# Modeling of $O_2$ Concentration in a Cellular Parallel–Plate Flow Bioreactor

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# 1 Introduction

As the field of biology progresses, there arises a need to apply specific culture conditions for the growth and proliferation of target cell types. This is particularly important in stem cell differentiation where external conditions ranging from gas concentration to the concentration of various reagents in solution can determine the efficiency of a cell to progress towards a target lineage.[1] Oxygen in particular is an important modulator of cellular function as it is a key element in metabolic processes. The manipulation of the oxygen concentration would then allow us to induce phenotypic changes of cells in culture.[2] For this project, we are analyzing the spatial oxygen distribution inside a bioreactor in which fluid is flowed across a cell culture at a constant rate. Modeling such a device provides an important step to control as it allows the visualization of oxygen concentrations throughout the bioreactor without the need for a complex sensor array and gives the ability to predict outcomes that could be correlated to apparent oxygen concentration for given cells.

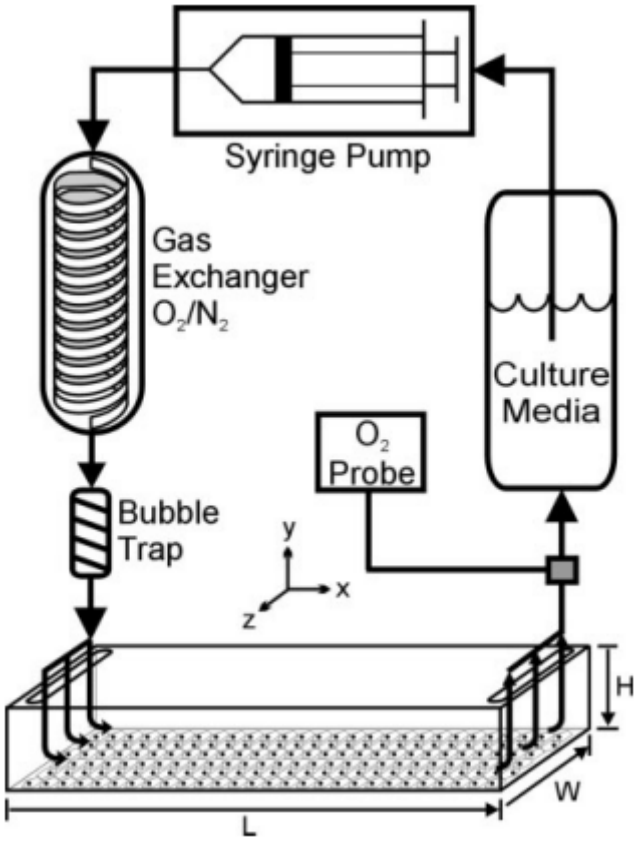


Figure 1: A parallel–plate flow bioreactor. Image source: [2]

# 2 Bioreactor Model

Figure 1 from Allen, *et al.*[2] shows a bioreactor scheme that we will analyze. This particular bioreactor is a parallel–plate type flow bioreactor. A fluid carries oxygen to the cell by flowing through the chamber parallel to a monolayer of cells on the bottom of the chamber. This fluid delivers the oxygen to the cell monolayer. Figure 2 shows a simplified schematic of the same bioreactor. Note the fluid enters the chamber from the left side.

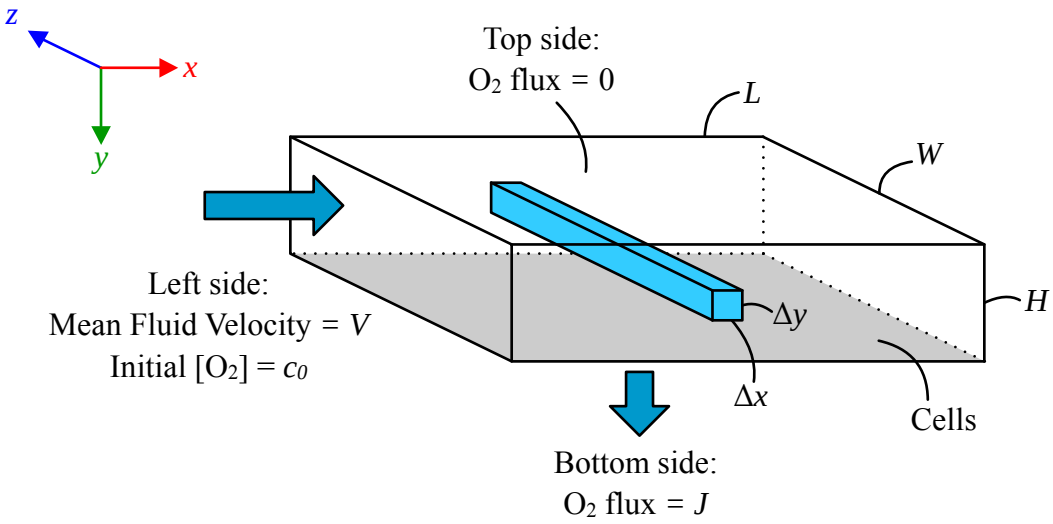


Figure 2: A simplified schematic of a parallel–plate flow bioreactor.

