DESIGN CONCEPTS FOR BIOREACTORS IN SPACE

P. K. Seshan, G. R. Peterson,
Jet Propulsion Laboratory
California Institute of Technology
Pasadena, CA

B. Beard. C. Boshe, and E. H. Dunlop
Washington University
St. Louis, MO

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INTRODUCTION

Bioprocessing in space and in extraterrestrial facilities is both a logical extension of bioprocessing needs on Earth and in some cases a better alternative to obtain biomass and biologicals rapidly and efficiently. In the context of controlled environmental life support systems (CELSS), trade-off studies on food regeneration strategies may point to microbial food production as the choice in terms of efficiency, economy, and fault-tolerance. In addition, their value as redundant and supplementary food sources cannot be overemphasized. Work needs to be done to identify and even genetically modify microbial strains to provide an optimum (70:20:10)[1] mixture of carbohydrates, proteins, and lipids. The promise of high energy efficiencies and weight/volume ratios does provide a tremendous incentive to undertake such work expediently and systematically. While this work is progressing, parallel efforts must be undertaken to address the problems of operating bioprocessing units in microgravity.

Microgravity is not expected to have any significant effect on basic biokinetic rates of biological reactions. However, the associated operations of mixing nutrients/reactants and separating products will greatly depend upon the magnitude of gravitation. These two, in turn, will affect significantly the production rate of the bioprocessing units. Any bioprocessing unit may contain one or more bioreactors. The mixing/separation operations are too complex to model from the first principles. There are no simple correlational procedures to convert engineering data and tools used for terrestrial designs for applicability to microgravity. One can choose between two approaches to arriving at a successful design of a bioreactor for use in microgravity: (1) build right away a candidate bioreactor and associated instrumentation based on terrestrial experience and test it in microgravity. Then conduct subsequent tests to modify the hardware and operating conditions/pro-

cedure to optimize the design; or (2) conduct an experimental program for obtaining key engineering data under conditions of microgravity and use this data base to develop design tools and procedures for the design of space bioreactor systems for a broad range of applications. The authors tend to prefer the second approach as the one providing the most value for the money.

BACKGROUND

A major research thrust of NASA's CELSS program is to develop practical and energy-efficient ways to recycle all of the materials involved in life processes so that a controlled life support system requires as little resupply and energy input as possible to sustain spacecraft crews for long-term space habitation.

Microorganisms as Food Sources

Conventional food sources consist of higher plants and animals. Unconventional food sources for human consumption are photosynthetic algae and bacteria and nonphotosynthetic bacteria, yeasts, and fungi. Conventional food sources are highly palatable, but require long lead times to produce. Under conditions of epidemic loss of conventional food sources, recovery may be prolonged or impossible. The photosynthetic energy efficiency of higher plants is less than 3%. Even though conventional food sources will be our best choice because of our excellent culinary experience with them, they are not the most abundant and dependable in the context of an enclosed extraterrestrial habitat with conventional food sources, and survival under conditions of "drought" during a long-term space mission can be realistically estimated to be near zero. Microbial food sources such as algae, yeast, and fungi are unconventional and have been considered for use as supplements to conventional food. Their nutritional content based on current data can be presumed to be adequate to meet human dietary needs. Microbial food production systems have the advantages of lower weight/ volume requirements[2] over conventional plant/animal production systems and they also account for superior energy utilization in the production of carbohydrates from CO_2 and H_2O. A quick summary of what it takes to produce an acceptable menu of food items for space habitats can be found in the literature[2] and the problems do not seem to be intractable. Therefore, it is not a far-fetched assumption that adequate nutritional consumption can be achieved using microbial food sources and the microbial mass can be made palatable to humans through development of suitable food processing techniques.

Microbial Growth Chambers

There have been efforts made by both U.S. and Soviet scientists to design microbial growth chambers. Two plans have been suggested by Martin-Marietta Corporation.[3,4] One involves a flow circulation loop and the other is a cylindrical fermenter design. The flow circulation model is designed for both production and collection of cell mass; however, it is more suitable for bioprocessing than cell harvesting. The cylindrical fermenter is more like the standard terrestrial fermenter adapted to accommodate microgravity environments. In the early days of CELSS, the use of hydrogen bacteria as regenerative food was considered.[5] An apparatus for operating such a system was suggested by scientists working for NASA. The Soviets also did some preliminary work on H_2 bacteria growth in their "flying oasis" which was reported to have flown on Soyuz 13 in 1973.[6] Both NASA and Soviet flight programs included algal growth chambers which were tested in various stages of development from ground-based studies to flight models.[7-10] Neither program generated sufficient data to evaluate their progress.

