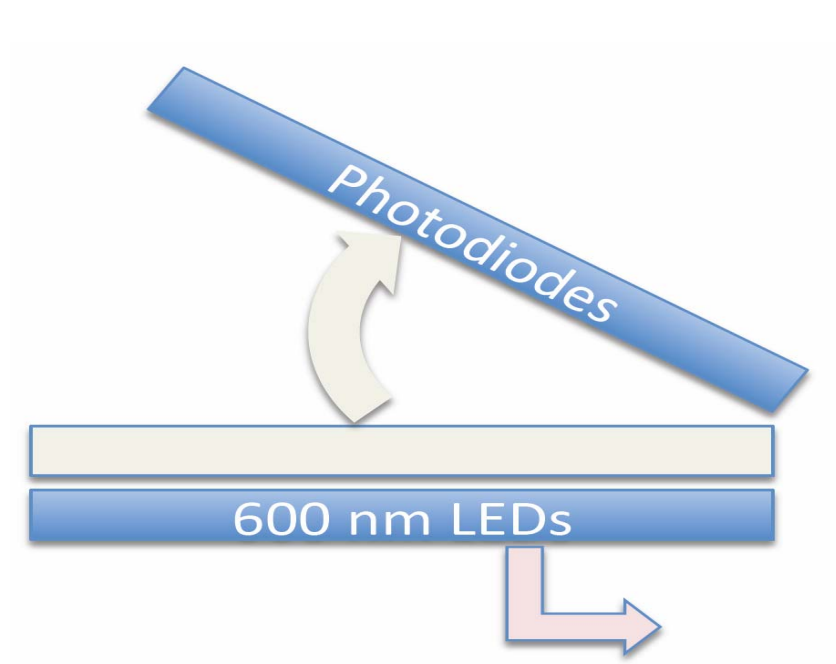


Turbidostat System Model

MS2



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1. INTRODUCTION

What we are trying to accomplish for this project is the functionality of a mini plate scanner turbidostat. There are essentially three function to a turbidostat. The first is to measure light absorbed by bacteria. For our purposes we know that bacteria absorb light at 600 nanometers. The second is for the scanner to provide feedback to the controller. For the plate scanner turbidostat we will be implementing a PI controller. Lastly we want the bacteria to remain at a constant optical density (OD), which is the output measurement of the scanner. The goal is to keep the bacteria population constant and control evolution. Evolution of bacteria can be modeled by two first order differential equations. The first is with respect to population growth, and the second is with respect to nutrients being added to the solution. This is a non-linear system.

For this specific milestone, we are modeling and simulating the system. This system must be correctly modeled using equations that pertain to the population growth and nutrient concentration in the individual wells or our mini plates. These equations need to be modeled and simulated to test for accuracy and whether they are applicable to our setup. We also want to know if the system is controllable and observable and how accurate our model is compared to the actual behavior.

2. SYSTEM MODEL

The turbidostat system model is composed of two first order differential equations. Each one is affected by the other as input values change causing different system responses.

2.1 BLOCK DIAGRAM

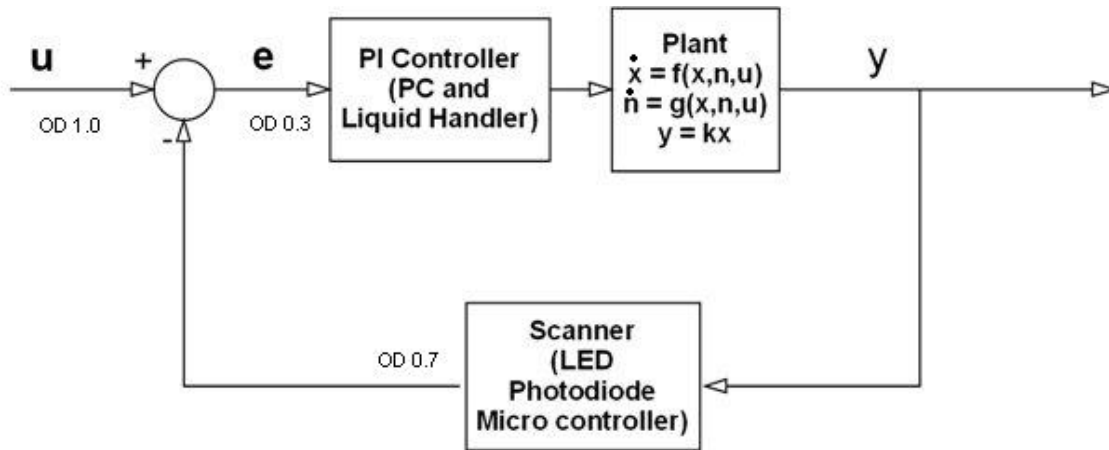


Figure 1: Block Diagram of turbidostat system

This is a total layout of what our system looks like and what we are trying to accomplish with the turbidostat. The inputs and variables are described as follows:

