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SPACE BIOREACTOR: DESIGN/PROCESS FLOW

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The design of the space bioreactor stems from three considerations. First and foremost, it must sustain cells in microgravity. Closely related is the ability to take advantage of the weightlessness of microgravity. Lastly, it should fit into a bioprocess such as shown in figure 2-1. This paper will describe the design of the space bioreactor in view of these considerations. The flow chart of the bioreactor will then be presented and discussed.

The conceptual space bioprocess in figure 2-1 yields pure product after six steps. The initial step is to select the cells that produce the most product. Studies already conducted in space have shown that some cell fractions selected by electrophoresis produce more of a given substance than others. The next step is to prepare a bioreactor inoculum from these cells.[1] This step is shown in a dotted box, because it has not been investigated in space. The third and fourth steps are those covered by the space bioreactor. The bioreactor design consists of two loops. The first loop contains the cell culture vessel and all the apparatus necessary to sustain the cells. The second loop, isolated from the first, has an ultrafilter or similar device for separation of the product from the cell culture media. The fifth step, again not tested in space, is concentration to the point the solution of product can be introduced into a continuous flow electrophoresis system for final purification. This last step has been quite well developed by the McDonnell Douglas Astronautics Company, Inc.

An important driving force for building a space bioreactor is clear from figure 2-1. The first and last steps are more efficient in space. Connecting the two with a space bioreactor makes possible a fuller exploration of the advantages of space for manufacturing pharmaceuticals. It should also be pointed out that the cells for the first step could be grown in space in the bioreactor.

The next consideration in the design is to take advantage of microgravity. Cells cultured in the weightlessness of space will not sediment. The only stirring required is to distribute oxygen and nutrients to the cells. This amount of stirring is expected to be considerably less than is needed to keep the cells suspended in 1-g. Less stirring means less turbulence and a lower rate of cell death. Consequently, it is hypothesized that in space higher cell concentrations can be cultured.[1] To allow for the best experimentation on this hypothesis, the cell culture vessel has few internal parts. The sensors and other parts are instead placed in the first loop mentioned above. The medium is pumped around the loop and passes over sensors for oxygen,

carbon dioxide, and pH. The oxygenator and other necessary vessels are also in this loop, called the main medium circulation loop. The reactor vessel has in it only a stirring vane and a spin filter, which keeps the cells in the reactor.

What type of cells would benefit most from a minimal stirring situation? After an assessment of different cell culture systems, it was concluded that the answer was mammalian cells on microcarrier beads. Specifically, human embryonic kidney cells have been selected for the first flights because they have been separated in space by electrophoresis. These cells produce urokinase, a pharmaceutical that dissolves blood clots. Many other types of mammalian cells could be cultured with minor procedural modifications. Details of the reactor design reflect the culture requirements of mammalian cells.

A second advantage of microgravity is expected to come from the stability of foams in space.[2] On Earth a foam of a gas in a liquid separates rapidly as the gas rises to the top. In space this density-driven separation does not occur and surface tension forces the gas bubbles from coalescing. One proposed experiment is to study oxygenation of a cell culture with a foam of tiny air or oxygen bubbles. The modular bioreactor design, a circulation loop connecting specialized vessels, allows for insertion of the apparatus needed for this experiment. Hopefully, this flexibility will prove to be general. Other experiments might be proposed after the data from several flights of the bioreactor clarify the effects of microgravity on cell culture.

The combination of operating in microgravity and in the Shuttle Orbiter vehicle places certain constraints on the bioreactor design (table 2-1).

Headspace will not exist in the cell culture vessel in microgravity. Many bioreactors use this headspace as a reservoir for oxygen and carbon dioxide. In the space bioreactor, the vessel is kept full of medium. Without headspace, the bearings for the spin filter and the stirring vane are immersed in the medium. Consequently, a potential exists for grinding cells between the bearing surfaces. Designs for the cell culture vessel include versions with sleeves that cover the bearings and versions that route cell-free medium through the bearings.

