Optimization of Oxygen Transport in a Media-based Bioreactor for Non-Adherent Cells

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1. Problem Statement

Improving cell culture techniques is of vital importance to countless biomedical and pharmaceutical industries, as minute increases in cell viability or decreases in reproduction cycles time can translate to hundreds of thousands of dollars in increased profit. Bioreactors are used to culture industrial quantities of cells. They regulate specific conditions such as pH, temperature, oxygen transfer, and agitation rate to allow for optimal growth. However, this scaled up approach comes with additional challenges. Oxygen regulation in bioreactors has traditionally been a crucial limiting factor in cell growth that has yet to be adequately addressed. As the cells take in oxygen and proliferate, a higher concentration of oxygen will be required to maintain the viability of cells. Optimizing the oxygen-cell interactions within a bioreactor will help to optimize the number of viable cells, decrease waste, and optimize the overall efficacy of the system.

2. Formulation of the Problem

2.1 - Design and Governing Equations

The bioreactor is a cylindrical chamber filled with cell culture media. At the bottom of the reactor, numerous inlets pump solute oxygen into the chamber which diffuses through the media. Within the bioreactor, non-adherent cells grow over time according to oxygen concentration and exponential growth. Dissolved oxygen crosses the cell membrane where it is utilized for metabolic function and cellular respiration among a myriad of other processes. However we will be modeling strictly cells use of oxygen due to cellular respiration.

2.2 - Decision of Governing Equations

The introduction of solute oxygen into the base of the bioreactor creates a concentration gradient that can be profiled through the equation of change according to set assumptions in our system. Additionally, the process of oxygen crossing the cell membrane is a chemical reaction that can also be profiled within the equation of change. The large concentration gradient between the start and end of the bioreactor, as well as the gradient between the outside and inside of the cell across the membrane, makes these systems heavily dependent on diffusion. However, convective forces are still present throughout these interactions. Oxygen is being transported from the media inside the cell, where the oxygen will be consumed by the cell. The transportation of oxygen involves diffusion but not convection.

2.3 - Initial and Boundary Conditions

In the cylindrical bioreactor, we are profiling solute oxygen transport in the positive z direction, from z to Δz, over time. Oxygen saturation in the reactor media is 100% at the bottom, where oxygen enters the reactor. Oxygen concentration in the angular and radial domains are constant, and concentration decreases as the z direction increases from the bottom of the reactor to the top. A Steady state scenario is being evaluated for this section of the oxygen transport. In steady state, a constant rate of oxygen transport enters the reactor, setting an oxygen-limited cap to the growth of the cell population.

3. Computed Equations and Solutions

3.1 - Oxygenation of the Bioreactor

We first start by considering the system which is composed of a bioreactor, oxygen, and cells. The bioreactor chamber is cylindrical, with a volume of cell culture media filling most of its inner volume. A smaller volume of air resides in the remaining volume at the top of the bioreactor. At the bottom of the bioreactor, an array of inlet ports pump solute oxygen into the bioreactor. This array can be assumed to pump oxygen into the media in the z direction homogeneously with respect to the radius of the cylinder. A generic bioreactor schematic, with the system of oxygen being added to the bioreactor can be seen in the illustration below.

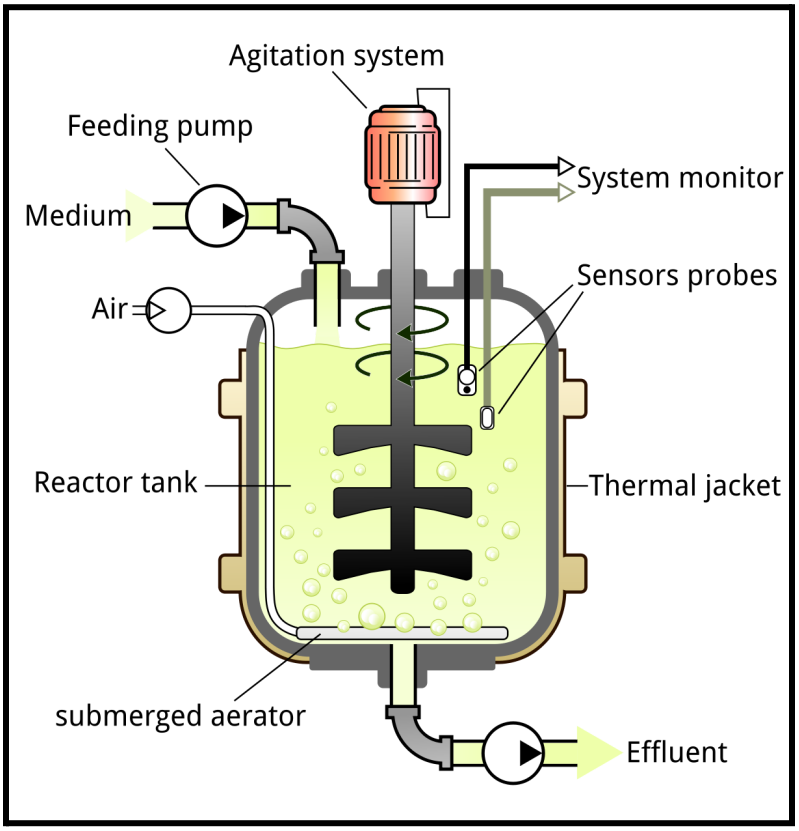

Figure 1 - [5] A schematic showing the basic layout of a bioreactor. At the bottom, a submerged aerator adds bubbles of pure oxygen to the system to allow for cell growth. In our system, these bubbles are replaced with oxygen as a solute. Factors such as agitation systems and effluent removal are not within the scope of this analysis

When profiling the oxygenation of the bioreactor, we outlined a number of assumptions that were used as the basis for our transport equations.