For over a decade, NASA personnel at JSC have directed the development of a bioprocessing system that includes both the production of pharmaceutical products and their separation in space. The bioseparation process has been demonstrated on successive STS missions over the past 2 years. Work in the bioproduction area has not progressed quite as rapidly due to concentrated effort on bioseparation. The project is designed to bring about the culture of mammalian cells to produce pharmaceutical products. Terrestrially, the culture of cells is compromised by sedimentation and oxygen transfer limitations. Microgravity can help overcome these problems. Over the past few years, U.S. and European flight experiments have shown positive microgravity effects on eucaryotic cell growth and cell size. A space bioreactor for cell culture has been proposed for operation in microgravity.[11] It is designed for eventual tandem operation with continuous flow electrophoresis. The elimination of sedimentation or bubble buoyancy is thought to aid in the growth and maintenance of mammalian cells which are extremely shear-sensitive. The purpose of these attempts is to enhance the production of pharmacologically important hormones and other medical products. It is important to note that design and performance data from the mammalian cell growth programs are not immediately applicable to the growth of microorganisms. The requirements for microbial food production units in a CELSS environment include high intensity cultures requiring significantly higher quantities of oxygen and mixing rates which would shear mammalian cells.

SPACE BIOREACTOR - DESIGN CONSIDERATIONS

Since the emphasis in this paper is on unconventional food production for CELSS, the following discussion will be concerned with fermenters as micro-

bial growth chambers. Bioreactors for other applications will have many characteristics in common with fermenters and their design and operation can have many similarities to the design and operation of fermenters.

A typical industrial fermenter on Earth will not operate in microgravity for the following reasons:

- Gas bubbles will not rise through the fermentation media due to absence of significant buoyancy forces.

- There will not be a single level separating the gas and the liquid. The gas bubbles may not disengage at these multiple interfaces.

- As gas transfer efficiencies of 100% are practically unachievable on Earth even in the absence of other fermenter constraints, there is not reason to believe that they will be attainable in microgravity. Phase separations are not spontaneous.

- Foaming is likely to be an even more severe problem as surface forces causing foaming will be more predominant in microgravity.

- The fermenter may oscillate between continuous liquid phase and continuous gas phase or both may coexist in various regions of the fermenter volume.

In submerged culture, aerobic microorganisms grow very rapidly until at a critical cell mass they are consuming all the oxygen that a fermenter can supply. For growth conditions of relevance to microbial food production, the biological kinetics are sufficiently fast that the rate of transfer of oxygen and rate of removal of carbon dioxide determine the microbial growth rate. Surface area of gas bubbles and internal convection within air bubbles control the rate at which the oxygen transfers to the growing cells. Buoyancy forces also act to enhance gas transfer by participating in more intense surface renewal and gross mixing. But in microgravity these forces are too small to be significant. Therefore, to provide efficient mixing of gas and liquid inside the fermenter, stable colloidal gaseous dispersions must be generated within the fermenter with the help of suitable surfactants.[12] Even though the surfactants will inhibit the mass transfer rate across the gas-liquid interface, through proper choice on concentration of surfactants it is possible to ensure that the increase in interfacial area more than compensates for the inhibitory role of surfactants. A second approach to overcome the problems of bubble size and lack of buoyancy is not to generate bubbles at all in the liquid medium, but to transfer the gases across a suitable membrane at a rate equal to the dissolution rate of gases in the liquid. A third approach will be to induce centrifugal body forces in the liquid medium and provide for buoyancy forces for gas bubbles to "rise" to the center. As

in terrestrial designs, baffles and other flow redirections can be provided inside the fermenter to augment the mixing intensity.

In microgravity, reluctance for dissimilar phases to separate is a serious design issue. If collidial gas bubble suspensions are employed, at the end of required cell growth, the surfactants must be disabled without toxicity and detriment to nutritional quality. Separation of gases from liquid and separation of cell mass from liquid will necessitate suitable membrane transfer or centrifugal separation units.

The problems associated with mass transfer can also be expected with heat transfer. Natural convection will be too feeble in microgravity and forced circulation over heating or cooling surfaces will be necessary which is also the preferred approach in terrestrial designs as well.