## 2.1 Assumptions

We will make several assumptions before we state the problem in quantitative terms. Here we will ignore edge effects and solve the problem in two dimensions. We assume gravity is negligible and diffusion in the $x$-direction is insignificant compared to convection in the $x$-direction.

With regards to the fluid, we will use the average fluid speed instead of deriving a flow velocity profile. We will assume that fluid flow is well–developed and is in steady–state at all times. In addition, we assume the oxygen concentration in the fluid is constant at all times and that the diffusivity of oxygen in the fluid is constant throughout the problem domain. The incoming fluid is the chamber's only source of oxygen; there is no oxygen generation within the chamber itself.

Finally, we assume that the cell monolayer consumes oxygen at a constant rate and that the top of the bioreactor is impermeable to oxygen. The cell monolayer is the only thing in the bioreactor that consumes oxygen. The right side of the chamber in Figure 2 is an open boundary.

## 2.2 Physics

We can now invoke the conservation of mass to obtain the governing equation of the system. The conservation of mass is stated as follows:

$$\underbrace{\frac{d}{dt}\iiint_R c(\mathbf{r},t)\,dV}_{\text{change of mass in } V} = \underbrace{\oiint_{\partial R} \mathbf{J}(\mathbf{r},t)\cdot\mathbf{n}\,dS}_{\text{mass flow into } V} + \underbrace{\iiint_R Q(\mathbf{r},t)\,dV}_{\text{mass generation in } V} \tag{1}$$

$$\Rightarrow \frac{\partial c}{\partial t}(\mathbf{r},t) = \nabla\cdot\mathbf{J}(\mathbf{r},t) + Q(\mathbf{r},t) \tag{2}$$

where $c$ is the concentration and $R$ represents a fixed volume (region). Equations 1 and 2 are the integral and differential forms of the conservation of mass law, respectively. We will use the differential form for our problem.

To solve for this problem in steady–state conditions, we set the time derivative to zero. Also, we set $Q = 0$ since there is no oxygen generation in the chamber:

$$0 = \nabla\cdot\mathbf{J}(\mathbf{r},t) \tag{3}$$

The expression for the flux $J$ is:

$$\mathbf{J}(x,y) = Vc(x,y)\hat{\mathbf{i}} - D\frac{\partial c}{\partial y}(x,y)\hat{\mathbf{j}} \tag{4}$$

where $V$ is the average fluid velocity and $D$ is the diffusivity of oxygen in the fluid. The flux takes into account the flux due to convection and the flux due to diffusion. We use Fick's law to describe the diffusion flux here.

The $\nabla$ operator in 2D Cartesian coordinates is:

$$\nabla = \frac{\partial}{\partial x}\hat{\mathbf{i}} + \frac{\partial}{\partial y}\hat{\mathbf{j}} \tag{5}$$

This results in the following expression:

$$0 = \nabla\cdot\mathbf{J}(\mathbf{r},t) \Rightarrow V\frac{\partial c}{\partial x} = D\frac{\partial^2 c}{\partial y^2} \tag{6}$$

Which gives us the partial differential equation to be solve for:

$$V\frac{\partial c}{\partial x} = D\frac{\partial^2 c}{\partial y^2} \tag{7}$$

This equation must be subject to the following boundary conditions:

$$c(0,y) = c_0,\ -D\frac{\partial c}{\partial y}(x,0) = 0,\ -D\frac{\partial c}{\partial y}(x,H) = J \tag{8}$$

Which follows from the previous set of assumptions. Namely, that the inlet fluid oxygen concentration is constant (first boundary condition), that the top of the chamber is impermeable to oxygen (second boundary condition), and that the cell monolayer consumes oxygen at a constant rate (third boundary condition).

# 3 Analytical Solution

To solve Equation 7 given the boundary conditions, we employ several mathematical techniques to simplify the problem further.