- The submerged aerator pumps solute oxygen into the bioreactor chamber. This added media is assumed to have an oxygen concentration of 60-80% in order to subvert the

analysis of phase equilibria in the system with the dissolution of oxygen gas bubbles into liquid media.

- The solute oxygen is inserted into the bioreactor chamber at multiple equally spaced inlet ports at the bottom of the bioreactor. These multiple inlet ports can be assumed to collectively create a uniform and homogeneous concentration profile in the z direction.
- The bioreactor media is static and has no initial velocity

A graphical representation of the oxygen diffusion into the bioreactor chamber can be seen in Figure 2, which shows z-direction diffusion with constant diffusion in the radial and axial direction.

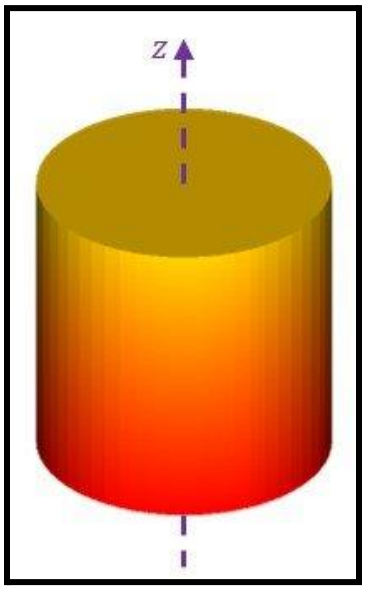

Figure 2: A visual representation of cylindrical diffusion oxygen in the z direction with no diffusion or concentration gradients in the radial or axial direction. Red denotes a higher oxygen concentration, while yellow indicates a lower oxygen concentration. [6]

The oxygen transport into the bioreactor is profiled as a steady state. In steady state, oxygen is pumped into the system at a constant rate. In this scenario cells will exponentially grow until reaching a natural upper limit where oxygen content in the media becomes a limiting factor for growth. Steady state transport can be defined by applying our outlined assumptions to the Equation of Change, shown in Equation 1.

$$\frac{dC}{dt} + Vr\frac{dC_{o_2}}{dr} + \frac{V\theta}{r}\frac{dC_{o_2}}{d\theta} + Vz\frac{dC_{o_2}}{dz} = D\left(\frac{1}{r}\frac{d}{dr}\left(r\frac{dC_{o_2}}{dr}\right) + \frac{1}{r^2}\frac{d^2C_{o_2}}{d\theta^2} + \frac{d^2C_{o_2}}{dz^2}\right) + R$$

Equation 1 - The standard Equation of Change

When taking into account that our media is static, and that our concentration and diffusion profiles are unchanging in the radial and axial directions, we can simplify this equation as shown in Equation 2. Variable R, for reaction related variables, is replaced by oxygen dependent cell respiration variables that model the oxygen consumption of cell populations.

$$Vz\frac{dC_{o_2}}{dz} = D\frac{d^2C_{o_2}}{dz^2} + R$$

Equation 2 - The simplified Equation of Change when taking set assumptions into account.

On a smaller scale, individual cells undergo chemical reactions to take in oxygen from the system and proliferate more cells. This equation shows non-reversible reaction however due to the complexity of the various stages of cellular respiration which is described here, it is difficult to establish an order of the reaction thus making the reaction equation more difficult to model so through making the assumption that it is a 3 step process for the glycolysis, Krebs cycle and the electron transport chain we can reduce the process to this 3 step reaction.

$$C_6H_{12}O_6 + 6O_2 \xrightarrow{k_1} 2C_3H_6O_3 + 6O_2 \xrightarrow{k_2} 8NADH + 6CO_2 + 2FADH_2 + 2ATP \xrightarrow{k_3} 8NAD^+ + 2FAD^+ + 32ATP$$

Equation 3 -

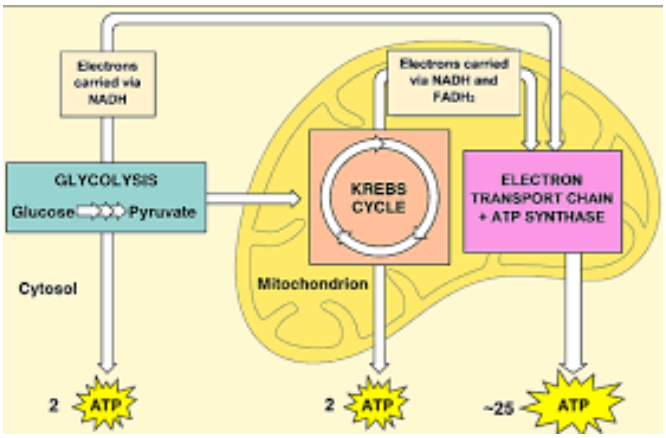

Figure 3 - Graphic of the 3 steps in the oxygen consumption process of the cells in our reactor and how this analysis goes about modeling the oxygen reaction occurring throughout the medium. Using each of the 3 steps rather than the smaller reactions in each one for the rate of consumption of oxygen

K1 represents the glycolysis reaction rate K2 represents the Krebs cycle reaction rate and K3 represents the Electron transport chain reaction. For the rate equation we are assuming that all cells are performing aerobic respiration rather than aerobic respiration. We have also removed the effect of the intermediate coenzyme A which appears during glycolysis and is removed during the Krebs cycle.

