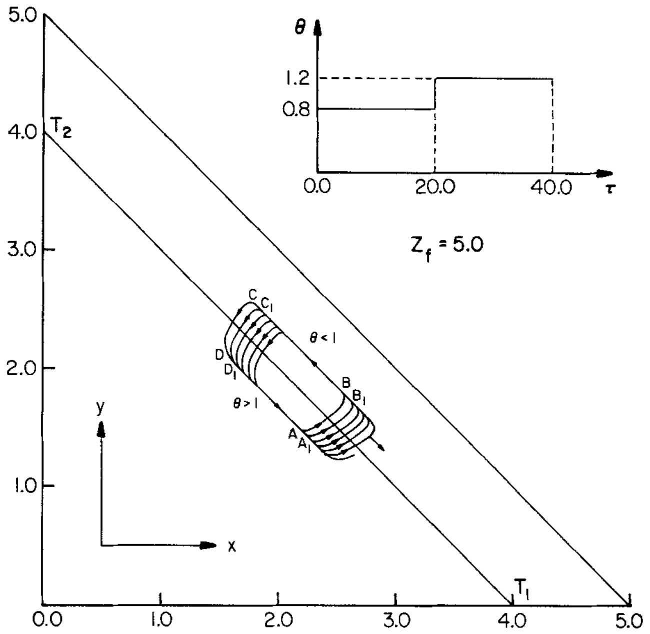


Figure 3(b). Drift of an unstable periodic trajectory obtained by cycling the dilution rate.

g/L where subscript 1 refers to *E. coli* and subscript 2 to *Spirillum sp.* The specific growth rate curves for the two populations intersect at the common point $s_c = 0.00471$ g/L and $\mu_c = 0.275$ h^{-1}. Yield coefficients of 0.11 g/g were assumed for both species.

The cycles obtained by cycling the concentration of the substrate in the feed alone at a constant dilution rate or by cycling the dilution rate between two values (one of which is much larger than unity, Figure 2b), were found to lead always to the washout of one of the populations. This is not very surprising, since in those cycles the competitive advantages does not alternate between the two populations.

Our calculations with the other patterns of forced cycling indicated that they can give stable periodic trajectories of coexistence.

Thus, in Figure 3a we show a stable periodic trajectory obtained by cycling θ at a constant z_f. Another cycle, which is unstable, is shown in Figure 3b for a similar cycling. This was constructed by searching for those values of z_f, period and upper and lower values of the dilution rate, for which the time spent on the upper part (ABC of the cycle, during which $\theta < 1$), exactly balances that spent on the lower part of the cycle (CDA, during which $\theta > 1$). Given, then, an initial point A, a value of z_f and two values between which θ oscillates, the period is varied until a value is found for which the system returns to point A at the end of the period. The trajectories of Figure 3 were obtained in this way with an analog computer which facilitates greatly the above search.

The value of z_f used is not important in obtaining the above stability behavior. A small value was chosen for a clearer and more informative representation of the cycle ABCD and its subsequent drift.

As mentioned before, cycle ABCD of Figure 3b is unstable. A small fluctuation of one of the parameters involved will cause the drift of the cycle from the steady state position ABCD to a new one, $A_1B_1C_1D_1$, and further down toward the washout of the second population. Applying the stability criteria to the case of Figure 3b shows that the cycle which lies on the boundary $x = 0$, $y > 0$ is unstable, while the one lying on the boundary $x > 0$, $y = 0$ is stable. Both of the above cycles are unstable when the case of Figure 3a is considered. Also, both Floquet multipliers have moduli less than unity for the cycle of Figure 3a, but not for the cycle of Figure 3b.

The large number of parameters prohibits a detailed parametric analysis. In general, we can state that for conditions clearly favorable to either population, a stable periodic trajectory of coexistence is not possible, and the washout of the population which grows under unfavorable conditions will take place. Examining only the upper value, θ_2, which the dilution rate takes during the second part of the period, a large value of θ_2 will result in washout of the second population, while a value very close to unity will result in the washout of the first. Furthermore, the region of θ_2 values which give coexistence is small. For the case of a period of 50, $\kappa = 0.50$ and a lower value of the dilution rate equal to $\theta_1 = 0.90$, stable coexistence could be achieved for θ_2 values between 1.073 and 1.086. Similar statements can also be made for the other parameters of the system.

