

CHEMOSTAT DYNAMICS OF PLASMID-BEARING, PLASMID-FREE MIXED RECOMBINANT CULTURES

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Abstract—The problem of plasmid stability and strain reversion in recombinant cultures is investigated through a complete stability analysis of a plasmid-bearing, plasmid-free mixed culture growing in a chemostat. Using a general method based on the index theory of a singular point, the complete stability portrait of all competitive interactions is obtained which can occur under all possible mutual dispositions of the specific growth rate curves and chemostat dilution rate. It is found that such a mixed culture can coexist in a chemostat only if there is a range of substrate concentrations where the plasmid-bearing cells grow at a specific rate which is larger than the specific growth rate of the plasmid-free cells. Realistic further genetic modifications that could possibly yield a culture with such properties are discussed.

INTRODUCTION

A problem often encountered in recombinant DNA experiments is the instability of the recombinant plasmid DNA. During growth, or even storage, a population of plasmid-bearing cells often segregates to give plasmid-free cells or cells that contain modified plasmids. Such revertant cells do not produce the desired foreign-gene product and, in most cases, they enjoy a growth advantage over the plasmid-bearing (parental) cells. In large-scale production of recombinant DNA products, instability of plasmids is a major problem because of the importance of the efficiency and productivity of the fermentation process. These fermentations involve a large number of generations (over 50 from the inoculum), allowing ample time for takeover of the population by the faster growing modified cells. In some cases, especially with pharmaceuticals which have to be manufactured under GMP (Good Manufacturing Practice) conditions, the drug regulatory authorities consider instability undesirable. Changes in the plasmid could cause changes in the amino acid sequence of a protein product or changes in the background from which it must be purified. It is vital to produce a uniform product if it is a drug intended for human use.

A number of studies to-date have reported on culture instabilities observed with recombinant *Escherichia coli* (Imanaka and Aiba, 1981; Imanaka *et al.*, 1981; Dwivedi *et al.*, 1982; Kim and Ryu, 1984) *Bacillus stearothermophilus* (Aiba and Koizumi, 1984) and *Bacillus subtilis* (Kreft *et al.*, 1978; Ehrlich *et al.*, 1982), among others. Especially in the case of *B. subtilis*, recombinant plasmids show a great tendency to undergo alterations and this has prevented the widespread use of this organism as a host of genetic recombination despite its many other advantages, such as its lack of pathogenicity and toxicity, its ability to excrete many proteins and its history as an industrial microorganism.

Two general mechanisms are responsible for plasmid instability: (1) Plasmids may be lost from some cells due to unequal segregation, and (2) Plasmids may be maintained in an altered form after undergoing structural changes. However, these initial modifications are usually rare events and altered plasmids or cells would not be detected in the absence of a growth advantage over the parental strain. Competition between revertant and parental cell populations is, therefore, a key issue in the stability of recombinant cultures and the analysis of the dynamics of such systems naturally falls in the realm of mixed culture research.

Theoretical models have been proposed by several investigators (Imanaka and Aiba, 1981; Kim and Ryu, 1984; Anderson and Lustbader, 1975) for inheritability of plasmids and population dynamics as well as modifications and extensions to include substrate consumption and product formation (Ollis and Chang, 1982) and to describe behavior in continuous culture (Dwivedi *et al.*, 1982). More complicated mathematical models for plasmid stability have been proposed to interpret data from: lambda-dv plasmid replication and analysis of copy number mutants (Lee and Bailey, 1984a); analysis of growth rate effects on plasmid content and productivity of *E. coli* plasmids, (Lee and Bailey, 1984b); analysis of the stability of plasmids in budding yeast populations at steady-state growth and during the dynamics following a shift to a nonselective medium (Hjortso and Bailey, 1984) and kinetics of loss of plasmids with initiation of replication as a Poisson process (Nordstrom and Aagaard-Hansen, 1984).

The purpose of this work is to present a comprehensive stability portrait of a recombinant culture growing in a chemostat. Following Imanaka and Aiba (1981), the biological system is modelled as a mixed culture consisting of a plasmid-bearing (parental) cell population and a cell population with no plasmids or

with modified plasmids (revertant cells). Certainly, a rigorous treatment of the subject must recognize the segregated nature of the cell population with respect to the different plasmid numbers and foreign-gene product formation of the various cells (Hjortso and Bailey, 1984). For the purpose of analysing, however, the general dominance and coexistence characteristics in recombinant cultures, the lumping, implicit in the modeling of such systems as mixed cultures consisting of two competing cell populations, seems to be well justified. A previous work (Ryder and DiBiasio, 1984) has presented a stability analysis of these systems through linearized eigenvalue analysis on arbitrary growth kinetics. Although general, results are specific to the parameter values used and no generalizations are allowed to all possible combinations of cell growth kinetics and chemostat dilution rates that may result in stability or instability of the culture. By invoking a method based on the theory of the index of a singular point, the complete steady state portrait of the above mixed culture can be determined by the shape and mutual disposition of the specific growth rate curves of the parental and revertant cell populations. An exhaustive stability analysis of all possible combinations of uninhibited Monod and substrate inhibited Andrews kinetics is presented and can be used as a guide for selecting the operating conditions or further genetic modifications that insure the stability and robustness of the culture.