For this analysis of oxygen consumption by cellular respiration, the K2 reaction is where this paper focuses as this step is where oxygen is converted into Carbon dioxide.

$$R = \frac{dC_{o_2}}{dt} = -K_2C_{o_2}$$

Equation 4

$$Ni = N_0\left(\frac{1}{2} + \frac{1}{2}\sum_{i=0}^{n}(2F)^i\right)$$

Equation 5

Where *Ni* is the total cell number, *N0* is the initial number of cells, n is number of reproduction cycles the cells has gone through since the initial population was obtained and *F* is the constant for cell growth. ni rather than Ni will be used to represent the cell number in the bioreactor so it is not confused with flux equations

$$R = -K_2 C_{O_2} N_i$$

Equation 6 - Breakdown of reaction variable. C02 denotes the concentration of diatomic oxygen in the system. K2 denotes the reaction rate constant of the Krebs Cycle, and Ni denotes the initial number of cells in the system.

Because $K_2$and N_i are constants, we will simplify this equation by setting these variable equal to

$$W = K_2 N_i$$

Equation 7 - Simplification of reaction constants

With this simplification, Equation 2 becomes:

$$Vz\frac{dC_{o_2}}{dz} = D\frac{d^2C_{o_2}}{dz^2} - C_{O_2} * W$$

Equation 8 - Equation of Change with condensed reaction constants

Next, we differentiated this equation using Wolfram Alpha, solving for concentration with respect to z

$$C(z) = G_1 e^{\left(z(V_z - \sqrt{4Dw + V_z^2})\right)/2D} + G_2 e^{\left(z(\sqrt{4Dw + V_z^2} + V_z)\right)/2D}$$

Equation 9 - Solved differential equation where G represents equational constants

For clarity, we can substitute W for its substituent constants back into the solved differential equation

$$C(z) = G_1 e^{\left(z(V_z - \sqrt{4DN_iK_2 + V_z^2})\right)/2D} + G_2 e^{\left(z(\sqrt{4DN_iK_2 + V_z^2} + V_z)\right)/2D}$$

Equation 10 - Solved differential equation with constants K and n substituted back in place of W.

Next, we will solve for the differential constants, G_1 and G_2.Through algebraic manipulation we can derive the following equation.

$$G_1 = \frac{C_2 - G_2 e^{\left(Z_{max}(\sqrt{4DN_iK_2+V_z^2}+V_z)\right)/2D}}{e^{\left(Z_{max}(V_z-\sqrt{4DN_iK_2+V_z^2})\right)/2D}}$$

Equation 11: Solving for G_1

We can further manipulate this equation by plugging in for G_2 as a function of C_1 and G_1 as seen in Equation 12. Thanks to this, we can finalize the equation for G_1 in terms of our established variables and boundary conditions as shown in Equation 13

$$G_1 = \frac{C_2 - ((C_1 - G_1)e^{\left(Z_{max}(\sqrt{4DN_iK_2+V_z^2}+V_z)\right)/2D})}{e^{\left(Z_{max}(V_z-\sqrt{4DN_iK_2+V_z^2})\right)/2D}}$$

$$G_1 = \frac{C_2 - C_1 e^{\left(Z_{max}(\sqrt{4DN_iK_2+V_z^2}+V_z)\right)/2D}}{e^{\left(Z_{max}(V_z-\sqrt{4DN_iK_2+V_z^2})\right)/2D} + e^{(Z_{max}(\sqrt{4DN_iK_2+V_z^2}+V_z))/2D}}$$

Equations 12 and 13, isolating constant G_1

Now that we have an equation for G_1, we can algebraically manipulate our equations to solve for G_2.Equation 14 is in terms of established variables as well as G_1, while Equation 15 is in terms of only established variables

$$G_2 = \frac{C_2 - G_1 e^{(Z_{max}(V_z-\sqrt{4DN_iK_2+V_z^2}+V_z)/2D)}}{e^{\left(Z_{max}\sqrt{4DN_iK_2+V_z^2}+V_z\right)/2D}}$$

$$G_2 = \frac{(e^{\left(Z_{max}(\sqrt{4DN_iK_2+V_z^2}+V_z)\right)/2D})(C_2 - C_1 e^{\left(Z_{max}(\sqrt{4DN_iK_2+V_z^2}+V_z)\right)/2D})}{(e^{\left(Z_{max}(V_z-\sqrt{4DN_iK_2+V_z^2})\right)/2D} + e^{(Z_{max}(\sqrt{4DN_iK_2+V_z^2}+V_z))/2D})^2}$$

Equations 14 and 15, isolating constant G_2

Our final equation of change with boundary conditions and solved coefficients of integration are as follows

$$C(z) = 1 - \left[\frac{C_2 - C_1 e^{\left(Z_{max}\left(\sqrt{4DN_iK_2+V_z^2}+V_z\right)\right)/2D}}{e^{\left(Z_{max}\left(V_z-\sqrt{4DN_iK_2+V_z^2}\right)\right)/2D} + e^{\left(Z_{max}\left(\sqrt{4DN_iK_2+V_z^2}+V_z\right)\right)/2D}} e^{\left(z\left(V_z-\sqrt{4DN_iK_2+V_z^2}\right)\right)/2D} + \ldots\right.$$

$$\left. \ldots + \frac{\left(e^{\left(Z_{max}\left(\sqrt{4DN_iK_2+V_z^2}+V_z\right)\right)/2D}\right)\left(C_2 - C_1 e^{\left(Z_{max}\left(\sqrt{4DN_iK_2+V_z^2}+V_z\right)\right)/2D}\right)}{\left(e^{\left(Z_{max}\left(V_z-\sqrt{4DN_iK_2+V_z^2}\right)\right)/2D} + e^{\left(Z_{max}\left(\sqrt{4DN_iK_2+V_z^2}+V_z\right)\right)/2D}\right)^2} e^{\left(z\left(\sqrt{4DN_iK_2+V_z^2}+V_z\right)\right)/2D}\right]$$

Equation 16 - Overall oxygen saturation equation with respect to z

Boundary Conditions:

- When z = 0; C_{o_2} = C2
- When z = z_max ; C_{o_2} = C1

3.2 Oxygen from media to cell

After the media with dissolved oxygen reaches the cells, we can then model the transport of oxygen into an individual cell. We can assume that the oxygen is transported into a cell by steady state diffusion through a porous membrane. This membrane would have a total area of “A” and be permeable to oxygen but not the media. Within the membrane, there are *n* pores with radius ‘R’, giving a total pore area of $n.\pi R^2$. The following assumptions were made to examine this transport process.

