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Dynamics of Mixed Cultures of Microorganisms: Some Topological Considerations

Structural relationships between the numbers of the various types of steady states exhibited by a mixed culture of microorganisms growing in a chemostat have been obtained. The analysis is based on the degree theory and utilizes Hopf's index theorem. Useful information concerning the culture growth and general conclusions pertaining to the nature of the possible steady states are obtained from these relationships. An alternate method of stability analysis, based on the above results, has also been developed and applications to several cases of growth of indirectly interacting populations which demonstrate the efficiency of the method are presented.

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SCOPE

A continuous flow biochemical reactor in which a mixed culture of microorganisms is growing very often exhibits multiple steady states. The analysis of the stability of these steady states has been the subject of many works so far. Invariably, these analyses have been based on Liapounov's first method, and in many cases they have produced very informative results concerning the dynamic behavior of interacting microbial populations. There are indirect interactions, however, where analytical solutions for the steady states cannot be obtained, and the stability of a steady state is determined by numerical evaluation of the steady states and, subsequently, the eigenvalues of the linearized system. One disadvantage of this approach is the limited generality of the so obtained results. Taylor and Williams (1974), for example, examined the growth in a chemostat of a culture consisting of two populations growing on two rate limiting substrates for which they competed. Among other possibilities, they also investigated the case of two coexistence steady states, and although their numerical calculations indicated that these steady states were never meaningful and stable at the same time, this result could not be shown in general through the linearized stability analysis.

Similar problems arise in the analysis of other types of indirect interactions, especially when the number of the environmental variables affecting the growth rates of the two populations becomes large. The objective of this work, then, has been to develop some means of facilitating the stability analysis and obtaining general results on the nature of the various steady states which are possible in such systems. This is accomplished through the development of some topological relationships between the numbers of the various types of steady states exhibited by a mixed culture of microorganisms growing in a chemostat. These relationships utilize the results of the degree theory and Hopf's index theorem and recognize some general mathematical laws governing the structure of the differential equations which describe the dynamics of growth of the mixed culture. The importance of these relationships lies in the ease with which one determines the type and therefore the stability of a steady state from relatively limited information and in the generality of the obtained results.

CONCLUSIONS AND SIGNIFICANCE

The above topological relationships admit a limited number of solutions because of the integer nature of the variables involved in them. For a small number of steady states, their type and stability can be determined directly from the relationships. As the number of steady states increases, the number of possible combinations that satisfy the relationships increases too, but still a number of general results can be obtained. Among them we find:

1. The origin is always a steady state, and its type depends on the total number of partial washout steady states (PWSS) only and not on the number of the coexistence steady states (CSS).

2. If the total number of PWSS is less than three and the total number of CSS is two, one of the CSS must be a saddle point and the other must be a node, a focus or a center.

3. If there are no PWSS, there can be only an even number of CSS, and half of them are saddle points while the other half are nodes, foci or centers.

A further screening of the solutions is possible by combining the topological relationships with a theorem that relates the types of the steady states that can be found along the same branch of the contour defined by the vanishing of the derivative of one of the state variables. Thus a new method of stability analysis was developed which can be used either by itself or combined with the traditional linearized stability analysis for more general results. This method was applied to several types of indirect interactions (competition for a single or double limiting substrates, commensalism, mutualism, etc.), and the ease by which the various stability analyses were carried out demonstrated the efficiency of the method.

A number of other chemical engineering systems can be analyzed in a similar manner, and the possibility of extending these ideas to the analysis of a CSTR is discussed.

Microorganisms are presently employed by man in quite a number of significant industrial operations. Fermentation technology or biochemical engineering refers to those situations in which the technologist uses the activities of microorganisms to increase the value of raw materials (production of foods, antibiotics, etc.). Sanitary or environmental engineering, on the other hand, refers to those situations in which the technologist uses the activities of microorganisms to decrease the negative value of aqueous waste materials discharged to the environment. The activities of microorganisms are also employed in the mining industry, and still another area where man might eventually utilize microbial activity on a very large scale is that of capture and conversion of solar energy.

With the exception of applications in environmental engineering, pure cultures are employed in almost all of the above applications. Nevertheless, improvements in the yield, productivity and substrate utilization have been witnessed with the use of mixed cultures in many applications which traditionally have been carried out with pure cultures. Furthermore, the proper exploitation of the potential of mixed microbial populations can lead to a whole new variety of biochemical applications, as it is already evident in some areas (Harrison, 1976; Trivedi and Tsuchiya, 1975; and many others). Clearly, increased knowledge of the behavior of mixed cultures is needed before these possibilities are realized.

Mixed culture phenomena are not merely composites of the pure culture behavior of the organisms present. The performance of a complex microbial process depends upon interactions between its species and strains. These interactions can be classified in the more general way as either indirect or direct. We will be mainly concerned with indirect interactions in this work, that is, those interactions which are exerted through the abiotic medium, (some population changes somehow the state of the abiotic environment and this in turn affects the rate at which another population carries on its life processes (Frederickson and Tsuchiya, 1977)). Direct interactions, that is, interactions requiring direct physical contact of individual organisms from the interacting populations, will not be examined here.

Accepting the notion that appropriate mathematical models can describe these growth processes in a quantitative way, we next address the question of the dependent variables which should be included in such models. Clearly, a model of indirect interactions that purports to have a mechanistic basis must necessarily include the state of the abiotic environment as a dependent variable; the concentrations of those substances, in particular, which exert stimulatory or inhibitory effects on the growing populations should be included. Considering the operation of a continuous flow stirred bioreactor (chemostat), the above substances can be either introduced in the bioreactor with the feed or produced by the metabolic activities of the microorganisms. If we denote then by s_i, $i = 1, \ldots, p$, the concentration of the growth limiting substrates which are introduced in the chemostat with the feed (primary substrates), by r_j, $j = 1, \ldots, q$, the concentration of those metabolic products which inhibit growth (inhibitors) and by u_k, $k = 1, \ldots v$, the concentration of those growth limiting substrates which are produced by the metabolic activities of the microorganisms (secondary substrates), the dynamics of growth in a chemostat can be described by the following set of equations:

$$\dot{C}_A = -DC_A + \mu_A C_A \tag{1a}$$

$$\dot{C}_B = -DC_B + \mu_B C_B \tag{1b}$$

$$\dot{s}_i = D(s_{if} - s_i) - \frac{1}{Y_{Ai}}\mu_A C_A - \frac{1}{Y_{Bi}}\mu_B C_B;\ i = 1, \ldots, p \tag{1c}$$

$$\dot{r}_j = -Dr_j + \alpha_{Aj}\mu_A C_A + \alpha_{Bj}\mu_B C_B;\ j = 1, \ldots, q \tag{1d}$$

$$\dot{u}_k = -Du_k + \beta_{Ak}\mu_A C_A + \beta_{Bk}\mu_B C_B;\ k = 1, \ldots, v \tag{1e}$$

Notice that the above equations are general in the sense that they include all possible cases of indirect interactions. The specific growth rates μ_A and μ_B are functions of the environmental variables s_i, r_j and u_k. The specific environmental variables that affect μ_A and μ_B depend upon the particular indirect interaction under examination. Thus, in the case of competition for a single primary substrate, $q = v = 0$, $p = 1$ and μ_A, μ_B are functions of s_1. In the case of pure mutualism, where the growth of population A is limited by the concentration of substrate u_2 which is the metabolic product of population B, and similarly the growth of population B is limited by the concentration of a substrate u_1 which is the metabolic product of population A, $p = q = 0$, $v = 2$ and μ_A and μ_B are functions of u_2 and u_1, respectively. If, in addition to the above, the growth of both populations A and B is limited by a primary substrate s_1 for which the two populations compete, then $\mu_A = \mu_A(s_1, u_2)$ and $\mu_B = \mu_B(s_1, u_1)$. If the two populations compete for a primary substrate s_1 and at the same time the growth of A is inhibited by a substance of concentration r_1 which is the metabolic product of population B, then $\mu_A = \mu_A(s_1, r_1)$ and $\mu_B = \mu_B(s_1)$. Other types of indirect interactions can be treated similarly.

The particular function which is used for μ_A and μ_B is of no importance for our analysis as long as it does not correspond to physically unrealistic situations (a constant specific growth rate for all values of the substrate concentration would be such a case resulting in negative values for the biomass concentrations). Usually the Monod (1949) model, $\mu = \mu_m s/(K_s + s)$, or the multiple saturation model (Megee et al., 1972), $\mu = \mu_m s_1 s_2/(K_1 + s_1)(K_2 + s_2)$, is used to describe uninhibited growth that involves one or two rate limiting substrates, primary or secondary; the Andrews (1968) model, $\mu = \mu_m s/(K_2 + s + s^2/K_i)$, is used if growth is inhibited at high substrate concentrations, and the model by Aiba and Shoda (1969), $\mu = \mu_m s/(K_s + s)(1 + r/K_r)$, is used for the specific growth rate of a population whose growth is inhibited by some metabolic product. It should also be pointed out that the stoichiometric coefficients Y_{Ai}, Y_{Bi}, α_{Aj} and α_{Bj} are non-negative, while the coefficient β_{Ak} (or β_{Bk}) is positive if the secondary substrate u_k is produced by population A (or B); it is negative if it is consumed by population A (or B), and is zero otherwise.

We can employ arguments similar to Aris and Humphrey (1977) to reduce the dimensions of the system (1) to two by replacing Equations (1*c*), (1*d*) and (1*e*) by the steady state relations

$$s_i = s_{if} - \frac{1}{Y_{Ai}} C_A - \frac{1}{Y_{Bi}} C_B \,; \; i = 1, \ldots, p \qquad (2a)$$

$$r_j = \alpha_{Aj} C_A + \alpha_{Bj} C_B \,; \quad j = 1, \ldots, q \qquad (2b)$$

$$u_k = \beta_{Ak} C_A + \beta_{Bk} C_B \,; \quad k = 1, \ldots v \qquad (2c)$$

In this way, instead of trajectories in the positive angle of the $(C_A, C_B, s_i, r_j, u_k)$ space, we consider their projections on the bounded domain:

$$0 \le C_A, C_B \quad \text{and} \quad 0 \le \frac{1}{Y_{Ai}} C_A + \frac{1}{Y_{Bi}} C_B \le s_{if} \,; \; i = 1, \ldots, p \qquad (3)$$

No justification is needed for the above substitution if the state of the system lies initially on the hyperplanes defined by Equation (2). This can be achieved by filling up the chemostat initially with fresh substrates of concentrations s_{if}, $i = 1, \ldots, p$ and then seeding the chemostat 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 to be considered in examining the dynamics of a CSTR. In our case there are two reactions, namely, those which transform the substrate into biomass of A and B.

The dynamic behavior of the system of Equations (1*a*), (1*b*) and (2) has been the subject of many studies in recent years (Jost et al., 1973; Taylor and Williams, 1974; Meyer et al., 1975; Yang and Humphrey, 1975; Yoon et al., 1977; Yoon and Blanch, 1977; Aris and Humphrey, 1977; De Freitas and Fredrickson, 1978; and others). In addition to providing easily implementable qualitative criteria for testing the validity of proposed models of growth, the stability analysis of the dynamics of mixed cultures of microorganisms is of great value in assessing the performance of a bioreactor in which microbial populations are utilized in a specific application.

The technique most frequently employed in determining the stability characteristics of a steady state is based on Liapounov's first method. It consists mainly of a linearization of the differential equations about the steady state under scrutiny and examination of the eigenvalues of the Jacobian matrix. With the exception of a few relatively simple cases, however, the steady state values of the state variables cannot be determined analytically so that the stability of a steady state is determined after the steady states and eigenvalues of the linearized system have been evaluated numerically. Although this approach has proven very useful in examining specific stability problems, the lack of explicit expressions for the eigenvalues prohibited detailed parametric analyses and resulted in conclusions of limited generality, since expensive numerical calculations were needed for this purpose.

In this paper we examine the topological structure of the singular points of Equations (1) with the objective of aiding the existing methods of analyzing the stability of a system. Our approach utilizes the degree theory. The concept of the degree was first introduced by Poincaré in a discussion of the behavior of the integral curves at infinity in the phase plane. These studies took place before the advent of the great computing devices and during a period when topological arguments were very important for the study of nonlinear differential equations. The results of degree theory are used in this paper to derive an equation relating the numbers of the various types of possible singular points of Equations (1*a*), (1*b*) and (2). This turns out to be a generally valid and useful equation, since the number of singular points is always an integer, and there are a limited number of singular points for the system. Furthermore, the above equation provides an additional test for the validity of a stability analysis since the results of the latter will have to satisfy this equation. Finally, as it will become apparent from subsequent discussion, the structure of the phase planes can be easily predicted, and the effect of the various parameters can be evaluated without the need of linearized eigenvalue analysis. It is emphasized, however, that although useful and in many cases self-sustained, the presented results are not intended to substitute the procedure based on Liapounov's first method; rather they should be regarded as complementary to this method whose the potential can thus be greatly increased to give answers to problems which could not be handled satisfactorily before.