## 3.1 Nondimensionalization

A particularly useful technique for physical problems involves rewriting Equation 7 into a dimensionless form (and thus also making the boundary conditions dimensionless). This can be done by performing the following change of variables:

$$\hat{c} = \frac{c}{c_0} \qquad \hat{x} = \frac{x}{L} \qquad \hat{y} = \frac{y}{H}$$
$$\alpha = \frac{L}{H} \qquad \mathrm{Pe} = \frac{VH}{D} \qquad \mathrm{Da} = \frac{JH}{Dc_0}$$

Using the chain rule for derivatives, we can rewrite both the partial differential equation:

$$\frac{\partial \hat{c}}{\partial \hat{x}} = \frac{\alpha}{\mathrm{Pe}} \frac{\partial^2 \hat{c}}{\partial \hat{y}^2} \tag{9}$$

And the boundary conditions:

$$\hat{c}(0, \hat{y}) = 1, \ \frac{\partial \hat{c}}{\partial \hat{y}}(\hat{x}, 0) = 0, \ \frac{\partial \hat{c}}{\partial \hat{y}}(\hat{x}, 1) = -Da \tag{10}$$

Note that $\mathrm{Pe}$ and $\mathrm{Da}$ really are dimensionless!

$$\mathrm{Pe} = \frac{VH}{D} \Rightarrow \left(\frac{\mathrm{m}}{\mathrm{s}} \times \mathrm{m}\right) \Big/ \left(\frac{\mathrm{m}^2}{\mathrm{s}}\right) = 1$$
$$\mathrm{Da} = \frac{JH}{Dc_0} \Rightarrow \left(\frac{\mathrm{mol}}{\mathrm{m}^2 \times \mathrm{s}} \times \mathrm{m}\right) \Big/ \left(\frac{\mathrm{m}^2}{\mathrm{s}} \times \frac{\mathrm{mol}}{\mathrm{m}^3}\right) = 1$$

Also note that the process of nondimensionalization produces some dimensionless numbers, such as the length–to–height ratio $\alpha$ and the dimensionless oxygen flux $\mathrm{Da}$ (the Damköhler number) The most important number for our case is the Péclet number, $\mathrm{Pe}$. The number is defined as:[3]

$$\mathrm{Pe} = \frac{\text{convective transport rate}}{\text{diffusive transport rate}} \tag{11}$$

Recall that we assumed that diffusion in the $x$-direction was insignificant compared to convection in the same direction. The Péclet number described above allows us to quantify how well the assumption holds in our problem. In other words, if $\mathrm{Pe} >> 1$, then the assumption holds for all intents and purposes.

## 3.2 Homogenization

We will continue to simplify the problem; specifically we will rewrite the partial differential equation and the boundary conditions to make the boundary conditions as homogeneous as possible. Through some trial–and–error, we come to using the following change of variables:

$$\hat{c} = \hat{\theta} - \mathrm{Da}\left(\frac{\hat{y}^2}{2} + \frac{\alpha}{\mathrm{Pe}}\hat{x}\right) \tag{12}$$

Which substituted into Equation 9 produces:

$$\frac{\partial\hat{\theta}}{\partial\hat{x}} = \frac{\alpha}{\mathrm{Pe}}\frac{\partial^2\hat{\theta}}{\partial\hat{y}^2} \tag{13}$$

We must also rewrite the boundary conditions to be consistent with the change of variables:

$$\hat{\theta}(0,\hat{y}) = 1 + \frac{\mathrm{Da}}{2}\hat{y}^2,\ \frac{\partial\hat{\theta}}{\partial\hat{y}}(\hat{x},0) = 0,\ \frac{\partial\hat{\theta}}{\partial\hat{y}}(\hat{x},1) = 0 \tag{14}$$

Note that we have effectively made all but one boundary condition homogeneous. This will be especially helpful for the next technique.

## 3.3 Separation of Variables

In the separation of variables technique, we assume the solution is of the form:

$$\hat{\theta}(\hat{x},\hat{y}) = X(\hat{x})Y(\hat{y}) \tag{15}$$

With this assumption, we rewrite the partial differential equation yet again:

$$\frac{1}{X}\frac{dX}{d\hat{x}} = \frac{\alpha}{\mathrm{Pe}}\frac{1}{Y}\frac{d^2Y}{d\hat{y}^2} = -\lambda^2 \tag{16}$$

And the boundary conditions:

$$X(0)Y(\hat{y}) = 1 + \frac{\mathrm{Da}}{2}\hat{y}^2 \tag{17}$$

$$X(\hat{x})\frac{dY}{d\hat{y}}(0) = 0 \Rightarrow \frac{dY}{d\hat{y}}(0) = 0 \tag{18}$$

$$X(\hat{x})\frac{dY}{d\hat{y}}(1) = 0 \Rightarrow \frac{dY}{d\hat{y}}(1) = 0 \tag{19}$$