MODEL EQUATIONS

The kinetic model is based on the following assumptions:

- (1) A culture consists of two types of cells: those harboring recombinant plasmids (parental cells), and "negative variants", i.e. cells that lose the plasmid or harbor modified plasmids. Cells harboring recombinant plasmids produce negative variants at a constant probability.
- (2) Negative variants cannot revert to give parental cells.
- (3) Parental cells and negative variants may have different growth rates and yields

The mass balances for parental cells, P , and negative revertant cells, N , in a chemostat with dilution rate D are:

$$\dot{P} = \mu_1(S)P(1-p) - DP \quad (1)$$

$$\dot{N} = \mu_2(S)N + p\mu_1(S)P - DN \quad (2)$$

where p is the probability that upon division a plasmid is lost or becomes modified. Hence, the plasmid loss for the P population is accounted for by the term $p\mu_1(S)P$ in eq. (1). The specific growth rates of the P and N cell populations are μ_1 and μ_2 , respectively, assumed to be functions of the limiting substrate concentration S . A mass balance for the latter gives

$$\dot{S} = D(S_f - S) - \frac{1}{\gamma}\mu_1(S)P - \frac{1}{\gamma}\mu_2(S)N \quad (3)$$

where the same growth yield has been assumed for the growth of both populations and S_f is the concentration of the limiting substrate in the feed.

For the purpose of analyzing the stability of the steady states of eqs (1) and (2), eq. (3) may be substituted by the corresponding steady state relationship:

$$S = S_f - \frac{1}{\gamma}(P + N). \quad (4)$$

The substrate concentration is bounded between 0 and S_f and both P and N are non-negative and less than γS_f .

Previous arguments (Aris and Humphrey, 1977) have justified the use of eq. (4) in the sense that if the state of the chemostat lies initially on the plane defined by eq. (4) (equivalent to filling the chemostat with medium at concentration S_f and subsequently seeding with a small inoculum of P cells) it will stay on this plane for all time. If a trajectory starts at a point off the plane, then it approaches the plane asymptotically and the behavior of the trajectory as it approaches the steady state is the same as if the trajectory was on the plane at all times. Therefore, local stability behavior for the full system can be obtained by considering the projections of the trajectories in the (P, N, S) space onto the plane defined by eq. (4). Such a dimensional reduction can be carried out in mixed culture systems with growth-associated indirect interactions and yields a much simpler system for analysis. Such a reduction is not possible in cases involving non-growth associated substrate consumption or product formation, or when the structure of eqs (1)–(3) is altered in ways that sometimes seem to be demanded by biological considerations (Baltzis and Fredrickson, 1983). Had the stability of the full system of eqs (1)–(3) been considered, the resulting eigenvalues would be the same as the eigenvalues of the reduced system with the extra dimension yielding an eigenvalue equal to $-D$.

There are three types of steady states for the system of eqs (1), (2) and (4). Total washout occurs for $P = N = 0$ and $S = S_f$. Parental cell washout occurs for $P = 0$ and $N > 0$, and coexistence steady state for $P > 0$ and $N > 0$. It should be noted that revertant cell washout is not possible for when $p \neq 0$ there is a continuous seed of N cells from the parental cell population so that the derivative of eq. (2) cannot be zero. In view of this, the analysis and discussion in this paper are geared towards achieving a stable coexistence steady state as the situation that can insure the presence of parental cells in the culture. As it will be shown in the sequel, some coexistence steady states approach the situation of parental cell growth quite closely.

STABILITY ANALYSIS

The usual method of stability analysis is through linearization of the dynamic equations about the steady state under scrutiny and eigenvalue analysis. A method based on the theory of the index of a singular

point is preferable because it can produce general results for given shapes and mutual disposition of the specific growth rate curves, and it is also faster yielding results on steady state stability by simple inspection of the growth rate curves. The drawback is that the method cannot differentiate between a node, focus or center, but rather between a saddle point and the three previous types of steady states as a group. However, general results exist which allow the unique determination of the nature of a steady state.

