BIOREACTOR DESIGN CONCEPTS

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INTRODUCTION

Serious development of a space bioreactor was begun at JSC in the spring of 1982 under direction of the Principal Investigator (D. R. Morrison, JSC). The work was an outgrowth of earlier studies by Martin Marietta, Arthur D. Little, and Fermentation Design. The results identified the concepts for suspension cell culture in a zero-g environment.

The first two years of the program were devoted to development and proving of concepts. Two of those concepts which received the most work were "zero headspace" and diffusion oxygenation. Other areas of research were sensor technology, pump operation, sterility, and culture maintenance. Work during those first years was conducted with mouse lymphocytes, strain L1210. Those cells were chosen because of their ease in culturing, then low cost and availability. We did not think it prudent to utilize an expensive, hard-to-obtain cell line during early development work.

The reasons and rational for the construction of a space bioreactor are not covered in this paper. Those topics are detailed in the first paper (Morrison) presented at this bioreactor workshop. This paper presents some of the developmental logic and considerations which have led to the present successfully operated laboratory version of the space bioreactor.

BIOREACTOR-EVOLUTION

Bioreactor systems techniques and equipment have undergone significant changes since initiation of the program. Due to the developmental nature of the reactor system, such changes can be expected and are a normal part of the system evolution.

Bioreactor equipment and technique changes have been incorporated in the following systems:

- Pressure control system
- Fluid flow
- Gas input control
- Sterilization
- Stir (agitation) system
- Oxygenation

- Culture density control
- pH control

The following are brief explanations of each system area.

a. Pressure control system - Early efforts at pressure control relied on pressure gages and subsequent adjustment of pumping rates or fluid bypass flows. However, pressure gages have proved to be unsatisfactory because of their inaccessibility via electronic systems. As long as the system was sterilized by heat, cavities in such devices presented no serious problems. If heat sterilization is not desirable, contaminating organisms trapped in the cavities cannot be killed or removed without the use of strong chemical sterilants. Sterilants such as ethylene oxide or glutaraldehyde which outgas enough to penetrate empty cavities. However, absorption of the sterilant by water trapped in the cavities leads to leaching of chemical contaminants into the process system. Consequently, a substantial effort was initiated to located pressure transducers with no dead end cavities. One such device was located and installed, but has failed to operate satisfactorily.

b. Fluid flow system - Rotometers of fixed cross-sectional areas were utilized originally for flow measurements. Proper functioning of such devices was dependent upon their orientation in a gravity field. Also they were not inherently accurate devices and resulted in more fluid path length than was desirable, thus increasing the system volume.

   Electronic flow transducers were ordered for the second stage reactor. Two different types of transducers were desired; one was dependent upon differential pressure across an orifice, while the other was a vane type turbine meter. The differential pressure device is used in the low flow loops where rates of 10 ml/min or less are encountered. Turbine meters are used in the higher flow loops where flow rates of 10's-100's ml/min are encountered. Both flow devices function in a zero-g environment.

c. Gas input control - Our original bioreactor system relied on gas flow rates set by metering valves on the input side of rotometers. As the system pH decreased due to cell metabolism, adjustments in the CO_2 and air input rates were recognized as necessary. A common observation was a shift to alkalinity when attempting to input relatively high (>20%) concentrations of oxygen. Such shifts could be corrected by increasing the CO_2 concentration. However, the system was constructed to accommodate only two gases. Thus, if greater than 20% oxygen were required it would have to be mixed prior to entering the control valve and meter. Such a gas mixing system was not available during the early phases of reactor construction.

A more advanced reactor incorporated a gas mixing system which controlled a component gas at a fixed level relative to a carrier gas. For instance, if 5% CO_2 in air were desired, the system would deliver 5% CO_2 relative to the air flow rate. Increasing the oxygen concentration over 20% was still a problem, but one that was partially solved. A one-way check valve was installed in the air input line upstream from the air controller. Oxygen was brought into the system between the check valve and the controller at a higher pressure than the incoming air. As the oxygen tension in the reactor vessel fell below some set point (i.e., 20%), a solenoid valve opened allowing a short surge of oxygen to enter the air line. The higher oxygen pressure closed the check valve, but also increased the flow rate and pressure sensed by the controller. Consequently, control measures were automatically instituted to maintain the previously set flow rate. Some transient bounce was observed in the resulting flow, but it did not affect the gas absorption dynamics of the reactor media.

