INFLUENCE OF ZERO-G ON SINGLE-CELL SYSTEMS AND ZERO-G FERMENTER DESIGN CONCEPTS

By Jerry V. Mayeux, Bio Innovar, Inc., Storm Lake, Iowa

INTRODUCTION

Exploratory biological studies on the effect of the space environment on biological systems, including microorganisms, have been carried out on the United States' Discoverer, Biosatellite, Gemini, Apollo, Skylab, and ASTP programs and on several Russian satellites. In many cases, the results were incomplete or inconclusive; however, significant observations with respect to microbial interactions with the space-flight environment have been made. The data obtained have evoked considerable interest to continue a space-flight experimental program (ref. 1).

The advent of the shuttle era presents the first multiple-flight opportunity for performance of comprehensive and well-controlled space-flight experiments. Early shuttle payloads such as the Long-Duration-Exposure Facility (LDEF) and Biomedical Experiment Scientific Satellite (BESS) may provide opportunities for bioscientists to initiate experimental programs that can be expanded and continued when the shuttle dedicated labs become operational. These first-generation experiments are a necessary step in the process of developing an orderly space microbiology application. Early investigations can be accomplished by passive growth experiments involving a selection of viable organisms over an extended period of time. The results of these experiments could lead to developments that would eventually provide beneficial exploitation of the space environment for unique application in a space microbiology program.

An analysis has been made to identify potential gravity-sensitive mechanisms that may be present in the single-cell growth system (ref. 2). Natural convection (density gradients, induced sedimentation, and buoyancy) is important in microbial systems. The absence of natural convection in the space-flight environment could provide an opportunity for new approaches for developments in industrial fermentation and agriculture.

Some of the potential influences of gravity (i.e., convection, sedimentation, etc.) on the cell will be discussed to provide insight into what experimental areas may be pursued in future space-flight research programs.

INFLUENCE OF A CONVECTION-FREE ENVIRONMENT ON SINGLE CELLS

Mass and energy transport depends primarily on diffusion and convection in single-cell systems (ref. 3). Migration or relocation of products and substrates from one point of production to a point of utilization can be critical to the cell, especially in synthetic processes (refs. 4, 5, and 6). Some of these requirements and their interdependence on diffusion and convection are shown in table I.

Further evaluation of the cell functions identifies other potential convection-dependent functions at the subcellular level. Examples of these functions at the enzyme reaction site, for example, are as follows: heat dissipation, substrate transport, product removal, boundary layer formation by concentration gradients, enzyme induction or inhibition, and active or passive transport across membranes.
Upon examination of the growth environment of the single cell, other convection-sensitive parameters can be identified. These parameters are in part determined by the nature of the growth system; i.e., air-water, air-water-particulate, air-water-hydrocarbon, or water-hydrocarbon. The major difficulty with these systems in unit gravity is maintaining homogeneity of the growth medium, because these nonmiscible materials are buoyant. The gravity-induced buoyancy affects nutrient transfer to the aqueous phase, sedimentation of cells, pH control, dissolved oxygen supply, and heat dissipation (refs. 4, 5, and 6).

The theoretical advantages of low gravity in the space-flight environment may provide the opportunity to work with stable multiphasic fermentation systems that are not currently available to the fermentation scientists. These multiphasic fermentation systems could provide the cell with a continuous maximum interfacial contact of oxygen, aqueous medium, and insoluble substrates, with a minimum of agitation (ref. 7). The lack of buoyancy should allow the residence time of oxygen bubbles and oil droplets to be increased, with a concomitant decrease in shear forces (due to reaction in the need for agitation)(ref. 8). This result may allow fragile cells or shear-sensitive enzyme systems to be utilized in production processes requiring high levels of oxygen.

The analysis of convection-dependent cellular functions and environmental growth factors has led to speculations about what would happen in the absence of gravity-induced convection (ref. 2). Intracellular biochemical reactions could exhibit an abnormal periodicity due to the time delay for translocation in regulatory substrates or metabolites. Multiphasic microbial fermentation systems that are stable, though not emulsified, could be prepared and maintained as a uniform mixture without the interference of natural convection (ref. 2). Single cells, suspended in a suitable liquid medium, could be made to grow and reproduce to form discrete colonies, originating from a single parent cell or clumps of cells (ref. 2).