1. Oxygen transport into the cell is due only to diffusion.
2. Oxygen transport into the cell is a steady state process.
3. Cell membrane is semi permeable and porous.

We first consider the flux of the oxygen going through one pore of the cell, which can be seen below in equation 9.

$$N_p = \frac{D}{L}\Delta C$$

Equation 17

Where N_p is the flux through one pore, *D* is the distance, *L* is the length, and ΔC is the concentration gradient between the interior and exterior of the cell. We can multiply both sides by πR^2 to formulate an equation that gives us the total number of moles (per unit time) entering the cell through one pore. This can be seen below in equation 10.

$$N_p * \pi R^2 = \frac{\pi R^2 D}{L}\Delta C$$

Equation 18

We can also use equation 10 to show the total moles of oxygen (per unit time) entering the cell through n pores. This equation can be transformed again to show the overall flux through the entire membrane of the cell.

$$N = \frac{n*\pi R^2 D}{A*L}\Delta C$$

Equation 19

We derived this equation by using the total moles of oxygen (per unit time) through the n pore and divide that by the total area of the cell membrane. The porous membrane's permeability is represented by equation 12.

$$P = \frac{\phi D}{L}$$

Equation 20

Where ϕ is the partition coefficient of the membrane. This is related to the ability of a solute to cross the membrane. In this case, the solute is oxygen.

$$\phi = \frac{n}{A}\pi R^2$$

Equation 21

When we substitute the expansion of ϕ into equation 13, we get:

$$P = \frac{\phi D}{L} = \frac{n*\pi R^2 D}{A*L}$$

Equation 22

Then, multiplying the permeability by the concentration gives us the overall flux through the entire membrane, which can be seen below in equation 11.

$$N = \frac{n*\pi R^2 D}{A*L}\Delta C = P\Delta C = \frac{\phi D}{L}\Delta C$$

Equation 23

The partition coefficient in the porous membrane can also be defined in terms λ, which can be seen in equation 14.

$$\phi = (1 - \lambda)^2$$

Equation 24

Where $\lambda = \frac{a}{R} = \frac{solute\ radius}{pore\ radius}$. If the radius of the solute is close to the radius of the pore, then it is harder for the solute to diffuse through the pore.

Since we were able to model the flux of the oxygen through the entire porous membrane, we can then model the flux of the solute overall, which is oxygen. This is shown in the following

equation where Vm is the solvent flux through the membrane, W is the hydrodynamic resistance coefficient for convection, C is the concentration, D is the diffusion coefficient, and $\frac{dC}{dz}$ is the concentration gradient in the z direction

$$N_s = -D\frac{dC}{dz} + WCv_m$$

Equation 24

Since oxygen enters the cell by simple diffusion through the membrane, convection isn't present in this equation, meaning that *W* is equal to zero.

$$N_s = -D\frac{dC}{dz}$$

Equation 25

Equation of change in spherical coordinates

$$\frac{dC}{dt} + Vr\frac{dC}{dr} + \frac{V\theta}{r}\frac{dC}{d\theta} + \frac{V\phi}{rsin\theta}\frac{dC}{d\phi} = D\left(\frac{1}{r^2}\frac{d}{dr}\left(r^2\frac{dC}{dr}\right) + \frac{1}{r^2 sin\theta}\frac{d}{d\theta}\left(sin\theta\frac{dC}{d\theta}\right) + \frac{1}{r^2 sin^2\theta}\frac{d^2C}{d\phi^2}\right) + R$$

Equation 26

Using the assumptions that oxygen transport into the cell is a steady state process due solely to diffusion, we can simplify the equation. Additionally, the concentration does not change in θ or ϕ directions and there is no reaction at this stage. The overall simplification is shown below.

$$0 = D\left(\frac{1}{r^2}\frac{d}{dr}\left(r^2\frac{dC}{dr}\right)\right)$$

Equation 27

Which can be rewritten as

$$0 = \frac{D}{r^2}\frac{d}{dr}\left(r^2\frac{dC}{dr}\right)$$

Equation 28

Boundary conditions for this equation would be as follows:

$@\, r = R_O;\ C = C_O$

$@\, r = R_i;\ D\frac{dC}{dr}\Big|_{r=Ri} = K_2 * C_0$

The second boundary condition comes from equation 25. The concentration gradient in the z direction of the cylindrical pore would be the same as the concentration gradient in the radial direction of the spherical cell. K_2 is related to the rate of oxygen metabolism of the cells and was further defined in a previous section. $K_2 * C_0$ is the reaction rate of oxygen, as shown by equation 3, and should be equal to the rate of oxygen entering the cell across the membrane.