The effect of periodic harvesting is shown in Figure 4a. For the cycle shown, 90% of the biomass is removed at the end of each period (which is equal to 50). The chemostat is filled up with fresh medium and the populations are allowed to grow again for another period at a constant dilution rate $\theta = 0.90$ with $z_f = 1000$.

If we represent by h the harvesting number, that is the fraction of the amount of biomass that is harvested away, then, for a period $\theta = 50$, decreasing h below 0.80 led to unstable coexistence cycles, with X_2 the dominant population. Increasing h above 0.96 led again to unstable coexistence cycles, but with X_1 the dominant population. This result is not specific to the particular value of the period or the dilution rate used. So we can state that, for a given dilution rate and period, there is an upper limit to the harvesting number h below which the cycle that lies on the boundary $y = 0$, $x > 0$ is unstable and a lower limit above which the cycle that lies on the boundary $x = 0$, $y > 0$ is unstable. For the values of h in the region defined by these limits, both cycles lying on the boundaries are

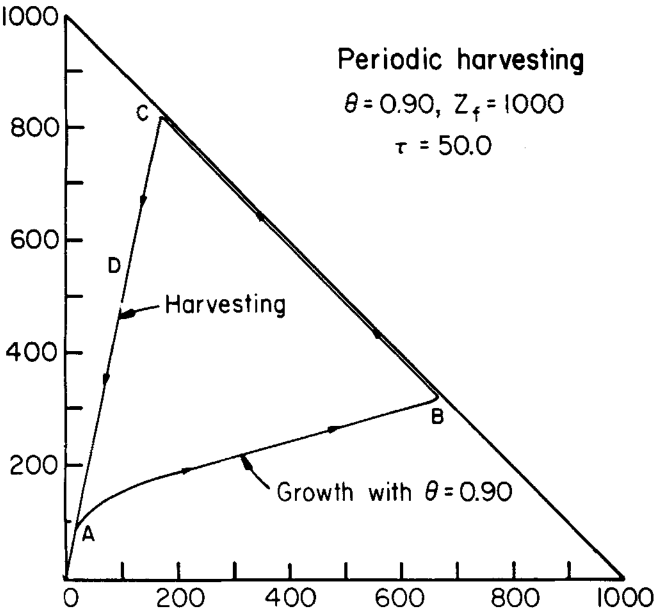


Figure 4(a). Stable periodic trajectory with periodic harvesting.

unstable, and then a stable periodic trajectory of coexistence is possible.

The above are summarized in Figure 4b, where the foregoing limits are represented by the curves $0A_1B_1$ and $0A_2B_2$ for $\theta = 0.90$. Thus, for h below the curve $0A_1B_1$ the cycle lying on $y = 0$, $x > 0$ is unstable and for h above the curve $0A_2B_2$ the cycle lying on $x = 0$, $y > 0$ is unstable. So, for h between $0A_1B_1$ and $0A_2B_2$, the possibility of coexistence clearly exists. Similar results were obtained for other values of θ as well. As θ decreases below 0.90, the two curves $0A_1B_1$ and $0A_2B_2$ change position, moving to $0C_1D_1$ and $0C_2D_2$, respectively, (Figure 4b, $\theta = 0.80$). But their mutual arrangement remains the same. In that way, for smaller values of θ, shorter periods are required for coexistence.

For even smaller values of θ, the periods which give coexistence further decrease until θ reaches a critical value above which the periods are increasing. At $\theta = 0$, the two curves $0A_1B_1$ and $0A_2B_2$ coincide at $h = 0$, so that no stable cycle of coexistence is possible. The same is true for $\theta = 1$. The conclusion is that 1) if we harvest biomass periodically from a batch reactor or from a continuous flow reactor which operates under the flow rate that 2) gives coexistence of the two competing populations in the absence of harvesting, then 3) coexistence is not possible and the outcome will be pure population 1 or 2. Another way to look at it is that only with an infinite period of harvesting, that is, no harvesting at all, is coexistence achieved in the above systems.

The above are better shown in Figure 4c, drawn for constant period 10. For h below the curve ABD, the cycle that lies on $y = 0$, $x > 0$ is unstable. For h above ACD, the cycle that lies on $x = 0$, $y > 0$ is unstable. So for h between ABD and ACD, a cycle of coexistence exists. Again we can see that as $\theta \to 0$ or $\theta \to 1$, the region of coexistence is reduced to zero, as indicated before.