Before we proceed with the development of some mathematical preliminaries, we present some properties of Equations (1*a*), (1*b*) and (2) which are later used in obtaining the detailed picture of the system's dynamics.

1. The solutions of Equations (1*a*), (1*b*) and (2) are confined in the positive quadrant or the axes of the (C_A, C_B) plane provided that the state of the system lies initially in the same region (Hsu et al., 1977). If the growth of at least one population is limited by primary substrates, then the equations

$$\frac{1}{Y_{Ai}} C_A + \frac{1}{Y_{Bi}} C_B = s_{if} \,; \; i = 1, \ldots, p \qquad (4)$$

constitute upper bounds for the values of C_A and C_B [see Equation (2*a*)]. If, on the other hand, no primary substrates are required for growth, no such upper bounds exist, and C_A and C_B can grow indefinitely. Singular points that lie on the axes can be only of the node or saddle point type; focal behavior about these singular points is not possible, since this would require the state variables C_A and C_B to assume negative values.

2. If $\beta_{Ak} = \beta_{Bk} = 0$ for all k (or if $v = 0$), that is, if there are no growth associated secondary substrates and if there is no inhibition at high substrate concentrations, then there can be no foci or centers (stable or unstable) in the interior of the domain defined by (3). Furthermore, a node in the interior of the same domain cannot be unstable (that is, not both eigenvalues can be positive at the same time). These conclusions are easily derived from an eigenvalue analysis of the linearized system of Equations (1*a*) and (1*b*).

We proceed now to the development of the equation that relates the number and the type of singular points of Equations (1*a*), (1*b*) and (2) after some mathematical preliminaries are discussed.

THEORETICAL BACKGROUND

Degree of a Mapping

Let $f_i(x_1, \ldots, x_n)$, $i = 1, \ldots, n$ be n continuous functions of the variables $(x_1, \ldots, x_n)$, and consider $f = (f_1, \ldots, f_n)$ as defining a mapping from real Euclidean n space R^n into itself.

Denote by $C(\bar{D})$ the set of continuous, vector valued functions defined on $\bar{D}\subset R^n$ with the topology of uniform convergence and by $C'(\bar{D})$ the subset of continuously differentiable vector valued functions on $\bar{D}$, where D is an open set in R^n. For $p\epsilon R^n$ we want to define a measure of the number of solutions of $f(\underset{\sim}{x}) = p$ in D by an integer $d(f, p, D)$ which depends continuously on both f and p. This integer is called the degree of the mapping f relative to the point p and the set D. The degree is first defined on a dense set of functions f in $C(\bar{D})$ and on a dense set of points p; then the definition is extended to all functions of $C(\bar{D})$ and all points p of the image of D by a limiting process. It is also assumed throughout the discussion that $\underset{\sim}{f}(\underset{\sim}{x}) = \underset{\sim}{p}$ has no solutions for $\underset{\sim}{x}\epsilon\partial D$.

Definition. Suppose $f\epsilon C'(\bar{D})$ and the Jacobian determinant of $\underset{\sim}{f}$, $\det J_f(\underset{\sim}{x}) = \det(\partial f_i/\partial x_j)$, does not vanish at any point $\underset{\sim}{x}$ in the solution set $\{\underset{\sim}{x}/\underset{\sim}{x}\epsilon D, \underset{\sim}{f}(\underset{\sim}{x}) = \underset{\sim}{p}\}$. Then we define

$$d(\underset{\sim}{f}, \underset{\sim}{p}, D) = \sum_{\underset{\sim}{x}\epsilon \underset{\sim}{f}^{-1}(p)} \operatorname{sgn} \det J_f(\underset{\sim}{x}) \tag{5}$$

For functions $f\epsilon C'(\bar{D})$ whose solution set contains some $\underset{\sim}{x}$ where the Jacobian determinant vanishes, $\det J_f(\underset{\sim}{x}) = 0$, the degree is defined by the limiting process $d(\underset{\sim}{f}, \underset{\sim}{p}, D) = \lim\limits_{m\to\infty} d(\underset{\sim}{f}, \underset{\sim}{p}_m, D)$, where p_m are points that approximate p and whose solution sets contain points $\underset{\sim}{x}$ at which $\det J_f(x) \neq 0$. Similarly, by noting that $C'(\bar{D})$ is dense in $C(\bar{D})$, the degree for all continuous functions $f\epsilon C(\bar{D})$ is defined as the limit $d(\underset{\sim}{f}, \underset{\sim}{p}, D) = \lim d(f_m, p, D)$, where $f_m\epsilon C'(\bar{D})$ and $f_m \to f$ uniformly in $\bar{D}$. The above definitions are, of course, justified provided that they are independent of the approximating sequences and the limits exist. The monograph by Berger and Berger (1968) can be consulted for further details.

We see from the above discussion that the degree of a function $\underset{\sim}{f}(\underset{\sim}{x})$ is concerned with the solutions of the equation $\underset{\sim}{f}(\underset{\sim}{x}) = \underset{\sim}{p}$. For a system of differential equations, $\dot{\underset{\sim}{x}} = \underset{\sim}{f}(\underset{\sim}{x})$, the properties of the singular points can be determined by studying the degree of the function f relative to the point $p = \underset{\sim}{0}$. To this connection and for two-dimensional systems, two geometric ways of finding the degree are available.

Consider the system

$$\dot{x} = X(x,y) \quad\text{and}\quad \dot{y} = Y(x,y) \tag{6}$$

where X and Y are assumed to be real and continuous functions of x and y in a domain D of the plane so as to ensure the uniqueness of the solutions and their continuous dependence on the initial values.

The solution of (6) defines a set of trajectories which may form closed cycles, unwind from a singular point and ultimately approach infinity in the form of an unstable spiral, approach a certain point, or, in general, behave in any of the characteristic ways which can be observed in the vicinity of a singular point. With every point $P = (x,y)$ of D we associate the vector $V(P) = (X,Y)$; with the singular points of (6) we associate the zero vector. In this way, the ratio $Y(x,y)/X(x,y)$ evaluated at any point of D gives the gradient of the integral curve of

$$dy/dx = Y(x,y)/X(x,y) \tag{7}$$

through that point.

The topological arguments of Poincaré, which subsequently led to the definition of the degree, apply to the direction of the vector rather than to its magnitude. Since this direction is related to the functions X and Y, qualitative information about this vector field can be obtained without need of integration of (6) or (7).

Now, if ∂D is the boundary of D such that the equations $X = Y = 0$ have no solution on ∂D, and if one traverses ∂D in the positive sense (that is, with the domain D to the left-hand side), then the degree of the mapping (X,Y) relative to the domain D and the point $\underset{\sim}{0}$ is

$$d = \tfrac{1}{2}(k - h) \tag{8}$$

where k is the number of times Y/X jumps from $+\infty$ to $-\infty$ and h the number of times Y/X jumps from $-\infty$ to $+\infty$ in moving around ∂D.

The definition in (8) is equivalent to the following, more descriptive, way of finding the degree. Starting at some point A of ∂D denote by θ the angle between the positive direction of the x axis (or any other fixed direction) and the vector $V(P)$ where point P traverses ∂D once in the positive sense. Since the final position of P coincides with its initial position, the final value of θ will differ from the initial by $2\pi d$, where d is again the degree of the vector field, an integer. Thus we have the following continuous definition of d:

$$d = \frac{1}{2\pi}\oint_{\partial D} d\theta = \frac{1}{2\pi}[\theta]_{\partial D} \tag{9}$$

In moving through the h positions where Y/X jumps from $-\infty$ to $+\infty$, θ moves in the negative direction through the position $3\pi/2$ or $\pi/2$ on each occasion, whereas in moving through the k positions where Y/X jumps from $+\infty$ to $-\infty$, θ moves in the positive direction through the position $3\pi/2$ or $\pi/2$. Since θ is a continuous variable, the total change in θ in moving around ∂D will be $k\pi - h\pi$, hence the equivalence of (8) and (9).

The definitions of the degree given above by Equations (8) and (9) refer to the degree of a vector field (X,Y) relative to the set D and the point $p = \underset{\sim}{0}$. Similar definitions can be given for the degree relative to any other point p by employing a parallel translation that brings point p to the origin.

A number of theorems on the properties of the degree have been proven. We state some of them below; the following references can be consulted for proofs and further details (Sansone and Conti, 1964; Lefschetz, 1957; Temme, 1976; Guillemin and Pollack, 1974; Milnor, 1976). Again, the degree relative to the point $\underset{\sim}{p} = \underset{\sim}{0}$ is considered.

T1. Excission property. If ∂D is deformed continuously so as not to pass through any singular point, the degree does not change.

T2. Homotopy invariance. Let the prime (′) denote a second system similar to (6). Then if $V'(P)$ and $V(P)$ denote the two vector fields at point P and ∂D is such that $V'(P)$ and $V(P)$ are never zero or at opposite directions at any $P\epsilon\partial D$, then the degrees of the two vector fields V and V' relative to D are the same. [One can see the validity of $T2$ if the vector $V'' = (1 - \lambda)V + \lambda V'$, for $0 \le \lambda \le 1$ is considered. V'' is continuous on ∂D and never zero there; thus its degree varies continuously with λ. Therefore, it is constant for any $\lambda\epsilon(0,1)$.]

T3. If the curve ∂D contains no singular points in its interior, then the degree relative to the domain surrounded by ∂D is zero. If the degree relative to D is not zero, then D contains at least one singular point.

Index of a Singular Point

Up to this point the discussion concerned itself with the degree of a mapping, its definition and properties. In studying a system of differential equations, however, the behavior of the integral curves about the singular points is of particular interest. A means of describing this behavior is clearly needed and this is the reason for introducing the index of a point.

Definition. The index of a function $\underset{\sim}{f}\epsilon C(\bar{D})$ at a point $\underset{\sim}{x}\epsilon D$ relative to a point $p\epsilon R^n$ is defined as

$$I(\underset{\sim}{f}, \underset{\sim}{p}, \underset{\sim}{x}) = d(\underset{\sim}{f}, \underset{\sim}{p}, D_x) \tag{10}$$

where $D_x\subset D$ is an open ball around $\underset{\sim}{x}$ that contains a single solution of $f(\underset{\sim}{x}) = p$ in its interior and such that no solutions of $\underset{\sim}{f}(\underset{\sim}{x}) = \underset{\sim}{p}$ exist on its boundary ∂D_x.

It is thus seen from the above definition that the index of a function f at a point $\underset{\sim}{x}$ concerns itself with a single solution of $\underset{\sim}{f}(\underset{\sim}{x}) = \underset{\sim}{p}$. If $\underset{\sim}{p} = \underset{\sim}{0}$, the index of f at $\underset{\sim}{x}_s\,\epsilon \underset{\sim}{f}^{-1}(\underset{\sim}{0})$ is a characteristic of the singular point $\underset{\sim}{x}_s$ and is usually referred to as the index of $\underset{\sim}{x}_s$.

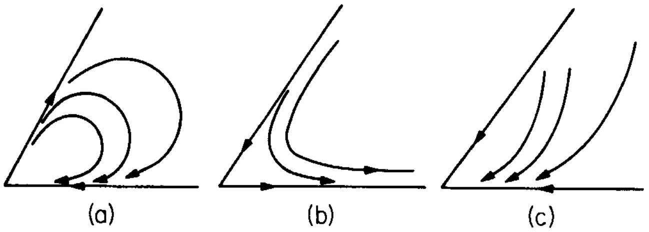

Figure 1. Behavior of integral curves in the neighborhood of a singular point: (*a*) elliptic type of sector, (*b*) hyperbolic type of sector and (c) parabolic type of sector.