Note that we have introduced a separation constant, $-\lambda^2$. This form was chosen for reasons explained later. The constant appears since the two sides of Equation 16 can only be equal if both

sides are in fact constant, and are both equal to the same constant. This allows us to rewrite the partial differential equation as two separate ordinary differential equations which are easily solved:

$$\frac{dX}{d\hat{x}} = -\frac{\alpha\lambda^2}{\mathrm{Pe}}X \Rightarrow X = k_0 e^{-\alpha\lambda^2\hat{x}/\mathrm{Pe}} \tag{20}$$

$$\frac{dY}{d\hat{y}} = -\lambda^2 Y \Rightarrow Y = k_1\sin(\lambda\hat{y}) + k_2\cos(\lambda\hat{y}) \tag{21}$$

The equations above and the physics of the problem effectively require that $\lambda$ is a positive quantity. If it was negative, then the exponential term in the solutions above would grow without bound. This is physically impossible in our system since we have no sources. Since we know that $\lambda$ is positive, we can simplify subsequent operations by using $-\lambda^2$ as the separation constant.

Substituting the above solutions back into Equation 15:

$$\hat{\theta} = e^{-\alpha\lambda^2\hat{x}/\mathrm{Pe}}\left(k_1\sin(\lambda\hat{y}) + k_2\cos(\lambda\hat{y})\right) \tag{22}$$

Note that the constants have been coalesced. This is the general solution.

## 3.4 Application of Boundary Conditions

To get the solution particular to our problem, we must apply the boundary conditions. First, apply the Neumann boundary conditions (18) and (19):

$$\frac{dY}{d\hat{y}} = k_1\cos(\lambda\hat{y}) - k_2\sin(\lambda\hat{y}) \tag{23}$$

$$0 = k_1\cos(0) + k_2\sin(0) \Rightarrow k_1 = 0 \tag{24}$$

$$0 = k_2\sin(\lambda) \Rightarrow \lambda = \pi n \quad n \in \mathbb{Z}^* \tag{25}$$

Where $\mathbb{Z}^*$ is the set of all nonnegative integers. Note that $k_2$ cannot be zero since that would lead to a trivial solution. Therefore, we instead find all $\lambda$ that satisfies the boundary conditions.

Because of the superposition principle we can write a single equation for all values of $n$:

$$\hat{\theta} = \sum_{n=0}^{\infty} k_n e^{-\alpha n^2\pi^2\hat{x}/\mathrm{Pe}}\cos(n\pi\hat{y}) \tag{26}$$

$$\hat{\theta} = k_0 + \sum_{n=1}^{\infty} k_n e^{-\alpha n^2\pi^2\hat{x}/\mathrm{Pe}}\cos(n\pi\hat{y}) \tag{27}$$

then to solve for the coefficients $k_n$, we apply the last remaining boundary condition (17):

$$1 + \frac{\mathrm{Da}}{2}\hat{y}^2 = k_0 + \sum_{n=1}^{\infty} k_n\cos(n\pi\hat{y}) \tag{28}$$

This is in fact a Fourier series and $k_n$ is the Fourier coefficients.

We use the orthogonality of cosine to remove the infinite sum and obtain an expression for $k_n$:

$$\int_0^1 \cos(n\pi x)\cos(m\pi x)\, dx = \frac{1}{2}\delta_{nm} \tag{29}$$

$$\int_0^1 \cos(n\pi x)\, dx = 0 \tag{30}$$

Where $\delta_{nm}$ is the Kronecker delta and $n \geq 1$. The expression for $k_0$ and $k_n$ are:

$$k_0 = \int_0^1 \left(1 + \frac{\mathrm{Da}}{2}\hat{y}^2\right) d\hat{y} = 1 + \frac{\mathrm{Da}}{6} \tag{31}$$

$$k_n = 2\int_0^1 \left(1 + \frac{\mathrm{Da}}{2}\hat{y}^2\right)\cos(n\pi\hat{y})\, d\hat{y} = \mathrm{Da}\frac{2}{\pi^2}\frac{(-1)^n}{n^2} \tag{32}$$

Finally, substituting (31) and (32) into (27), then substituting the result into (12), we get the solution for the concentration:

$$\boxed{\hat{c} = 1 + \mathrm{Da}\left[\frac{1-3\hat{y}^2}{6} - \frac{\alpha}{\mathrm{Pe}}\hat{x} + \frac{2}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}e^{-\alpha n^2\pi^2\hat{x}/\mathrm{Pe}}\cos(n\pi\hat{y})\right]} \tag{33}$$

# 4 Numerical Solution via MATLAB

MATLAB's built–in `pdepe()` function can solve partial differential equations of the form:

$$c\left(x,t,u,\frac{\partial u}{\partial x}\right)\frac{\partial u}{\partial t} = x^{-m}\frac{\partial}{\partial x}\left(x^m f\left(x,t,u,\frac{\partial u}{\partial x}\right)\right) + s\left(x,t,u,\frac{\partial u}{\partial x}\right)$$