No stable cycle of coexistence is possible for $\theta > 1$. This result, as well as the ones obtained above for $\theta < 1$, can be explained by considering the direction and the form of the phase plane trajectories obtained for a chemostat operating at a constant dilution rate θ. Thus, from Equations (6) and (7) we see that:

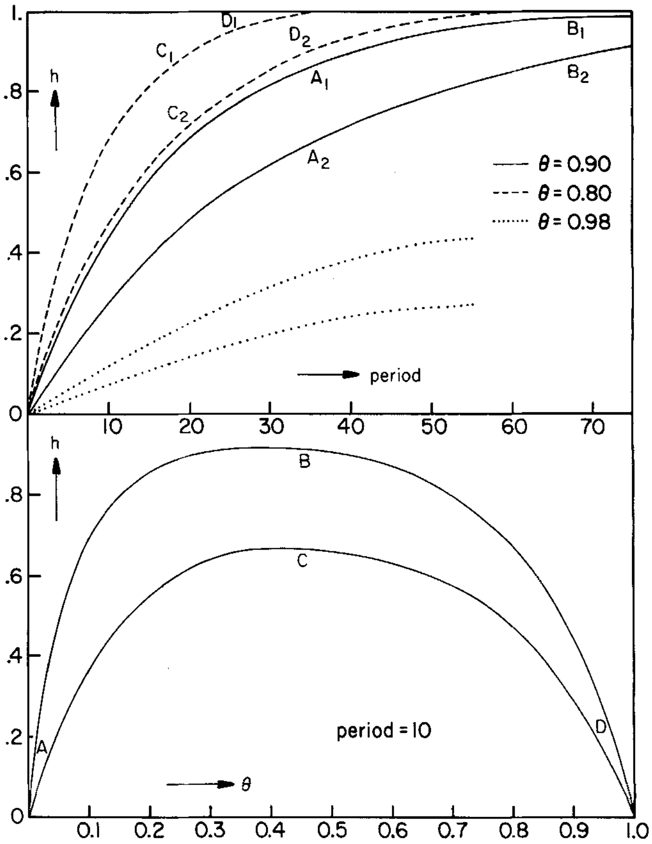


Figure 4(b), and (c). Operating diagram of a chemostat with periodically harvested biomass for constant values of the dilution rate, (b), and constant period, (c).

$$\frac{d}{dt}\left(\frac{x}{y}\right) = \frac{x}{y}\,[f(z) - g(z)]. \qquad (30)$$

For $\theta > 1$, the steady state value of z is larger than 1. So, starting with a large value of z (always true considering the operation of periodic harvesting, that is, harvesting the biomass and filling up the reactor with fresh substrate), the value of the latter stays always above 1, so that $f > g$ and the ratio x/y increases monotonically according to Equation (30). The phase plane trajectories then are always convex curves. And, a cycle lying on $x = 0$, $y > 0$ is always unstable, while the ones on $x > 0$, $y = 0$ are always stable.

On the contrary, for $\theta < 1$, the steady state value of z is smaller than unity. For large initial values of z, then $f > g$ and the ratio x/y increases as long as z is larger than unity. For $z < 1$, $f(z) < g(z)$, so that when z becomes smaller than unity, the ratio x/y starts decreasing. This decrease continues until the steady state is reached. The phase plane trajectories are initially convex, but they become concave after z drops below unity. It is this complex feature that causes the interesting behavior described previously for the case $\theta < 1$.

A final point of interest is the effect of the period on the relative abundance of the two populations for a given θ and h. It was found that smaller periods cause an increase of the biomass concentration of population 1 relative to that of population 2, while larger periods cause just the opposite effect. This result is general and does not depend on the particular value of $\theta < 1$ or h, as long as they are so chosen to guarantee that no population becomes extinct, according to Figures 4b and 4c.

