Steady state and the chemostat in ecology

Wer wird nicht einen Klopstock loben, 
doch wird ihn jeder lesen? Nein.
Wir wollen weniger erhoben 
doch um so mehr gelesen sein.

G. E. Lessing

If translated with a slight bias: “Who wouldn’t praise a laureate, but would you read him? No. Accepting praise, he still prefers you’d understand him though,” Lessing’s lament appears to apply to science as well as to poetry and seems to be as timely today as it was in the days of Klopstock. It deprecates assuming an eminent man’s idea without really taking the pain of reading his original writings and perceiving their meaning in the proper perspective. As a corollary, concepts arise and turn into methodologies which, although patched with respectable names, often do not hold water.

The laureate in question here is Jacques Monod who reported (Monod 1942) that the growth rate of bacteria in certain culture media was controlled by a substrate (carbon or nitrogen source) as the sole limiting factor within a certain range of concentration. For a “data-fitting” mathematical function he proposed the most simple one, containing the constants $a$ and $b$: $x = ay/b + y$, or in its common form: $\mu = \frac{\mu_m S}{K_s + S}$ (where $\mu$—growth rate, $\mu_m$—maximum growth rate, $S$—concentration of the growth-limiting substrate, $K_s$—saturation or affinity constant—substrate concentration at which half of the maximum growth rate is attained: see Fig. 1).

Thus, under certain conditions, growth appears to behave similarly to enzyme reactions, or their integral, described by the nominally identical Michaelis-Menten equation. The emphasis is on “under certain conditions,” and these are very restricted in closed (batch) culture systems. In efforts to describe growth more realistically, a number of other more complex equations, today often called models, have been proposed, which incorporate a variable death rate, effects of population density, excretion of metabolites, etc.; it is a matter of judgment and practicality which equation to use in a particular situation. Monod’s is the most simple and practical, but can only be used for equally simple and well defined systems.

Closed culture systems have the advantage of technical simplicity but the disadvantage of ever-changing growth conditions. This is especially true in the case of the ecologically important low concentrations of the limiting substrate: here the two artificially introduced growth phases (lag and stationary phase) may leave only an insignificant portion of the analytically required exponential growth phase between them or may even, more commonly, overlap (Fig. 2). Monod’s (1950; Novick and Szilard 1950) motive in defining the chemostat was to keep the culture conditions constant and one known substrate truly growth limiting over an indefinite period of time. Under these conditions the application of his intentionally simple equation becomes more meaningful than in batch culture. The continuous addition of fresh medium and the removal of spent medium and cells at constant rates maintain the conditions under which exponential growth occurs.

It is important to remember that even a continuous culture system is generally very
different from a chemostat. While an undefined open culture system will undergo population changes dependent on a variety of factors, the chemostat is constructed to fulfill the requirements that lead to the self-adjustment of a time-independent steady state. This distinction is vital because the applicability of manageable mathematical expressions is limited to conditions of a well-defined steady state.

Herbert et al. (1956) have given a very clear explanation why only this time-independence of steady state permits the use of a few simple mathematical expressions, derived from Monod's equation, relating population density ($x$), dilution rate ($D$), and the concentrations of the limiting substrate in the chemostat ($s$) and in the reservoir ($S_0$): $x = y(S_0 - s)$ and $s = K_s D / \mu_m - D$ ($y$ being the yield coefficient $dx/ds$). To achieve a steady state, a continuous culture system has to become a chemostat by fulfilling the following requirements: 1) the organism (pure culture) must be capable of exponential growth and should be limited by only one known factor (i.e., kinetic response to changes of this factor, largely the concentration of essential nutrients), 2) growth conditions must be constant (i.e., culture volume, flow rate, and physicochemical conditions such as temperature, composition of medium etc.), 3) homogeneous mixing must be maintained (e.g., no wall growth).

Before the steady state is reached in the chemostat, the population is in a transient state, increasing or decreasing, where the mathematical relations of steady state do not apply. This last point is of special importance to the ecologist. While the chemostat is an almost ideal and a legitimate tool in microbial physiology, its induced time-independence of steady state represents an utterly unnatural situation. It is neither meant to reproduce nor capable of reproducing a natural habitat. Its sole purpose is to make physiological, biochemical, and genetic responses of whole populations amenable to arithmetic analysis. There is no equivalent for the "dilution rate" in a natural population. All forces eliminating organisms from a population in a natural environment are individually selective: sedimentation, grazing, sporulation, die-off, etc. In a chemostat run with a mixed bacterial population, the indiscriminate removal of cells by dilution results in simple selection for that species attaining the fastest growth rate under the particular conditions given. During this artificially induced transient state of such a population (normally approaching the complete displacement of those species that compete unsuccessfully), no information can be gained on its behavior under natural conditions, namely: no constancy of physicochemical conditions, no
homogeneous distribution of cells, and probably no simple growth limitation of an individual species by a single factor for any length of time.

The steady state of the rain forest ecologist is not the same as that of the microbiologist. It is significant that the definition of steady state in continuous culture was originally based on thermodynamic terms as in chemical reaction kinetics. Hinshelwood (1946) bridged the two areas by putting the term steady state on the population level: while the individual cells are passing through division and age cycles, the integrated rate of growth (biomass increase) and metabolism in the population are constant, i.e. independent of time. The rain forest or parts of the ocean appear, as a whole, to be in steady state and yet population density or growth of no one of the individual species will be in steady state. To make the term steady state useful, it has to be defined at its specific and proper level of complexity.