The problems of mixing and separation in microgravity will have to be overcome in suitable ways depending on the type of fermenter operation. Fermenters can be operated in batch mode, feed-batch mode, and continuous mode. In the continuous mode, the choice is between a single plug flow design and a multiple CSTR design with cocurrent or countercurrent gas flow.

Fermenter configurations vary owing to emphasis on one or more of the following key parameters:

- Oxygen transfer intensity
- Power economy
- Cell growth rate
- Production rate of other products

NEED FOR ENGINEERING DATA

In addition to physical configuration of a space fermenter, a designer must determine quantitatively the following:

- Power required for mixing.

- The space velocity or space-time which are measures of fermenter volume or the amount of time the nutrients and gases must remain in the fermenter for the required production rate of cell mass.

- The mass transfer rate achievable and the associated gas bubble size, mixing intensity, and interfacial area.

- The heat transfer rate necessary to maintain the temperature within the optimum range.

For terrestrial designs, power demand for agitators is determined from a correlation of a dimensionless quantity called power number with the Reynolds number based on the impeller distance. Data for this experimentally determined correlation were obtained in terrestrial agitators. The Froude number (the ratio of convectional acceleration to gravitational acceleration) associated with these data was less than 2 in most cases. This correlation cannot be applied without corrections to conditions of microgravity where the Froude numbers are very large. And the corrections must be experimentally determined.

The size of fermenter volume for a given rate of production of cell mass will depend on the mass transfer rate of oxygen through the liquid film which is determined with the help of the quantity, K_1a, which is a product of the mass transfer coefficient and the associated interfacial area. A number of factors determine K_1a which include bubble and cell dimensions, fluid density and rheological properties, agitator and fermenter geometry, and power input for agitation. Among these, bubble shape and dimensions, dynamics of bubble movement, and hence, the gas-liquid interfacial area and the agitator power input are affected by the absence of gravity. As pointed out by Oldshue,[13] K_1a does not scale in the same way as reactor size and agitation rate do. The design tools involving correlations of K_1a with the other factors must then be recreated for microgravity conditions.

Only two key parameters in the design of fermenters for microgravity application have been discussed above. The purpose was to illustrate a design engineer's concern in having to use the terrestrial data and correlations to design space bioreactors. On the other hand, it may turn out that for some of these parameters the corrections for microgravity application are indeed small, but it is not possible to know that without conducting experiments specifically for obtaining values of these key parameters in the region of high growth rate of cell mass. It is also necessary to determine experimentally the parametric region where bubble formation, fouling, foaming/entrainment occur under conditions of microgravity.

Once the database for microgravity operation of fermenters is established, a clear and dependable design methodology can be established to design space fermenters for any configuration, size, and product. Even if the database is not extensive, it is a great help to the design process to obtain good estimates of the magnitude of corrections to be applied to terrestrial design data.

TWO CANDIDATE SPACE FERMENTERS

The problem of intimately mixing oxygen with the fermentation broth or separating product gases in the absence of gravity can be overcome by designs

that are "gravity independent." Two such design concepts are presented here. In the first, direct gas-liquid contact is eliminated thus obviating the need for dealing with three-phase hydrodynamics in microgravity. In the second, the required gas-liquid contact and disengagement are forced in a controllable and predictable manner.

The "Gasless" Fermenter

The "gasless" fermenter is a closed sterilizable vessel through which an equally spaced bundle of polydimethyl silicone (PDMS) tubes pass. The PDMS tubes carry the oxygen required for fermentation and the carbon dioxide to be removed from the fermentation broth. Fermentation medium consisting of salts, carbon substances such as sucrose, and vitamins surround the tubes and fill the vessel. The fermentation fluid can be circulated through an external loop or agitated with an internal marine type propeller to keep uniform concentrations inside the vessel.

PDMS is six times as permeable to oxygen as polyfluorosilicone and 25 times as natural rubber and 600 times as high density polyethylene. PDMS preferentially transfer carbon dioxide over oxygen by a factor of 6 to 1. Oxygen diffuses rapidly through PDMS tubes to enter the fermentation broth by dissolution. Silicone hollow fiber tubes are a good choice because they, in addition to permitting high gas transfer rates, exhibit excellent biocompatibility and nonadherence to biological materials as testified by the choice of the same material for human and animal surgical implants. However, due to nonuniformity in tube thickness or tube packing density and in regions of stagnation of the fermentation broth, a small number of oxygen bubbles may form inside the vessel. These bubbles should be not allowed to build up and create problems of direct gas-liquid contact. As a safeguard against this, a small fraction of the fermenter contents will be degassed in a low-speed centrifugal separator and the separated liquid returned to the fermenter.

