SOME PROCESS CONTROL/DESIGN CONSIDERATIONS IN THE DEVELOPMENT OF A MICROGRAVITY MAMMALIAN CELL BIOREACTOR

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INTRODUCTION

The purpose of this paper is to review some of the physical/metabolic factors which must be considered in the development of an operating strategy for a mammalian cell bioreactor. Particular emphasis will be placed on the dissolved oxygen and carbon dioxide requirements of growing mammalian epithelial cells. Literature reviews concerning the oxygen and carbon dioxide requirements of mammalian cells will be presented first. This will be followed by the presentation of a preliminary, dynamic model which encompasses the current features of the NASA bioreactor. This paper concludes with a discussion of the implications of this literature survey and modelling effort for the design and operation of the NASA bioreactor.

DISSOLVED OXYGEN AND EPITHELIAL CELL GROWTH: LITERATURE SURVEY

Few relevant literature articles are available concerning the oxygen utilization of cell cultures and the influence of the dissolved oxygen concentration on cell growth and product information. The principal focus of this limited literature has been the metabolism of fibroblast cell lines, such as WI-38. It is now generally recognized that the growth and plating efficiency of fibroblast cells are inhibited by dissolved oxygen concentrations corresponding to 140 to 160 mm Hg. The optimal dissolved oxygen concentration is of the order of 40 mm Hg or less, which corresponds to the physiological level of dissolved oxygen.[1,10,11] Balin et al. (1984) have noted that dissolved oxygen inhibition is particularly pronounced when fibroblast cells are grown against 20% oxygen at a low seeding density.

Published oxygen utilization rates (OUR) for fibroblast cell cultures vary over a broad range--from 0.06×10^{-12} to 0.5×10^{-12} moles of oxygen utilized per cell per hour.[4] This variation may be partially due to a variation in the OUR between growing and confluent cells. Balin et al. (1976) noted that WI-38 cells in logarithmic growth utilized oxygen at a rate of 0.5×10^{-12} moles per cell per hour, whereas stationary phase WI-38 cells utilized oxygen at a rate of 0.2×10^{-12}. Variation in medium composition may also contribute

to the broad range of OUR values which have been observed, but no literature references were found which addressed this question.

The literature concerning the effect of dissolved oxygen concentration on the growth of epithelial cells is extremely limited. The most authoritative reference in this area is by Taylor and Camalier (1982). These authors assessed the effect of dissolved oxygen concentration on the growth of epithelial cells from rhesus monkey kidney (cell line LLC-MK) and epithelial cells from human neonatal foreskin. Their results were in contrast to those which have been obtained for fibroblast cells. Epithelial cells were noted to proliferate without inhibition at dissolved oxygen concentrations corresponding to 130 to 140 mm Hg, but were severely inhibited at dissolved oxygen concentrations in the vicinity of 40 mm Hg. The authors hypothesize that a dissolved oxygen concentration of at least 70 mm Hg is necessary for continued epithelial cell proliferation.

No specific OUR values were provided by Taylor and Camalier, but they did demonstrate that the kidney epithelial cells in vitro consumed dissolved oxygen more rapidly than do fibroblast cells.

CARBON DIOXIDE AND EPITHELIAL CELL GROWTH: LITERATURE REVIEW

It has been clearly established that mammalian cells have a carbon dioxide requirement for growth. This has been particularly evident in experimental systems in which pH is controlled via a buffering system other than the carbon dioxide-bicarbonate system. In these cases it has been demonstrated that cells grown in the presence of a carbon dioxide overlay proliferate faster than cells grown in the absence of a carbon dioxide overlay.[3,5,6,8] The carbon dioxide requirement stems from its incorporation in the anabolic pathways of cells, including the purine and pyrimidine pathways.

In addition to its absolute requirement for molecular synthesis, carbon dioxide has been postulated to serve a major important role as a regulator of cell activity.[7]

The literature would suggest the existence of an optimum external carbon dioxide concentration(s) for cell growth and product formation. In this literature review no data were found concerning this optimum concentration value. It will probably be necessary to determine this value experimentally for each cell line of interest.