Theorem T1 indicates that the definition (10) does not depend on the particular subset D_x. Also, according to T3, the index of a regular point is equal to zero. Another useful relationship is

$$d(\underset{\sim}{f}, \underset{\sim}{0}, D) = \sum_{\underset{\sim}{x}_s} I(\underset{\sim}{f}, \underset{\sim}{0}, \underset{\sim}{x}_s) \tag{11}$$

that is, the degree of a function $\underset{\sim}{f}$ relative to a domain D, and the point $\underset{\sim}{p} = 0$ is equal to the sum of the indexes of the singular points of $\underset{\sim}{f}$ that lie in the interior of D. Finally, it can be seen from the definition of the degree (5) that the index of a singular point is equal to the sign of the Jacobian determinant evaluated at this point; the index of an elementary singular point then [that is, such that $\det J_f(\underset{\sim}{x}_s) \neq 0$] is equal to +1 or −1. This is not true for a nonelementary singular point [$\det J_f(\underset{\sim}{x}_s) = 0$], however, for in this case the degree $d(\underset{\sim}{f}, \underset{\sim}{0}, D_x)$ of Equation (10) is defined as the limit of $d(\underset{\sim}{f}, \underset{\sim}{p}_m, D_x)$, where $\underset{\sim}{p}_m$ are points that approximate the point $\underset{\sim}{0}$ and whose solution sets contain only $\underset{\sim}{x}$ at which $\det J_f(\underset{\sim}{x}) \neq 0$, and now the possibility exists that more than one solution of $\underset{\sim}{f}(\underset{\sim}{x}) = \underset{\sim}{p}_m$ lies inside D_x. The way to circumvent this problem is by employing the two geometric ways of finding the degree [Equations (8) and (9)] which are applicable for elementary and nonelementary singular points of two-dimensional systems.

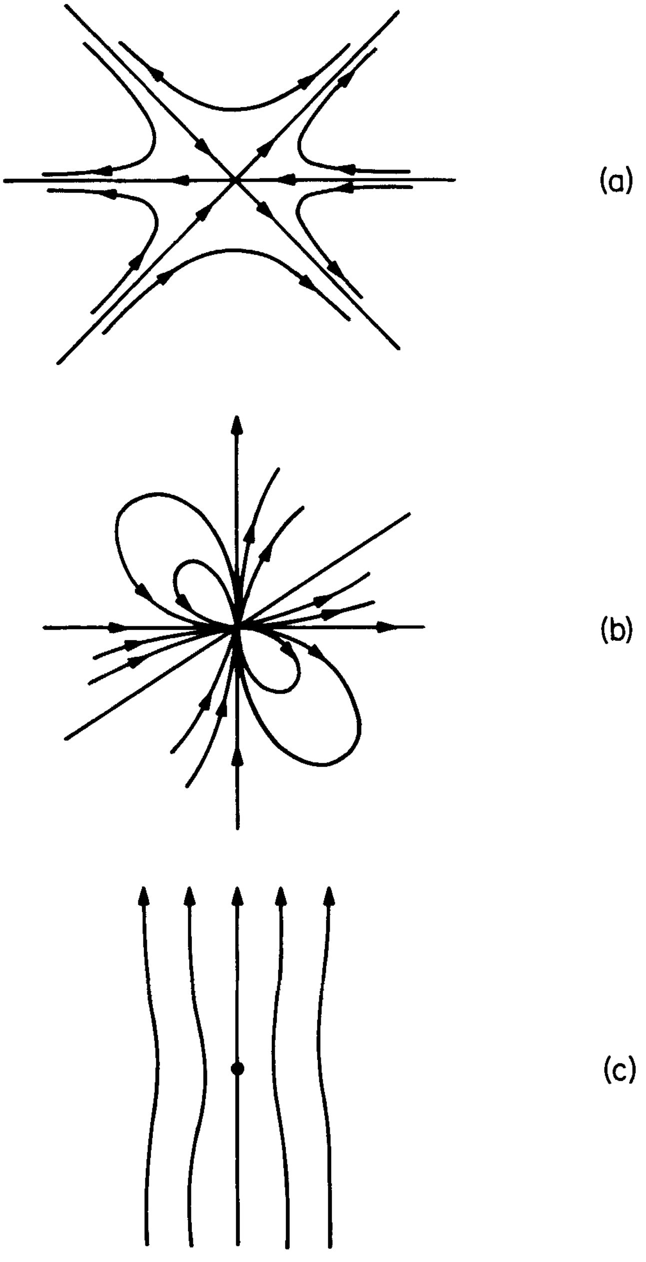

Figure 2. Index of nonelementary singular points, (*a*) $I = -2$, (*b*) $I = 2$, (c) $I = 0$.

For elementary singular points, the index of a node, a focus or a center is +1, while the index of a saddle point is −1 (see previously cited references). When the Jacobian determinant vanishes, nonelementary singular points may arise; that is, a necessary condition for nonelementary singular points to exist is that the Jacobian matrix is singular. Following Brouwer (1909), the behavior of the integral curves in a neighborhood of a singular point will be that of a focus, a center or a combination of three types, the elliptic, the hyperbolic and the parabolic type (Figure 1).

If $C(0,r)$ denotes a circle of radius r centered at the singular point 0, then the circle $C(0,r)$ can be divided into a finite number of sectors through every point of which pass elliptic, hyperbolic or parabolic arcs. Then if n_e denotes the number of elliptic sectors and n_h the number of hyperbolic sectors, the index of the singular point 0 is given by Bendixson's formula:

$$I = 1 + \frac{n_e}{2} - \frac{n_h}{2} \cdot \tag{12}$$

Equation (12) can be applied for the index of elementary points (except foci and centers) as well as of nonelementary ones. Thus, for a node, $n_e = n_h$ and $I = 1$, while for a saddle, $n_e = 0$ and $n_h = 4$ so that $I = -1$. For the singular points of Figure 2, $I = -2$ for the point of Figure 2*a* and $I = 2$ for the point of Figure 2*b*. The index of the singular point of Figure 2*c* is $I = 0$, indicating that the converse of *T*3 is not true if nonelementary points are involved.

A RELATIONSHIP BETWEEN THE VARIOUS TYPES OF SINGULAR POINTS

It was noted previously that the degree theory outlined in the previous section would be utilized in deriving a relationship between the various types of singular points of Equations (1*a*), (1*b*) and (2) from which useful conclusions about the dynamics of a mixed culture of microorganisms can be deduced. We take up this point now.

In the following discussion we denote by D the domain described by Equation (3) and by ∂D its boundary. One possibility could be to use the relationship between the degree of a field relative to a set $D' \subset D$ and the indexes of the singular points surrounded by $\partial D'$, as expressed by Equation (11). The degree can be calculated by one of the two geometric ways mentioned earlier, and Equation (12) can be used for the index of each singular point. Actually, if d is the degree of a field $\underset{\sim}{f}$ relative to D' and the latter contains N nodes, C centers, F foci and S saddle points of the differential equation $\dot{\underset{\sim}{x}} = \underset{\sim}{f}(\underset{\sim}{x})$ then

$$d = N + F + C - S \tag{13}$$

provided that $\partial D'$ does not pass through singular points.

The above approach, however, cannot give a global relationship between the singular points of Equations (1*a*), (1*b*) and (2) for the following reasons.

For such a global relationship to be obtained, the degree of the vector field relative to a set that includes all singular points of Equations (1*a*), (1*b*) and (2) that may arise must be considered. This set cannot exceed the domain where the differential equation is defined and which is given by Equation (3) for the case of the system described by Equations (1*a*), (1*b*) and (2). Examination of Equations (1*a*) and (1*b*), however, shows that singular points lie on the boundary of this domain, and, therefore, these points cannot be included in applying Equation (12) since the degree was defined relative to a set such that no singular points exist on its boundary. One way to deal with this problem is to extend continuously the vector field defined by Equations (1*a*), (1*b*) and (2) outside the closure of D, making sure that no new singular points are introduced by this extension. The degree relative to the extended domain is defined and can be calculated. By *T*1 it is also equal to the degree relative to the initial domain D an estimate of which is thus obtained to be used in Equation (12). This method has been applied by Gavalas (1968).

Another problem with the use of Equation (12) is that the point $p = \underset{\sim}{0}$ lies on the image of the boundary ∂D so that the degree relative to it is not defined.

In order to overcome the above difficulties, we will employ a method first proposed by Doherty (1977), which is based on a theorem by Hopf (see Guillemin and Pollack, 1974, chapter 3) that relates the sum of the indexes of the singular points of a system of differential equations defined on a manifold to the Euler number of the manifold. According to Equation (4), if the growth of at least one of the populations is limited by at least one primary substrate, then the solutions of Equations (1*a*), (1*b*) and (2) are confined to lie on the boundary of or the interior of the domain defined by (3). Equivalently, we can state that the solutions of Equations (1*a*), (1*b*) and (2) are confined to lie on the boundary of or interior of the right triangle of side equal to R, where R is large enough so that the above triangle contains all the straight lines described by Equation (4) for $i = 1, \ldots, p$. This is illustrated in Figure 3. The idea then is to put together eight such triangles to form a closed, hollow octahedron in the three-dimensional space (see Figure 4). In this way, Equations (1*a*) and (1*b*) define a vector field on the above octahedron, and since the latter is homeomorphic to the two-dimensional sphere, a vector field is thus defined on the surface of the 2 sphere. The Hopf theorem now states (Berger and Berger, 1968):

Theorem (Hopf). Let $V(\underset{\sim}{x})$ be a tangent vector field on a compact differentiable manifold M which vanishes at isolated points of M. Then the sum of the indexes at such isolated points (a) is independent of the vector field $V(\underset{\sim}{x})$, and (b) is equal to the Euler-Poincaré number of M, $\chi(M)$.

The Euler number is an invariant characteristic of the manifold equal to (number of triangles—number of sides + number of vertexes) in any triangulation of the manifold. For a two-dimensional sphere, $\chi(M) = 2$.

All that remains now is to find the possible singular points on the surface of the octahedron of Figure 4, multiply each one by the corresponding index and equate the sum of these products to the Euler number $\chi(M) = 2$. Since the singular points that can be found on the surface of the octahedron (or the surface of the 2-sphere) are mirror images of the singular points that lie in the domain of Figure 3, a global relationship between the various types of singular points of (1*a*), (1*b*) and (2) is thus obtained. We derive this relationship below assuming that only elementary singular points exist. The case of nonelementary singular points reflects rather pathological cases and will not be of concern to us in this discussion.

Referring to the domain of Figure 3, we let N, F, C, S be the numbers of nodes, foci, centers and saddle points, respectively, that lie in the interior of the above domain. We let N_x, S_x be the numbers of nodes and saddle points, respectively, that lie on the axis $C_B = 0$. We let N_y, S_y be the numbers of nodes and saddle points, respectively, that lie on the axis $C_A = 0$, and we let N_0, S_0 be the numbers of nodes and saddle points at the origin. (Note that no foci or centers can exist on the axes $C_A = 0$ and $C_B = 0$ of the domain).

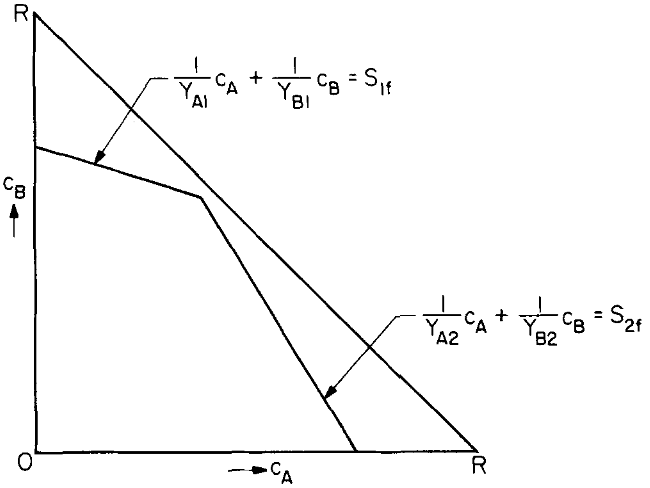

Figure 3. Domain of biomass concentrations of two microbial populations growing in a chemostat.

Noting that a singular point in the interior of the domain gives rise to eight similar points on the surface of the sphere, a singular point on one of the axes gives rise to four similar singular points, and a singular point at the origin gives rise to two similar singular points on the surface of the sphere, we can write, invoking Hopf's theorem $2N_0 - 2S_0 + 4N_x - 4S_x + 4N_y - 4S_y + 8N + 8F$

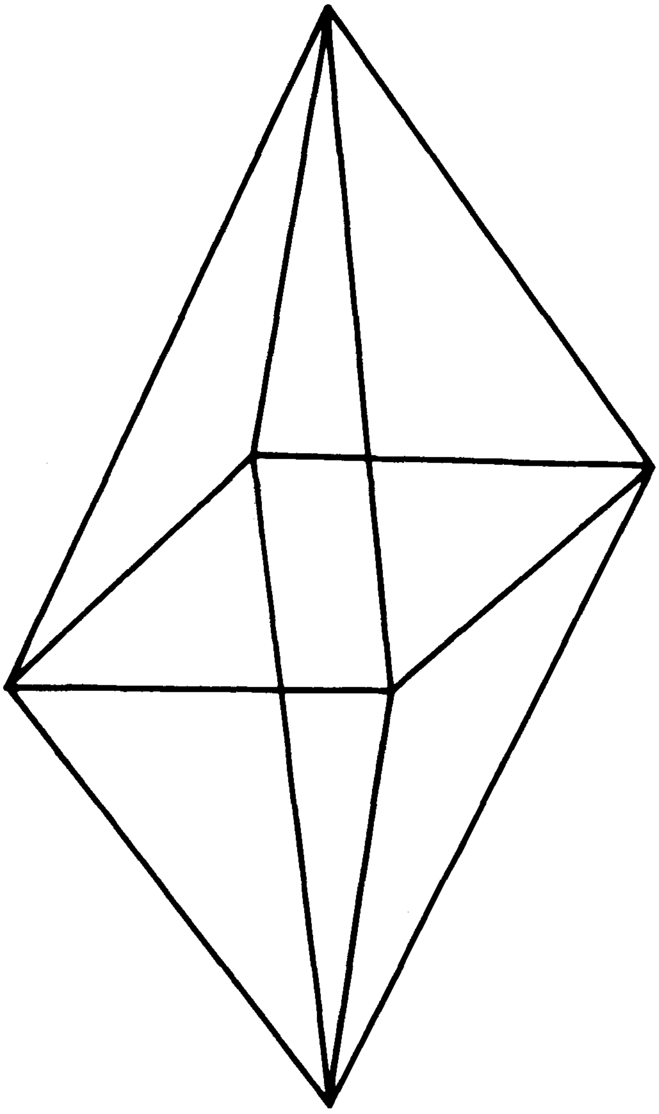

Figure 4. A closed hollow octahedron constructed from the right triangles of Figure 3 and homeomorphic to the 2-sphere.