Carbon dioxide passes even more freely than oxygen across the silicone tube walls. However, the removal rate will also be governed by solubility of carbon dioxide in fermentation broth which again is a function of the pH. Carbon dioxide could be removed from the same tubes containing oxygen, or a portion of the tubes in the bundle may be dedicated for carbon dioxide removal. The use of a carrier fluid such as amines in these dedicated bundles in also a possibility.

Using a 1.5 liter Braun fermenter and a single strand of PDMS tubing, a yeast culture was grown successfully as a preliminary demonstration of this concept at Washington University. Air at 1 atmosphere pressure was supplied to the fermenter through the PDMS tube. The tube outlet was connected to a mass spectrometer gas analysis system which allowed the uptake of oxygen by the fermenter to be measured. The tube occupied 0.08% of the fermenter volume.

Transfer of oxygen into water and a fermentation medium as well as sustained yeast growth were demonstrated. Oxygen transfer rate into water at 37° C was found to be directly proportional to the oxygen driving force. Transfer of oxygen into the fermentation was five times slower than into water. An inoculum of yeast (S. cerevisiae) grew to produce a culture with a cell density of about 1g/1 in 2 hours. Visual and microscopic examination of the PDMS tubing showed no evidence of fouling after being left in the fermenter for several days.

The "gasless" fermenter was modelled as three CSTR's through which the fermentation broth circulates in cyclic fashion. Through two of these CSTR's PDMS tubes pass carrying oxygen to the broth and carbon dioxide out of it. Monod's model for cell growth was adopted. With this model, a computer program was written at Washington University and the effects of liquid flow rate, gas flow rate, and fraction of total number of tubes dedicated to CO_2 removal. At gas flow rates below 0.5 1/min, dedicating tubes to CO_2 removal actually helped increase the final cell mass. However, above 0.5 1/min of gas flow all the tubes had to carry oxygen to meet the demand for cell growth. Further, it was found that at flow rates of gas above 1 1/min cell yield does not significantly increase. When five percent of the fermenter volume was dedicated to CO_2 removal tubing, a very sizable decrease in dissolved CO_2 concentration was obtained. This result is significant for prevention of bubble formation inside the fermenter.

The absence of direct gas-liquid contact is a unique feature of this design concept eliminating problems of three-phase hydrodynamics. This fermenter does not require a gas disengagement volume and since the volume occupied by the silicone tubing is not expected to exceed 10%, volume available for cell culture is 90% compared to 60-70% for conventional fermenters. High oxygen transfer intensities, fewer moving parts, and low shear rates are some of the major advantages of this design. Potential problem areas to be addressed during the design and operation of this fermenter are (1) possibility of membrane fouling, (2) regions of stagnation, and (3) formation of oxygen bubbles.

The Rotating Packed Bed (RPB) Fermenter

The RPB shown in figure 11-1 has a cylindrical housing and, proceeding inward, has an annular region for air/oxygen distribution followed by a region of small packings and then a central region for entry of liquid and exit of gases. The whole assembly rotates about the axis of the cylinder. The rotation rate can change the throughput rate or, conversely, for a given throughput rate the mass transfer rate can be changed significantly. The RPB fermenter will not run at high speeds associated with the "Higee" units for fear of disintegration of cell mass. However, the packed bed will provide a more intense renewal of gas-liquid interface so that it may be possible to