The existence of the carbon dioxide requirement does not necessarily mean that a canister of carbon dioxide must be carried on board the Space Shuttle. It is possible that the confluent cells to be carried into space will produce sufficient carbon dioxide to satisfy their own carbon dioxide requirement.

This must be determined experimentally. It is possible that the lag phase associated with the innoculation of cells into the bioreactor could be reduced by the introduction of carbon dioxide from an external during the initial period of cell growth.

MODELLING OXYGEN TRANSPORT AND UTILIZATION

A flow diagram of the NASA bioreactor is presented in figure 6-1. The elements in the main flow loop of the reactor system include the bioreactor, two sensor blocks (containing measurement electrodes for dissolved oxygen, carbon dioxide, and pH), an in-line filter, a mixing chamber, and an oxygenator (designated O_2). A separate system for protein concentration is also indicated in figure 6-1. This loop includes a hollow fiber protein concentration unit and the reservoir for the protein concentrate (designated "protein").

Subscript definitions for each of the volume elements are presented in figure 6-2. Variable assignments for flow rate (F), dissolved oxygen concentration (O), carbon dioxide (C), and volume (V) are presented in figure 6-3. Note that the concentrations of oxygen and carbon dioxide in the liquid stream are specified at the exit of the oxygenator (i.e., Oo and Co) as an indication that the composition of that stream will change as a function of position in that device.

The oxygenator and the reactor are the only significant volume elements in the main flow loop. At the current time it would appear that these two elements would need to be considered in developing the dynamic oxygen and carbon dioxide mass balances for the system (fig. 6-4). Variable definitions for the inlet and outlet gas streams are included in this figure.

A dynamic model of oxygen transport and utilization is presented in figure 6-5. The assumptions involved in generating these equations will be outlined below.

Equation 5-1

This is a standard dynamic mass balance equation for a continuous stirred-tank reactor (CSTR). It is assumed that the contents of the tank are well-mixed. That is, the liquid contents of the reactor are homogeneous with respect to dissolved oxygen, dissolved carbon dioxide, and cells. The first term of the right side of the equation corresponds to the input and output of oxygen to the reactor via the input and output flow streams. The second term on the right side of the equation corresponds to the oxygen utilization by the cells for growth and maintenance.

Equation 5-2

As a first approximation, the oxygen utilization rate (OUR) is modelled empirically by a simple Michaelis-Menton-type relationship. The choice of this relationship is based on the assumption that the growth of the cells will be dependent on the dissolved oxygen concentration when the concentration is low, and that there will be no inhibition of growth at high levels of dissolved oxygen. The validity of this relationship must be determined through experimentation with the cells of interest. The presence of oxygen inhibition of growth at high concentration levels or the finding of a lower threshold of dissolved oxygen necessary for growth would necessitate the substitution of a more complex relationship for equation 5-2.

The constant K1 in equation 5-2 corresponds to the maximum possible oxygen utilization rate. As mentioned previously, the experimental values which have been determined (principally for fibroblasts) range from 0.05×10^{-12} to 0.5×10^{-12} moles of oxygen utilized per cell per hour. In the absence of specific experimental data, it would appear to be the most prudent to utilize a value of K1 of 0.5×10^{-12} moles per cell per hour. This would correspond to a value at the high end of the range which has been observed, in acknowledgment to the findings of Taylor and Camalier (1982) concerning the high oxygen consumption rate of epithelial cells.

The parameter K2, the dissolved oxygen concentration at which the OUR is half-maximum, is even more difficult to estimate from the available literature data. In the absence of specific experimental data, a value of 50 mm Hg might be used in simulation--that is K2 = 0.0625, assuming a Henry's Law constant of 0.2 mM/160 mm Hg.