The remainder of the items in table 2-1 are operational constraints. Complete liquid containment is mandatory, because spills in space cannot be easily cleaned up; the bioreactor uses magnetic drives to minimize points for leaks and stainless steel tubing instead of plastic tubing. To minimize the possibility of contamination during operation in a nonsterile environment, the bioreactor is autoclaved as a sealed unit. It is opened only to charge it with medium and cells. The bioreactor must be compact and energy efficient to allow the maximum number of experiments to be carried out on each

Shuttle flight. The lessons learned from these design constraints may give industrial biochemists clues for reducing capital and operating costs. Automatic operation of the bioreactor is another consequence of trying to maximize the number of experiments on every flight; the crewmembers can spend only so much time with every experiment. The space bioreactor design includes an advanced process control scheme (described later) that provides for operating the system, logging data, and controlling the dynamics of a biological system.

This completes the discussion of the design considerations for the bioreactor. The next subject is a description of the flow chart. To begin this, table 2-2 summarizes the essential elements of any bioreactor. The following discussion will show how these elements are expressed in this particular design. Detailed descriptions of individual components are given in the paper by William Bowie.

The culture vessel, labeled reactor vessel, is a 500 ml perfused vessel with a new version of a spin filter from Virtis Company (fig. 2-2 and table 2-3). The cells are, as stated, anchored on microcarrier beads and suspended in the culture vessel. To get the desired gentle mixing, a stirring vane similar to that described by Feder and Tolbert[3] will be tested initially. The reactor is designed to operate with no headspace. Medium from the main medium circulation loop, Stream 1, is pumped into the reactor vessel and withdrawn through the spin filter, which allows the medium to pass, but retains the microcarrier beads with the cells. Locating sensors for pH, dissolved oxygen (dO_2), CO_2, and redox potential before and after the reactor in the medium circulation loop provides for differential measurements across the reactor and reduces turbulence and shear within the reactor.

In Stream 1 are vessels for oxygenation, separation of depleted medium (upper left, Waste or Sample Tap), replenishment of medium, and pH control. A connection to the product separation loop, Stream 3, is also provided. A dialysis unit to separate wastes is contemplated, but has not been incorporated. Provision is made to maintain the entire loop at 37° ± 1°C. The oxygenator has a membrane that is permeable to oxygen and carbon dioxide. A gaseous mixture of nitrogen and oxygen (possibly room air) is passed through one side of the membrane. Carbon dioxide from metabolism passes from the medium to the gas mixture and is carried away. This design allows for addition of CO_2 if desired. System pressure is designed for 5 psig with a maximum of 15 psig; four transducers are incorporated in the system. Concentration of the medium is done in Stream 3 by ultrafiltration or hollow fiber filtration. This loop will be run every 3 or 4 days to provide a solution of urokinase and other cell products for analysis. (In a complete bioprocess, this loop would be designed to provide crude urokinase to a purification unit, figure 2-1, steps 5 and 6.)

The process control scheme has two levels of control (fig. 2-3). The level depicted at the top maintains set levels of pH, dO_2, CO_2, and temperature. These variables determine the environment of the cells. The environmental controller is being built in the JSC labs from a STD bus system. This system has been used successfully for the McDonnell Douglas continuous flow electrophoresis system and the 3M crystallization unit. In addition to operating the system, the STD bus system logs data from the biochemical sensors, the flow meters, and the pressure transducers. This part of the process control system will fly. In many processes, controlling the environmental variables would control the process. A biological system, however, is more complex. To obtain the extra control required, a second level of control has been designed to monitor the metabolism of the cells.

This function, termed an "expert system," will supervise the process controller by adjusting the environmental set points. The metabolic parameters now thought to be the most important are the respiratory quotient (dO_2/CO_2 concentration ratio), concentration of NADH, and the type and quantity of cell secretory products, such as urokinase. Some of the data used by the expert system will be from online sensors, but results from reactor samples and subjective evaluations of previous experience will be incorporated using "fuzzy modelling."[4] An IBM AT computer will be used. The expert system computer will be used for development and other ground-based operations. More details are given in the paper by Bill Hall.

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4. Turunen, I., Nyberg, T., Jarvelainen, J., Linko, Y-Y., Lindo, P., and Dohnal, M., "Fuzzy Modeling in Biotechnology: Sucrose Inversion," The Chemical Engineering Journal, 30:B51-B60, 1985.