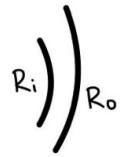

Figure 4 - Graphic depicting the difference in inner and outer membranes

Integrating equation 28 once gives us the following.

$$\frac{dC}{dr} = \frac{A}{r^2}$$

Equation 29

Using the second boundary condition we get:

$$D\frac{dC}{dr}\Big|_{r=Ri} = K_2 * C_0$$

$$\frac{DA}{R_i^2} = K_2 * C_0$$

$$\frac{K_2 * C_0 * R_i^2}{D} = A$$

Equation 30

Integrating again results in:

$$C = -\frac{A}{r} + B$$

Equation 31

We can plug in A for A and rearrange for B.

$$C = -\frac{K_2 C_0 R_i^2}{Dr} + B$$

$$B = C + \frac{K_2 C R_i^2}{Dr}$$

Equation 32

Plugging in the first boundary condition allows us to solve for a more specific B.

$$B = C_o + \frac{K_2 C_o R_i^2}{DR_o}$$

Equation 33

We can then plug in these values for A and B to get a concentration profile in the radial direction, C(r).

$$C(r) = -\frac{K_2 C_o R_i^2}{Dr} + C_0 + \frac{K_2 C_o R_i^2}{D R_0}$$

$$C(r) = C_0 - \frac{K_2 C_0 R_i^2}{D}\left(\frac{1}{r} - \frac{1}{R_o}\right)$$

Equation 34

C_O is the concentration of oxygen in the media, which will be controlled by the rate that soluble oxygen is pumped into the bioreactor. R_i would be the inner radius of the cell membrane while R_O is the outer radius of the membrane, so $R_O - R_i$ is the thickness of the cell membrane. K_2 is determined in a previous section and r is the particular location that concentration is being analyzed with respect to the center of the cell.

4. Theoretical and Practical Analysis

4.1 - Theoretical Analysis of Oxygen into the Bioreactor

For the oxygen transport into the reactor, the variables analyzed in this model are V_Z, D, K_2, and N_i.These values are analyzed against the variable z direction from 0 to Z_{max}. Additionally, we must set a maximum value for our Z dimension in order to use Z_{max}. $C_1$and C_2 are set concentration constants according to our boundary conditions, where oxygen concentration is higher near the bottom of the reactor (C2) and lower near the bottom (C1). We set rough estimates for our values according to external research, with our chosen values as follows:

- V_Z**: 0.0005m/s - 0.0015m/s [1]**
  - Constant value: 0.001m/s
- D: 3.2*10^-5 (m^2/s)
- K_2: Between 7.91*10^-19 mol*cell^-1*s^-1 and 1.19*10^-17 mol*cell^-1*s^-1 [2]
- N_i**: Between 5*10^9 and 5*10^13**
  - Constant value: 5*10^9
- Z_{max}: 1m
- C2= .8
- C1= .14

Because K2 is such a small and narrow range of values, it will effectively be drowned out by other variables in the equation. Therefore, we can assume it is a negligible constant. The

diffusion coefficient of oxygen in cell culture media will also be assumed to be constant in this model. Therefore, our two variables being analyzed against reactor height, z, will be N_i and V_Z.

When analyzing one variable against z, the third variable will be kept constant at the value shown above. For example, when analyzing N_i against z, V_Z will be set to .001m/s. Table values for z will be distributed on an exponential curve. All table matrix values are represented as a scaled oxygen concentration ratio, where 1 represents total oxygen saturation in the medium and 0 represents no oxygen saturation. Values approaching infinity are scaled to 1 with other values being scaled proportionally for clarity.

Vz = .001m/s		N_i - Initial number of cells present in bioreactor (cells)				
		$5 * 10^9$	$5 * 10^{10}$	$5 * 10^{11}$	$5 * 10^{12}$	$5 * 10^{13}$
Z Vertical distance within bioreactor (meters)	0	.99999	.99999	.99999	.99999	.99999
	0.5	.99932	.99922	.99921	.99921	.99921
	0.75	.97394	.97210	.97191	.97188	.97188
	0.875	.83857	.83297	.83239	.83232	.83232
	0.9375	.59821	.59131	.59059	.59051	.59051
	1.000	$3.41 * 10^{-1}$	$5.87 * 10^{-1}$	$6.21 * 10^{-1}$	$6.24 * 10^{-1}$	$6.24 * 10^{-1}$

Table 1 - Analysis of oxygen saturation with variable height and cell population- Values in the table are oxygen saturation percentages in the medium

$N_i = 2.5 * 10$		V_Z - Convective velocity (m/s)		
		$5 * 10^{-4}$	$1 * 10^{-3}$	$1.5 * 10^{-3}$
Z Vertical distance within bioreactor (meters)	0	.99842	.99999	.99999
	0.5	.97114	.99921	.99998
	0.75	.83180	.97188	.99529
	0.875	.59038	.83232	.93134
	0.9375	.36031	.59052	.73797
	1.000	$4.93 * 10^{-10}$	$6.24 * 10^{-7}$	$7389 * 10^{-4}$

Table 2 - Analysis of oxygen saturation with variable height and convective velocity. This table shows some constant parameters and some variables gradually increasing to show the effects of these selected values on the oxygen saturation percentage in the incubator.

It is apparent that oxygen saturation decreases with increased cell number, and that it remains higher at greater z values when convective velocity is higher. These trendlines outline ways that a bioreactor can be optimized according to these variables. This would indicate that increased stirring and mixing will increase oxygen concentration at the top of the bioreactor. As with the current conditions the very top of the reactor will have very low concentration of oxygen.