Luckily, our partial differential equation can be written in this form by letting:

$$c = 1, \quad m = 0, \quad f = D\frac{\partial u}{\partial x}, \quad s = 0$$

thus we can use `pdepe()` to numerically solve our problem and compare the results with our analytical solution. A somewhat confusing change of variables is needed to fit the form `pdepe()` expects:

$$\text{our model's } x \Rightarrow \texttt{pdepe()}\text{'s } t$$
$$\text{our model's } y \Rightarrow \texttt{pdepe()}\text{'s } x$$

This is taken into account in our MATLAB code. The code used to generate solution plots can be found in Appendix A. Equation 7 and its associated boundary conditions were used in implementing the `pdepe()`–based solution.

# 5 Results

For the purpose of plotting the results, we have used the following parameters:

| Parameter | Value | Description |
|---|---|---|
| $L$ | $5.5\,\text{cm}$ | Bioreactor length |
| $W$ | $2.8\,\text{cm}$ | Bioreactor width |
| $H$ | $0.01\,\text{cm}$ | Bioreactor height |
| $c_0$ | $140\,\text{nmol cm}^{-3}$ | Inlet oxygen concentration |
| $Q$ | $1\,\text{mL min}^{-1}$ | Volumetric flow rate |
| $D$ | $2 \times 10^{-5}\,\text{cm}^2\,\text{s}^{-1}$ | Oxygen diffusivity |
| $J$ | $0.0646\,\text{nmol cm}^{-2}\,\text{s}^{-1}$ | Oxygen flux into cell monolayer |
| $V$ | $0.5952\,\text{cm s}^{-1}$ | Average flow velocity $= Q/(W \times H)$ |
| $\alpha$ | 550 | Length/height ratio $= L/H$ |
| Da | 0.2307 | Damköhler number $= (J \times H)/(D \times c_0)$ |
| Pe | 297.6190 | Péclet number $= (V \times H)/D$ |

The last four parameters are calculated from the previous parameters. We used the numerical values presented in Allen, *et al* for the parameters.[2] We plotted all analytical solutions using the first 10 terms in the infinite series.

Shown below are the solutions obtained by using `pdepe()` and by evaluating the analytical solution across the entire problem domain: The two solutions are in good agreement. There is,

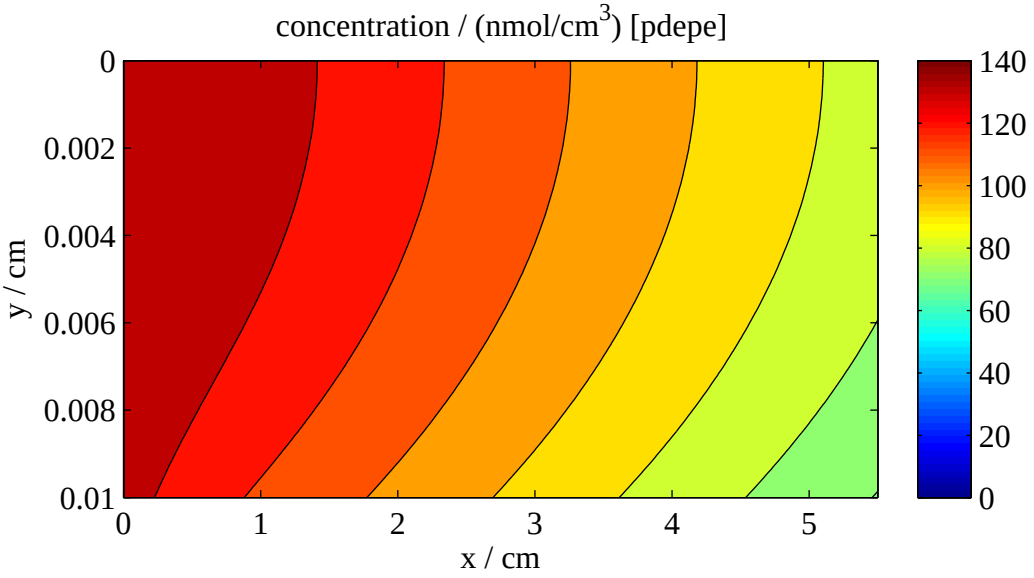


Figure 3: Numerical solution using the parameters presented above.

however, a bit of oscillation at the very left edge of the domain. This is a manifestation of the