Equations 5-3 and 5-4

In the steady state the oxygen concentrations of the gas and liquid streams of the oxygenator will vary with position in the oxygenator. The dynamic material balances for oxygen on the gas (eq. 5-3) and liquid sides (eq. 5-4) of the oxygenator must therefore be partial differential equations with two independent variables, time (t) and position (z).

These equations describe the situation where the gas and liquid phases pass in "plug flow" through the oxygenator, with negligible back-diffusion or back-mixing (the validity of the plug flow assumption is under examination). The term on the right side of each of these equations represents the transport of oxygen between the gas and liquid phases; as the term indicates, this transport is proportional to the difference at any point between the oxygen concentrations in the gas and liquid streams.

Equations 5-3 and 5-4 reduce to equations 5-3b and 5-4b in the steady state and if it can be assumed that the liquid and gas velocities are constant throughout the oxygenator. Equations 5-3b and 5-4b can be solved analytically to determine the steady state profile of oxygen in the gas and liquid streams, given the entering concentrations of oxygen in gas and liquid streams (see appendix A). Equations 5-3 and 5-4 require a more time-consuming numerical (i.e., computer) solution. Equations 5-3 and 5-4 contain three parameters Ag, Al, and Uo. The first two parameters, which relate to the area available for mass transport can be computed from the manufacturer's literature. The overall mass transport coefficient for oxygen, Uo, is not available and must be determined through experimentation.

It is possible that the oxygenator will possess such overcapacity for oxygen transfer that the rigorous equations 5-3 and 5-4 can be replaced by a simple equilibrium relationship (eq. 5-5).

A MODEL OF CARBON DIOXIDE PRODUCTION AND TRANSPORT

The dynamic model for carbon dioxide transport in the oxygenator is presented in figure 6-6. Equation 6-1 is the dynamic model describing the dissolved carbon dioxide concentration (Cr) in the reactor. The last term in that equation is the carbon dioxide generation term. The value of the important parameter n, the carbon dioxide production rate (CDPR) per cell, is unknown. It is assumed that the CDPR will be related in some way to the dissolved oxygen concentration--that is, if the dissolved concentration is reduced, then the cell would be expected to produce less carbon dioxide and more lactate. Equation 6-2 represents a first crude approximation of the relationship between the carbon dioxide production rate and the dissolved oxygen concentration.

Equations 6-3 and 6-4 in figure 6-6 represent the dynamic carbon dioxide balances for the liquid and gas streams passing counter-currently through the oxygenator. These equations are analogous in form to equations 6-3 and 6-4 of figure 6-5 for oxygen, and assume negligible back-diffusion or back-mixing of the gas and liquid streams.

Equations 6-3b and 6-4b in figure 6-6 represent the steady state relationships for carbon dioxide on the gas and liquid sides of the oxygenator, assuming that the liquid and gas flow rates in the oxygenator can be assumed to be constant. The same analytical solution technique presented in appendix A for oxygen can be applied to solve these equations for the steady state carbon dioxide profiles in the oxygenator.

For carbon dioxide, no set of conditions is expected to exist which will permit a simplification of the balance equations 6-3, 6-4, 6-3a and 6-4a.

IMPLICATIONS OF THIS INITIAL LITERATURE SURVEY AND MODELLING EFFORT

Based on the model presented previously, it is possible to roughly compare the potential rate of oxygen utilization by cells in the bioreactor with the maximum rate of oxygen transport to those cells in the incoming liquid stream from the oxygenator. Solving equation 5-1 of figure 6-5 in the steady state:

$$0 = F1*(Oo - Or) - m*X*Vr$$

Assuming values for m, X, Vr, Oo, and Or of

$m = 5.0 \times 10^{-13}$ moles per cell per hour
$X = 3.0 \times 10^{-9}$ cells per liter
$Vr = 0.5$ liters

$Oo = 2.0 \times 10^{-4}$ (saturated solution for pO = 160 mm Hg)
$Or = 1.0 \times 10^{-4}$ (assumption of desired oxygen concentration at 50% of saturation in the bioreactor)