TABLE 2-1.- HARDWARE CONSTRAINTS

No Headspace
Complete Liquid Containment
Autoclave
Compact
Energy Efficient
Automatic

TABLE 2-2.- ESSENTIAL ELEMENTS

Oxygenate
Resupply Nutrients
Remove Wastes
Remove Product
Collect Biochemical Data

TABLE 2-3.- STREAM DESCRIPTIONS FOR FIGURE 2-2

Stream No.	Description	Composition	Flow Rate	Remarks
1	Main medium circulation loop	Cell free medium	50-100 ml/min	This stream provides all the cell maintenance requirements. Composition adjusted by process controller.
2	Feed to product separation loop	Cell free medium	As needed	
2a	Flow restriction device	Below device, same as 1. Above, same as 3.	-	Device provides for adding Stream 1 to Stream 3.
3	Product separation loop	Medium, relatively high product concentration	180-200 ml/min	Operated every few days to concentrate medium sample, which is withdrawn for in-flight product assay.
4	Return stream from product separation loop	Medium, depleted of product	Low, 20-30 ml/min	Can be diverted to waste vessel.
5	Oxygenator feed	5-100% O_2 0-5% CO_2 N_2 balance	Flow essentially unrestricted	-
6	Oxygenator effluent	O_2 (excess) CO_2 from metabolism N_2	Flow rates same as 5	-

