Kinetic Modeling and Process Control of Cytotoxic T Cell Expansion in a Centrifugal Bioreactor System

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BACKGROUND

- Cancer is a leading cause of death worldwide
- Traditional cancer treatments destroy healthy tissue. The alternative: immunotherapy, using patients' own T cells to fight their cancer, but it has high costs and the culture methods are inefficient
- To solve this issue, our lab developed a centrifugal bioreactor (CBR) system, which can quickly expand cytotoxic T lymphocytes (CTLs) to a high density
- The CBR balances drag, buoyancy and centrifugal force to retain a cell suspension (Figure 1)

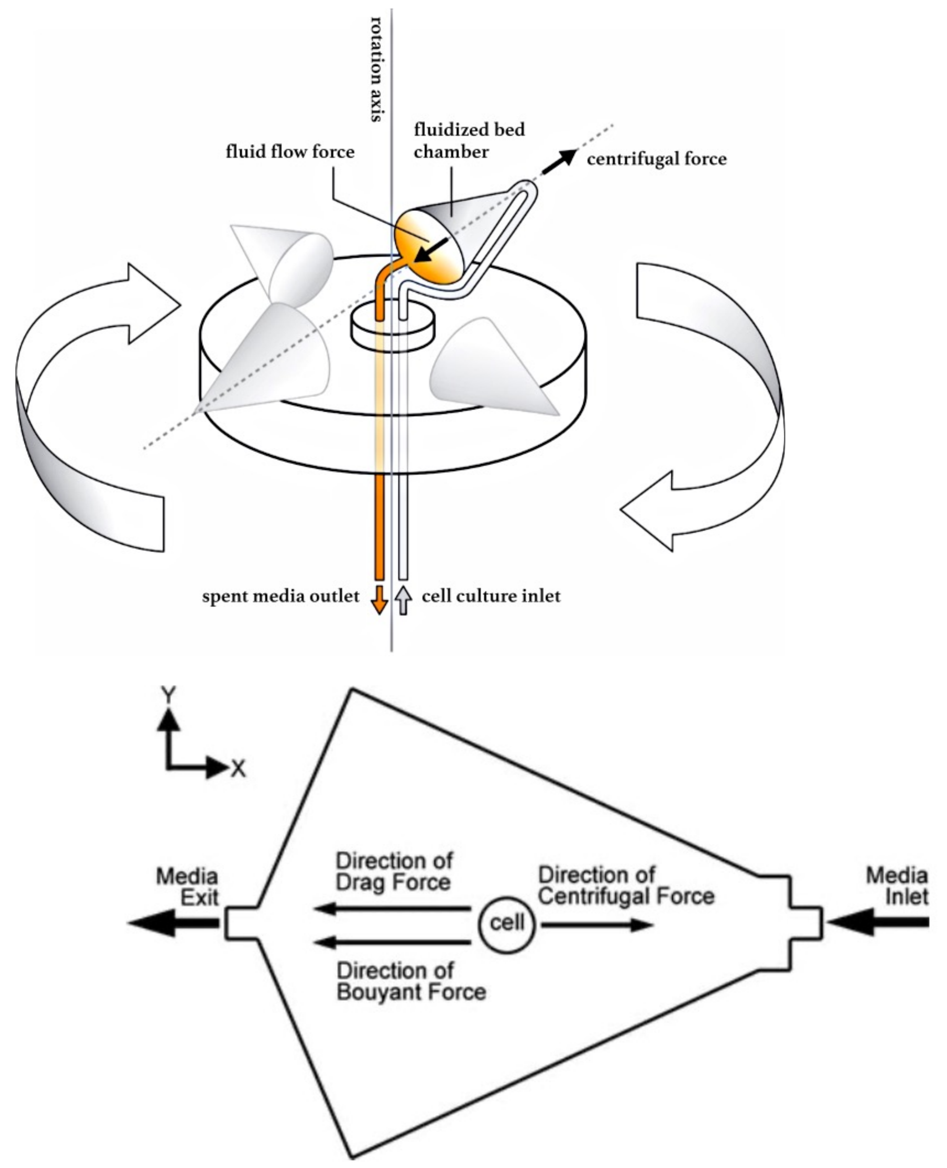

Figure 1: CBR chamber on top of centrifuge disc; balance of forces on each cell within the chamber

- Hypothesis: batch growth parameters can be used to predict cell growth in the CBR (Figure 2)
- Optimization can be achieved through a model used by Detzel et al (2009), which can predict substrate and inhibitor concentration. Modified Monod kinetics can be used to find the kinetic parameters μ_{max} and K_M
- Recent work has optimized the base CBR, upcoming efforts will focus on developing a control system to prevent cyberattacks on the CBR