4.2 - Theoretical Analysis of Oxygen into the Cell

We then found literature with data and then plugged the values into our equations to do a practical analysis of our equations. We are using data for Red Blood Cells in this section, and were assuming they are spherical.

$$N = \frac{n*\pi R^2 D}{A*L}\Delta C$$

Equation 19

$n = 3,000,\ R = .7\ nm -> .0007\ \mu m,\ D = 3.2E-5\ cm^2/s -> .32\ \mu m,\ A = 140\ \mu m^2, and\ L = 2\ \mu m.$ We're assuming for this situation that the concentration of oxygen outside of the cell is 100% and the cell has no oxygen inside, making $\Delta C = 100\ cm^{-3} -> 1E6\ \mu m$. Since ΔC would be the only variable changing in this context, that our equation would have a linear relationship with ΔC; as ΔC increases, flux increases, and as ΔC decreases, flux would decrease.

$$N = \frac{(3,000)*\pi(.0007)^2(.32)}{(140)*(2)}(1E6) = 5.278$$

This means there are about 5 moles of oxygen transported into the cell per unit time. If the concentration gradient is reduced, we get:

$$N = \frac{(3{,}000)*\pi(.0007)^2(.32)}{(140)*(2)}(5E5) = 2.639$$

This means if we reduce the concentration gradient by half, the amount of oxygen per unit time being transported into the cell is also reduced by about half.

$$N = \frac{(3{,}000)*\pi(.0007)^2(.32)}{(140)*(2)}(0) = 0$$

If we have a concentration gradient of 0, then we would have 0 flux. This is due to ΔC canceling out the rest of the equation.

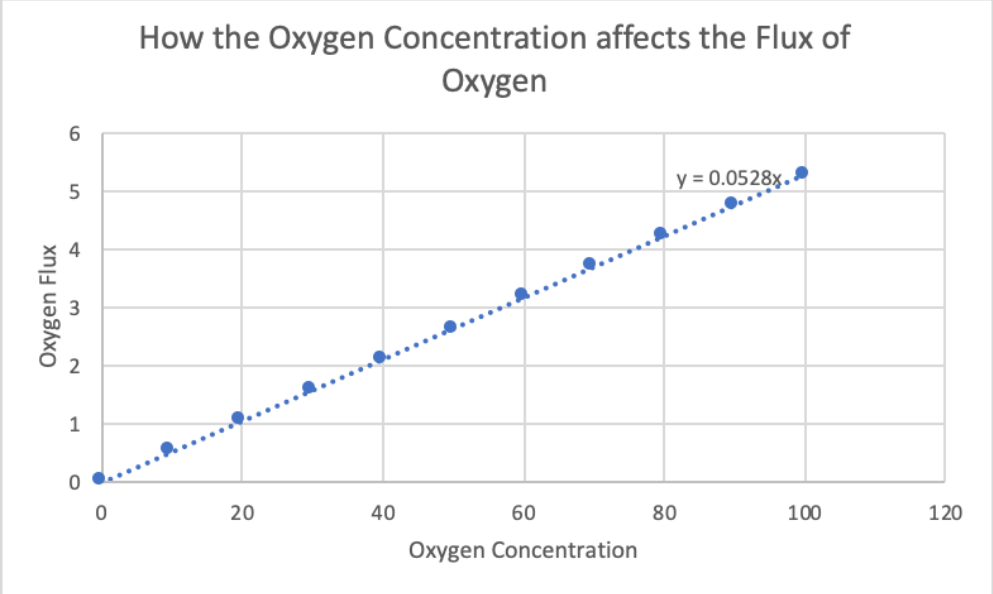

Figure 5 - Oxygen Flux (moles of Oxygen) vs. Concentration (dimensionless)

Figure 5 agrees with our statement that the concentration gradient and oxygen flux have a linear relationship.

We then performed a theoretical analysis of solvent flux in the system, which can be seen below:

$$N_s = -D\frac{dC}{dz}$$

Equation 26

How the Concentration Gradient in the Z-Direction Affects the Solute Flux

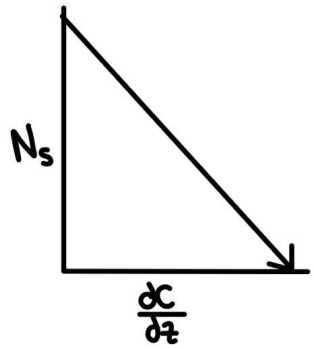

Figure 6 - Flux of the Solute (moles of Oxygen) vs. the Concentration Gradient in the Z-Direction (dimensionless)

Figure 6 shows that as $\frac{dC}{dz}$ increases, the solvent flux decreases. This occurs because *D*, the diffusion coefficient is always $3.2E-5\ cm^2/s$. *D* can change with respect to temperature, but in this system we are assuming the temperature is 37C. Since *D* is negated, we would have an inverse relationship between N_s and $\frac{dC}{dz}$, which is theoretically modeled by Figure 6.

We did theoretical analysis of our solution by setting everything in equation 34 to 1 then changing just one variable, which can be seen below.

$$C(r) = C_0 - \frac{K_2 C_0 R_i^2}{D}\left(\frac{1}{r} - \frac{1}{R_o}\right) \qquad (34)$$

The following graphs were made using Desmos. In order to properly use this program, R_i, *C(r)*, and *r* were changed to R_1, *y*, and *x* respectively. Since R_1 was set to 0.9 in order to be less than R_0 since it is the inner radius of the cell. X can vary from 0 to 1 since it represents the radius within the cell.

The variables that we are analyzing are C_0, K_2, R_1, and R_0. We aren't looking at the effects of changing D since the oxygen diffusion coefficient changes mainly based on temperature which is constant in a bioreactor. K_2, R_1, and R_0 will all change based on the chosen cell.

All of the following graphs show dimensionless oxygen concentration on the y axis and dimensionless radius on the x axis. The middle of the cell is r=0 and the outer radius of the cell is at r=1.