A more desirable method of gas control would have been an additional control unit dedicated to oxygen. Thus, three controllers would provide CO_2, air or nitrogen, and oxygen gas as required. Their total flow rates would be compared to the output of a flowmeter upstream from the reactor and the flow of each gas would be adjusted to maintain a desired oxygen-CO_2-air or nitrogen level.

d. Sterilization - Considerable difficultly was encountered in the development of system sterilization techniques. Several components (i.e., probes and oxygenator) were heat labile and suffered severe performance decrements after steam autoclaving. Nevertheless, the first prototype system was sterilized via a combination of autoclaving and ethylene oxide. The system was dismantled and physically moved in pieces to the various facilities. Such procedures proved to be awkward and time consuming, but were successful.

   Several chemical sterilants or disinfectants were tried and of those, a 2% glutaraldehyde proved to be successful. Thus, it was not necessary to dismantle the system except to wash it and even that could be successfully accomplished by the use of an appropriate detergent circulating through the reactor plumbing. However, a major disadvantage to the in-place washing and sterilization is the long period of post sterilization washes with sterile water to remove sterilant residuals.

e. Stir (agitation) system - A Corning laboratory stirrer was used as the first reactor stirrer. The device had a small rotor that was prone to decoupling from the vessel stirrer. It also was difficult or impossible to regulate slow stirrer speeds (in the area of 30 rpm or lower) due to the motor characteristics.

As a consequence, a commercially available motor and controller were purchased. The motor was equipped with a feedback generator which provided shaft rpm data to the controller, thus accurately regulating stirrer speed at some dialed-in valve. A large, heavy magnet was affixed to the motor shaft, thus lessening the possibility of stirrer decoupling. Speed control was very precise from very low rpm (<10) to very high rpm (>500).

f. Oxygenation - A 4.5 m^2 membrane external oxygenator was first utilized; however, the device proved too large and resulted in very low through media flows. Such low flows through the oxygenator in turn caused pockets of media stagnation. A 0.4 m^2 membrane device was subsequently purchased and produced better results than larger units. Flow rates were higher through the device, which resulted in little or no media stagnation.

Low L1210 cell densities (to about 200,000 per ml) could be adequately oxygenated with air at 100 ml/min and liquid flow rates of about 0.5 ml/min. Higher density cell population (to about 500,000 per ml) required 100% O_2 at the above flow parameters while cell densities over about 500,000 cells/ml required an increased fluid flow.

A more efficient oxygenator system was required because of pump rate limitators through the spin filter. Consequently, a silicon membrane envelope was applied to the inside of the reactor vessel. Thus, no media pumping was required to contact the membrane. Culture densities of 1.8×10 cells/ml were achieved with air plus 5% CO_2.

g. Culture density control - Cell culture densities depend on the supply of nutrients and respiratory gases. Oxygen supply control has been discussed previously and will not be presented in this section.

Control of cell densities has been accomplished in other systems (i.e., algal and bacterial) via light attenuation or scattering techniques. As the culture increased in density more attenuation or scattering would occur and at a predetermined value, a pump would be activated to remove old culture and infuse fresh media. Such a system can be made functional on the bioreactor provided suspension cells are grown.

Use of pH indicators such as phenol red in the media complicates the light sensing circuit by introducing a color change as the culture matures. Consequently, elimination of the indicator dye is recommended for bioreactor studies. With no color change, a simple turbidistat will control suspension cell densities.

h. pH control - Cell culture media (Fischers media for leukemic cells of mice) has a pH of about 7.2 (6.9-7.3). However, during cell growth, that pH will normally shift toward acid. An opposite shift toward alkalinity occurs in the pressure of high dissolved oxygen or at room atmosphere equilibration.

Neither pH extreme can be tolerated long by the cells. So some means of controlling the hydrogen ion concentration was necessary. Such control was complicated by the 5% CO_2 routinely used in the input gas. As the culture increased in density, more oxygen and less CO_2 became necessary. Thus, the ratio of oxygen to CO_2 was changed throughout the life of the culture and that change resulted in undesirable shifts in the culture pH. In order to counteract gas and growth-induced pH changes, an external pH control loop was devised.

The pH control loop received its input from a sealed combination pH electrode immersed in the growth media. That signal was amplified by Orion pH meters and fed into a double comparator circuit. As the pH shifted toward acid or base, the voltage sensed by the culture electrode was continuously compared to a reference voltage. When the sensed voltage drifted higher than the reference voltage, a pump was activated through a solid state relay and acid (.25N HCL) was slowly added to the culture. Conversely, when the pH fell below its reference value, .25N NaOH was pumped into the culture.