A basic discussion of the theory can be found elsewhere (Stephanopoulos, 1980) along with applications to various types of indirect interactions (Stephanopoulos, 1981). Presented here is an important result that will be used for the construction of the full stability diagram of the recombinant culture.

Result from the index theory

Let the dynamics of two state variables be described by

$$\frac{du}{dt} = X(u, v)$$

$$\frac{dv}{dt} = Y(u, v).$$

A steady state of the system is defined to be a point (u, v) which is an intersection of the contours of $X = 0$ and $Y = 0$. Let A_1 and A_2 be two consecutive steady states formed by the intersection of $Y = 0$ ($X = 0$) with the same branch of $X = 0$ ($Y = 0$). If there are 0, 2, 4, . . . branches of $X = 0$ ($Y = 0$) passing between A_1 and A_2 , then one of the A_1, A_2 is a saddle point and the other is a node, focus or center. If there are 1, 3, 5, . . . branches of $X = 0$ ($Y = 0$) passing between A_1 and A_2 , then both are saddle points or both are nodes, foci or centers.

Figure 1 presents two sample results from the theorem. In Fig. 1(a), since there are no branches of $X = 0$ between A_1 and A_2 , one of the steady states is a saddle point, and the other is a node, focus or center. In Fig. 1(b), since there is one branch of $X = 0$ between A_1 and A_2 , both of the steady states are saddle points, or both are nodes, foci or centers. This result is quite powerful for analysing the stability of a system. Given the contours of $X = 0$ and $Y = 0$ in a solution space and the type of one of the steady states, the type of some or all of the other steady states may then be determined by the application of this result.

In order to apply the above result to the recombinant culture dynamics, one needs, first, to define the steady state contours $X = 0$ and $Y = 0$ for the system defined by eqs (1), (2) and (4), and second, to determine the stability of the washout steady state by an eigenvalue analysis. Starting from the latter steady state and moving along the contours $X = 0$ and $Y = 0$, the stability of the possible steady states is determined by using the above results.

Using the steady state eq. (4) for S, μ_1 and μ_2 can be expressed as functions of P and N , so that the

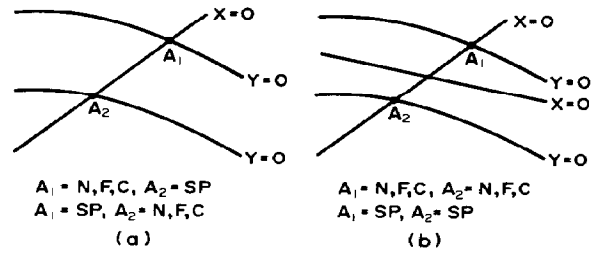


Fig. 1. Illustration of the result from the index theory to two different sets of contours. N = node, F = focus, C = center, SP = saddle point.

recombinant culture dynamics is written as:

$$\dot{P} = X(P, N) \quad (5)$$

$$\dot{N} = Y(P, N) \quad (6)$$

with the functions X and Y defined as

$$X(P, N) = (1-p)\mu_1(P, N)P - DP \quad (7)$$

$$Y(P, N) = \mu_2(P, N)N + p\mu_1(P, N)P - DN. \quad (8)$$

The contours $X(P, N) = 0$ and $Y(P, N) = 0$ are located in the (P, N) plane as follows:

Construction of the contour $X = 0$

One branch of the contour of $X = 0$ is $P = 0$. The other branches of $X = 0$ are given by the solutions of $(1-p)\mu_1(P, N) = D$ which is just a solution to $(1-p)\mu_1(S) = D$. Denoting such solutions \hat{S} , the branch of the contour in P, N space is the straight line

$$P = -N + \gamma(S_f - \hat{S}). \quad (9)$$

The number of solutions \hat{S} will determine the number of branches of the contour $X = 0$. For the growth models considered here, we expect zero, one or two branches of $X = 0$ from eq. (9), in addition to the branch given by $P = 0$. If the dilution rate is greater than $(1-p)\mu_1(S)$ for all S less than S_f , then there are no additional branches. If the dilution rate is less than $(1-p)\mu_1(S)$ for some S less than S_f , then with an uninhibited growth curve there is one additional branch while if the growth curve is substrate inhibited there are two additional branches. Figure 2 presents a specific growth curve and chemostat dilution rate, along with the associated branch of $X = 0$ from eq. (9) on the bounded domain.