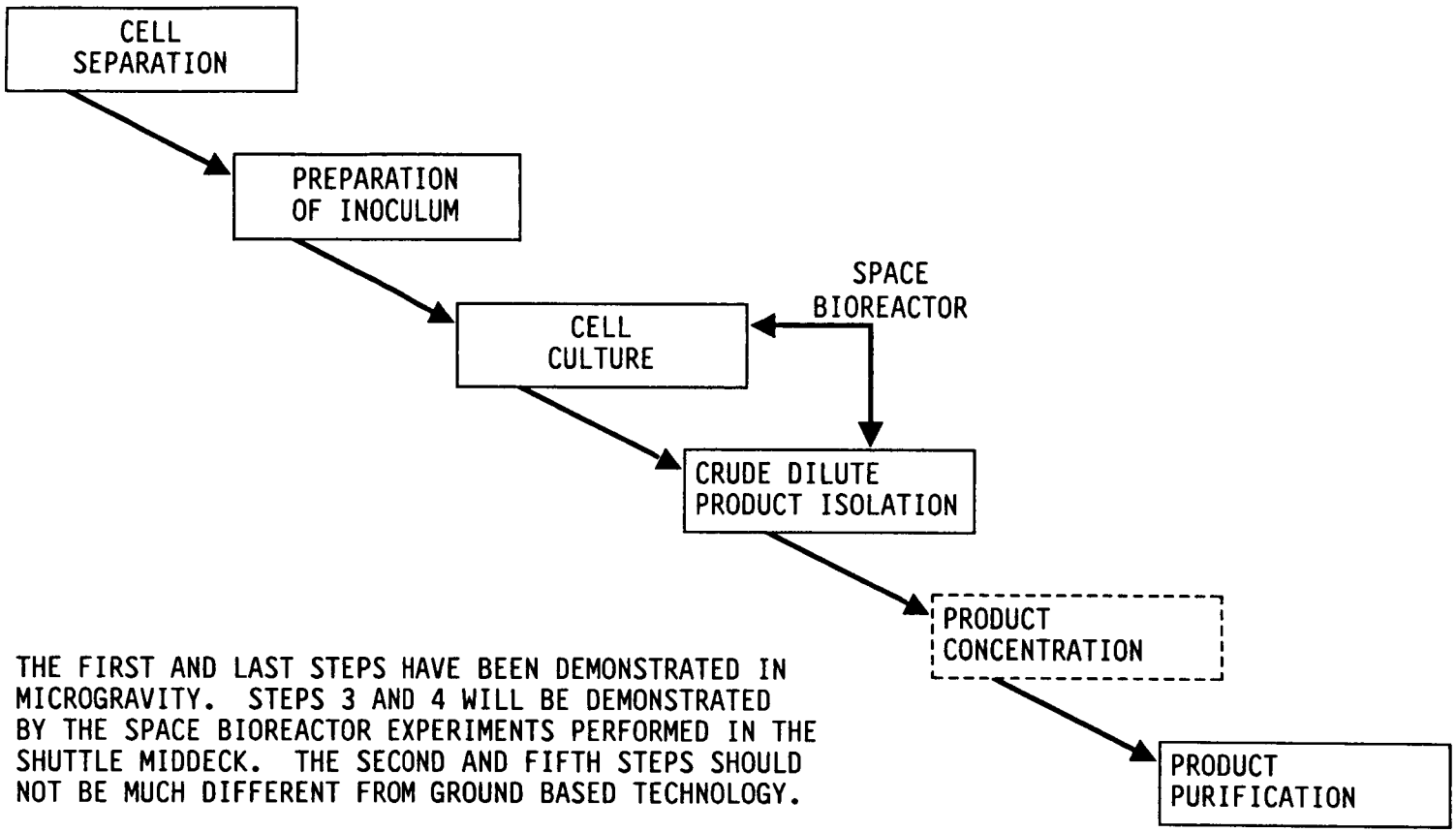

Figure 2-1.- Conceptual bioprocess.

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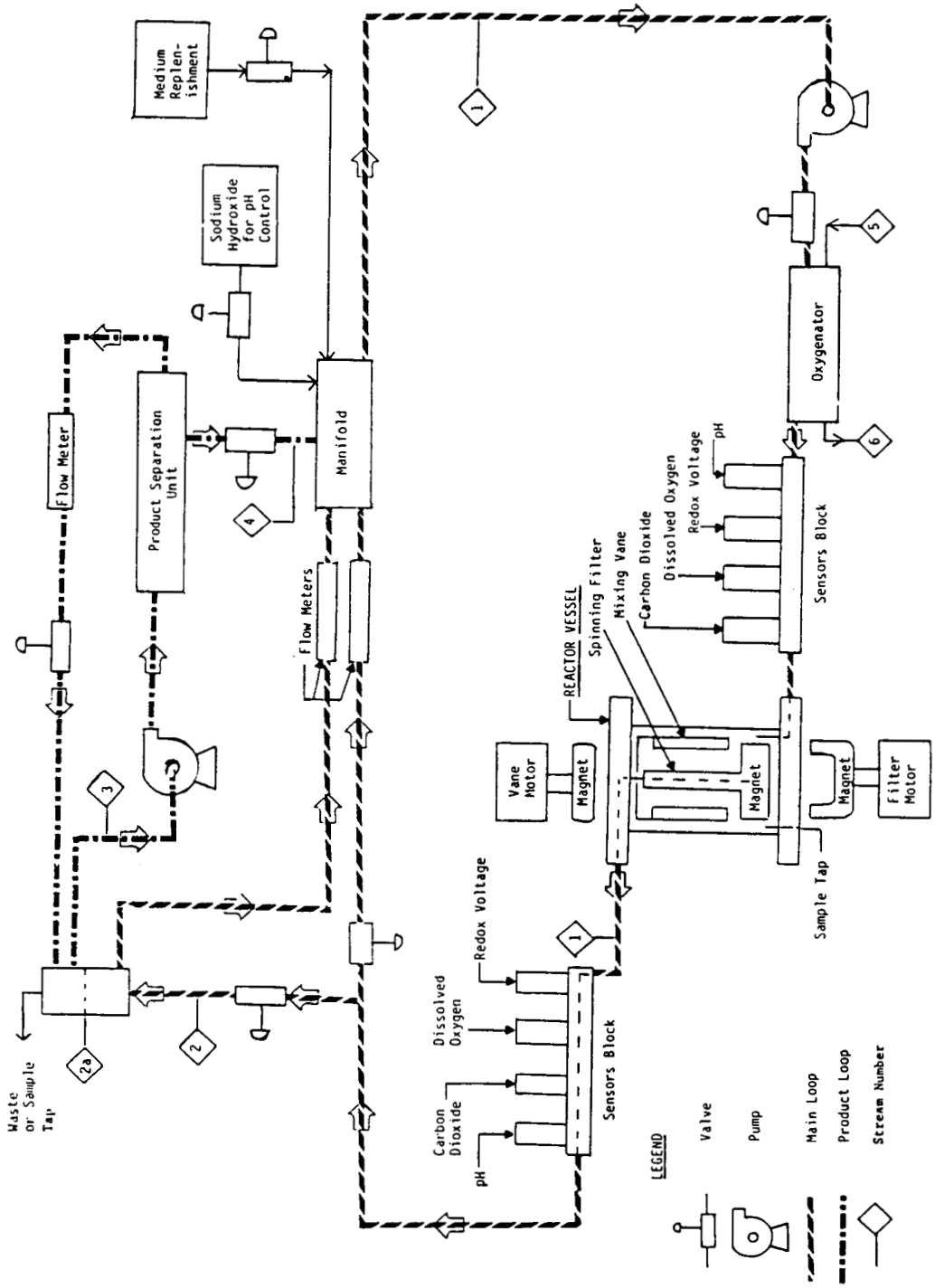