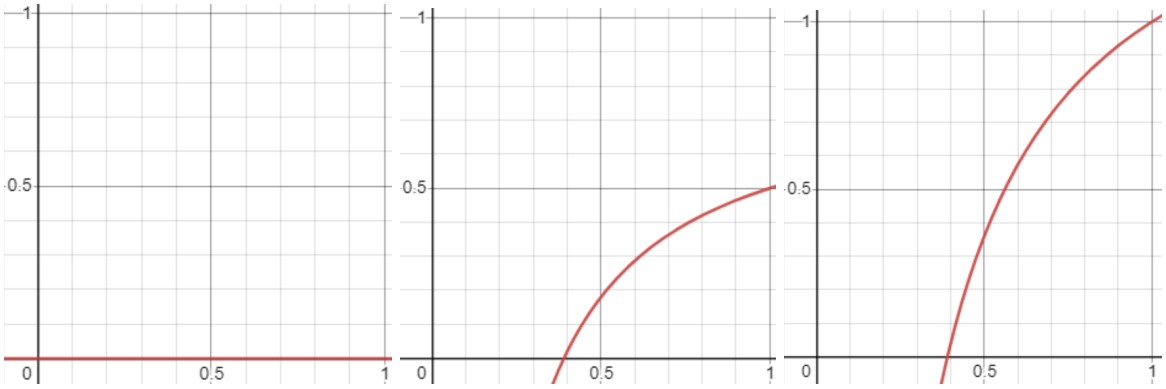

Figure 7 - Oxygen Concentration (dimensionless) vs Radius (dimensionless)
C_0 at 0, 0.5, and 1

These graphs show that as you move from the center to the outside of the cell, oxygen concentration increases to C_0.

When demonstrating the effects of other variables, we set C_0 to 0.5 in order to create a more readable graph when looking from 0 to 1 on the x axis.

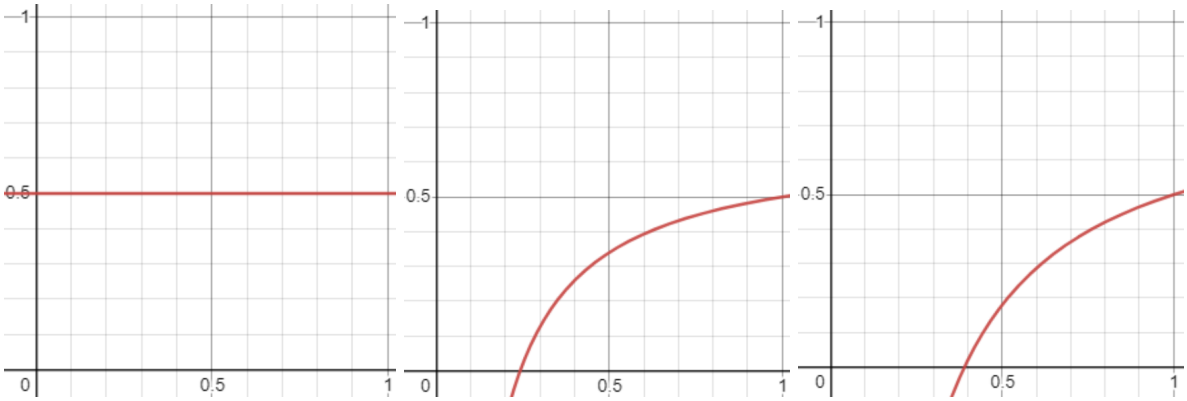

Figure 8 - Oxygen Concentration (dimensionless) vs Radius (dimensionless)
K_2 at 0, 0.5, and 1

These graphs show that as you move from the center to the outside of the cell, oxygen concentration increases to C_0. Changing K_2 affects the rate that concentration increases.

R_0 is kept as 1 and R_1 can vary from 0 to 1. In reality, R_1 has to be less than R_0 since the inside radius can't be greater than the outside radius.

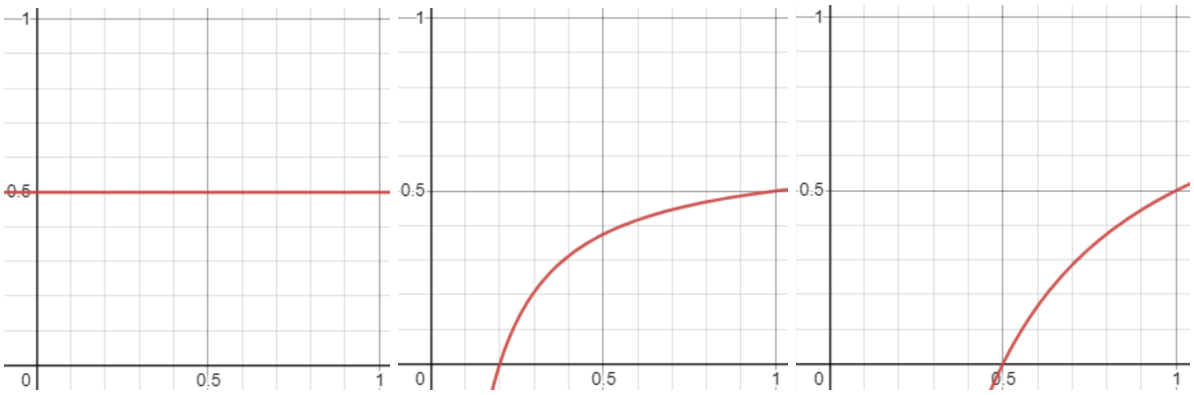

Figure 9 - Oxygen Concentration (dimensionless) vs Radius (dimensionless)
R_1 at 0, 0.5, and 1

These graphs show that as you move from the center to the outside of the cell, oxygen concentration increases to C_0. Changing R_1 affects the rate that concentration increases.

To analyze the effects of changing R_0, we set R_1 to 0.5 and varied R_0 from 0.5 to 1. This ensures that R_1 is still less than R_0. Since R_0 is the outside radius of the cell membrane, this value would only change if different cells are being grown in the bioreactor.