- x is the population of bacteria (g/L)
- n is amount of nutrients (g/L)
- u is amount of fresh nutrients being added to the system (g/L)
- Output from scanner is measured in optical density (OD) which is: $OD_{600} = \text{Log}_{10}(I_0 / I)$

It is also important to understand from this diagram that there will be a reference point in OD to set the level at which we want to keep the population constant. As the scanner reads the optical density the summer will calculate the error and feed that signal to the controller. The controller will then execute pipeting out bacteria in order to maintain that

population. The execution of this pipeting will be operated on the liquid handler (see figure 2).



Figure 2: University of Washington SOS lab liquid handler

The very same liquid handler with its embedded software will be implementing the PI controller used for this system. This is due to the fact that all the necessary functions needed in order to control the population will be running in real time and using a micro controller will only make things more complicated. We're not sure at this point exactly how long the scanning/liquid handler functions will take but we know that using the PC software will enable for more accuracy and fewer bugs in the control portion.

2.2 SYSTEM EQUATIONS

$$\begin{aligned}\dot{x} &= \frac{vnx}{k+n} - ux \\ \dot{n} &= -\gamma \frac{vnx}{k+n} + u(n_0 - n) \\ y &= kx\end{aligned}$$

Figure 3 - Equations for change in population (x dot) change in nutrition (n dot) and y (system output)

The equations given in figure 3 are used to model the behavior of the bacteria and the nutrition given to them in a single well of a plate used in our turbidostat. The equations

$\dot{x}$ and $\dot{n}$ are functions of x , n , and u ($\dot{x}=f(x,n,u)$ and $\dot{n}=g(x,n,u)$) and are used to

measure the change in the population and nutrition, respectively. The following are all constants in these equations that are decided by the individual environment that is being worked in and the type of bacteria that is being worked with: v , k , γ , and n_0 . The constants are described as following:

- v is used for the maximum growth rate and has the units of generations per hour
- k is the half saturation constant which designates half of the maximum population that can be achieved and is measured in grams per liter
- γ is the nutrient mass used per bacteria mass grown and this is unitless because it's a ratio
- n_0 is the nutrient concentration in fresh media and is measured in grams per liter.

The states x and n represent the amount of bacteria and amount of nutrition in a well and are both measured in grams per liter. The variable u is used to signify the amount of nutrition that is put into the system and is measure in grams per liter. All 24 wells will behave in this same fashion and can be modeled with these equations.

2.3 SIMULATIONS

To simulate our model, we used Simulink in Matlab to model our equations and then ran these setups with various inputs to u . Figure 4 shows our model simulation in Simulink.

