Hydrodynamic Effects on Cell Growth in Agitated Microcarrier Bioreactors

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Abstract

The net growth rate of bovine embryonic kidney cells in a microcarrier bioreactor is the result of a variable death rate imposed on a cell culture trying to grow at a constant intrinsic growth rate. The death rate is a function of the agitation conditions in the system, and increases at higher agitation because of increasingly energetic interactions of the cell-covered microcarriers with turbulent eddies in the fluid. At very low agitation rates bead-bead bridging becomes important; the large clumps formed by bridging can interact with larger eddies than single beads, leading to a higher death rate at low agitation. The growth and death rates have been correlated with a dimensionless eddy number which compares eddy forces to the buoyant force on the bead.

Introduction and Approach

The negative effect of excessive agitation on cells in culture is well known and has in the past been ascribed to vaguely defined "shear". Because the microgravity of space may allow the use of less agitation, there may be some operating improvement for a bioreactor in space. More importantly however, the study of cellular physiological differences between one g and microgravity requires that the agitation effects in each environment be known and quantified to eliminate them as uncontrolled variables. This project seeks to identify and describe quantitatively the mechanisms of cell damage in a stirred microcarrier bioreactor.

Because a microcarrier bioreactor is a complex turbulent two-phase (beads and liquid) system, a detailed analytical solution for all the forces imposed on the bead is not possible. We have tackled this problem by analyzing simplified models of potentially damaging bead-system interactions: bead-impeller collisions, bead behavior in a boundary layer, and interaction of the beads with turbulent fluid eddies [1]. The specific mechanisms of cell damage that are potentially significant based on the amount of shear stress or energy transmitted to the cell are bead-impeller collisions, bead-bead collisions, and direct bead-fluid eddy interactions. For each of these we derived a mathematical parameter which characterizes how much of that mechanism is occurring.

To test these ideas experimentally, we grew bovine embryonic kidney cells (analogous to NASA's human embryonic kidney cells but more readily available) on Cytodex 3 microcarriers in a well-controlled 1 liter stirred reactor [2]. Agitator geometry, speed, and diameter, bead diameter and medium viscosity and total volume were varied in different experiments, and the growth rate of the cells under each set of conditions was measured. In addition, the growth medium was replaced with a serum-free medium after some runs to measure the rate of decrease in the number of cells under non-growing conditions with specific agitation conditions. This gave us death rate information.

Results and Discussion

Plotting our data against the various parameters for each potential damage mechanism showed that the eddy number gave the best and most consistent correlations. Figure 1 shows that the first order death rate of cells can be correlated with the log of the eddy number, a dimensionless number which compares the forces from turbulent eddies to the force a bead feels due to its buoyancy. Higher eddy numbers imply greater turbulent fluid forces, and the death rate increases correspondingly. Figure 2 shows growth rate data also correlates although there is a maximum in the curve. Above an eddy number of about 1, the trend decreases with the same slope as the death rate curve, which was an independent measurement. This implies that the decreased net growth rate is caused by an increase in the death rate acting on a population of cells trying to grow at some constant intrinsic rate.

At eddy numbers below about 1.0, the decrease in growth rate is apparently due to an increase in the amount of bridging in the system (Figure 3). Bridging causes cell death because the larger clumps formed by bridging can interact with more and larger eddies than can single beads, hence can suffer more damage. This result can also be shown mathematically with our derivations of the eddy number and the linear regressions of data.

The plan for future work in this area is to investigate more thoroughly the effect of such variables as volume fraction of beads in the system, other impeller configurations, and a broader range of medium viscosities. Now that the range of damage mechanisms to consider has been narrowed, a more detailed theoretical analysis of the fluid mechanics actually occurring is feasible. In addition, experiments measuring the death rate of cells in a well defined environment such as in a viscometer are planned. The goal of this work is to better and more accurately understand the interaction between a cell on a bead and its fluid environment.

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References

1. Cherry, R. S. and E. T. Papoutsakis, Bioproc. Eng., **1,** 29 (1986)

2. Cherry, R. S. and E. T. Papoutsakis, submitted to Biotechnol. Bioeng., (1987)

FIGURE 1.

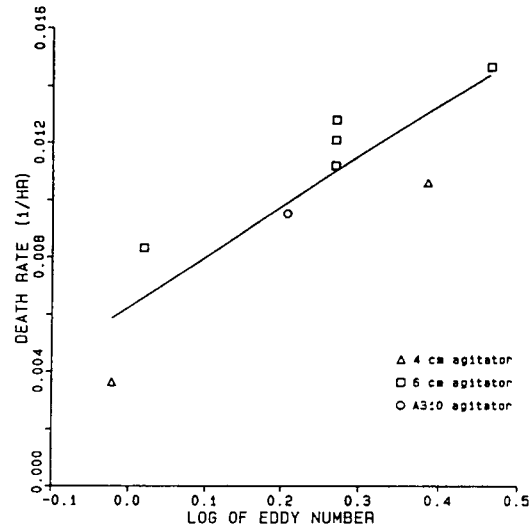

FIGURE 2.

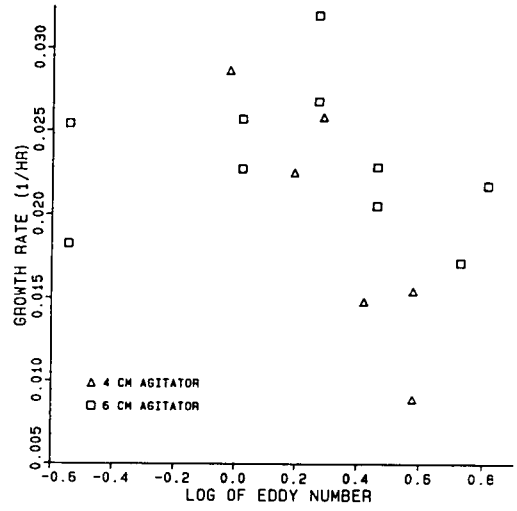

FIGURE 3.