These values lead to a required flow rate, F, of

$$F = \frac{(5.0 \times 10^{-13}) * (3.0 \times 10^{9}) * (.5)}{1.0 \times 10^{-4}} = 7.5 \text{ liters/hour} = 125 \text{ ml/min}$$

The above computation emphasizes the very low capacity of the liquid stream to carry oxygen to the cells in the reactor, due to the low solubility of oxygen in water. A number of factors could result in the necessity of an even higher flow rate:

- If the oxygenator is not capable of saturating the liquid stream
- If it is necessary to have a value of Or greater than 0.1 mM (e.g., if the optimum dissolved concentration for cell growth or product formation is greater than 0.1 mM.)
- If the solubility of oxygen in cell culture media is less than 0.2 mM for an oxygen partial pressure of 160 mm Hg. This is likely, since the solubility of oxygen in pure water at 37 degrees is 0.2 mM, and the solubility of oxygen is known to decrease with increasing electrolyte concentration. For example, 1.0 M NaCl reduces the solubility of oxygen in water by 30%. The value of 0.2 mM used in the above calculation is therefore close to the maximum possible solubility.
- If the cell concentration is greater than 3.0×10^9 cells per liter

- If the OUR of the kidney cells is greater than 0.5×10^{-12} moles per cell per hour

These factors underscore the importance of reliable experimental data in deciding on a final reactor design. These are several operational and design alternatives which could alleviate any problem which might arise with the current NASA bioreactor configuration:

- Increase the partial pressure of the oxygen in the gas stream to the oxygenator (i.e., by increasing the concentration of oxygen in the feed).

- Redesign the spin filter so that a higher liquid flow rate can be tolerated. The current oxygenator could tolerate much larger flow rates.

- Reduce the vessel size, which would reduce the number of cells which must be supplied with oxygen.

- Design a new oxygenation system which introduces oxygen directly into the reaction vessel (e.g., silicone tubing or sheets in the bioreactor).

- Investigate the possibility of using an artificial oxygen carrier to increase the oxygen-carrying capacity of the liquid stream.

A related issue worthy of consideration is the total gas flow through the oxygenator during long-term operation in space. It is recommended by the manufacturer that the gas flow rate through the oxygenator be roughly two times greater than the liquid flow rate to achieve oxygen saturation. At 50 to 100 ml/minute of liquid flow, the gas flow would be 1000 to 2000 liters per week. If that gas was carried in compressed form at 150 atm, then the gas would occupy about 10 liters. The associated containers and valves would probably raise the total volume to about 20 liters.

The alternative is to use an air pump to circulate cabin air through the oxygenator. This would reduce, but not eliminte, the volume of compressed gas that would have to be carried on the Shuttle. It will be necessary to carry some compressed gas for the following reason. It will probably be desirable to control both the dissolved oxygen and the dissolved carbon dioxide concentrations in the bioreactor. The probable technique for controlling these gases would be to control dissolved carbon dioxide by regulating the total gas flow through the oxygenator (thereby controlling the rate at which carbon dioxide is flushed from the system), and to control the dissolved oxygen concentration by controlling the oxygen concentration in the feed gas stream to the oxygenator. It would therefore be necessary to carry some oxygen and/or nitrogen to achieve the desired oxygen concentration in the gas stream. A final decision concerning which of the two gases would have to be carried on the Shuttle will await experimental results concerning the OUR of the

epithelial kidney cells and the optimum dissolved oxygen concentration for their growth.

It may, additionally, be desirable to carry some carbon dioxide on the Shuttle. Growing cells at low density may not initially produce sufficient carbon dioxide to maintain the carbon dioxide concentration at the desired level. Carbon dioxide would therefore be introduced into the feed gas stream to the oxygenator to avoid an unnecessary lag in cell growth rate. For a confluent culture this may not be a problem.