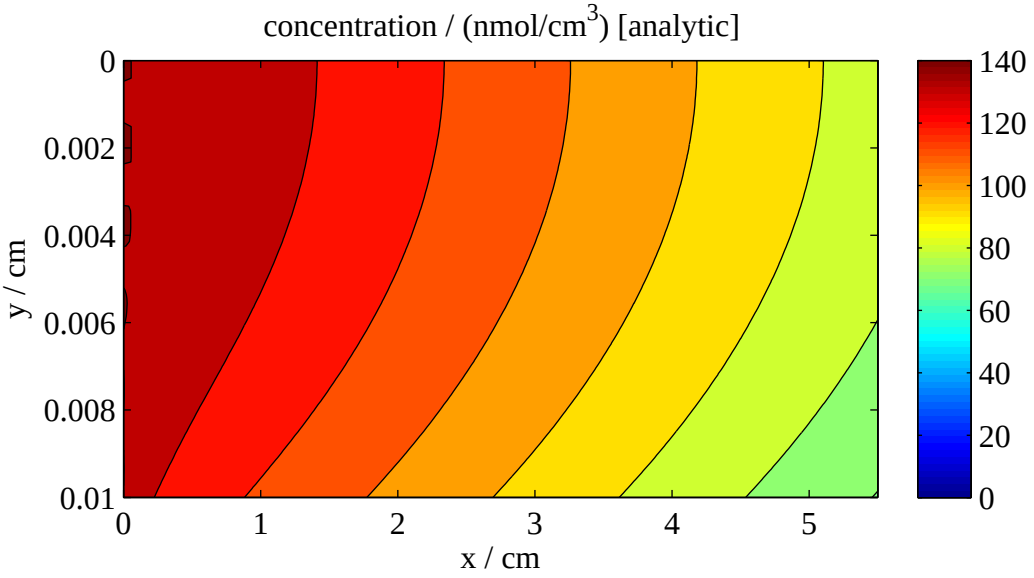


Figure 4: Analytical solution using the parameters presented above.

Gibbs phenomenon associated with the Fourier series. Increasing the number of terms reduces the severity of the oscillations, but will always persist as we cannot practically evaluate the infinite series.

We varied some of the parameters to see the effect it produces on the solution. The parameters we changed were the initial inlet concentration $c_0$, the oxygen consumption rate $J$, and the volumetric flow rate $Q$.

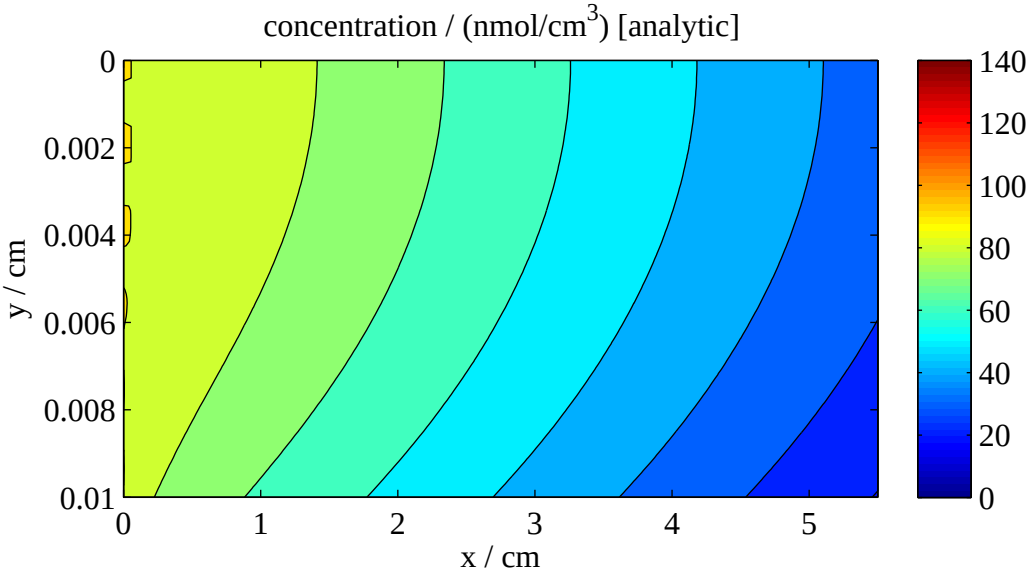


Figure 5: Analytical solution with $c_0 = 90\,\text{mmHg}$.