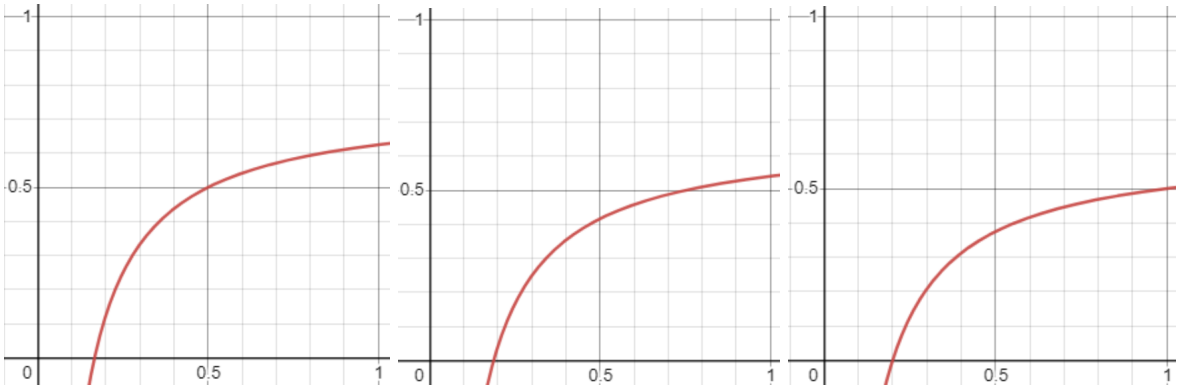

Figure 10 - Oxygen Concentration (dimensionless) vs Radius (dimensionless)
R_0 at 0.5, 0.75, and 1

These graphs show that as you move from the center to the outside of the cell, oxygen concentration increases to C_0. Changing R_0 affects the radius at which oxygen concentration is equal to C_0. Oxygen concentration will equal C_0 when radius is equal to R_0.

For practical analysis, we found values for K_2, *D*, R_i, and R_0 to use in equation 34. We are continuing to use red blood cells for our example analysis. These cells have a diffusion coefficient (*D*) of $3.2(10^{-5})\ cm^2/s$, diameter of 7.5um, and membrane thickness of 5nm. R_0 is

7.5um / 2 = 3.75um and R_i is 3.75um - 5nm = 3.745um. K_2 varies from $7.91(10^{-19})\ \frac{mol}{cell*sec}$ to $1.91(10^{-17})\ \frac{mol}{cell*sec}$. For this analysis we are going to use the smaller value of K_2.We can set r to be anywhere inside the cell, for example 3um. Since C_0 is determined by the rate that oxygen is added to the bioreactor we can use arbitrary values to show the relationship between C_0 and C(r), with the chosen values.

$$C(r) = C_0 - \frac{K_2 C_0 R_i^2}{D}\left(\frac{1}{r} - \frac{1}{R_o}\right) \qquad (34)$$

$$C(r) = 0 - \frac{(7.91(10^{-19})\ \frac{mol}{cell*sec})(0)(3.745um)^2}{3.2(10^{-5})\ cm^2/s}\left(\frac{1}{3um} - \frac{1}{3.75um}\right)$$

$$C(r) = 0$$

If the initial concentration is 0 then there is no diffusion across the cell membrane.

$$C(r) = 1 - \frac{K_2 C_0 R_i^2}{D}\left(\frac{1}{r} - \frac{1}{R_o}\right)$$

$$C(r) = 1 - \frac{(7.91(10^{-19})\ \frac{mol}{cell*sec})(1)(3.745um)^2}{3.2(10^{-5})\ cm^2/s}\left(\frac{1}{3um} - \frac{1}{3.75um}\right)$$

$$C(r) = 1 - 2.311(10^{-20})$$

If we set the initial oxygen concentration to 1, this equation tells us that there is a slightly lower concentration of oxygen within the cell at a radius of 3um.

5. Significance

This design focuses on a bioreactor that is transporting oxygen into the cell, allowing the cells to proliferate. We expect to have results that model the flow of oxygen into the system and the transport of oxygen from the media into the cell. Our solutions allow us to better understand exactly how bioreactors function and how we can potentially improve their efficiency based on how convective velocity, cell number, maximum height and many other variables were modeled in the system. By solving these problems in a steady state, as opposed to an unsteady state, we can see that having a continuous flow of oxygen into the bioreactor is more efficient than having a varying flow. This continuous flow allows for more rapid transport of oxygen into the bioreactor and media, which then allows for the cells to proliferate faster. Since there is a large and constant flow of oxygen into the system, a higher concentration of oxygen can cause more diffusion into the cells per unit of time, therefore allowing the cells to gain ATP faster. When the cells have an ample amount of oxygen they can use all of that oxygen in respiration, which aids

them in proliferation. If we used an unsteady state approach to this problem we would run into the issue of being dependent on time and oxygen concentration.

Improving bioreactor design matters to society because of the growing need for systems to proliferate animal cells, bacteria, and yeast in a controlled system. These organisms are then used to create products such as antibodies, vaccines, or pharmaceuticals such as insulin. Since oxygen is a cell growth rate limiting solute in a bioreactor, its optimization is crucial for any pharmaceutical company. It would be more cost-effective to run a constant flow of oxygen into the bioreactor because the cells would be proliferating at a large and constant rate. Our solution shows methods to improve the efficiency of oxygen diffusion significantly, which will allow for higher yields of pharmaceuticals, vaccines, and antibodies using roughly the same inputs, which in turn allows for more patients to have easier access to drugs and treatments that they need. This also allows for these products to be more accessible to the public at a lower cost as even a minute increase in efficiency of these processes being produced at such a large scale will lead to a large reduction in costs.

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