SHORT COMMUNICATIONS

Effect of Dilution Rate on the Outcome of Chemostat Mixed Culture Experiments

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Despite the facts that micro-organisms do not often grow as pure cultures in natural environments, and that growth of microbial cultures is likely to be profoundly affected by the presence of other species, most laboratory research has been concentrated on pure cultures. The value of continuous culture for studying microbial physiology is now widely recognized, but this technique has been little used to study the complex interrelationships that exist in mixed microbial populations.

The chemostat is particularly suited to the study of situations in which different organisms compete for a limited supply of an essential nutrient. Powell (1958) discussed the growth of contaminants and mutants in continuous cultures at fixed dilution rates. Subsequently Tempest, Dicks & Meers (1967) and Meers & Tempest (1968) showed that *Aerobacter aerogenes* or *Pseudomonas fluorescens* outgrew and totally replaced several Gram-positive organisms in magnesium-limited mixed cultures in a manner predicted by the mathematical analysis of Powell (1958). However, the experiments of the above workers were conducted at a fixed dilution rate (0.3 h⁻¹), whereas Powell (1958) had argued that changes in dilution rate might alter the outcome of mixed-culture experiments. However, only in one instance was the outcome of mixed-culture experiments affected by variation in dilution rate between 0.05 and 0.6 h⁻¹ in potassium or magnesium-limited chemostat cultures (Meers, 1970). This result is of theoretical and practical interest and is described below.

The methods and organisms used have been described previously (Meers & Tempest, 1968).

The result of an experiment in which *Bacillus subtilis* var. *niger* and *Torula utilis* were growing together under magnesium-limited conditions in a chemostat are shown in Fig. 1. The dilution rate was alternated between 0.05 and 0.08 h⁻¹, both dilution rates being well below those at which the organisms would otherwise be washed from the culture (i.e. 0.7 and 0.5 h⁻¹, respectively). *Bacillus subtilis* replaced the yeast at the higher dilution rate, but the reverse was true at the lower dilution rate. Thus complementary fluctuations, or oscillations, in the density of the bacterial and yeast populations occurred in response to changes in flow rate. In subsidiary experiments the dilution rate was not alternated, but was kept constant at a value of 0.05 or 0.08 h⁻¹. It was then observed that either the *B. subtilis* or *T. utilis* organisms continued to be washed from the fermentor at an exponential rate until, after approximately 6 days, cultures were obtained that on microscopic examination appeared to be pure. Even after this length of time, however, if the dilution rate was changed from 0.08 to 0.05 h⁻¹ the undetectably small population of *T. utilis* organisms could gradually

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increase in number. But, because of the predictably exponential nature of this increase, the yeast population only became large enough to influence the growth of the bacterial population after a period of several days. Hence one could predict the observed types of asymptotic curves that are illustrated by Fig. 1.

This result implies that, under magnesium-limiting growth conditions, the saturation curves for the growth limiting substrate (Mg²⁺) for these two organisms cross between the specific growth rates of 0.05 and 0.08 h⁻¹ (see Fig. 1 of Meers & Tempest, 1968). No attempt was made to establish the unstable ‘steady state’ condition at which, presumably, the two species could coexist indefinitely in the chemostat culture (Powell, 1958).

![Diagram](image)

Fig. 1. Growth of *Bacillus subtilis* (●) and *Torula utilis* (○) in a Mg²⁺-limited simple salts medium in a chemostat. The initial concentration of *B. subtilis* was 6 × 10⁶ organisms/ml. and of *T. utilis* 1 × 10⁶ organisms/ml. The pH was 6.4 ± 0.1 throughout the experiment, and the temperature was 33°C. At the start of the experiment the dilution rate was set at 0.05 h⁻¹. This rate was increased to 0.08 h⁻¹ after 55 h. and returned to 0.05 h⁻¹ after 143 h.

Microbial populations frequently oscillate in natural environments (Hobson, 1969), and many of these natural environments can be simulated in a chemostat. Oscillations in microbial populations could be caused in two ways. Oscillations caused by such factors as predation (Gause, 1935) are due to interactions between the component species and could occur with regular periodicity in a constant environment. Oscillations could also be caused by changes in the environment. For example, the flow rate into a biological waste treatment system would be expected to fluctuate periodically. This could result in changes in the dominant species of the microflora, precisely as illustrated by Fig. 1. It follows from this argument that (contrary to the commonly asserted opinion) in many natural environments a rapid maximum rate of growth is not fundamental to evolutionary success. This is because in open systems the rate of flow and supply of essential nutrients will frequently be substantially lower than the maximum growth rate for the micro-organisms in that environment, and the growth advantage will then be with the species best able to concentrate the growth-limiting nutrient under the prevailing growth conditions. Thus *Torula utilis* would replace *Bacillus subtilis* var. *niger* in a magnesium-limited system that was operating with a mean residence time of 20 h., despite the fact that the bacterium had the higher maximum growth rate.
The apparent randomness of fluctuations in natural environments (Cassell, Sulzer & Lamb, 1966) is probably caused by the simultaneous operation of biotic and physical factors causing irregular fluctuations that are difficult to rationalize. Cassell et al. (1966) suggested that these fluctuations 'contradict the implicit assumptions made in theoretical analyses of completely mixed systems, namely that cultures (pure or mixed) reach a stable equilibrium or the so-called steady state'. However, for a steady state to be established the environment must remain unaltered with time, and the organisms in the environment must not interact with one another. These conditions are unlikely to be observed in many natural environments, and certainly would not have been obtained in the systems examined by Cassell et al. (1966). It seems likely that the, as yet largely uninterpretable, behaviour of natural ecosystems can be explained by a complex interaction of simple processes. In most cases, the most effective way of demonstrating these simple processes is to grow microorganisms under well-defined conditions in chemostats. The more commonly adopted procedure of trying to rationalize ecological data from complex ill-defined environments would appear to be premature, at least until some basic data has been obtained that can be interpreted unequivocally.

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REFERENCES