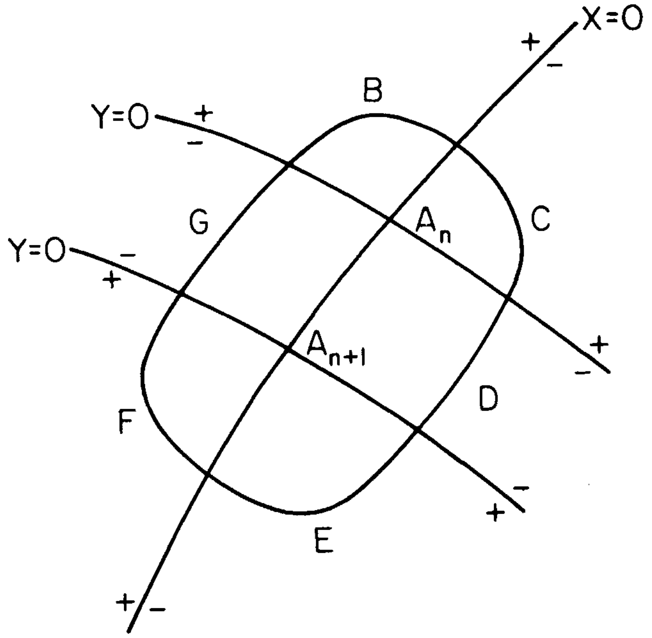

Figure 5. Calculation of the degree of a closed curve which contains a finite number of singular points lying along the same branch of the contour defined by the vanishing of the derivative of one of the state variables.

$+ 8C - 8S = 2$, where, of course, the fact that the index of a saddle point is -1 and that of a node, focus or center is $+1$ has been used.

We also have the constraint equation, $N_0 + S_0 = 1$, since there is only one singular point at the origin. Eliminating N_0 between the two equations, we obtain

$$N_x - S_x + N_y - S_y + 2(N + F + C) - 2S = S_0 \quad (14a)$$

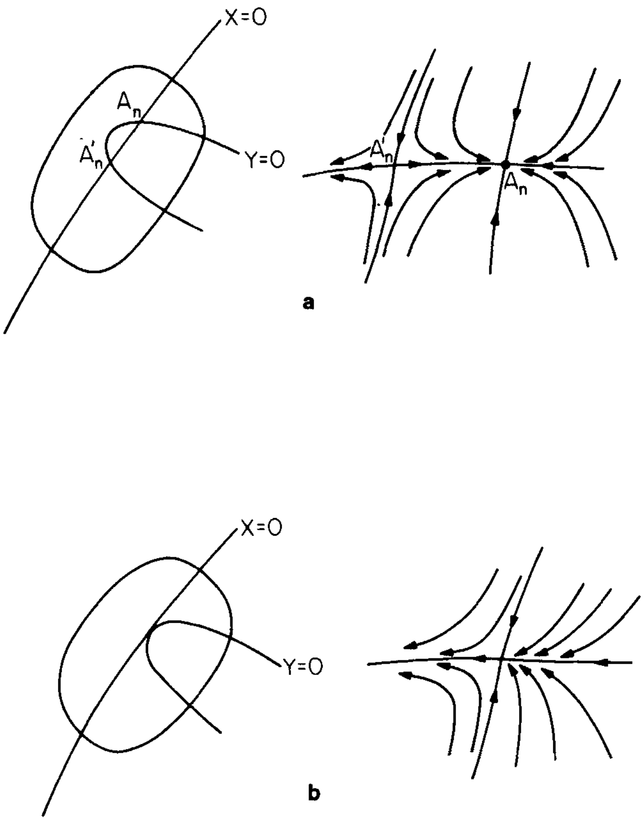

Figure 6. Schematic representation of the formation of a nonelementary singular point at the tangential point of the contours $X = 0$ and $Y = 0$.

If there are no growth limiting secondary substrates ($v = 0$), there can be no foci or centers, and Equation (14a) reduces to

$$N_x - S_x + N_y - S_y + 2N - 2S = S_0 \quad (14b)$$

Equations (14a) and (14b) are the desired relationships. We will show how they can be utilized in specific applications in the following section. Meanwhile, we prove a theorem which is useful in conjunction with the above relationships, thus increasing their potential and applicability.

Theorem. Referring to the system of Equations (6), let A_n and A_{n+1} be two consecutive elementary singular points obtained from the intersection of the curve $Y = 0$ (or $X = 0$) with the same branch of the curve $X = 0$ (or $Y = 0$). If there is no branch of the curve $X = 0$ (or $Y = 0$) passing between A_n and A_{n+1}, then one of the A_n, A_{n+1} will be a saddle point and the other will be a node, a focus or a center.

Proof. Consider the case shown in Figure 5. The two singular points are obtained as the intersections of $X = 0$ with two branches of $Y = 0$. Let the signs of X and Y in the regions B, C, D, E, F and G be as shown. Then, applying Equation (8) for the degree of the vector field relative to the set defined by the closed curve BCDEFGB, we obtain $k = 1$ and $h = 1$ so that the degree relative to this set is zero. Then, according to Equation (11), one of the A_n, A_{n+1} must have an index equal to $+1$ and the other an index equal to -1, or, equivalently, one must be a saddle point and the other a node, focus or a center.

If an odd number of branches of the curve $X = 0$ passes between A_n and A_{n+1}, the theorem is no longer true, and both A_n and A_{n+1} will have the same index. However, if an even number of branches of $X = 0$ passes between A_n and A_{n+1}, the results of the theorem are valid. It can also be seen that the particular choice of signs at the two sides of the curves $X = 0$ and $Y = 0$ is of no importance as long as it is remembered that they change when the corresponding branch is crossed. Finally, if we interchange X and Y in Figure 5, we obtain $k = h = 2$, so that again the degree is zero; therefore, it makes no difference the way A_n and A_{n+1} are formed as long as they lie on the same branch of one of the curves $X = 0$ or $Y = 0$.

A special case is shown in Figure 6b, where one of the branches of $Y = 0$ is tangent to the $X = 0$. This situation is the limiting case of the arrangement shown in Figure 6a as the points A_n and A_n' move closer to each other. A portrait of the local phase planes is given in the same figures. It can be seen that the situation of Figure 6b involves nonelementary singular points and is not included in the above theorem. The theorem is applicable to the case of Figure 6a, and the degree relative to the domain surrounded by the shown closed curve is zero. It is of interest to note that the index of the singular point of Figure 6b is, according to Bendixson's formula, zero too.

APPLICATIONS

General Observations

Inspection of Equations (1a) and (1b) shows that the system of differential equations that describes the dynamics of growth of a mixed culture of two microorganisms in a chemostat can have steady states (singular points) at the origin, the axes or in the interior of the domain (3). These steady states are located as follows:

1. $C_{As} = C_{Bs} = 0$. There is always a steady state at the origin; it represents the total washout of the culture.
2. Steady states on the axis $C_A = 0$. Their location is determined by the solutions of $\mu_B = D$; population A is washed out at these steady states.
3. Steady states on the axis $C_B = 0$. Their location is determined by the solutions of $\mu_A = D$; population B is washed out at these steady states.
4. Coexistence steady states. They are obtained as the solutions of $\mu_A = \mu_B = D$ and lie in the interior of the domain (3).

The possibility of multiple steady states clearly exists, and Equations (14) will be employed to aid in the stability analysis.

Various situations involving zero to four steady states on the axes (partial washout steady states, PWSS) combined with none, one or two coexistence steady states (CSS) are examined below:

1. Zero PWSS. There can be none or two CSS. In the latter case, one has index +1 (node N, focus F or center C), and the other is a saddle point. The origin is always a stable node.
2. One PWSS. If there is no CSS, the PWSS is a stable node. If there is one CSS, it must be N, F or C and the PWSS a saddle point. If there are two CSS, one is a saddle point and the other a N, F or C, and the PWSS is a stable node. The origin is always a saddle point.
3. Two PWSS. If there is no CSS, one of the PWSS is a node and the other a saddle point. If there is one CSS, it can be either N, F or C or a saddle point; the PWSS will be saddle points in the former case and nodes in the latter. If there are two CSS, one is a saddle point and the other a N, F or C; of the two PWSS, one is a node and the other a saddle point. Also, if the two PWSS are located on different axes and one or both are nodes, they are stable. The origin is always a node, stable if both the PWSS lie on the same axis, unstable otherwise.
4. Three PWSS. If there is no CSS, two of the PWSS are nodes, and the other is saddle point; at least one of the nodes is stable. If there is one CSS , it can be either a saddle point or N, F, C; in the former case, the PWSS are all nodes, two of which are stable. In the latter case, one of the PWSS is node, and the other two are saddle points. If there are two CSS, they can both be N, F or C, with three saddle points on the axes (PWSS), or one is N, F, C and the other a saddle point. In this case, one of the PWSS is saddle point and the other two nodes with at least one being stable. The origin is always a saddle point.
5. Four PWSS. If there is no CSS, two of the PWSS are saddle points and two are nodes, one of which, at least, is stable. If there is one CSS, it will be either N, F, or C, in which case three of the PWSS are saddle points and the fourth is node or the CSS will be a saddle point with one of the PWSS being a saddle point too and the other PWSS being nodes, at least one of them stable. If there are two CSS we, have the following possibilities: both CSS are N, F or C, and all PWSS are saddle points; both CSS are saddle points and all PWSS are nodes, or one CSS is N, F or C and the other is a saddle point, while two PWSS are nodes and two saddle points. The origin is always a node, stable if there is an even number of PWSS on each axis, otherwise unstable.

One can proceed in an analogous manner to analyze other cases which involve larger numbers of CSS and PWSS. Such cases, however, are very rare, with interactions involving two populations. Therefore, we pause for a moment to summarize some general conclusions which are derived from the above analysis.

First the type of the origin does not depend on the number of CSS and is a saddle point if the total number of PWSS is odd; it is a node otherwise. In the latter case, it is a stable node if the number of PWSS on each axis is even.

Second, when the total number of PWSS is less than three and there are two CSS, one of them must be a saddle point and the other a node, a focus or a center.

Third, if there are no PWSS, there can be only an even number of CSS, half of which are saddle points and half nodes, foci and centers.

Although a significant number of possible situations can be eliminated with the aid of Equations (14), more information is needed if a unique answer on the type of the various steady states of a given system is to be given. One possibility is to apply the usual stability analysis. We present here another approach which utilizes the results of the theorem that was proved in the previous section.