THE CURRENT SYSTEM

The bioreactor consists of two major fluid loops. A third loop may be envisioned by connecting the two major loops. All three loops are shown by heavy, dark lines in figure 3-1. The primary loop routes fluid through the reactor vessel (RV) and its support equipment, while the secondary loop routes fluid through the protein extraction unit. A connecting fluid line between the extraction unit and the filtrate vessel completes the fluid circuit.

A number of different fluid pumps were considered for the bioreactor. Any pump that produced detectable pulses in the fluid flow was eliminated in order to achieve tighter control through the feedback circuits. An additional elimination factor was the presence of dynamic seals; such seals have the potential of leaking and cannot be tolerated in a sterile pressurized system. The pumps chosen and shown as P1 and P2 in figure 3-1 were positive displacement, essentially pulseless units with static seals. However, some question has been raised as to the effect of the gears on long chain, complex molecules. Studies into that area are planned, but are not yet underway.

Shown in figure 3-1 on the output side of P1 is a feature called a "high dialysis refeed" and the notation TBD. That feature represents one potential location for a media conditioning unit to be composed of some type of dialysis equipment.

Commercial fermentors have, in the past, utilized headspace and sparging for media oxygenation. Headspace cannot be tolerated in zero-g, but some sparging with microbubbles and controlled foams may be usable concepts. Our efforts to date, have been directed toward diffusion oxygenation. One such diffusion device involves the placement of a thin silicone membrane bag, envelope, or coil inside the reaction vessel, thus, diffusing oxygen directly into the cell support media. That method works well for small vessels (thus far up to about 1 liter) and eliminates the need for fast fluid flow; it also simplifies the system pumping. A second method utilizes oxygenators external to the reactor vessel.

An extracoporeal blood oxygenator is downstream from the proposed dialysis unit in figure 3-1. The oxyenator functions to provide cells in the reactor vessel with a vital oxygen supply.

The oxygenator chosen for our bioreactor is manufactured in several different sizes and is a diffusion device. Incidentally, we discovered that the same unit is used by McDonnell Douglas to degas and remove bubbles from their continuous flow electrophoresis system. However, the commercial version does not tolerate repeated use and cannot be autoclaved, thus, some modifications were necessary to adapt it to the bioreactor. Those modifications consisted of repackaging the membrane in a rigid, temperature-resistant sleeve and rerouting the gas input and output lines.

Gases are provided to the oxygenator via a set of three mass flow controllers; one each for nitrogen, oxygen, and carbon dioxide. The total gas flow is set and controlled via a mass flowmeter on the oxygenator input. Oxygen, nitrogen, and carbon dioxide ratios are adjusted via computer commands to provide an optimal environment for the cells.

The block labeled SB1, downstream from the oxygenator is a device called a "sensor block." It consists of a delrin block with a through-channel and at least four electrochemical sensors. The channel allows media to pass through the block. Each electrode is positioned such that its sensing element is in the media channel. The block contains one each oxygen sensor, carbon dioxide sensor, pH electrode, and reduction-oxidation electrode.

Several oxygen, carbon dioxide, and pH/Redox electrodes have been investigated. The oxygen electrode installed in the bioreactor was chosen by a combination of default and recognition of the state of fermentation technology. All other electrodes tested or investigated failed in some respect

to satisfy our requirements. Also, the concensus among fermentation users was that the electrode chosen was perhaps the best available. The carbon dioxide electrode was chosen because no other suitable electrode was available. Redox and pH electrodes were available from several manufacturers, but only Phoenix electrodes worked with us to develop the type of electrode required for our bioreactor system.

The heart of the bioreactor consists of the RV. That vessel contains 500 ml of cell bead suspension and the media extraction filter. The principle of tangential filtration is employed to remove media from the RV. Specifically, a spinning filter is used which, due to the sweeping motion of the fluid across the filter, results in much less filter clogging than would be encountered in a stationary filter.

A spinning filter alone will not suspend the cell bead mass, so small propellers or vanes have been used for that purpose.

A second sensor block (SB2) identical to SB1 is located on the output side of the RV.

Cell debris conceivably could find its way through the spin filter body or seals and thus be forced into the reactor fluid stream. Consequently, a stationary filter has been installed before the mixer vessel to trap particulate material before it enters the protein extraction unit.

A second primary loop vessel is a mixer vessel and serves to provide a buffer and reservoir between the relatively low flow RV system and the higher flow of the high molecular weight (HMW) extraction system.