Construction of the contour $Y = 0$

As stated earlier, $N = 0$ is not a possible steady state, and it is not a branch of the contour $Y = 0$. Rearranging eq. (8) with the left hand side equal to zero shows that the contour of $Y = 0$ satisfies the relation

$$\frac{P}{N} = \frac{D - \mu_2(S)}{p\mu_1(S)}. \quad (9)$$

Since P/N and $p\mu_1(S)$ are strictly positive, then the contour of $Y = 0$ is defined within the bounded domain $(0 \leq P < \gamma S_f, 0 < N < \gamma S_f)$, only for D greater than $\mu_2(S)$. For any S such that $D > \mu_2(S)$, we denote

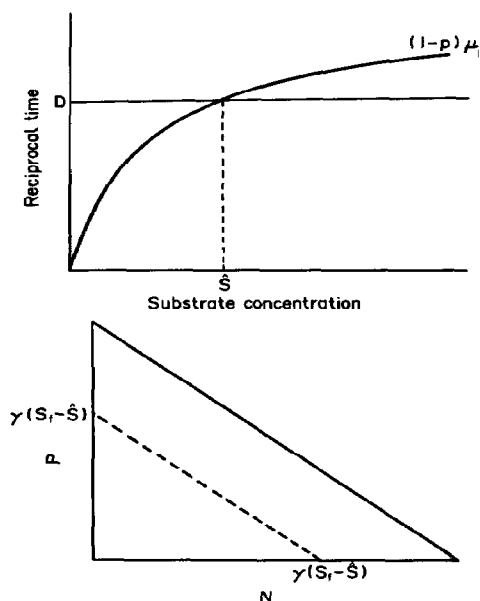


Fig. 2. Schematic illustration of the construction of the $X = 0$ contour (-----) for uninhibited growth kinetics.

the value of P/N from eq. (9) as β . A point on the contour $Y = 0$ is then given by the intersection of the lines $P = -N + \gamma(S_f - S)$, and $P = \beta N$. It is clear then that S parameterizes the contour of $Y = 0$.

The construction of the contour can be obtained from an inspection of the specific growth curves for the plasmid-bearing and plasmid-free species. In both Monod and Andrews kinetics, the two models considered here, as S approaches zero, $\mu_1(S)$ and $\mu_2(S)$ approach zero. Therefore as S approaches zero, β approaches infinity, and the point on the contour corresponding to $S = 0$ is at the upper vertex on the P axis of the bounded domain. Suppose there are no solutions to $\mu_2(S) = D$, that is, D is greater than $\mu_2(S)$ for all S between zero and S_f . When $S = S_f$, the corresponding point on the contour is the intersection of the lines $P = -N$ and $P = \beta N$ where β is evaluated at S_f . We see then that the contour will leave the bounded domain through the origin, see Fig. 3(a).

Suppose that there is one solution to $\mu_2(S) = D$ and denote this solution \bar{S} . This would be typical of an uninhibited growth curve such as a Monod behavior where D is less than the maximum growth rate. For $S = \bar{S}$, $\beta = 0$, and the contour will leave the bounded domain across the N axis at the point $N = \gamma(S_f - \bar{S})$. For S greater than \bar{S} , $\mu_2(S)$ is greater than D , and the contour will lie outside the bounded domain. There are two types of constructions of the contour outside the domain, and the type exhibited depends on whether a value of $\beta = -1$ is reached for some S . If β is strictly greater than -1 , the contour is restricted to the fourth quadrant, and the contour will curl back towards the origin. For $S = S_f$, the corresponding point on the contour is the intersection of $P = -N$ and $P = \beta N$, and the contour will pass through the origin. It cannot,

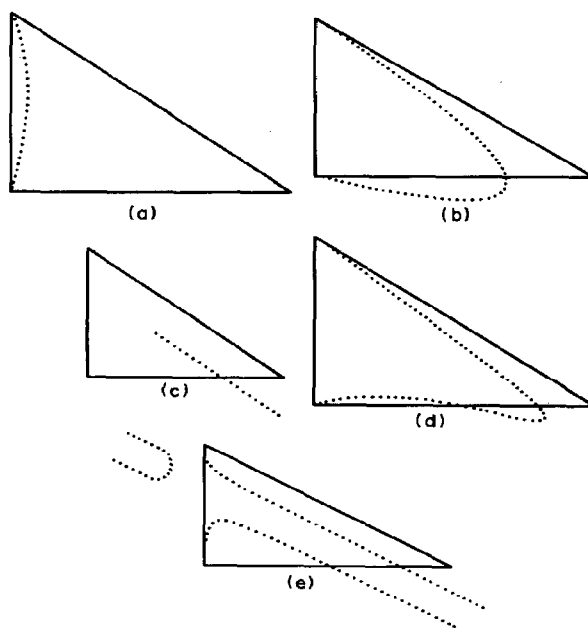


Fig. 3. The five possible constructions of contour $Y = 0$ (....) for various specific growth curves, dilution rates, and plasmid loss probabilities.

however, re-enter the bounded domain because S parameterizes the contour, see Fig. 3(b). If $\beta = -1$ for some S denoted S^* , then the contour must approach the line $P = -N + \gamma(S_f - S^*)$ asymptotically. For S greater than S^* , β is less than -1 and the contour must lie in the third quadrant. The contour will approach the bounded domain from the asymptotic line. When $S = S_f$, the contour will pass through the origin for the same previous arguments, see Fig. 3(c).