CONCLUSIONS

A review of the available literature concerning the dissolved oxygen and carbon dioxide requirements of mammalian cells has been presented, and the importance of this data in the design and operation of a mammalian cell bioreactor has been demonstrated. It is noted that little literature data is available for mammalian cells in general, and for epithelial cells in particular. The available literature is dominated by data for fibroblast cell cultures, which appear to be substantially different from epithelial cells in their metabolic requirements. This literature survey underscores the importance of procuring reliable experimental data for the particular cell type of interest.

A preliminary dynamic model has been presented in this report for the simulation of cell growth and oxygen/carbon dioxide transport and utilization. This model will be refined on the basis of experimental data collected from the bioreactor system using the cell type of interest. It is expected that this model will be of utility in the simulation of oxygen and carbon dioxide control algorithms. The model will ultimately be expanded to include product formation by the cells.

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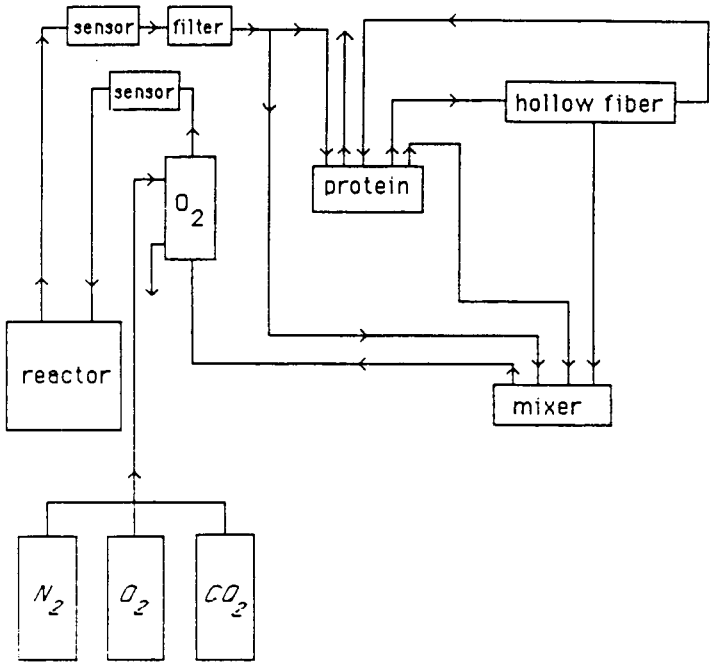

Figure 6-1.- General flow diagram of the NASA bioreactor.

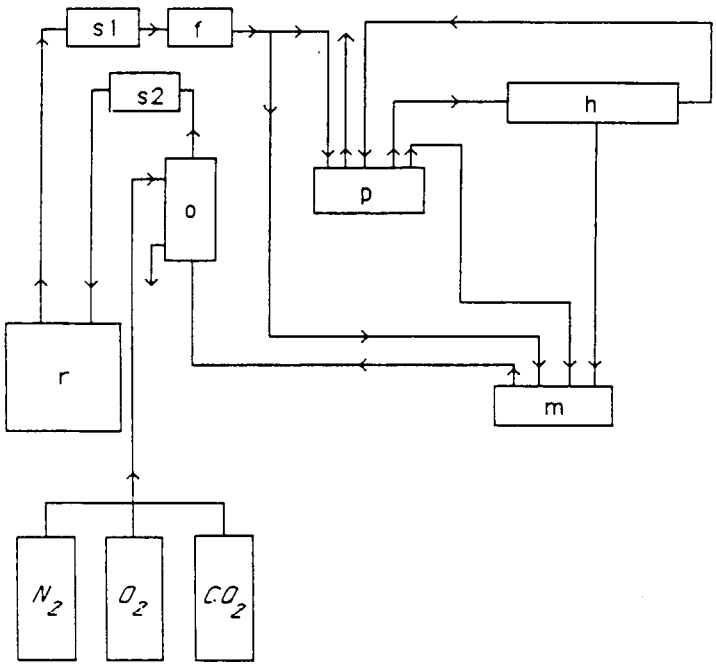

Figure 6-2.- Volume element designations.