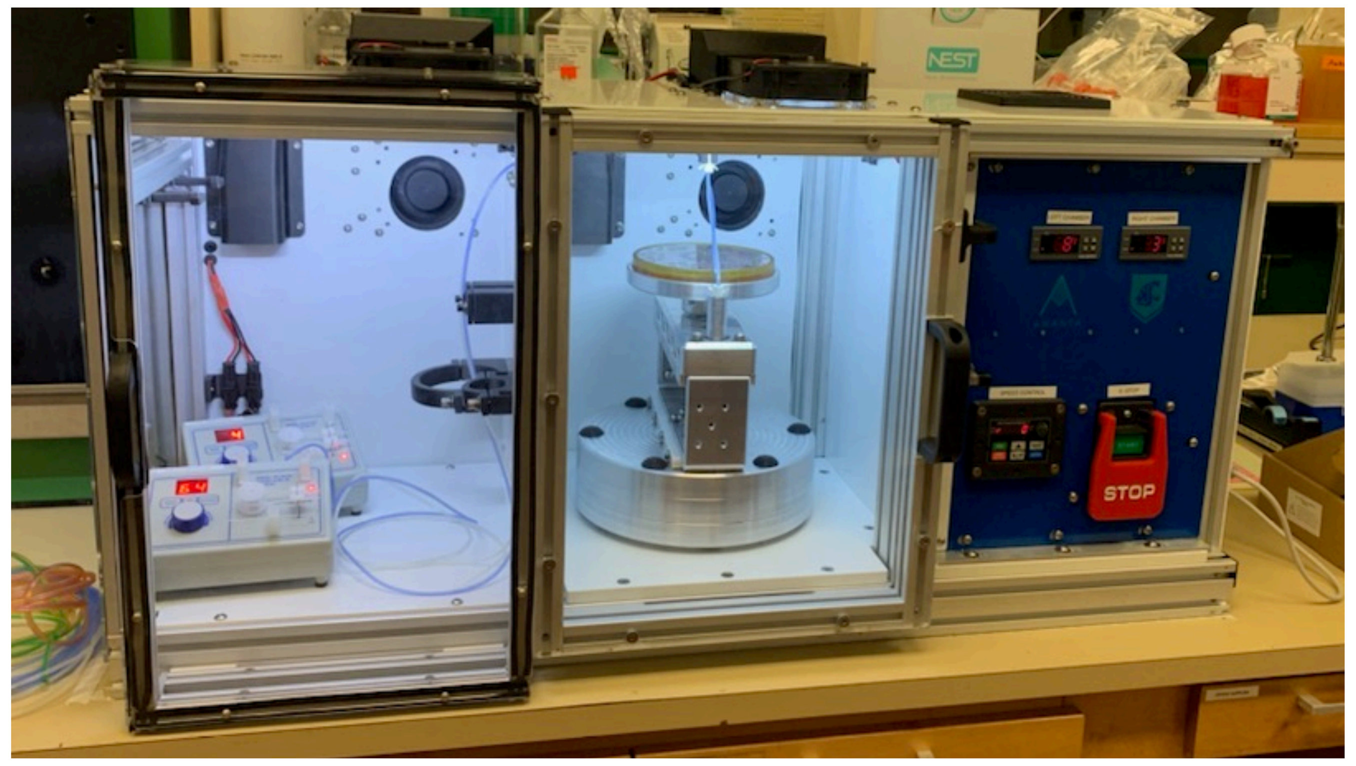

Figure 2: Current CBR prototype with centrifuge chamber visible in the center

KINETIC MODELING

- Model accounts for lactate/ammonium (L,A) inhibition, as well as glucose (G) and cell concentration (C_{cell})
- $\frac{\partial C_{cell}}{\partial t} = \mu_{max} * (1 - \frac{C_L}{C_{L\_max}})^n * (1 - \frac{C_A}{C_{A\_max}})^m * C_{cell}$

  and
  - $\frac{\partial C_G}{\partial t} = D(C_{G0} - C_G) - Y_{GC} * \left(\frac{\partial C_{cell}}{\partial t}\right)$
  - $\frac{\partial C_A}{\partial t} = D(C_{A0} - C_A) + Y_{AC} * \left(\frac{\partial C_{cell}}{\partial t}\right)$
  - $\frac{\partial C_L}{\partial t} = D(C_{L0} - C_L) + Y_{LC} * \left(\frac{\partial C_{cell}}{\partial t}\right)$
  - Where D is dilution rate (feed rate / culture volume)
  - Y_{GC}, Y_{AC}, and Y_{LC} are yield coefficients, calculated by measuring the amounts of each component in a culture over time.
  - n, m, C_{Lmax} and C_{Amax} can be found by measuring cell growth at various lactate or ammonium concentrations
- μ_{max} and K_M are found from the Monod equation, for substrate-dependent growth, where μ_{max} is the maximum specific growth rate and K_M is the glucose concentration at ½ of μ_{max}