Suppose there are two solutions to $\mu_2(S) = D$ denoted \bar{S}_1 and \bar{S}_2 where \bar{S}_1 is less than \bar{S}_2 . This would be typical of an inhibited growth curve such as Andrews behavior, where D is less than the maximum growth rate. The construction of the contour for $0 < S < \bar{S}_1$ is the same as previously described, and the contour will leave the bounded domain through the point $N = \gamma(S_f - \bar{S}_1)$. The construction outside the domain again depends on a critical value of $\beta = -1$. For β strictly greater than -1 , the contour remains in the third quadrant and curls back towards the origin. When $S = \bar{S}_2$, the contour will re-enter the bounded domain across the N axis at the point $N = \gamma(S_f - \bar{S}_2)$. The contour moves through the domain and for $S = S_f$, it passes out of the domain through the origin, see Fig. 3(d). If for some S , β is less than -1 , the contour will display the previously mentioned asymptotic behavior. There are now at least two values of S denoted S_1^* , S_2^* such that $\beta = -1$. The contour is asymptotic to both of the curves $P = -N + \gamma(S_f - S_i^*)$, $i = 1, 2$. The contour will re-enter the domain across the N axis at $N = \gamma(S_f - S_2^*)$, and leave the domain through the origin for $S = S_f$, see Fig. 3(e).