Let $X_A(C_A,C_B)$ and $X_B(C_A,C_B)$ denote the right-hand sides of Equations (1a) and (1b); that is

$$X_A(C_A,C_B) = C_A(-D + \mu_A)\ ,\ X_B(C_A,C_B) = C_B(-D + \mu_B) \tag{15}$$

Clearly, the steady states of Equations (1a) and (1b) are the

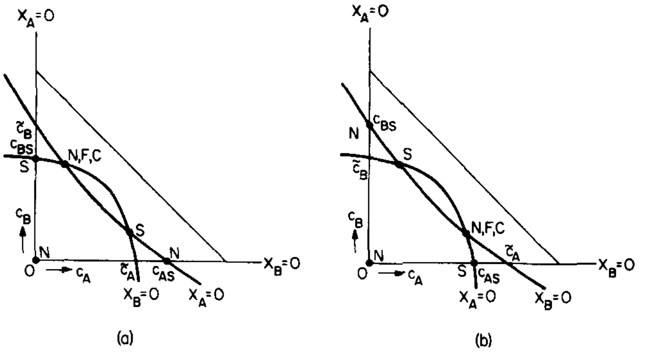

Figure 7. Example of a stability analysis which utilizes Equation (14) and information on the mutual disposition of the contours $X_A = 0$ and $X_B = 0$. The types of the various steady states are interchanged when the two contours are interchanged (Figure 7*b*).

intersections of the contours $X_A = 0$ and $X_B = 0$. The axis $C_A = 0$ is a branch of the contour $X_A = 0$ and the axis $C_B = 0$ is a branch of the contour $X_B = 0$, so that the origin is a steady state. Also, the PWSS are found as solutions of $C_{AS} = 0$, $\mu_B = D$ and $C_{BS} = 0$, $\mu_A = D$. In an indirect interaction, the specific growth rates μ_A and μ_B are functions of the environmental variables s_i, r_j and u_k. By employing the steady state relationships (2) for s_i, r_j and u_k, μ_A and μ_B are functions of C_A and C_B; that is, $\mu_A = \mu_A(C_A,C_B)$ and $\mu_B = \mu_B(C_A,C_B)$. The biomass concentrations of population B, C_{BS}, at a steady state where A is washed out ($C_{AS} = 0$) are found as the solutions of $\mu_B(0,C_{Bs}) = D$. Similarly, the biomass concentrations of population A, C_{AS}, at a steady state where B is washed out ($C_{BS} = 0$) are found as the solutions of $\mu_A(C_{AS},0) = D$.

Now the solutions of $\mu_A(0,\tilde{C}_B) = D$ give the ordinates $\tilde{C}_B$ along the $C_A = 0$ axis of those points at which various branches of the $X_A = 0$ contour intersect the axis $C_A = 0$, that is, the same branch of $X_A = 0$. Similarly, the solutions of $\mu_B(\tilde{C}_A,0) = D$ give the abscissas $\tilde{C}_A$ along the $C_B = 0$ axis of those points at which various branches of the $X_B = 0$ contour intersect the axis $C_B = 0$, that is, the same branch of $X_B = 0$. In this way the points $(0,C_{BS})$, $(0, \tilde{C}_B)$ can be located on the $C_A = 0$ axis as well as the points $(C_{AS},0)$, $(\tilde{C}_A,0)$ on the $C_B = 0$ axis, and then the theorem of the previous section can be applied. Note that the type of the origin is determined by the total number of the PWSS, so that starting from that point the type of the other PWSS can be determined by using the above theorem. The type of the CSS, if they exist, is then determined by applying Equations (14).

As an example, consider the two situations depicted in Figures 7. Steady states are indicated by full dots; the points $(0,\tilde{C}_B)$ and $(\tilde{C}_A,0)$ as well as the branches $X_A = 0$ and $X_B = 0$ are shown. The origin is a node because there are two PWSS. The steady state on the axis $C_B = 0$ of Figure 7a is a stable node because a branch of $X_B = 0$ passes between the two steady states lying along the same branch of $X_B = 0$. On the other hand, the steady state on the axis $C_A = 0$ of the same figure is a saddle point because no branch of $X_A = 0$ passes between this steady state and the origin. If the two contours $X_A = 0$ and $X_B = 0$ are interchanged, the types of the steady states are interchanged as well, as it is shown in Figure 7b.

We proceed below with the analysis of various types of indirect interactions.

Competition for a Single Rate Limiting Substrate

This is the interaction of two populations which grow in a chemostat utilizing a common substrate S which is supplied with the feed (primary substrate). With reference to Equations (2), $p = 1$, $q = 0$, $v = 0$. If the growth of populations is not inhibited at high substrate concentrations, the Monod model $\mu = \mu_m s/(K_s + s)$ is used for the specific growth rates; if one of the species is

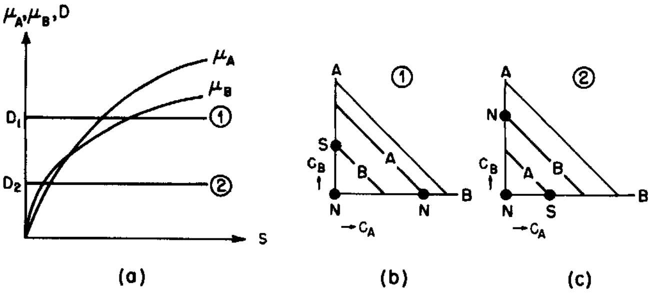

Figure 8. Competition for a single rate limiting substrate: (*a*) specific growth rate curves for uninhibited growth, (*b*), (*c*) the contours $X_A = 0$ and $X_B = 0$, (marked *A* and *B*, respectively), in a (C_A, C_B) diagram.

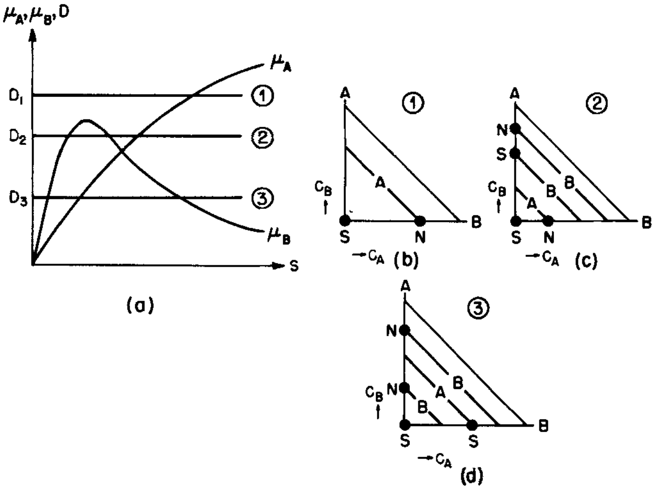

Figure 9. Competition for a single rate limiting substrate: (*a*) specific growth rate curves when the growth of one population is inhibited, (*b* to *d*) schematic representation of the mutual disposition of the contours $X_A = 0$, $X_B = 0$, in a (C_A, C_B) diagram for three different values of the dilution rate. The types of the steady states are as shown.

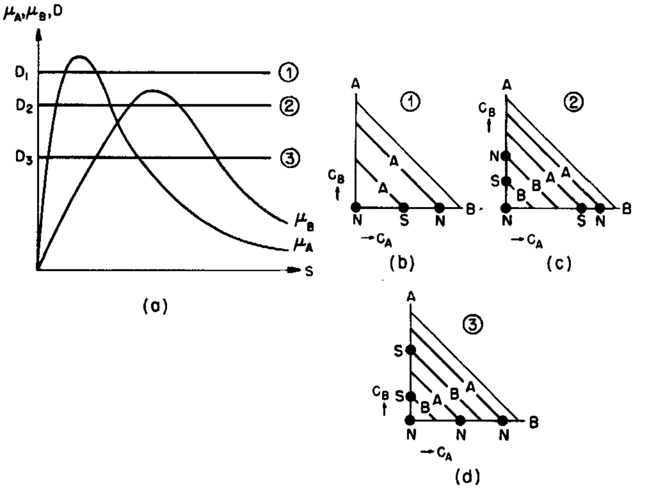

Figure 10. Competition for a single rate limiting substrate: (*a*) specific growth rate curves for inhibited growth, (*b* to *d*) the contours $X_A = 0$, $X_B = 0$ in a (C_A, C_B) diagram.

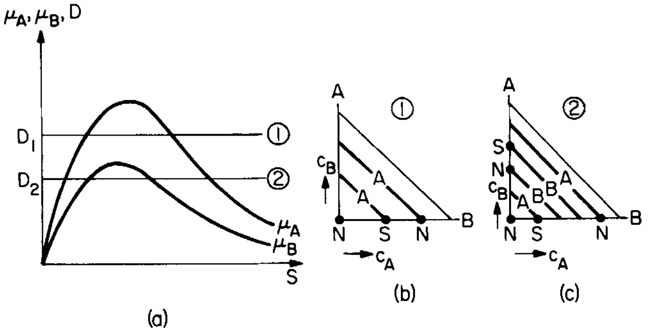

Figure 11. Competition for a single rate limiting substrate: (*a*) specific growth rate curves for inhibited growth with population *A* growing faster than *B* for all substrate concentrations, (*b* to *d*) the contours $X_A = 0$, $X_B = 0$ in a (C_A, C_B) diagram.

inhibited, we use Andrews model $\mu = \mu_m s/(K_s + s + s^2/K_i)$. In the latter case, μ has a maximum as a function of s.

Several possibilities exist, and the outcome of the competition depends on the shape of the specific growth rates, their mutual disposition and the choice of the operating parameters D and s_f. We examine some situations depicted in Figures 8, 9, 10 and 11.

If the growth of the two populations is uninhibited, the specific growth rates are increasing functions of s (Figure 8*a*). For a value of the dilution rate equal to D_1, the PWSS are found as the intersections of μ_A and μ_B with D_1. These intersections define the value of the substrate concentration at the steady state s_s, and then it is seen from Equation (2*a*) that the contours $\mu_A = D_1$ and $\mu_B = D_1$ are parallel straight lines described by

$$\frac{1}{Y_{A1}} C_{AS} + \frac{1}{Y_{B1}} C_{BS} = s_{1f} - s_s \qquad (16)$$

provided that s_{1f} is large enough for the right-hand side of (16) to be positive. These contours are shown schematically in Figures 8*b* and *c* for D equal to D_1 and D_2, respectively, where the contours $X_A = 0$ and $X_B = 0$ are marked by the letters A and B, respectively. Notice that the larger the substrate concentration at the steady state, the closer these contours are located to the origin. Applying the previously outlined procedure, we find that the types of the steady states are as shown in Figures 8*b* and *c*. It should be noticed that the origin is an unstable node, so that for $D = D_1$, population B washes out, while the reverse occurs for $D = D_2$. For a value of D equal to the common value of μ_A and μ_B, the Jacobian determinant vanishes at the coexistence steady state, so that this case cannot be analyzed by the degree theory.

If the growth of one population is inhibited, we have the situation of Figure 9*a*. Three possible cases obtained for the three values of D (D_1, D_2 and D_3) are analyzed in Figures 9*b*, *c* and *d*, respectively. Notice that for cases 2 and 3 we have three contours defined also by Equation (16). The nodes of Figures 9*b* and *c* are stable; only one is stable in Figure 9*d*. Again, the Jacobian determinant vanishes at the intersection of μ_A and μ_B.

The situation of inhibited growth for both populations is analyzed similarly, and the results are shown in Figures 10 and 11 where the types of the various steady states are as indicated. For other possible dispositions of μ_A and μ_B, we can follow the same procedure to derive the results presented in the anslysis of Aris and Humprey (1977).

Competition for Two Rate Limiting Substrates

This is the interaction of two populations which grow in a chemostat utilizing two common substrates which are supplied with the feed (primary substrates), $p = 2$ and $q = v = 0$ for this case. Since two primary substrates are involved, the trajectories will be confined in the domain defined by the two axes and the straight lines $1/Y_{Ai}\, C_A + 1/Y_{Bi}\, C_B = s_{if}$; $i = 1,2$.

We use the multiple saturation model $\mu = \mu_m s_1 s_2/(K_1 + s_1)(K_2 + s_2)$ for the specific growth rates of the two populations (s_1, s_2 are the concentrations of the two substrates). With this model, the contours $\mu_A = D$ and $\mu_B = D$ are hyperboles which are

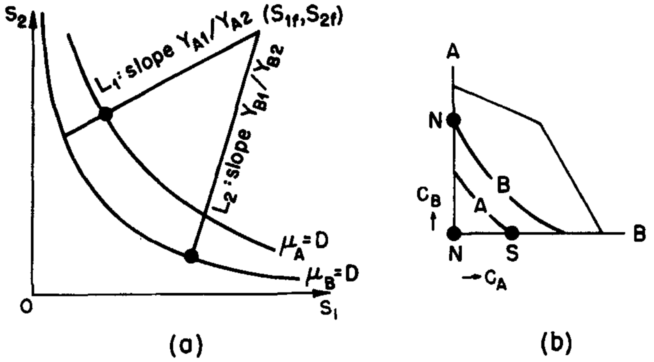

Figure 12. Competition for double rate limiting substrates: (*a*) the contours $\mu_A = D$ and $\mu_B = D$ in a (s_1, s_2) diagram; a CSS does not exist, (*b*) the contours $X_A = 0$, $X_B = 0$ in a (C_A, C_B) diagram.

shown schematically in Figure 12*a*. If these contours intersect with each other, there exist points in the (s_1, s_2) plane where the specific growth rates of both populations equal to D, and the possibility of coexistence clearly arises. Whether these points correspond to meaningful and stable CSS depends on the feed concentrations and the mutual disposition of the contours as discussed below.