**ZERO-G FERMENTER DESIGN CONCEPTS**

The unique opportunity that zero gravity provides for fermentation developments also provides some unique problems in equipment design for zero g.

**Design Considerations**

The oxygen bubbler (or aerator) generally depends upon bubble buoyancy and agitation for dispersion. Because there is no buoyancy, the bubbles will not automatically shear when a critical size is achieved. A shear device must be developed that will provide generation of fine bubbles without vigorous agitation of the growth liquids (refs. 2 and 9).

The mixer used in the fermenter must be capable of uniform dispersion of bubbles as well as other nonmiscible substrates. But most important, it must avoid promoting an increase in bubble coalescence, and a means must be provided to prevent bubble attachment to solid surfaces.

The oxygen, or other gas bubbles provided to the growth system, will serve as a reservoir for waste gases produced by the cells. Although carbon dioxide (CO₂) has a high solubility in water, it will establish an equilibrium with the gas bubble and thus prevent the bubble from decreasing in volume as the oxygen is utilized. A
mechanism for collecting and removing CO$_2$ from either the aqueous phase or the gas bubbles must be developed.

A necessary assumption is that, during the early stages of zero-g fermenter development, most of the preparation will be made on the ground and the events sequence will be programmed and automated. Some of the factors that must be considered in overall fermenter design for zero-g use are discussed briefly below.

Mixer control and agitator size will depend on the nature of the growth environment. If a true fermentation system is used, substrate mixing and CO$_2$ removal are the main problems to be considered (ref. 9). The addition of oxygen or a gaseous substrate provides other unique problems already mentioned.

The logistics for handling the inoculant, the substrate, and other nutrients must be considered. If the cells should be in a freeze-dried state, they must be uniformly resuspended, added to the fermentation medium, and uniformly dispersed. The same holds for the substrate and other nutrients. Some of the questions that arise are as follows: How and when should the nutrients premixed in the reaction tank; should all the substrate be added initially, or should substrate be added gradually over the growth phase; and if the nutrients and substrates are added incrementally, how is it accomplished?

At the other end of the fermentation program, the opposite sequence of events must occur. The fermentation products, metabolites or cells, must be accumulated or stored. Can these be selectively recovered while the fermentation is in progress? Or, must they be allowed to accumulate for later recovery? If so, how are they handled - as a batch or can a continuous system be developed?

The question of sterilization and contamination controls is one that has received much attention from NASA as well as the fermentation industry. It is probable that the technology for these problem areas is already developed for use in zero g. The operational controls, startup and handling procedures, and monitoring of the oxygen supply, pH, pressure, and other environmental parameters have been developed to a highly sophisticated level by the fermentation industry and for other space-flight programs.

It is conceivable that the technology developed for handling liquid fuels in zero g will be directly applicable to the handling and storage of nutrients, fermentation liquids, and metabolites.

Zero-G Fermenter Design: Cylindrical Chamber Concepts

A considerable amount of design work on zero-g fermentor concepts has been conducted by the Martin Marietta Aerospace Corporation in Denver. The information on these designs has been provided by courtesy of Martin Marietta's Denver Division (ref. 9).

A cylindrical chamber concept of a zero-g fermentor is shown in figures 1 and 2 (ref. 9). As conceived, the fermentor chamber will initially contain water and possibly the required initial concentration of dissolved nutrients. The cell inoculant would be presuspended and pressure fed into the tank, together with the nutrients and substrate to start the process.
Slow-speed paddles would provide uniform mixing of the cells and substrate. It is probable that the paddles need only operate at intervals. The oxygen would be fed with a special bubbler head and dispersed by the paddles. Bubble coalescence could be a problem in this design.