Figure 2-2.- Space Bioreactor flow chart.

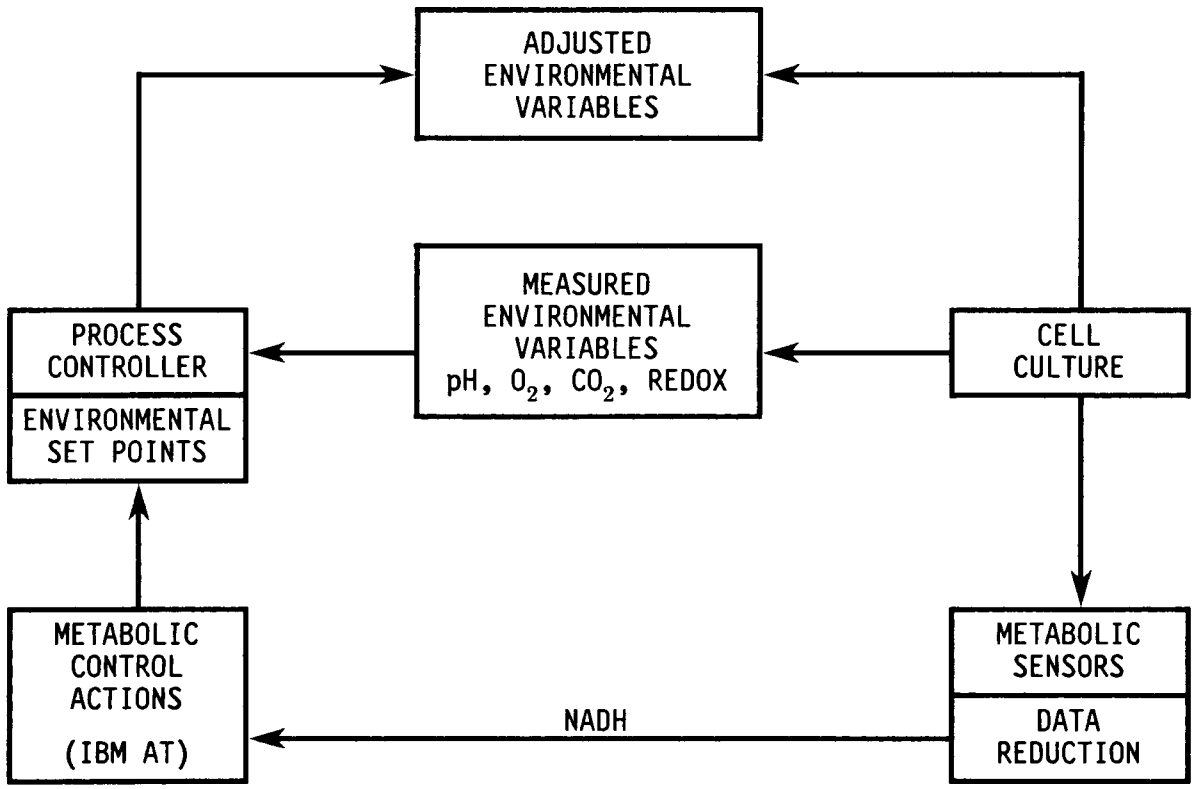

Figure 2-3.- Process control scheme.