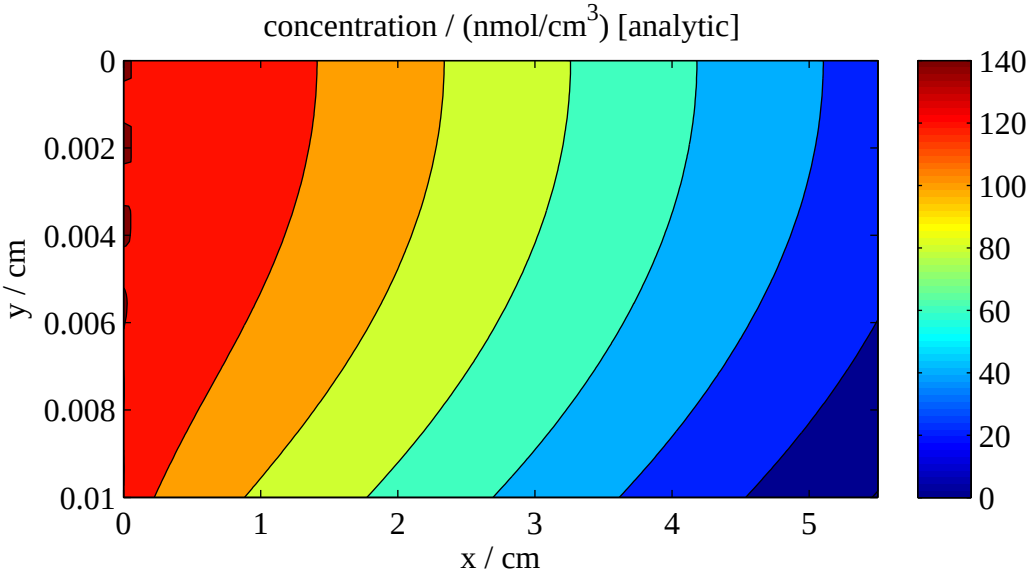


Figure 6: Analytical solution with doubled oxygen consumption rate.

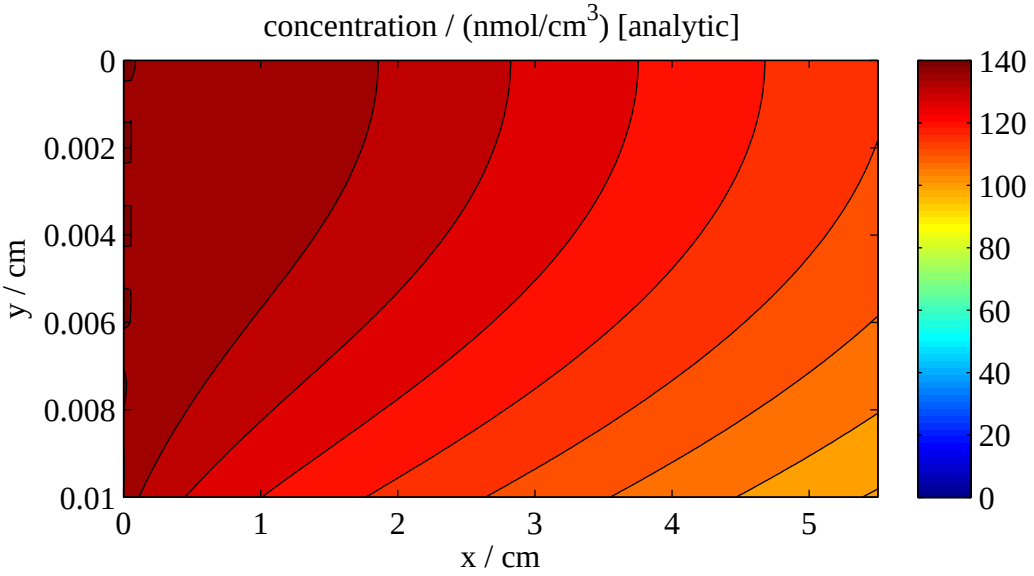


Figure 7: Analytical solution with doubled flow rate.

# 6 Discussion

As can be seen from Figures 3 and 4, a concentration gradient is achieved at the cell monolayer with contours that extend upwards to the top of the bioreactor. We can see that there are distinct regions of $O_2$ concentration at the standard flow rate and that it cannot be assumed that with a constant $O_2$ inlet concentration that all cells would be exposed to the same $O_2$ concentration. However, as can be seen from Figure 7, when the flow rate is doubled, the apparent $O_2$ concentration to the cells becomes more and more similar across the bottom of the bioreactor. This would lead to the

conclusion that there is an optimal flow rate (provided that it doesn't generate too much physical stress on the cells and inherently cause phenotypic changes) that would result in a minimal gradient from one end of the bioreactor to the other. We also noticed that halving the inlet concentration yielded the predicted results of having a lower $O_2$ concentration across the entire bioreactor and if the cells were to consume $O_2$ at a doubled rate, the gradient becomes sharper and more apparent. This would mean that the flow would need to be adjusted to compensate for the doubled rate to ensure a more even $O_2$ distribution in the bioreactor.