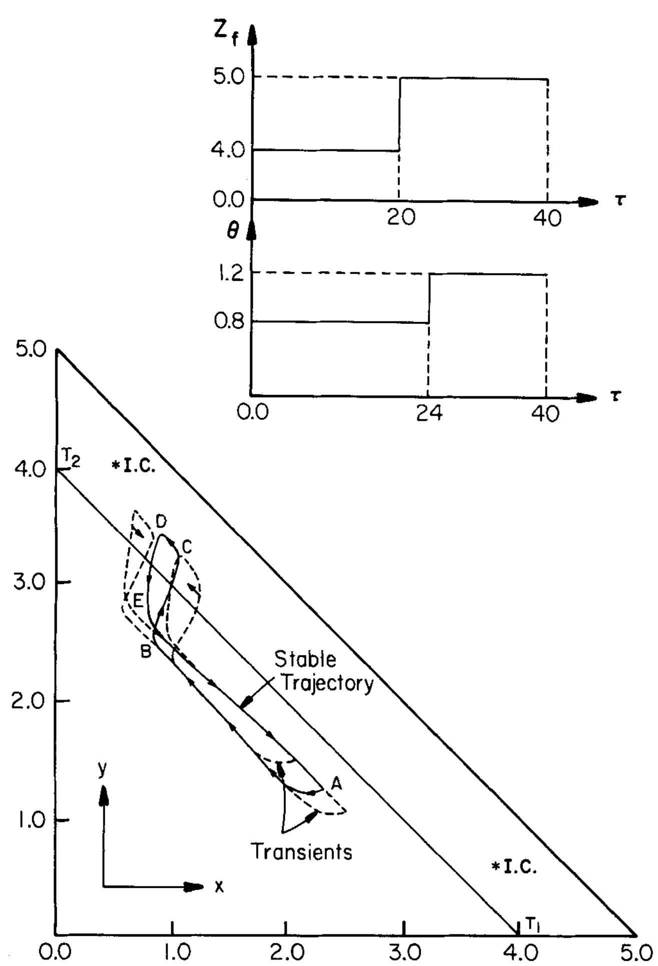


Figure 5. Stable periodic trajectory for simultaneous cycling of θ and z_f with phase difference.

The simultaneous cycling of the dilution rate and the concentration of the substrate in the feed can also produce stable cycles, sometimes even in those cases, for which the cycling of θ or z_f alone cannot do so. By cycling one more operating parameter, an extra degree of freedom is added, increasing the versatility of the system. Some of these cycles are shown in Figures 5 and 6 for the indicated periodic variation of θ and z_f. In Figure 5, the time at which the dilution rate is switched to a larger value lags behind the time of switching z_f. The cycle starts at A, at point B z_f is switched to a larger value, and at point D, the switching of θ to a larger value takes place. The dashed lines show how the stable position, ABCDE, is approached from two different directions, corresponding to two different initial conditions. Such a stable cycle does not exist if only one of the θ or z_f is varied periodically.

Another stable cycle for which the switching of both θ and z_f takes place at the same time is shown in Figure 6. The cycle starts at point A, and at point C both θ and z_f are switched to larger values.

Previous results were based on the assumption that Monod-type kinetics describe the growth of the populations during both transient and steady states, and that after each change in θ and/or z_f, the two populations are instantaneously adjusted, physiologically, for growth under the new conditions, according to Monod model. This is clearly not true. It is certain that there will be a lag between the time at which the growth conditions are changed until the physiological state of the population is completely adjusted to the new conditions and growth resumes according to Monod model.