In order to apply the procedure outlined earlier, we need to locate the contours $X_A = 0$ and $X_B = 0$ in the (C_A, C_B) plane. Analytically, this can be done by substituting Equations (2*a*) for $i = 1,2$ into the steady state relationships $\mu_A = D$ and $\mu_B = D$, respectively, and the corresponding contours for $X_A = 0$ and $X_B = 0$ are then easily plotted. However, since the exact location of these contours is not essential, and only qualitative information on their mutual disposition is needed for the stability analysis, we can employ the following arguments to obtain this information.

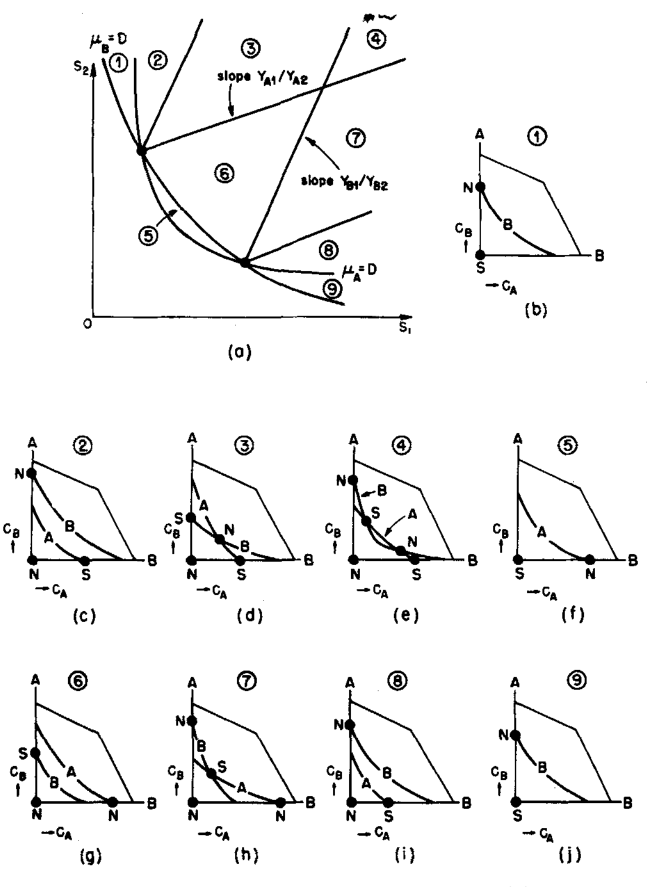

Figure 14. Competition for double rate limiting substrates: (*a*) the contours $\mu_A = D$ and $\mu_B = D$ in a (s_1, s_2) diagram; two CSS exist, (*b* to *j*) mutual disposition of the $X_A = 0$, $X_B = 0$ contours for nine possible feed concentrations of the limiting substrates.

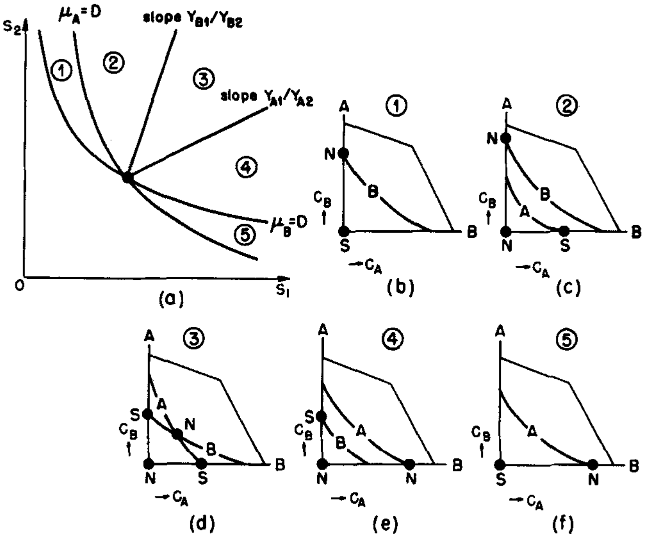

Figure 13. Competition for double rate limiting substrates: (*a*) the contours $\mu_A = D$, $\mu_B = D$ in a (s_1, s_2) diagram; one CSS exists, (*b* to *f*) mutual disposition of the $X_A = 0$, $X_B = 0$ contours for five possible feed concentrations of the limiting substrates.

We examine the relative position along the $C_A = 0$ axis of the points $(0, C_{BS})$ and $(0, \tilde{C}_B)$. [Note that C_{BS} and $\tilde{C}_B$ are the solutions of $\mu_B(0, C_{BS}) = D$ and $\mu_A(0, \tilde{C}_B) = D$, respectively.] For $C_A = 0$, we obtain from Equations (2*a*), for $i = 1,2$, after eliminating C_B:

$$s_2 = \left(s_{2f} - \frac{Y_{B1}}{Y_{B2}} s_{1f}\right) + \frac{Y_{B1}}{Y_{B2}} s_1 \tag{17}$$

This is a straight line in the (s_1, s_2) plane with slope Y_{B1}/Y_{B2} starting from the point (s_{1f}, s_{2f}). Call it L_2. The intersections of L_2 with the contours $\mu_A = D$ and $\mu_B = D$ determine the values of s_1 and s_2 at the points $(0, \tilde{C}_B)$ and $(0, C_{BS})$, respectively. The values of $\tilde{C}_B$ and C_{BS} are then obtained as equal to $Y_{B1}(s_{1f} - s_1)$, with s_1 given the values just found. Referring to Figure 12*a*, we see that $C_{BS} > \tilde{C}_B$ because the value of s_1 corresponding to the point $(0, C_{BS})$ is smaller than that corresponding to $(0, \tilde{C}_B)$. Thus we obtain the arrangement shown in Figure 12*b* along the $C_A = 0$ axis. A similar reasoning gives the arrangement shown along the $C_B = 0$ axis. It is easy, then, to conclude that population A is washed out in this particular case. If the position of the contours $\mu_A = D$ and $\mu_B = D$ is interchanged, the result is that population B will be washed out.

If the two contours $\mu_A = D$ and $\mu_B = D$ intersect at one point, as shown in Figure 13*a*, more possibilities exist depending on the location of the point (s_{1f}, s_{2f}). The five possible cases are analyzed in Figures 13*b* to *f* with the shown results. As an example, we examine the case of Figure 13*d* corresponding to the point (s_{1f}, s_{2f}) being in region 3. We see that any straight line (17) originating from a point in this region and with slope Y_{B1}/Y_{B2} intersects the contour $\mu_B = D$ first and the contour $\mu_A = D$ second. (Note that $C_A = 0$ along the above line, so that a PWSS exists at its intersection with the contour $\mu_B = D$). Consequently, the value of s_1 corresponding to the point $(0, C_{BS})$ is larger than that corresponding to $(0, \tilde{C}_B)$, so that $C_{BS} < \tilde{C}_B$. Similarly, it is found that $C_{AS} < \tilde{C}_A$ for the points $(C_{AS}, 0)$ and $(\tilde{C}_A, 0)$. The contour $X_A = 0$ in the (C_A, C_B) plane passes through the points $(0, \tilde{C}_B)$ and $(C_{AS}, 0)$, while the contour $X_B = 0$ passes through the points $(0, C_{BS})$ and $(\tilde{C}_A, 0)$. These contours are marked by A and B, respectively, and are shown in Figure 13*d*. It is easy to find that the two PWSS are saddle points, the origin an unstable node and the CSS a stable node.

In Figures 14 the same procedure is applied to the case where the contours $\mu_A = D$ and $\mu_B = D$ intersect at two points. The stability analysis is performed very easily, without the need of any linearization or solution of algebraic equations for the steady states. Furthermore, it should be pointed out that the latter

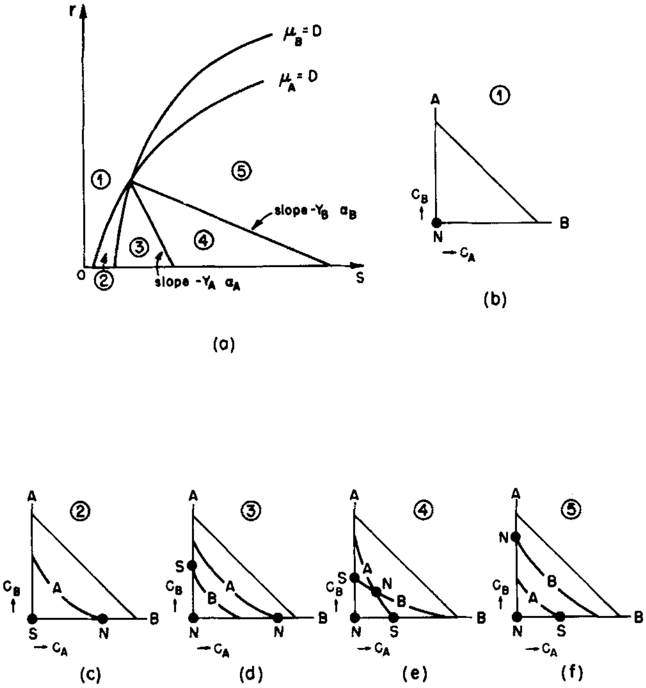

Figure 15. Competition for a single rate limiting substrate between two populations which also produce a common autoinhibitor; (*a*) the contours $\mu_A = D$, $\mu_B = D$; a CSS exists, (*b* to *f*) the contours $X_A = 0$, $X_B = 0$ for five possible feed concentrations of the limiting substrate.

cannot be done analytically so that Taylor and Williams (1974), who first examined this interaction, were unable to conclude in general from their numerical calculations that the two CSS cannot be both stable at the same time. It is easy to see from our analysis that this will be always the case.

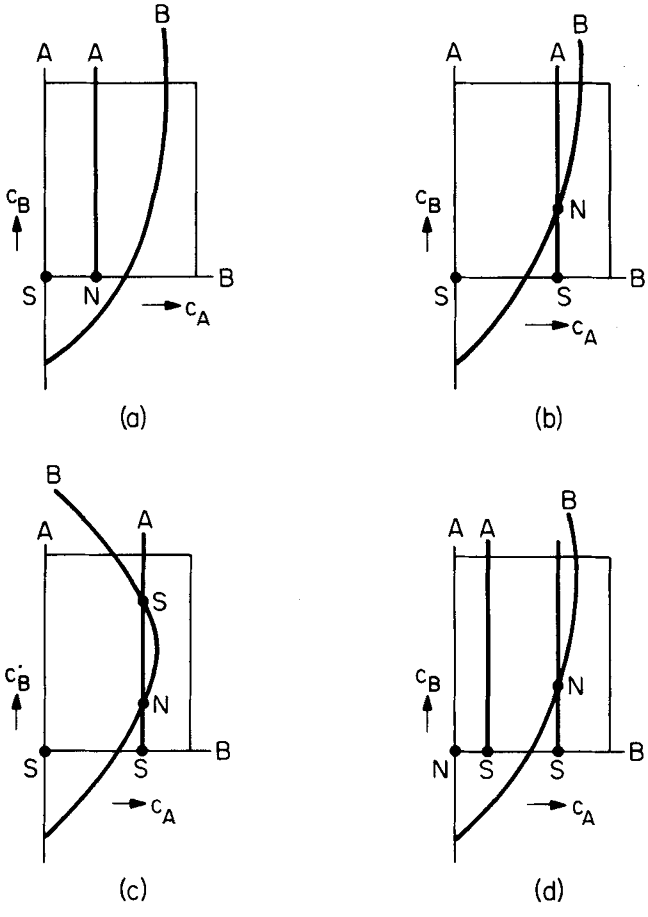

Figure 16. Commensalism: (*a*), (*b*), (*d*) three possible dispositions of the contours $X_A = 0$, $X_B = 0$; the situation shown in (c) is not possible.

Competition with Inhibition

This interaction involves two populations growing on a common primary substrate for which they compete and whose growth is inhibited at the same time by the metabolic products of their activities. The various possibilities that exist (as, for example, the two populations producing specific autoinhibitors or the populations producing inhibitors that do not act on the populations that produce them, etc.) have been analyzed in detail by DeFreitas and Fredrickson (1978). As another application we examine here the case of the two populations producing a common autoinhibitor.

There is one primary substrate in this interaction, ($p=1$), one inhibitor that is produced by both populations and inhibits the growth of both, ($q=1$), and no secondary substrates, ($v=0$). We use the modification of Monod model proposed by Aiba and Shoda (1969) for the specific growth rate: $\mu = \mu_m s/(K_s + s)(1 + r/K_r)$. Proceeding then in a manner analogous to the previous application, we plot the contours $\mu_A = D$ and $\mu_B = D$ in the (s,r) plane. One possible arrangement is shown in Figure 15*a*, where the two contours intersect at a common point. Next we examine the relative position of the points $(0,C_{BS})$, $(0,\tilde{C}_B)$ and $(C_{AS},0)$, $(\tilde{C}_A,0)$ along the axes $C_A = 0$ and $C_B = 0$, respectively.