METHODS

- Bovine cytotoxic T cells (CTLs) and human lymphoblastic leukemia cells (CEMs) were used as test cell lines for the CBR system
- Three different studies were performed in static culture to assess the cell growth: a glucose study, a yield coefficient study, and an inhibition study.
- Euler's method was used to develop a model simulating growth of the cells in the CBR

RESULTS AND DISCUSSION

- **Glucose Study**: The maximum specific growth rate and Monod constant were measured.
- **Yield Coefficient Study**: Glucose, lactate, and ammonium were measured along with cell growth over a period and the slopes of the resulting trendlines provided yield coefficients.
- **Inhibition Studies**: Maximum concentrations of lactate and ammonium were found, and the orders of inhibition n and m were determined.
- In summary, all kinetic parameters and coefficients were found with static culture experiments
- Euler's method was used to design a model that simulates growth of cells in the CBR. Model results for the CEM line are shown in Figures 3a and 3b.

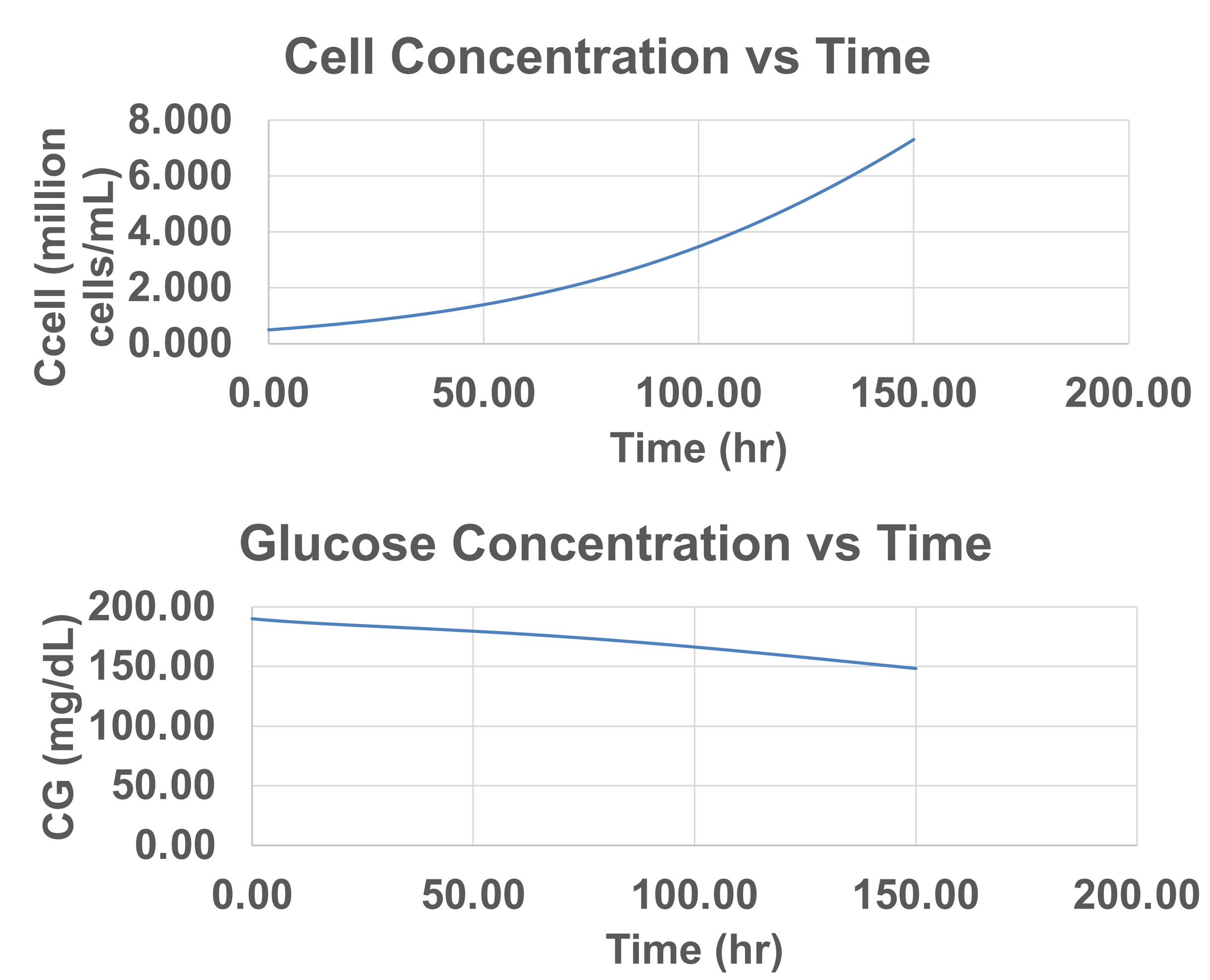

Figure 3a: Model results for cell and glucose concentration

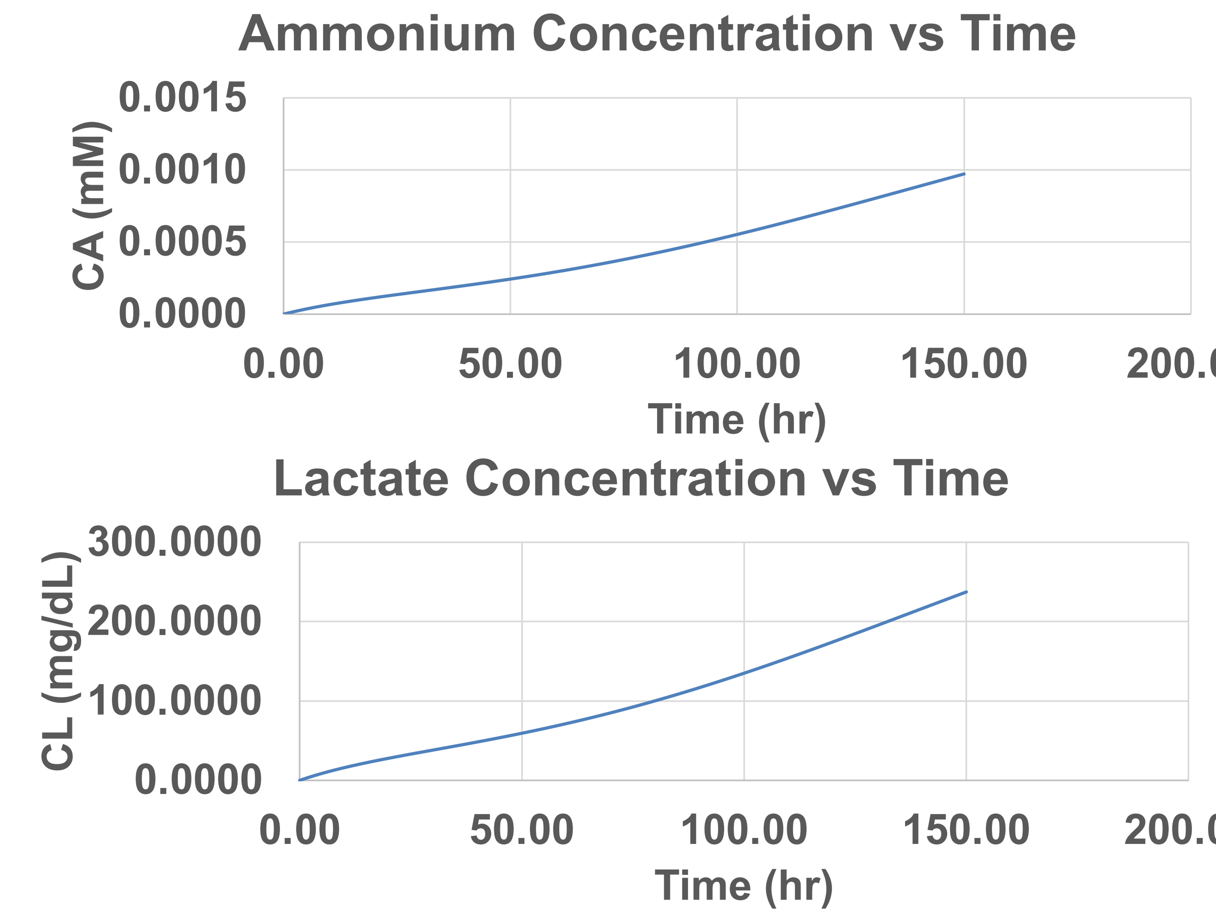

Figure 3b: Model results for lactate and ammonium concentration

FUTURE WORK

Future work will focus on development of a process control system that will prevent cyberattacks on the CBR.

- Durand et al. 2021 proposed a control model for continuous stirred-tank reactors (CSTR), here:

  $$\dot{C}_A = \frac{F}{V}(C_{A0} - C_A) - k_0 e^{-\frac{E}{R_g T}} C_A^2.$$

- In conjunction with Prof. Arda Gozen of the School of Mechanical and Materials Engineering, a process control system will be designed in Simulink
- The system will account for changes the cell, glucose, and lactate concentrations in the CBR as a result of cyberattacks

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