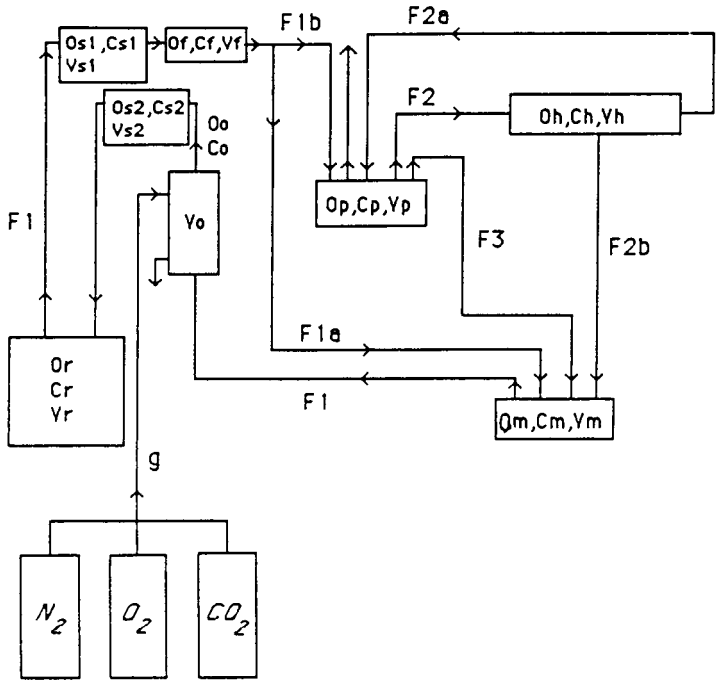

Figure 6-3.- Variable assignments.

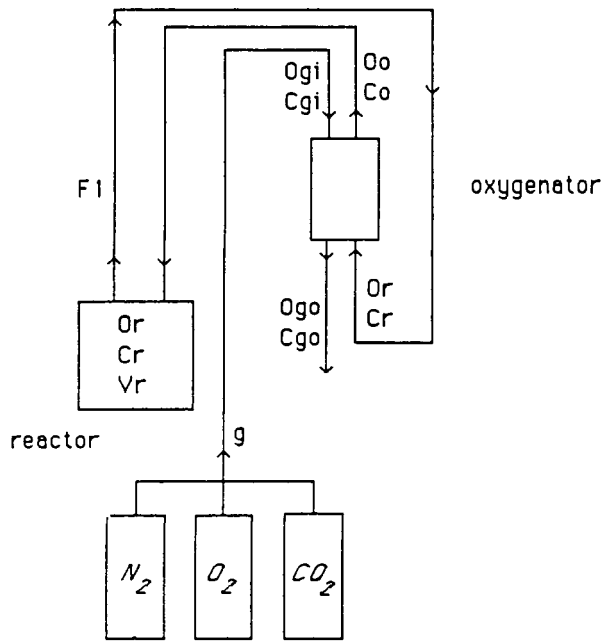

Figure 6-4.- Modelling the dynamics of the main flow loop: dissolved oxygen and carbon dioxide.

Reactor:

$$\frac{Vr\ dOr}{dt} = F1\ (Oo - Or) - m*X*Vr \tag{5-1}$$

where

$$m = \frac{K1*Or}{K2 + Or} \tag{5-2}$$

Oxygenator:

Gas side

$$\frac{\partial Og}{\partial t} + \frac{\partial (Vg*Og)}{\partial z} = -Uo*Ag*(Og - Ol) \tag{5-3}$$

Liquid side

$$\frac{\partial Ol}{\partial t} + \frac{\partial (Vl*Ol)}{\partial z} = +Uo*A1*(Og - Ol) \tag{5-4}$$

where:

$Ol = Or$ at $z = O$
$Ol = Oo$ at $z = L$

$Og = Ogi$ at $z = L$
$Og = Ogo$ at $z = O$

In the steady state:

Gas side

$$Vg*\frac{dOg}{dz} = -Uo*Ag*(Og - Ol) \tag{5-3B}$$

Liquid side

$$V1*\frac{dOl}{dz} = +Uo*Al*(0g - Ol) \tag{5-4B}$$

$$Oo = \frac{Po}{K} = \frac{Yo*Pt}{K} \tag{5-5}$$

where:
- Vr = reactor volume
- Or = dissolved oxygen concentration in the bioreactor
- Oo = dissolved oxygen concentration leaving the oxygenator
- F1 = flow rate through the bioreactor
- m = the oxygen utilization rate (OUR) in units of moles of oxygen per cell per hour
- X = cell density in units of cells per liter
- K1 = the maximum oxygen utilization rate

Figure 6-5.- Version 4: Dynamic oxygen balances-main loop components only.

K2 = the dissolved oxygen concentration at which the OUR is half of maximum
Og = the dissolved oxygen concentration in the gas phase at a particular point in the oxygenator
Vg = the gas phase velocity through the oxygenator
Ag = the area for gas phase mass transfer per volume of gas in the oxygenator
Uo = overall mass transfer coefficient for oxygen transport in units of cm/hr
O1 = the dissolved oxygen concentration in the liquid phase at a particular point in the oxygenator
V1 = the liquid phase velocity through the oxygenator
A1 = the area for liquid phase mass transfer per volume of liquid in the oxygenator
Po = the partial pressure of oxygen in the gas phase passing through the oxygenator
Yo = the mole fraction of oxygen in the gas phase
Pt = the total pressure of the gas passing through the oxygenator
K = the Henry's Law constant for oxygen solubility

Figure 6-5.- Version 4: Dynamic oxygen balances-main loop components only (Cont'd).

Reactor:

$$\frac{Vr\ dCr}{dt} = F1\ (Co - Cr) + n*X*Vr \tag{6-1}$$

where

$$n = Kco2*Or(?) \tag{6-2}$$

Oxygenator:

Gas side

$$\frac{\partial Cg}{\partial t} + \frac{\partial (Vg*Cg)}{\partial z} = -Uc*Ag*(Cg - Cl) \tag{6-3}$$

Liquid side

$$\frac{\partial Cl}{\partial t} + \frac{\partial (Vl*Cl)}{\partial z} = +Uc*Al*(Cg - Cl) \tag{6-4}$$

where:

$Cl = Or$ at $z = 0$
$Cl = Oo$ at $z = L$

$Cg = Cgi$ at $z = L$
$Cg = Cgo$ at $z = 0$

In the steady state:

Gas side

$$Vg*\frac{dCg}{dz} = -Uc*Ag*(Cg - Cl) \tag{6-3B}$$

Liquid side

$$Vl*\frac{dCl}{dz} = +Uc*Al*(Cg - Cl) \tag{6-4B}$$

where:

- Vr = reactor volume
- Cr = dissolved carbon dioxide concentration in the bioreactor
- Co = dissolved carbon dioxide concentration leaving the oxygenator
- F1 = flow rate through the bioreactor
- n = the carbon dioxide production rate in units of moles of carbon dioxide per cell per hour
- Kco2 = a proportionally constant for the hypothetical relationship of equation 6-2 above
- X = cell density in units of cells per liter
- Cg = the dissolved oxygen concentration in the gas phase at a particular point in the oxygenator

Figure 6-6.- Version 4: Dynamic carbon dioxide balances-main loop components only.

Vg = the gas phase velocity through the oxygenator
Ag = the area for gas phase mass transfer per volume of gas in the oxygenator
Uc = overall mass transfer coefficient for carbon dioxide transport in units of cm/hr
Cl = the dissolved oxygen concentration in the liquid phase at a particular point in the oxygenator
Vl = the liquid phase velocity through the oxygenator
Al = the area for liquid phase mass tranfer per volume of liquid in the oxygenator

Figure 6-6.- Version 4: Dynamic carbon dioxide balances-main loop components only (Cont'd).