The nutrients could be in the fermentation tank initially, and the nutrient level could be maintained by nutrient diffusion through the dialysis tube, with a partial shroud formed around the paddles. The metabolites could be removed by a separate set of dialysis membrane tubes.

Carbon dioxide and other gaseous wastes could be removed by diffusion through Teflon, silicone, or other selectively permeable membrane walls. This gas could be collected and vented as appropriate.

A microbial cell harvester is provided to collect cells for further processing in flight or for return to the ground-based laboratory. Preservation of the cells or extracellular metabolites could be accomplished by use of space vacuum for freeze drying.

Most of the handling and movement of fluids would be accomplished by the use of bladder tanks that can be subjected to a pressure gradient to force fluid flow in or out of the reservoir or collector.

This concept, although designed for use with oxygen in aerobic systems, may be more applicable to anaerobic systems. It does not appear that the concept will provide sufficiently small bubbles without vigorous initial agitation. The closed-loop concept discussed next appears to be more suitable for use with oxygen.

**Zero-G Fermenter: Flow Circulation Loop**

The flow circulation loop concept of a zero-g fermenter design is shown in figure 3 (ref. 9). This unit is designed with a continuous flow fermentation chamber that would be initially filled with water and that could contain a basal nutrient medium. The water is gently circulated by two pumps operating in synchrony.

The cell inoculum, substrate, and nutrients are introduced into the chamber vessel through a pressure-feed system. The oxygen required for growth of aerobic organisms is provided through the two sparger screens. These sparger screens are grids containing multiple bubbling sites. As the liquid flows across the bubbling sites, the bubbles are sheared off. The size of the bubbles can be regulated by the flow rate through the screen.

The nutrient supply in the fermenter is maintained by pumping the concentrates to the counterflow mass exchanger, which consists of a series of membrane tubes, to increase cross diffusion.

The carbon dioxide produced will be collected by a separator screen into a single large pocket at the acute angle between the screen and the closed-loop fermenter wall. Accumulation of the carbon dioxide will automatically activate an optical sensing device that will vent the CO₂ to the outside.

The metabolites will be removed by a second counterflow mass exchanger and accumulated for later use. The cells can be harvested continuously when growth reaches a steady state. These cells can then be stored in the collection reservoir or processed further in flight or preserved for return to a ground-based lab.
CONCLUSION

These concepts are preliminary and have not been proven to work, or fail to work, in flight. However, they do serve as a point of departure for discussion and planning of space-flight mission objectives in the field of applied biology. There are many steps that need to be taken before embarking on a space-flight fermentation program. The most critical question still remains to be answered. "To what degree does space flight influence single-cell systems?" Many projections have been made, but the experimentation to verify these predicted responses still remains to be conducted.

When the microbial behavior is determined and the facts bear out the predictions, then it is time to determine the advantages that may be gained from the space environment. Yet, it is not expedient to wait until the experimental results are collected and tabulated before proceeding with the conceptual design of the necessary hardware and the exploration of these new, theoretical possibilities for solving some very difficult engineering problems in the biosynthesis industry.
REFERENCES


### TABLE I. MECHANISM FOR MASS AND ENERGY TRANSPORT IN CELLULAR PROCESSES

<table>
<thead>
<tr>
<th></th>
<th>Conduction</th>
<th>Diffusion</th>
<th>Radiation</th>
<th>Convection&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nutrient procurement</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Passive diffusion</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Active transport</td>
<td></td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td><strong>Nutrient utilization</strong></td>
<td>X</td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Heat dissipation</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy conversion</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biosynthesis</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Waste-product removal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Passive diffusion</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Active transport</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Respiration</strong></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Gas exchange</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Gravity-dependent natural convection.
Figure 1.- Zero-g fermenter design, cylindrical chamber concept. (Courtesy Martin Marietta Corp.)
Figure 2.- Concept for carbon dioxide removal by dialysis in the cylindrical fermenter design. (Courtesy Martin Marietta Corp.)
Figure 3. - A zero-g fermenter design, flow circulation loop concept (courtesy of the Martin Marietta Corp.).