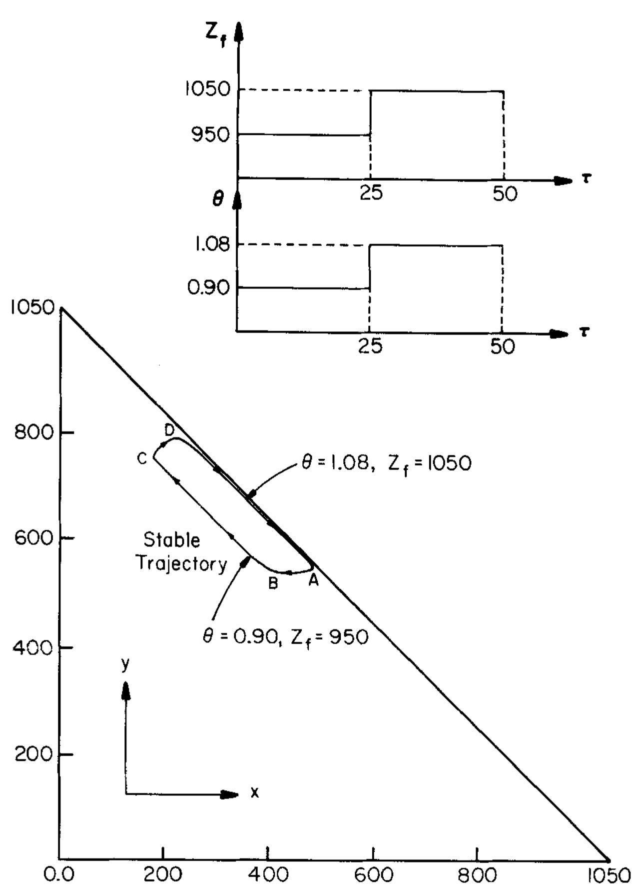


Figure 6. Stable periodic trajectory for simultaneous cycling of θ and z_f without phase difference.

Such a lag has not been accounted for in our model. This makes an experimental verification of these theoretical results a very interesting subject. It will be noticed also in Figures 3, 5 and 6 that θ and z_f alternate between values not too far apart from each other, so that physiological changes to be brought about after each change in θ or z_f should be minimal. Another point is that the yield coefficients for the growth of the two populations were assumed to be constant (although they are known to vary with the dilution rate, Aiba et al. 1973). The variations of the dilution rate, however, are generally small and occur over long periods of time, so that we do not anticipate that non-constant yield coefficients will affect the results presented above. In any case, cycling the chemostat as described does not require any involved experimental set up, and obtaining data on a real system with forced oscillations imposed on it will be our next step along this line of research.

Clearly, there is a large number of possible cycles for the operating parameters of the system, or combinations of them, that can be tried for the stabilization of the microbial competitors. In this work we presented some criteria that, when satisfied, guarantee, at least, that none of the populations become extinct, but that both coexist in a dynamic environment. Whether or not a stable periodic trajectory of coexistence will be approached remains an open question, answerable only when sufficient theoretical results become available. This leaves numerical integration, along with the application of the previous criteria, as the only way to investigate the existence and the stability characteristics of these cycles. The results so obtained cannot be claimed as of general applicability. It is, nevertheless, the first time to our knowledge that periodically varying external influences has been shown to stabilize two competing microbial populations in the same spatially homogeneous continuous flow system (see also Koch 1974). This result is of particular importance, since it provides an easily implemented scheme for sustaining competing populations in the same environment. Further, an explanation is provided for their coexistence in natural ecosystems, since periodic variations of growth conditions are the rule in most ecological situations.

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NOTATION

a_i, b_i = growth rate parameters in the dimensionless specific growth rate expressions
f = dimensionless specific growth rate of population 1
g = dimensionless specific growth rate of population 2
h = harvesting number
$\mathbf{h}$ = vector function, Equation (11)
$\underset{\sim}{\mathbf{I}}$ = identity matrix
$\underset{\sim}{\mathbf{J}}$ = Jacobian matrix corresponding to Equation (11)
K_s = Michaelis constant
$\underset{\sim}{\mathbf{P}}$ = matrix defined by Equations (14a) and (14b)
p_{ij} = elements of matrix $\mathbf{P}$
q = flow rate
S = substrate
s = concentration of substrate
s_c = value of s at which $\mu_1 = \mu_2 = \mu_c$
t = time (dimensionless)
$\mathbf{u}$ = vector describing the periodic inputs, Equation (11)
V = volume of chemostat
$\mathbf{w}$ = vector of the state of the system, Equation (11)
x_i = concentration of biomass of population i
x = dimensionless concentration of biomass of population 1
Y_i = yield coefficient of population i
y = dimensionless concentration of biomass of population 2
y_{si} = steady state biomass concentrations of population X_2 growing in a chemostat under dilution rate θ_i
z = dimensionless concentration of substrate

Greek Letters

α, β, γ = defined by Equation (17)
θ = dimensionless dilution rate
θ_1, θ_2 = the two values between which θ oscillates
μ_i = specific growth rate of population 1
μ_m = maximum specific growth rate
μ_c = common value of μ_1 and μ_2 at $s = s_c$
τ = period

Subscripts

1, 2 = populations X_1, X_2
f = feed conditions
s = steady state

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