The steady state equations for s and r are obtained from Equations (2):

$$S = s_f - \frac{1}{Y_A} C_A - \frac{1}{Y_B} C_B \quad , \quad r = \alpha_A C_A + \alpha_B C_B \qquad (18)$$

Letting $C_A = 0$ and eliminating C_B between the resulting equations, we obtain

$$r = Y_B \alpha_B s_f - Y_B \alpha_B s \qquad (19)$$

Equation (19) represents a straight line of slope $-Y_B\alpha_B$, passing from the point $(0,s_f)$. $C_A = 0$ along this straight line so that a PWSS is obtained at the intersection of (19) with the contour $\mu_B = D$. If r is the concentration of the inhibitor at this intersection, the value of C_{BS} at this steady state is obtained from $C_{BS} = r/\alpha_B$. We use the same equation for $\tilde{C}_B$ but with r being the ordinate of the intersection of (19) with the contour $\mu_A = D$ this time.

Similarly, we can let $C_B = 0$ and eliminate C_A between Equations (18) to obtain

$$r = Y_A \alpha_A s_f - Y_A \alpha_A s \qquad (20)$$

Equation (20) represents a straight line of slope $-Y_A\alpha_A$ passing from the point $(0,s_f)$ and its intersections with the contours $\mu_B = D$ and $\mu_A = D$ define the points $(C_{AS},0)$ and $(\tilde{C}_A,0)$, respectively, through the relation $C_A = r/\alpha_A$.

It can be seen from the above discussion that the relative location of the points $(0,C_{BS})$, $(0,\tilde{C}_B)$ and $(C_{AS},0)$, $(\tilde{C}_A,0)$ and therefore of the contours $X_A = 0$ and $X_B = 0$ in the (C_A,C_B) plane depends on the mutual disposition of the contours $\mu_A = D$ and $\mu_B = D$ and the point $(0,s_f)$. Drawing the straight lines with slopes $-Y_B\alpha_B$ and $-Y_A\alpha_A$ from the point of intersection of $\mu_A = D$ and $\mu_B = D$, we see that there are five possible regions where the point $(0,s_f)$ can lie (Figure 15*a*). Each of these cases is analyzed in Figures 15*b* to *f*. The results of the stability analysis are obtained easily by applying the theorem of the previous section and Equation (14). The origin is a stable node when it is the only meaningful steady state. In the other cases, it is a saddle point or an unstable node. All the other nodes indicated in Figures 15*b* to *f* are stable. With the arrangement shown, a CSS exists and is stable. If the contours $\mu_A = D$ and $\mu_B = D$ or the lines (19) and (20) are interchanged, the CSS becomes a saddle point and the PWSS stable nodes. Nothing changes in Figure 15*e* if both the contours and the lines are interchanged.

Commensalism

An interaction between two populations which benefits one population (the commensal population) but does not affect the other (the host population) is an example of commensalism. The word benefit is taken here to mean that the commensal population cannot survive in the chemostat if the host population is not present. As another application we examine the case where the host population (A) grows on a primary substrate S_1, and the commensal population (B) grows on two substrates, one primary S_2 and one secondary U which is the metabolic product of the growth of population A. Thus, $p = 2$, $q = 0$ and $v = 1$ for this interaction.

The steady state relationships (2) reduce to

$$s_1 = s_{1f} - \frac{1}{Y_A} C_A \quad , \quad s_2 = s_{2f} - \frac{1}{Y_B} C_B \qquad (21a,b)$$

$$u = \beta_A C_A - \beta_B C_B \qquad (21c)$$

The solutions of the differential equations for this interaction are confined in the rectangle: $0 \le C_A \le Y_A s_{1f}$, $0 \le C_B \le Y_B s_{2f}$. For a given dilution rate D, the steady state primary substrate concentration s_{1s} is found as the solution of $\mu_1(s_{1s}) = D$. The contours $X_A = 0$ in the (C_A, C_B) plane are the vertical lines $C_A = 0$ and $C_A = Y_A(s_{1f} - s_{1s})$. As usual, one branch of the contour $Y_B = 0$ is the axis $C_B = 0$. The other branch of this contour is obtained by substituting s_2 and u from the steady state relationships (21b) and (21c) into the steady state equation $\mu_B(s_2, u) = D$. If one uses the multiple saturation model $\mu_B = \mu_{mB} s_2 u/(K_1 + s_2)(K_2 + u)$ for μ_B, this gives a quadratic equation in C_B with C_A as a parameter:

$$\begin{aligned} \Pi(C_B) \equiv (\mu_{mB} - D)\frac{\beta_B}{Y_B} C_B{}^2 + \Big\{ - (\mu_{mB} - D)\frac{\beta_A}{Y_B} C_A - \\ (\mu_{mB} - D)\beta_B s_{2f} + DK_1\beta_B + DK_2\frac{1}{Y_B}\Big\} C_B \\ + \{(\mu_{mB} - D)s_{2f}\beta_A C_A - DK_1K_2 - DK_1\beta_A C_A - DK_2 s_{2f}\} = 0 \end{aligned} \qquad (22)$$

The points of intersection of the contour $\mu_B = D$ with the $C_A = 0$ axis are found as the solutions of $\Pi(C_B) = 0$, for $C_A = 0$. There are two such solutions, one negative and one positive, but always larger than $Y_B s_{2f}$ and therefore meaningless. This is in accordance with our definition of the commensal interaction, where the commensal population B cannot survive if the host population A is not present.

Two possible arrangements of the contours $X_A = 0$ and $X_B = 0$ are shown in Figures 16a and b. The types of the steady states are found by following the same as before procedure. Since $\Pi(C_B)$ is a quadratic equation, one might think that the possibility of two coexistence steady states exists; such an arrangement is shown in Figure 16c. It can be seen, however, that Equation (14a) and the theorem of the previous section cannot be satisfied simultaneously. The types shown in Figure 16c, for example, do not satisfy Equation (14). This is taken as an indication that the arrangement of Figure 16c cannot exist; indeed, examination of Equation (22) shows that $\Pi(Y_B s_{2f}) < 0$ for any $C_A \epsilon [0, Y_B s_{2f}]$ so that there is only one intersection of the contours $X_A = 0$ and $X_B = 0$ in the domain of meaningful solutions.

If the growth of A is inhibited at large substrate concentrations, there are two solutions to $\mu_A(s) = D$; Figure 16d depicts one possibility for this case.

Classicial Mutualism: Singular Points at Infinity

An interaction between two populations which benefits both populations is an example of mutualism. The term benefit is taken here to mean that each population cannot live in the absence of the other in a chemostat; it can also be used to describe other situations where the mutualistic interaction is not so strong. In the interaction of our example, population A grows on a limiting substrate, which is the metabolic product of population B, and B grows on a limiting substrate produced by population A. Thus there are no primary substrates or inhibitors ($p = q = 0$) but only two secondary substrates $v = 2$.

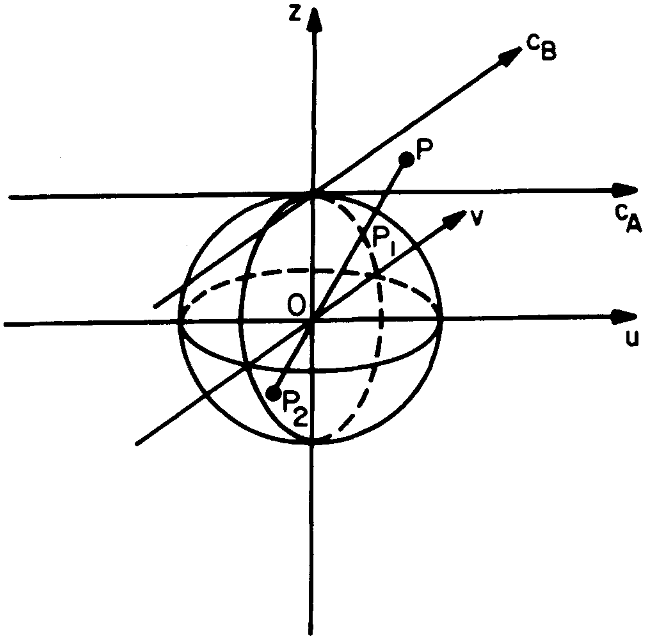

Figure 17. Projection of the (C_A, C_B) plane on a sphere.

A washout steady state can be obtained for all values of D. Also, if D is smaller than the maximum specific growth rates of both populations, and if, in addition, $\beta_{B1}\beta_{A2} > \beta_{A1}\beta_{B2}$ (that is, if the production of secondary substrates exceeds their consumption), a mutualistic steady state exists for which C_A, $C_B \neq 0$. Linearized stability analysis, however, shows that the washout steady state is always a stable node, while the CSS is a stable point (Meyer, et al., 1975), and then we see that Equations (14) are not satisfied. It should be pointed out that the above results are obtained regardless of whether the growth of one population is inhibited at high substrate concentrations or not.

The reason that Equations (14) cannot be used for the types of the steady states of a mutualistic interaction is that Equations (14) were developed for bounded domains, and therefore they cannot be applied to cases which involve singular points at infinity. In the interaction of mutualism there exists no growth limiting primary substrate so that the solutions of the differential

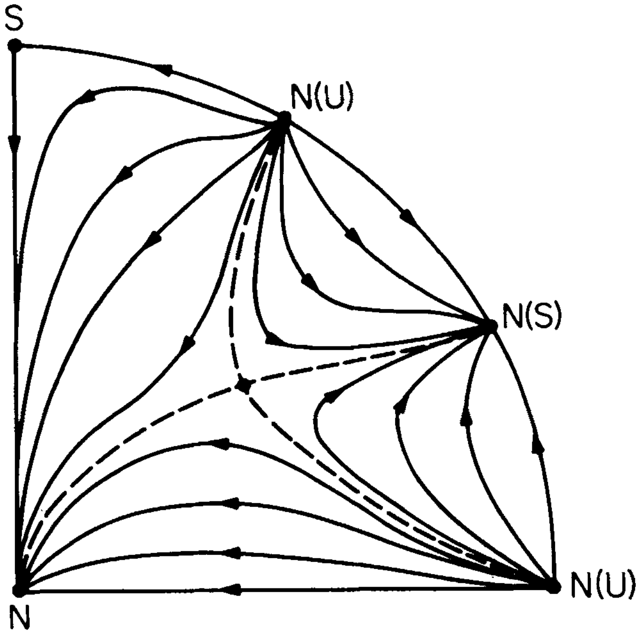

Figure 18. Phase plane portrait for the interaction of mutualism including singular points at infinity.

equations lie anywhere in the positive quadrant of the (C_A, C_B) plane extending all the way to infinity. Clearly, singular points *exist at infinity*, and, to facilitate the analysis in this case, it is convenient to project the (C_A, C_B) plane on a sphere as follows

Consider together with the (C_A, C_B) plane a Cartesian system (u, v, z) such that the (C_A, C_B) plane is the plane $z = 1$ for this system. The C_A, C_B axes are parallel to the u, v axes and have their origin at (0,0,1) as shown in Figure 17. Consider also a sphere of radius $\rho = 1$ centered at the origin of the (u, v, z) system.

If P is a point in the (C_A, C_B) plane, the ray OP intersects the sphere at two points $P_1 = (u_1, v_1, z_1)$ and $P_2 = (u_2, v_2, z_2)$. Clearly, $u_1^2 + v_1^2 + z_1^2 = u_2^2 + v_2^2 + z_2^2 = \rho^2 = 1$. Also

$$\frac{u_i}{C_A} = \frac{v_i}{C_B} = \frac{z_i}{1} = \frac{\sqrt{u_i^2 + v_i^2 + z_i^2}}{\sqrt{C_A^2 + C_B^2 + 1}} = \frac{1}{\rho} \quad ; \quad i = 1, 2 \tag{23}$$

and $C_A = u_i/z_i$, $C_B = v_i/z_i$, or, after dropping the indexes

$$C_A = u/z, \ C_B = v/z, \ u^2 + v^2 + z^2 = 1 \tag{24}$$

Transformation (24) takes a curve on the (C_A, C_B) plane to two symmetrical with respect to 0 curves on the sphere, straight lines through the origin of the plane to great circles on the sphere, etc. Any finite point on the (C_A, C_B) plane has two images on the two hemispheres. Points at infinity are projected on the equator of the sphere and conversely.