It seems ecologically significant that the complex natural situation does not select for steady state conditions. It has often been suggested that the most constant natural environment, the pelagic open ocean, possesses chemostat qualities and is in a steady state. Since no complete mixing occurs, however, a steady state in the microbiological sense could exist only if the consumption of nutrients were perfectly balanced by diffusion and growth by cell die-off. This unlikely situation elucidates another property of the chemostat: nutrient supply (growth stimulant) and dilution (cell removal) are closely linked. In a natural system they are not.

The restricted use of steady state growth kinetics as achieved in the chemostat (homogeneous culture) does not mean that the less controlled (hetero-) continuous culture systems are useless. They have been the only way to culture certain fastidious microorganisms that occur under similar conditions in nature. Since such organisms are characterized by a high sensitivity to environmental changes as, for instance, oxygen depletion or accumulation of waste materials, their autecology has been studied in open flow systems. In cases where certain microorganisms refused to grow in pure culture, metabolic or nutritional interrelationships with the required “contaminant” remained largely undetectable in batch culture systems but have been successfully untangled in continuous culture experiments. Applications in microbial technology have demonstrated a number of interesting synecological features which can be exploited, concerning the maximum production of an intermediate metabolite, or the optimal rate in the decomposition of complex organic matter involving controlled species succession and nutrient feedback (see Jannasch and Mateles 1974).

In certain cases, true steady states of mixed populations in a chemostat have been observed. However, the common phenomena in chemostat experiments with mixed populations of microorganisms are, first, enrichments, i.e. selective exclusion as a result of competition for some limiting factor and, second, oscillations. The former is an artifact of the indiscriminate dilution which, on the other hand, offers the advantage of separating two characteristically different nutritional types of microorganisms by varying experimentally the concentration of the limiting nutrient and the dilution rate (Jannasch 1967). Described by a high substrate affinity (small $K_s$) and low growth rates at high substrate concentrations (small $\mu_m$) and vice versa (Fig. 3), these two types correspond to Winogradski’s “autochthonous” and “zymogenous” microorganisms, or the $K$ (or $K_s$)-selected and $r$ (or $\mu_m$)-selected species of the phytoplanktologists (P. Kilham and R. E. Hecky unpublished).

The sustained diversity and variability of species composition is the main reason why a natural habitat cannot be equated with a chemostat.

Species oscillations observed in the chemostat have been studied under controlled conditions, resulting in information on a variety of intricate nutritional relationships that would escape detection under less controlled conditions. In the natural habitat,
these internally created oscillations are apt to be obscured by environmental inconstancies such as seasonal, diurnal, or tidal changes. The typical behavior of a natural population of microorganisms in its natural habitat is probably best characterized by these internally as well as externally maintained oscillations in a continual transient state.

The often mentioned capacity of a natural population to "buffer" or to "dampen" oscillations caused by an external disturbance cannot be viewed as a parallel to the self-adjustment processes inherent in chemostat culture, but rather represent the re-establishment of the former transient state. Again, the selective forces that lead to a steady state in the chemostat are not the same as those controlling a natural population. While the batch culture represents one and the chemostat the other extreme of artificial culture systems, a natural population in its habitat could be viewed as having characteristics of both, i.e. an open system under varying conditions.

To come back to the practical point of this comment, the application of a mathematically simplified model based on steady state kinetics obtained in the chemostat cannot lead to realistic analyses of and predictions for the behavior of complex natural populations. There are two interesting parallels where the actual data obtained seem to refute such a categorical condemnation. Gaudy and Gaudy (1966) discuss the kinetics of the heterogeneous populations of whole sewage plants in terms of Monod's relation and define a modus vivendi for its use. Wright and Hobbie's (1965) suggestion that Michaelis-Menten kinetics be used for calculating potential rates of substrate uptake by natural populations also relies on an apparent fit of data whenever obtained. Although the theoretical basis of determining enzyme activities in the required well defined systems.
Comment

does not apply to this approach, it has been used with a certain justification as long as the heterogeneous natural population appears to behave like a pure culture. On the other hand, we must keep in mind that the relation between growth or uptake and the concentration of any essential nutrient has to result in a pseudo-hyperbolic curve, even in mixed populations and at variable rate limitation. But it is difficult or impossible to prove statistically from the scatter of data points whether they truly relate to the assumed single-reaction kinetics that permit extrapolation of valid constants as obtained, for instance, graphically from Lineweaver-Burk plots (Fig. 3). The significance of “average” growth or uptake constants for natural populations appears doubtful, since prolonged exposure to any nutrient concentration will result in unpredictable changes of the population and its uptake characteristics. Finally, even if growth and uptake are both described by the same simplified kinetics, their constants can be used in the same calculation only if the yield coefficient is known.

The viewpoint that I give here is not presented in a mood of undue purism or pedantry but appears vital to avoiding a smothering accumulation of data that, for the time being, cannot be proven or disproven. An unambiguous terminology is needed for the proper use of simply “continuous culture” or the more sophisticated “chemostat” whenever applicable. In this area of model building it is essential to observe the compatibility of tools and materials, and, coming back to the introduction, to be well informed about brand names.

Holger W. Jannasch
Woods Hole Oceanographic Institution
Woods Hole, Massachusetts 02543

References