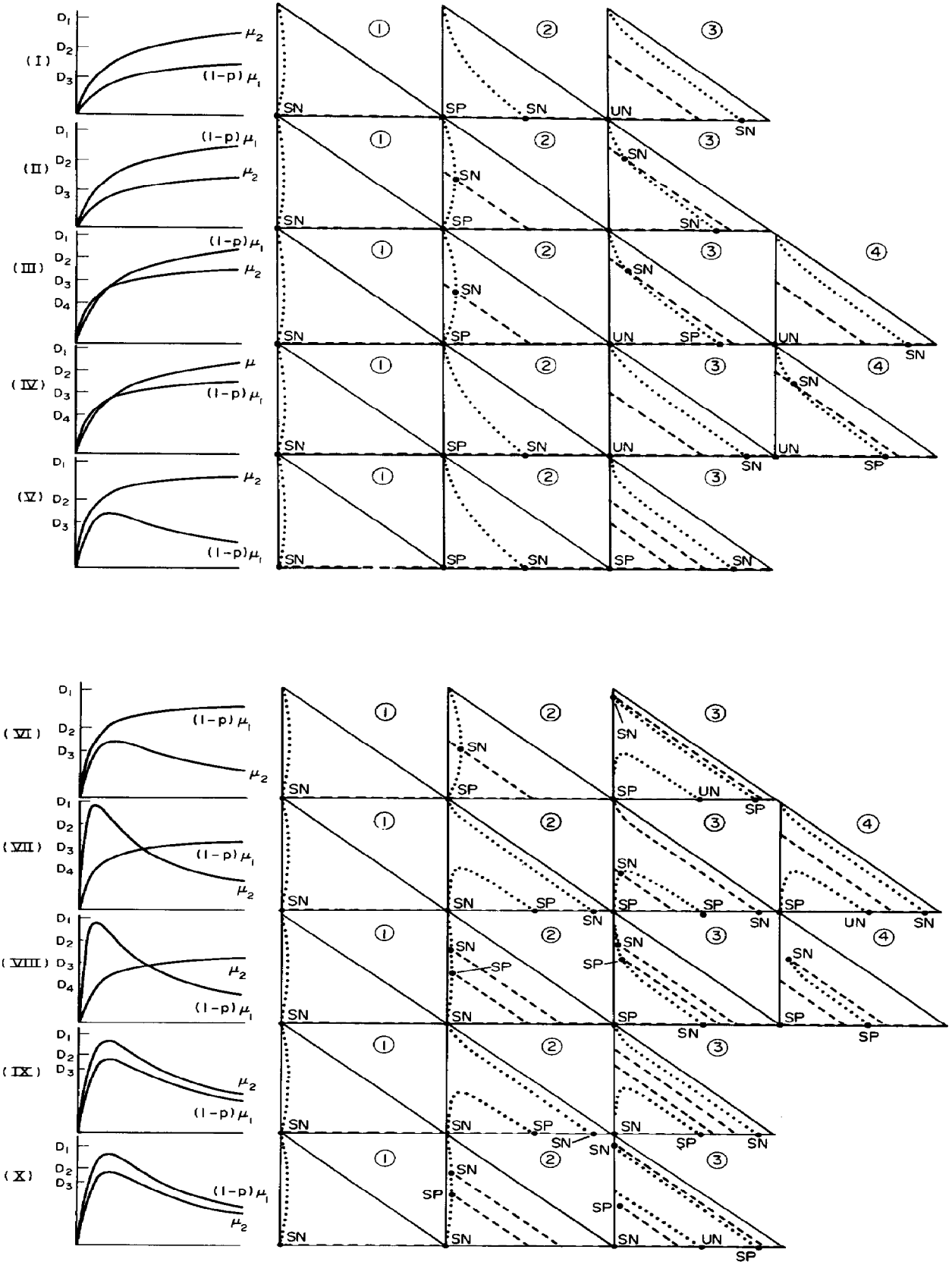


Fig. 4. (a) and (b).

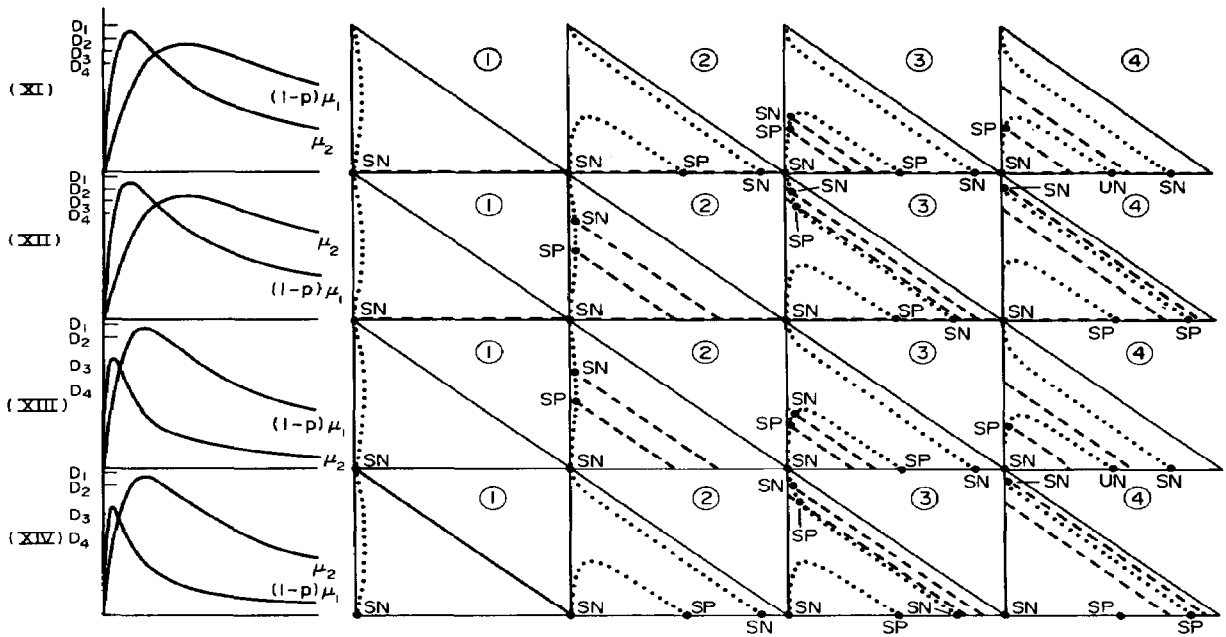


Fig. 4. Comprehensive stability portrait for a plasmid bearing plasmid-free mixed culture growing in a chemostat. . steady state, - - - - : contour $X = 0$, : contour $Y = 0$, SN: stable node, UN: unstable node, SP: saddle point.

This construction of the contours of $X = 0$ and $Y = 0$ is completely general, and for the purposes of the stability analysis is exhaustive of all the possible situations. These contours will depend on the shape and mutual disposition of the specific growth curves for the plasmid-bearing and plasmid-free cells. They also depend on the value of the plasmid loss probability. Inspection of eq. (9) shows that when $\mu_1(S)$, $\mu_2(S)$ and D are of the same magnitude, then a small value of p will guarantee a value of $\beta = -1$. Following the lead of previous researchers (Imanaka and Aiba, 1981; Dwivedi *et al.*, 1982), the plasmid loss probability