PROPOSED MODEL SYSTEMS

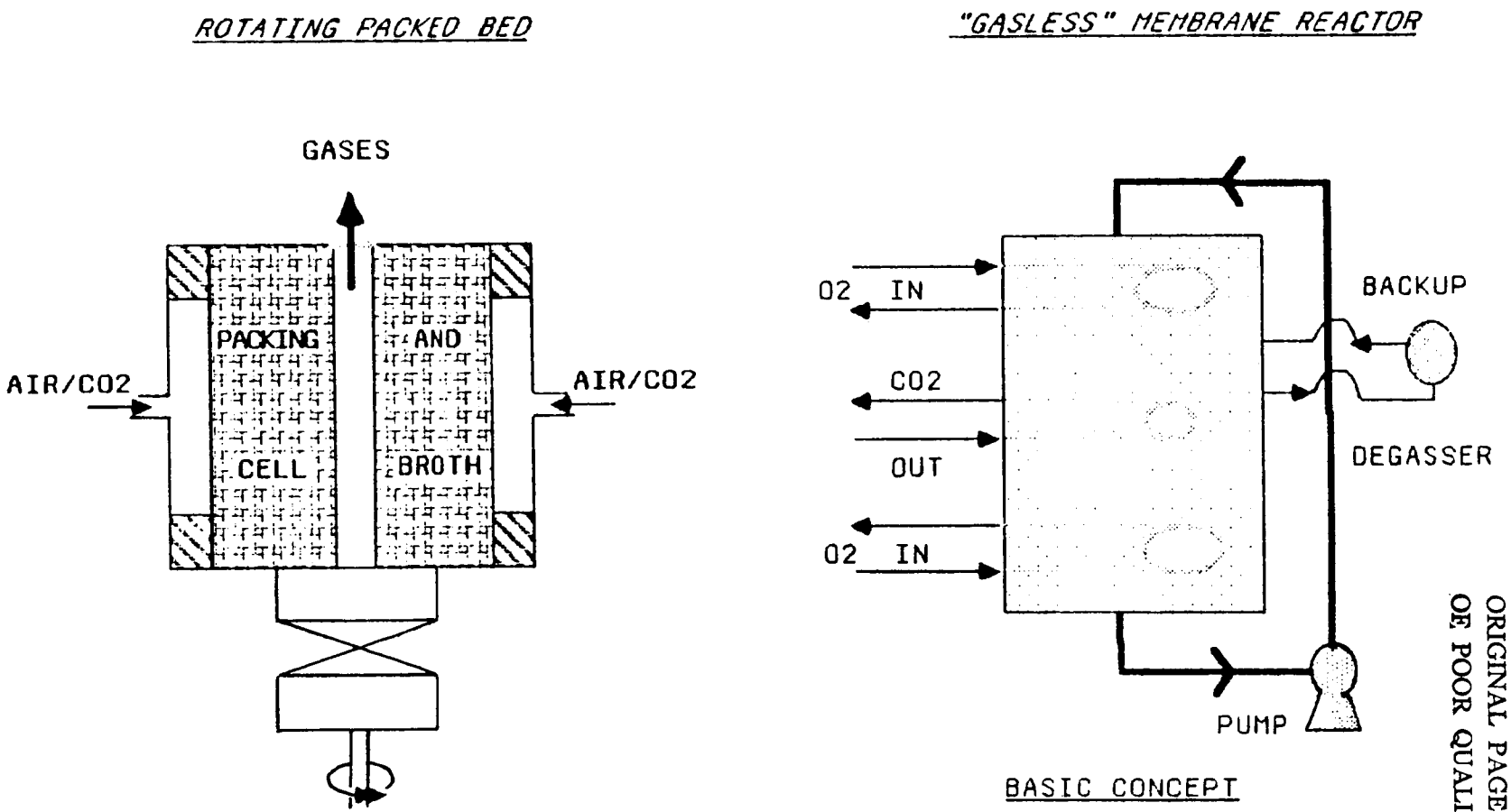

Figure 11-1.- Microgravity bioreactors for CELSS.

forestall any mass transport limitation on overall cell growth which is an important consideration in the design and operation of fermenters. The radial depth of packing will depend on the space-velocity (or space-time) required to achieve a certain growth rate. Since yeast culture is a low temperature operation, fragile ceramic packing need not be used. The packing material selected will withstand the high g's developed during STS liftoff.

The RPB was adapted from the commercial "Higee" unit of ICI Chemicals. This "Higee" unit was used to intensify chemical separations and its operating principles adapted to microgravity use provide a novel fermentation mode that could prove very efficient. Intensification through induced gravity provides a means of dealing with two-phase fermentation. A brief description of the operating principles and how this can be adapted to microgravity fermentation follows.