If the growth of the two populations is described by the equations $\dot{C}_A = X_A(C_A, C_B)$ and $\dot{C}_B = X_B(C_A, C_B)$, one can eliminate C_A and C_B by applying the transformation (24) to the above equations and to

$$u du + v dv + z dz = 0 \tag{25}$$

to obtain the system

$$\dot{u} = U(u, v, z) \tag{26a}$$

$$\dot{v} = V(u, v, z) \tag{26b}$$

$$\dot{z} = Z(u, v, z) \tag{26c}$$

The mechanics of this transformation can be found in Sansone and Conti (1964), Lefschetz (1963) or any other of the references mentioned earlier. The point to notice here is that the trajectories of Equations (26) which have a common point with the sphere $u^2 + v^2 + z^2 = 1$ actually lie on this sphere since they satisfy (25); furthermore, they are images of the trajectories in the (C_A, C_B) plane because they satisfy $\dot{C}_A = X_A$ and $\dot{C}_B = X_B$.

The points (u, v, z) at which $U = V = Z = 0$ are the singular points of (26). Those for which $z \neq 0$ correspond to singular points of the original system; those for which $z = 0$ and therefore lie on the equator are the images of the singular points of the original system that lie at infinity.

Now if we associate with every point of the sphere P the vector $W(P) = (U, V, Z)$, then we have a continuous distribution of vectors on the sphere analogous to the one defined previously on the plane (C_A, C_B). At a singular point, the vector W reduces to the zero vector. Note also that $W(P)$ lies on the tangent plane at P because it satisfies Equation (25).

At this point we can introduce the concept of the index of a point on the sphere. Thus, if P is a point on the sphere, we project a neighborhood of P from the center of the sphere to the plane that is tangent to the sphere at P. This projection takes the vectors $W(P)$ into the vectors $W'(P)$ on the tangent plane, and the index of P with respect to $W(P)$ is defined to be the index of P with respect to $W'(P)$.

Again, the index of a regular point is zero. The index of a singular point not on the equator is equal to the index of the singular point in the original system. The index of an isolated singular point on the equator defines the index of the singular points of the original system at infinity.

If the singular points on the equator do no fill it completely, then they are finite in number and are called isolated singular points. If P_0 is such an isolated singular point on the equator, then two arcs of trajectories of (26) meet at P_0, and they are precisely two arcs of the equator. Because of the uniqueness of the solutions of the system (26), singular point P_0 cannot be a focus or a center. Therefore, only nodes and saddle points exist on the equator.

Now if $N_{x\infty}$ and $S_{x\infty}$ are the numbers of nodes and saddle points, respectively, at the intersection of the $C_B = 0$ axis with the equator, $N_{y\infty}$ and $S_{y\infty}$ similarly are the numbers of nodes and saddle points at the intersection of the $C_A = 0$ axis with the equator and N_∞, S_∞ the numbers of nodes and saddle points on the part of the equator that belongs to the first quadrant, application of Hopf's theorem gives:

$$\begin{aligned} 2N_0 - 2S_0 + 4N_x - 4S_x + 4N_y - 4S_y + 8N + 8F + 8C \\ - 8S + 2N_{x\infty} - 2S_{x\infty} + 2N_{y\infty} - 2S_{y\infty} + 4N_\infty - 4S_\infty = 2 \end{aligned} \tag{27}$$

We also have the constraints:

$$N_0 + S_0 = 1 \quad , \quad N_{x\infty} + S_{x\infty} = 1 \quad , \quad N_{y\infty} + S_{y\infty} = 1 \tag{28}$$

Eliminating N_0, $S_{x\infty}$ and $S_{y\infty}$ between (27) and (28), we obtain

$$\begin{aligned} N_x - S_x + N_y - S_y + 2(N + F + C) \\ - 2S + N_{x\infty} + N_{y\infty} + N_\infty - S_\infty = 1 + S_0 \end{aligned} \tag{29}$$

Equation (29) must be satisfied by the numbers of the possible singular points if singular points at infinity exist. For the interaction of mutualism under examination, we obtain

$$N_{x\infty} + N_{y\infty} + N_\infty - S_\infty = 3. \tag{30}$$

Examining the behavior of Equations (1*a*), (1*b*) and (2*c*) at infinity, (again, the references given earlier can be consulted for the procedure involved), we find that there are two singular points on the quarter of the equator between the positive axes, in addition to the ones at the intersection of the equator with the axes $C_A = 0$ and $C_B = 0$. Equation (30) shows that the singular points of the equator that do not lie on the axes must be nodes ($S = 0$, $N = 2$), and one of the singular points at infinity that lie on the axes must be a node while the other one is a saddle point. A schematic representation of the phase plane portrait is given in Figure 18. Thus, by utilizing the degree theory we have been able to obtain information on the nature of the singular points at infinity without going through the troublesome procedure of projecting the neighborhood of each singular point to the tangent plane and analyzing the characteristics of the projected trajectories for stability.

CONCLUDING REMARKS

In this paper we have obtained some structural relationships between the pure component and mixed culture steady states occurring in an interacting mixture of microorganisms which grow in a chemostat, and at the same time we have tried to present a method for the stability analysis of the steady states exhibited by such a system.

These structural relationships allow one to obtain general results on the possibility of coexistence in a continuous flow system and also give useful information on the behavior of growth of mixed cultures in a chemostat. The method of stability analysis, which is based on the degree theory, is a combination of the above structural relationships [Equations (14) and (29)], and a theorem that relates the types of the steady states that can be found along the same branch of the contour defined by the vanishing of the derivative of one of the state variables. This method can be used by itself, in combination with the traditional linearized stability analysis, or as a means of verification of the results of the latter.

Using these tools, we first derived some general results concerning the nature of the possible steady states of a mixed culture of microorganisms. Subsequently, several cases of indirect interactions were analyzed. Comparison of the obtained information demonstrated the speed and efficiency of the method as well as the generality of the derived results, even in cases where the previous methods failed to provide such general results because of the lack of analytical solutions. Despite these results, however, it is not our intent to offer a substitute to the linearized stability analysis but rather to complement it with useful tools which will extend its applicability and emphasize its usefulness by strengthening the validity and increasing the generality of its results.

The degree theory can probably be applied to the analysis of other chemical engineering systems, as, for example, to the analysis of the steady states of a CSTR. No singular points exist on the concentration, temperature axes so that it will be relatively simpler to obtain the degree relative to a domain that encloses all steady states, since the introduction of an equivalent vector field on the 2-sphere will not be necessary for this case.

A final point should be discussed regarding the dimensions of the systems that can be analyzed in an analogous manner. Only two-dimensional systems were considered in our analysis. The bases of the theory, namely, the definition of the degree of a singular point through the sign of the Jacobian determinant and Hopf's theorem, are valid for higher-dimensional systems too. There are, however, several problems in deriving equations similar to Equations (14) for higher-dimensional systems stemming mainly from the difficulties of constructing the n sphere that corresponds to an n-dimensional system and that of picturing the behavior of the trajectories around a singular point whose index is defined by the sign of a determinant. The extension of the results presented in this paper to higher dimensional systems is the subject of current research.

ACKNOWLEDGMENT

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NOTATION

C_A, C_B = biomass concentration of species A, B, respectively
$C(\bar{D})$ = set of continuous vector valued functions defined on $\bar{D} \subset R^n$
$C'(\bar{D})$ = subset of continuously differentiable vector valued functions on $\bar{D} \subset R^n$
D = dilution rate; also an open set in R^n and, for a two-dimensional system, the domain in the positive quadrant where the solutions of the differential equations are confined
$\bar{D}$ = closure set of D
$d(\utilde{f}, \utilde{p}, D)$ = degree of function $\utilde{f}$ relative to the point $\utilde{p}$ and the set D
∂D = boundary set of D
$\utilde{f}$ = vector function with components f_i
h = the number of times Y/X jumps from $-\infty$ to $+\infty$
$I(\utilde{f}, \utilde{p}, \utilde{x})$ = index of function $\utilde{f}$ at the point $\utilde{x}$ relative to the point $\utilde{p}$
J_f = Jacobian matrix of function $\utilde{f}$
k = number of times Y/X jumps from $+\infty$ to $-\infty$
K_i = constant in the Andrews model of inhibition
K_r = constant in the Aiba and Shoda model
K_{sA} = Michaelis constant of the specific growth rate of A
K_1, K_2 = constants in the multiple saturation model
n_e, n_h = number of elliptic and hyperbolic sectors of a singular point
p = number of primary substrates
$\utilde{p}$ = point in R^n
q = number of inhibitors
R^n = Euclidean space of dimension n
r_j = concentration of a metabolic product that inhibits growth
s_i = concentration of the i^{th} growth limiting primary substrate
u_k = concentration of the k^{th} growth limiting secondary substrate
V = vector field with components (X, Y)
v = number of secondary substrates
X, Y = real continuous functions of x and y, Equation (6)
X_A, Y_A = defined by Equations (15)
$\utilde{x}$ = abstract state vector
Y_{Ai} = yield coefficient of population A growing on rate limiting substrate i

Greek Letters

α_{Aj} = stoichiometric coefficient for the production of the inhibitory substance j as a metabolic product of A
β_{Ak} = stoichiometric coefficient for the production (consumption) of the secondary substrate k as a metabolic product (limiting substrate) of population A
μ_A = specific growth rate of population A
μ_{mA} = maximum specific growth rate of population A
θ = angle between the vector $V(P)$ and a fixed direction

Subscripts

f = feed
s = steady state

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Hindered Settling of a Suspension at Low Reynolds Number

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The purpose of this paper is to analyze how the settling velocity of a dispersion of spherical particles (that is, drops of arbitrary viscosity) depends on concentration. The procedure used entirely avoids the divergent integrals which previous workers in this field have been forced to deal with and yields explicit formulas for the sedimentation velocity. These formulas, accurate to first order in the particle volume fraction, depend on both the physical characteristics of the particles (size, buoyant density, viscosity ratio) as well as on any long range forces (for example, electrical double layer repulsions or van der Waals attraction) which may exist between particles. Sample calculations are given for globular proteins subject to double layer repulsions and for micron size colloids which experience van der Waals attractions in addition to the electrostatics. In the latter case, it is shown how the Hamaker constant can be extracted from sedimentation data. The analysis is extended to more concentrated suspensions by assuming that the hydrodynamic interactions among particles are pairwise additive; comparison with published data shows this analysis to be reasonably accurate, even for dense suspensions, without any adjustable parameters.

SCOPE

The question addressed in this paper is: How does the presence of other particles dispersed in a Newtonian fluid influence the settling velocity of a typical particle? The problem is to find α_{ij} of Equation (2) and then to extend the model to include higher-order concentration effects. By particle we mean a spherical drop of arbitrary viscosity that is sufficiently small to be in the Stokes flow regime ($Re << 1$). The driving force for the settling is an externally controlled body force due to, say, gravity or centrifugal acceleration. We begin the analysis by developing a virial expansion of the concentration effect and focus on the first-order coefficient which represents the summation of hydrodynamic interactions between pairs of particles. Evaluation of this coefficient is made possible by utilizing constraints on the suspension, such as incompressibility (zero volume flow), which have been recognized by Batchelor (1972) and others (for example, Pyun and Fixman, 1964) as necessary to use the velocity fields generated by particles in an unbounded fluid when computing the average hydrodynamic interactions between particles in a bounded fluid. The results of the first-order analysis for concentration effects in a polydisperse suspension [Equations (24) and (41)] clearly show how physical properties such as drop viscosity, density and size must be accounted for and, further, how important a factor the microscopic spatial distribution of particles is to the settling rate of the suspension. In our analysis this microscopic distribution is assumed to be controlled by local equilibria.

Although the virial expansion described in Appendix C represents a formal method to include C^2 and higher-order concentration effects, it is not useful at present because the n particle hydrodynamic problem is not completely solved for $n \geq 3$. Instead, we extend the analysis to more concentrated suspensions by assuming pairwise additivity of the hydrodynamic interactions and by preaveraging the interactions among all particles except the typical one (called the test particle). The hindered settling rate of the suspension is then determined by allowing the test particle to sample positions within the suspension without perturbing it. Published experimental data for the settling of dense suspensions are compared with the results of this model to test the accuracy of this reasonable, although still ad hoc, preaveraging step in the analysis.

CONCLUSIONS AND SIGNIFICANCE

The first-order hindrance coefficient α_{ij} [see Equation (2)] is given approximately by Equation (43) if all particles have the same density. Its value is greatest for rigid particles ($\lambda = 1.5$) and smallest for gas bubbles ($\lambda = 1.0$); larger neighbors ($a_j > a_i$) tend to hinder the settling of type i particles more than do smaller neighbors. Contrary to intuition, repulsive forces acting between particles, which cause greater average separations between particles than in the case of a uniform distribution, result in a greater hindrance to settling. This surprising result is explained by noting that at large separations a pair of settling particles experience more of each other's (retarding) backflow generated by the boundaries of the suspension,

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