Eigenvalue analysis for the total washout steady state

The result from the index theory allows us to determine the type of all of the steady states if we know the type of one of them. As previously mentioned, this is most convenient for the total washout steady state since the eigenvalue analysis is very much simplified for this case. The linearized system for eqs (5) and (6) in terms of deviation variables, ΔP and ΔN , is

$$\frac{d}{dt} \begin{bmatrix} \Delta P \\ \Delta N \end{bmatrix} = \Theta \begin{bmatrix} \Delta P \\ \Delta N \end{bmatrix} \quad (10)$$

where

$$\Theta = \begin{bmatrix} (1-p)\mu_1 + (1-p)\bar{P} \frac{\partial \mu_1}{\partial P} - D & (1-p)\bar{P} \frac{\partial \mu_1}{\partial N} \\ p\mu_1 + p\bar{P} \frac{\partial \mu_1}{\partial P} + \bar{N} \frac{\partial \mu_2}{\partial P} & p\bar{P} \frac{\partial \mu_1}{\partial N} + \bar{N} \frac{\partial \mu_2}{\partial N} + \mu_2 - D \end{bmatrix}_{ss}$$

$$\Delta P = P - \bar{P}$$

$$\Delta N = N - \bar{N}$$

is assumed to be on the order of 10^{-2} to 10^{-5} . For this reason it is expected that the contour of $Y = 0$ will admit the form in Fig. 3(a), (c), and (e). It should also be noted that for small p , \bar{S} and S^* are necessarily very close in size to each other. This means the contour of $Y = 0$ will leave the bounded domain approximately along the asymptotic line, and will also re-enter the domain along the second asymptotic line. This is evident in Fig. 3(c) and (e).

where \bar{P} , \bar{N} are the values of P and N , respectively, at the steady state of scrutiny. The eigenvalues for the total washout steady state are given by

$$\lambda_1 = (1-p)\mu_1(S_r) - D \quad (11)$$

$$\lambda_2 = \mu_2(S_r) - D. \quad (12)$$

Thus, conditions for stability of the washout steady state are easily obtained from eqs (11) and (12).

The specific growth models used for the analysis are the two most common uninhibited and inhibited growth models. These are the Monod model (uninhibited growth) and the Andrews model (inhibited growth), and are given by

$$\mu(S) = \frac{\mu_{\max} S}{K_s + S} \quad \text{Monod}$$

$$\mu(S) = \frac{\mu_{\max} S}{K_s + S + S^2/K_I} \quad \text{Andrews.}$$

For a given set of specific growth curves, and a dilution rate, the contours of $X = 0$ and $Y = 0$ can be located in the bounded domain. This determines the number of steady states, and their locations. Eigenvalue analysis for the total washout steady state determines the type of steady state at the origin, e.g. node, saddle point, etc. The type of all of the steady states is then determined by moving through the bounded domain and applying the result of the index theory.

An exhaustive analysis for all of the situations which can occur for different combinations of $\mu_1(S)$, $\mu_2(S)$, and D appears in Fig. 4. This is thus a complete portrait of all of the interactions which can occur in a plasmid-bearing plasmid-free mixed recombinant culture growing in a chemostat.

DISCUSSION

As stated earlier, index theory does not differentiate between nodes, foci and centers, nor does it indicate the stability of the nodes and foci. Further information can, however, be obtained from some general phase-plane characteristics and the sign of functions X and Y of eqs (7) and (8) that uniquely define the nature of all possible steady states. First, no focal or oscillatory behavior is allowed around the steady states lying on the N axis, for this would give rise to negative values of the plasmid-bearing, P , cell population. Consequently, steady states on the N axis can be of the nodal type only, stable or unstable. Second, the stability of such steady states can be determined by the sign of function X (eq. 7). The signs of both functions X and Y in the various regions of the phase plane defined by the contours $X = 0$ and $Y = 0$ are shown in Fig. 5. If the steady state on the N axis lies in a region where $X < 0$, then the steady state is a stable node, otherwise unstable. Third, using the signs indicated in Fig. 5, it can be shown (Lapidus, 1985) that all coexistence steady states which are not saddle points are stable nodes. If multiple coexistence steady states are possible, the one that is stable will correspond to the steady state substrate concentration which is the smaller of the S values satisfying $(1-p)\mu_1(S) = D$. On the basis of the above general observations, possible steady states of eqs (5) and (6) have the stability indicated in Fig. 4.