Extraction of high molecular weight products is accomplished in the secondary loop which derives its feedstock from the mixer vessel. Pump P2 is similar to pump P1 and provides the required flow through the protein extraction unit. An optimal pressure is required for the extraction unit to successfully extract product; that pressure is provided by a bellows valve downstream from the extractor. During operation, 80 to 90% of the media passing through the extraction unit is shunted back into the mixer while the remaining few percent is bled off as waste or shunted into the filtrate vessel.

Between the extractor and the filtrate vessel is a second potential site for a dialysis unit. Both dialysis units are undefined and will remain so until analytical data detailing media depletion and waste product buildup is available.

The filtrate vessel is functionally a manifold into which fresh media is input and from which pump P1 draws media for the main loops.

A number of support vessels are necessary. Those vessels are the media reservoir, sodium hydroxide waste vessel, and pressure relief-air vessel waste vessel. No acid reservoir is utilized because during the life cycle of a cell culture, a strong trend toward acidity is normal.

The media storage, NaOH, and waste vessels employ a rolling diaphragm to provide compliance. Diaphragm position and vessel volume is determined via linear variable differential transformers attached to the piston. All vessels have the feature of being detachable from the system via quick disconnects. However, the media reservoir and NaOH vessel reside in the sterilized bioreactor system, while the waste vessel is a component of the service module and remains outside the system.

Pressure relief and air waste is provided by a small vessel, about 250-300 ml, loosely filled with hydrophilic wicking material. Filter F1 consists of two 0.2 um hydrophobic filters in series and provides air to the system for filling, draining, and venting.

BIOREACTOR PLANS

The bioreactor system now under development for the immediate future is intended to support microcarrier culture.

Development efforts now underway consist of the following:

- Minimal media replacement and maximal water recycling
- Stirring/agitation techniques
- Automation
- Sensor development and application
- Maximizing product recovery
- Developing ways to culture specific, high producer cell fractions from the continuous flow electrophoresis system (CFES)

The following is a brief statement of our plans in each of the developmental areas.

a. Minimal media - Use of a bioreactor or the CFES system in microgravity depends on the availability of water. Transport of that water to orbit is expensive, so media exchange or conditioning via dialysis is being explored to lessen that total transported water load. However, to de-

termine what in the media must be exchanged and how well that exchange occurs will require an analytical capability. A high pressure liquid chromotography system (HPLC) or equivalent system is proposed as the nucleus of that facility.

b. Stirring/agitation - The purpose of stirring is to provide cell-media contact. Such contact can be achieved through the use of various blades or propellers submerged in the cell suspension. However, the use of mechanical devices can create severe turbulence and shear forces that may strip cells from their anchorage surfaces.

c. Automation - In a space platform or even in a space station, crewmember time is at a premium; therefore, the bioreactor systems must be capable of limited unattended operation. Such systems depend on complex interrelationships between the living cell and its environment. All the controlling factors of a cell culture are not known. Nevertheless, the system must be capable of self-monitoring and must have some decision-making capability.

   We must first learn what needs to be controlled; second, we must learn the relationship between the controllable factors and, finally, we must learn how to apply electronic sensing and control techniques to manipulation of those factors.

d. Sensor development - Most sensors (pH, O_2, CO_2, ROX, etc.) in use today are products of and for the laboratory. Sensor technology that is closest to bioreactor use is that used in the process control industry. In addition, some fermentor manufacturers market sensors; however, those sensors from both markets are generally inadequate for our use. They usually cannot be adequately sterilized, they may be too large, or they may leak electrolyte. Consequently, the sensors now is use in our bioreactor are regarded as marginal. Much additional sensor development must be undertaken before semi-automatic, computer controlled devices can be lifted into orbit.

e. Product recovery - Bioreactors produce a relatively small amount of product in a large volume of media; therefore, methods must be investigated to extract as pure and as concentrated a product as possible. That product may then be routed to a CFES for purification, but only after significant concentration.

   The method of choice to begin such a concentration regimen is ultrafiltration. A multi-staged filtration system composed of perhaps hollow fibers or thin channel apparati is envisioned to accomplish the initial concentration.

f. Specific fraction cultures - The CFES has the ability to separate specific cells from a large heterogenous population of cells. Those cells can be characterized as high or low producers of some specific product. If such high producers can be obtained in sufficient quantity, they can be grown in-mass in the bioreactor.

However, the mass rejuvenation of a CFES separated cell population has not yet been achieved. Additional CFES experience and time is necessary, and characterization of cell damage or changes due to electrophoresis must be conducted.