To create a more robust model, a velocity profile can be derived from the Navier–Stokes equations instead of using average fluid velocity as was seen in the model presented above. Also, the above model assumes constant cellular $O_2$ consumption which is not necessarily the case in living organisms and would be better modeled with the help of Michaelis–Menten kinetics. Another case that can be considered is the fact that the cells might not be a perfect monolayer or the bioreactor is used to culture a thicker tissue slice. Modeling could be performed to check the $O_2$ concentration at each layer in the tissue. Finally, this model benefits from the assumption that convection is much greater that diffusion (i.e. the Péclet number is much greater than 1). Although this should generally be the case, a more robust model that allows for analysis of the scenario where this assumption doesnt hold true may be of use.

# Appendix A: MATLAB Code

The following MATLAB code was used to generate the solution plots: *[Begin code—*

```
function bioreactor_pde()
    % Bioreactor parameters
    L = 5.5;     % bioreactor length, cm
    W = 2.8;     % bioreactor width, cm
    H = 0.01;    % bioreactor height, cm
    c_0 = 140;   % inlet oxygen concentration, nmol/cm^3
    Q = 1/60;    % inlet volumetric flow rate, cm^3/s
    D = 2e-5;    % oxygen diffusivity, cm^2/s
    J = 0.0646;  % oxygen flux due to cells, nmol/(cm^2s)

    % Computed from above parameters
    V = Q/(W*H); % mean fluid velocity, cm/s
    alpha = L/H; % length-to-height ratio, unitless
    Pe = V*H/D;  % Peclet number, unitless
    Da = (J*H)/(D*c_0); % Damkohler number, unitless

    % Solve using MATLAB's pdepe solver.
    % Note: The documentation for pdepe expresses the PDE
    % in terms of x and t. To match the form of our PDE to
    % what pdepe expects, we assume our x = pdepe's t and
    % our y = pdepe's x. We transpose pdepe's solution afterwards to fix
    % this.
    x = linspace(0, L, 100);
    y = linspace(0, H, 100);
    u1 = pdepe(0,...
        @(~, ~, ~, DuDx) pde_problem(DuDx, V, D),...
        @(~) pde_bc_x(c_0),...
        @(~, ~, ~, ~, ~) pde_bc_y(J),...
        y, x);
    u1 = u1(:,:,1)';

    % Compare with the analytic solution.
    [xg, yg] = meshgrid(x./L, y./H);
    u2 = pde_solution(xg, yg, alpha, Pe, Da);
    % The solution is dimensionless.
    u2 = u2.*c_0;

    % Plot contour plots of the two solutions.
    figure(1);
    contourf(x, y, u1);
    set(gca, 'FontName', 'Times New Roman', 'FontSize', 12);
    set(gca, 'YDir', 'reverse');
    caxis([0 140]);
    c = colorbar();
    set(c, 'FontName', 'Times New Roman', 'FontSize', 12);
    title('concentration / (nmol/cm^3) [pdepe]');
    xlabel('x / cm');
    ylabel('y / cm');
```

```
    figure(2);
    contourf(x, y, u2);
    set(gca, 'FontName', 'Times New Roman', 'FontSize', 12);
    set(gca, 'YDir', 'reverse');
    caxis([0 140]);
    c = colorbar();
    set(c, 'FontName', 'Times New Roman', 'FontSize', 12);
    title('concentration / (nmol/cm^3) [analytic]');
    xlabel('x / cm');
    ylabel('y / cm');
end

function [c, f, s] = pde_problem(dudy, V, D)
    c = V;
    f = D*dudy;
    s = 0;
end

function u = pde_bc_x(c_0)
    u = c_0;
end

function [pl, ql, pr, qr] = pde_bc_y(J)
    pl = 0;
    ql = 1;
    pr = J;
    qr = 1;
end

function c = pde_solution(x, y, alpha, Pe, Da)
    sum = 0;
    % We can't actually evaluate an infinite sum, but the series
    % converges very quickly due to the exponential term. Thus,
    % we don't need to sum over many terms.
    for n = 1:10
        sum = sum + ((-1)^n/n^2.*exp(-alpha*n^2*pi^2/Pe.*x)...
            .*cos(n*pi.*y));
    end
    c = 1 + Da.*((1-3.*y.^2)./6-alpha/Pe.*x+2/pi^2.*sum);
end
```

*—End code]*

# References


[1] Rudel, D. & Sommer, R. J. The evolution of developmental mechanisms. *Developmental Biology* **264**, 15-37 (2003).

[2] Allen, J. W. & Bhatia, S. N. Formation of steady-state oxygen gradients in vitro: Application to liver zonation. *Biotechnology and Bioengineering* **82**, 253-262 (2003).

[3] Truskey, G. A., Yuan, F. & Katz, D. F. *Transport Phenomena in Biological Systems*. (Prentice Hall, 2009).