The Sherwood flooding correlation for packed beds provides a good estimate of the highest gas velocity which can be obtained for a given value of the ratio of liquid to gas flow rates (L/G).[14] The gas velocity (U) appears in the expression as U^2a/ge^3 where a is the specific area of the packing and e is the packing voidage. The term g, which normally represents acceleration due to Earth's gravity, has been recently generalized[15] to mean ambient acceleration to extend the correlation conceptually to other body force fields. When this is done, the correlation opens up new possibilities of packed bed operation through what has come to be called process intensification. For a given value of L/G and the flooding limit predicted by the correlation, by decreasing the specific area of the packing (a), or increasing the packing voidage (e), higher gas velocities could be achieved. However, even the latest improvements in packing design could not dramatically provide increases in gas velocity. Dramatic increases in gas velocity in a packed bed meant correspondingly high shear rates across the gas-liquid interface resulting in a great intensification of interfacial activity. This was indeed absolutely necessary for efficient interphase mass transport. With the announcement of ICI's "Higee" units, the engineering profession became keenly aware of the g-term in the Sherwood correlation which has been taken for granted as a constant to be used to compute the gas velocities correctly. By opening up the possibility that g could be varied by reconfiguring an absorption or distillation unit into a high-speed rotating cylinder packed with much finer packing than could be used before, ICI engineers demonstrated a dramatic reduction in equipment size and weight for a given separation operation. By increasing g in a rotary mode, one had a choice between increasing throughput rates for a given packing size and type or increasing the mass transfer rate by enabling the same throughput rate through much finer packing.[15]

The RPB fermenter will not quite operate at the level of intensification achieved by the "Higee" units for two reasons: (1) there is no advantage in

supplying dissolved oxygen any faster than the consumption rate of oxygen by the growing cell population, and (2) very high interfacial shear rates obtained in "Higee" units can damage cell walls and terminate fermentation or cell growth process. However, an optimal rotation rate and radial distance will be adopted to take advantage of the intensification effects of centrifugal acceleration while maintaining healthy cell growth. There is another important difference between RPB and "Higee" units. "Higee" units are very efficient countercurrent gas-liquid flow generators for rapid separation operations. The RPB fermenter is, on the other hand, a fermentation reactor and is not a mere component separator. Therefore, the RPB fermenter is limited in its liquid throughput rate to allow the long space-times required for growth of biomass. In fact, this fermenter may fall on one extreme end of the Sherwood correlation corresponding to very low liquid throughput rates and very high gas velocities.

The "Higee" units in operation either in industry or in the laboratory are exclusively designed and built for separation operations such as distillation and absorption. No "Higee" unit has yet been built and operated as a chemical rector or fermenter. A RPB fermenter combines gas-liquid mixing, cell growth and gas liquid separation in one operation with the advantages of lower volume and weight requirements compared to conventional fermenter types. Potential problem areas to be dealt with during design and operation of RPB fermenters are entrainment of liquid and foam by the rapidly countercurrent gases.

GROUND AND FLIGHT TESTS

Our approach to developing these bioreactor concepts is to ground-test and then flight-test identical fermenters. Two or more sizes would be initially tested with a view to specifically measuring mixing effectiveness, bubble sizes, mass transfer coefficients, power required for agitation, etc., in addition to growth rate of cell mass. By this approach a sound database is generated, reliable scale-up factors are derived, and procedures for applying corrections to terrestrial designs, so as to obtain designs for microgravity conditions.

The design procedure developed will enable the design engineer to calculate, for a given cell mass production rate, optimum gas velocities in a "gasless" fermenter, optimal rotation rates and radial distances, and intensification factors in a RPB fermenter. In addition, the design engineer will have the information to guide his choice of fermenter configuration, tubing size and arrangement, degassing requirements, carrier fluids, control of membrane fouling, secondary metabolite production rates and their disposal, extent of foaming, extent of channeling of gases, and cell disruption as a function of operating conditions.

Since both designs are essentially gravity independent, their development can largely be done terrestrially. This will provide cost savings by elimination of the need for numerous flight tests.

CONCLUSIONS AND RECOMMENDATIONS

Microbial food sources are becoming viable and more efficient alternatives to conventional food sources especially in the context of CELSS in space habitats.

Since bioreactor designs for terrestrial operation will not readily apply to conditions of microgravity, there is an urgent need to learn about the differences. These differences cannot be easily estimated due to the complex nature of the mass transport and mixing mechanisms in fermenters. Therefore, a systematic and expeditious experimental program must be undertaken to obtain the engineering data necessary to lay down the foundation of designing bioreactors for microgravity. This may be the harbinger of a major subdiscipline called variable gravity process engineering.

Two bioreactor design concepts presented here represent two dissimilar approaches to grappling with the absence of gravity in space habitats and deserve to be tested for adoption as important components of the life support function aboard spacecraft, space stations, and other extraterrestrial habitats.

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