Inspection of the stability portraits of Fig. 4 shows that a necessary condition for stability of the coexistence steady state is that the parental cells grow faster than the revertant cells in, at least, some region of the

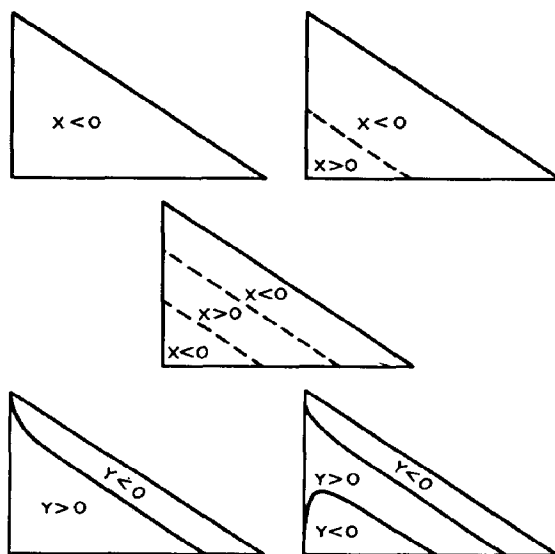


Fig. 5. The sign of $X(P, N)$ and $Y(P, N)$ in relationship to the contours of $X = 0$ and $Y = 0$.

limiting substrate concentration. The stability is then determined by the value, p , of the frequency of segregational and structural plasmid instabilities that determines the mutual disposition of the $(1-p)\mu_1$ and μ_2 curves, and the value of the dilution rate. In general, it is expected that parental cells will grow slower than revertant cells (Imanaka and Aiba, 1981) so that the above condition is not satisfied. There have been several reports, however, where the opposite situation was observed, as in (Nagahari *et al.*, 1980) where the host *E. coli* C600 containing the recombinant plasmid pBR322-T-Leu grew faster than the revertant cells and the culture stability increased as the fermentation progressed.

Growth conditions can have a profound effect on plasmid stability and the relative growth rates of the two cell populations. In general, plasmid stability is decreased when growth is under phosphate or glucose limitations (Godwin and Slater, 1979; Melling *et al.*, 1977) but exceptions exist. Growth under nitrogen limitation has a stabilizing effect on some plasmids (Wouters and van Andel, 1983; Noack *et al.*, 1981) which was attributed largely to a growth advantage that parental cells enjoy over revertant cells under such conditions. In view of such variability, the stability portraits of Fig. 4 can offer additional insight about the possible effects that various limiting substrates can have on recombinant culture stability and about the desired rate properties to be looked for in screening media formulations.

Looking into further plasmid modifications that could enhance the stability of parental cells in addition to coding for antibiotic resistance for growth in selective media, cases III, IV, VII, and XI of Fig. 4 are suggestive of four possibilities. In cases III and IV, both cell populations have Monod type, uninhibited

growth, kinetics with parental cells growing faster at larger (III) or lower (IV) limiting substrate concentrations. This could be achieved by cloning the gene for the enzyme responsible for growth limitation in the recombinant plasmid. In cases VIII and XI the growth of revertant cells is inhibited at high substrate concentrations presumably because of accumulation of metabolites which are inhibitory or toxic to cells. The coding of enzymes in the plasmid that accelerate the processing of such intermediates could eliminate (case VIII) or reduce (case XI) such inhibition and enhance the stability of the culture. Such a fermentor could operate at the resulting stable node or, through the application of proper controls (DiBiasio *et al.*, 1982) at the unstable saddle point, should the latter be judged desirable for various reasons.

In this work attention was focused on the stability of coexistence steady states for growth in a chemostat. In a real application, this type of device is less likely to be employed than a batch or fed-batch fermentor, at least with the presently available technology. However, analysis of chemostat stability offers a very clear picture of the dynamics of competitive growth and expected dominance characteristics for growth in any type of fermentor. Due to the ever-present probability for cell reversion, a stable, partial washout steady state with parental cell growth only is not possible and coexistence is the best outcome that one can hope for. As it can be seen from Fig. 4, some of these coexistence steady states involve minimal revertant cell densities and, for all practical purposes, could be selected for the propagation of recombination cultures with high product formation rates. Achieving such an operation will depend on the media used, plasmid construction and chemostat conditions and the present analysis offers some additional insights towards meeting this objective.

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NOTATION

A_1, A_2	steady state points in Fig. 1
D	chemostat dilution rate
N	plasmid-free cell population density
\bar{N}	steady state value of N
P	plasmid-bearing cell population density
\bar{P}	steady state value of P
p	probability of plasmid loss upon division
S	limiting substrate concentration
S_f	feed substrate concentration
\bar{S}	solution to $\mu_2(S) = D$
\hat{S}	solution to $(1-p)\mu_1(S) = D$
S^*	value of S such that $\beta = -1$
t	time
u	general state variable
v	general state variable
X	real, autonomous function describing the dynamics of P cells

Y real, autonomous function describing the dynamics of N cells

Greek symbols

β	ratio P over N
γ	biomass growth yield
λ	eigenvalue
μ_1	specific growth rate of plasmid-bearing cells
μ_2	specific growth rate of plasmid-free cells
Θ	Jacobian matrix

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