CONCLUSIONS

Two parallel lines of work are underway in the bioreactor laboratory. One of the efforts is devoted to the continued development and utilization of a laboratory research system. That system's design is intended to be fluid and dynamic. The sole purpose of such a device is to allow testing and development of equipment concepts and procedures. Some of the results of those processes have been discussed in this paper.

A second effort is designed to produce a "flight-like" bioreactor contained in a double middeck locker. The result of that effort has been to freeze a particular bioreactor design in order to allow fabrication of the custom parts. We expect the system to be ready for flight in early 1988. However, continued use of the laboratory system will lead to improvements in the space bioreactor. Those improvements can only be integrated after the initial flight series.

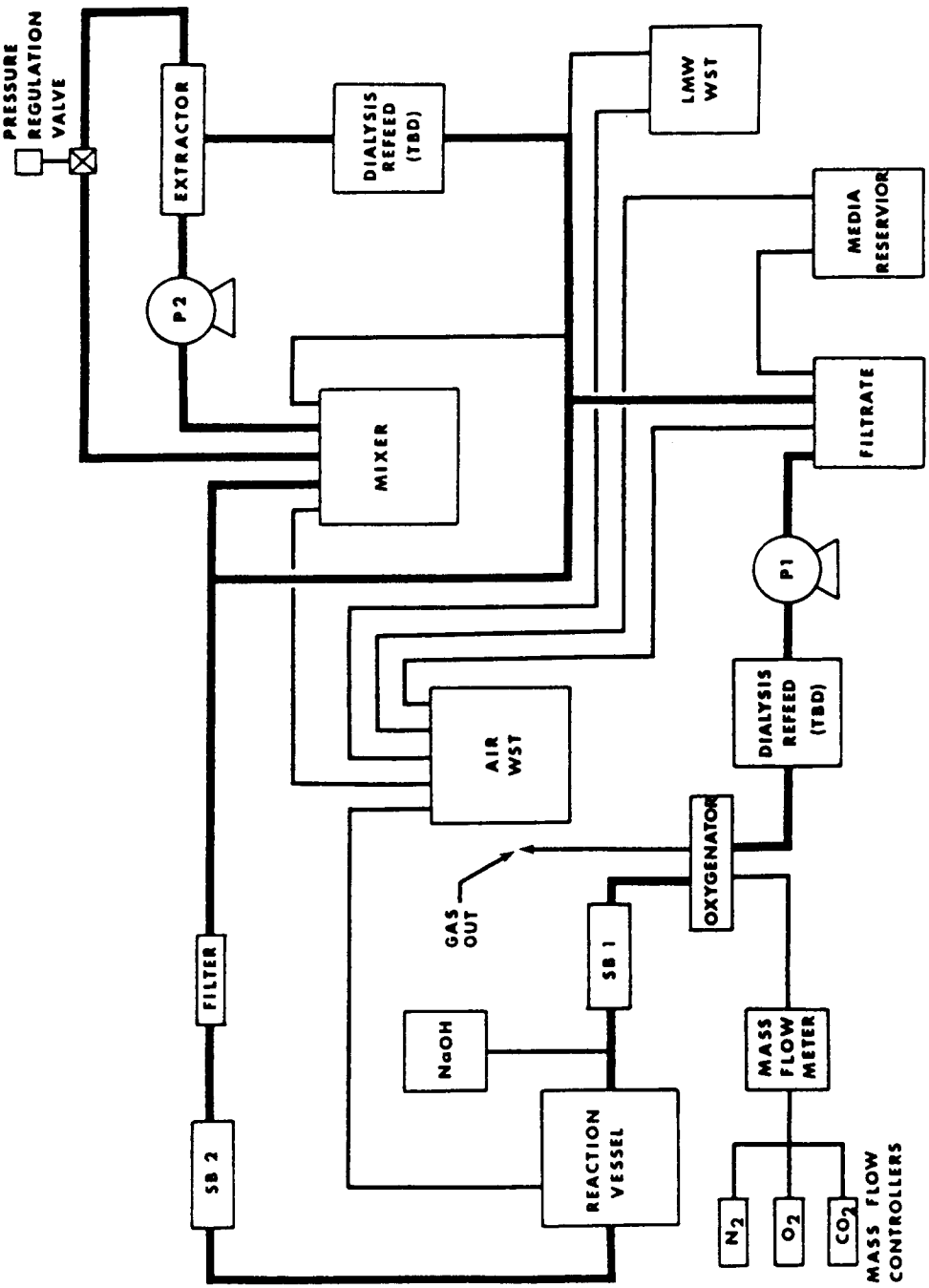

Figure 3-1.- Bioreactor block diagram.