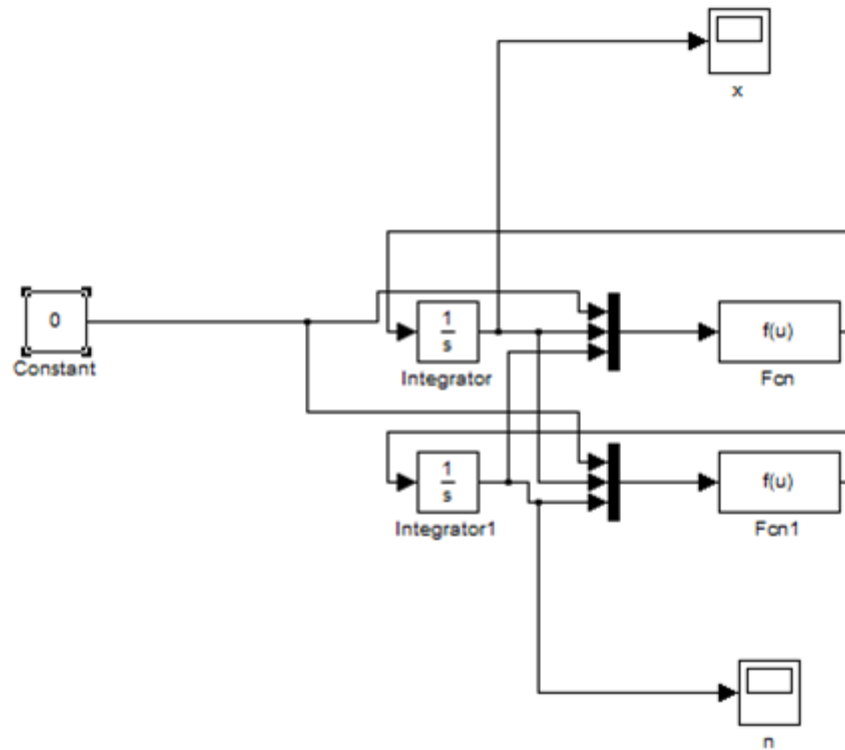


Figure 4 - Our System modeled in Simulink

The box on the left that is labeled “constant” is our u value, the box labeled “Fcn” on the right is our $\dot{x}$, and the box labeled as “Fcn1” is our $\dot{n}$. We used oscilloscope blocks to monitor the outputs of x and n . In order to get the values of x and n , we used an integrator block on our $\dot{x}$ and $\dot{n}$ blocks. The values x , n , and u were all then, in-turn, sent to our $\dot{x}$ and $\dot{n}$ blocks due to the fact that these blocks are functions of x , n , and u . We decided to set the constants to the following values after speaking with the graduate students that are coordinating with us on this project:

$v = 2$ generations per hour $k = 1$ g/L $\gamma = 0.5$ $n_0 = 1$ $x_0 = 0.5$

Where n_0 and x_0 are used for the initial values for the integrators (and n_0 was also used in the equation for $\dot{n}$).

We ran our simulations with u set to 0, 0.5 and 2. The graphs that result have y-axes that correspond to grams per liter and the x-axes that correspond to hours of the simulation. These simulations are shown as follows:

Simulation with u equal to 0:

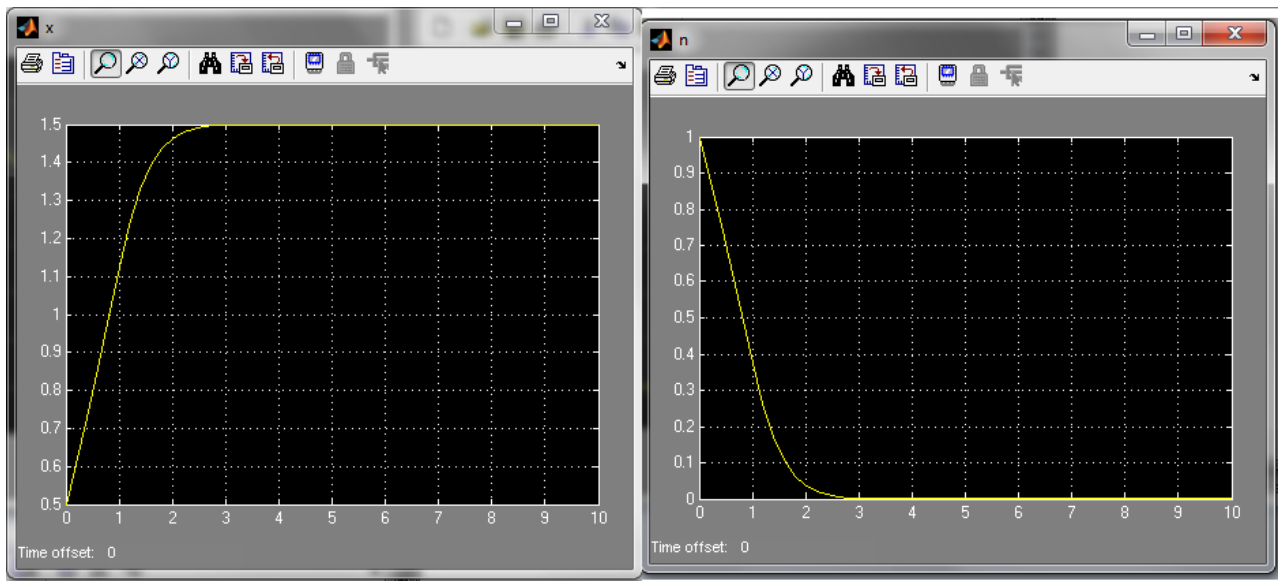


Figure 5 - Simulink Simulation with u set to 0

As you can see in the simulation, because u is set to 0, no new nutrients are being added to the system and the bacteria grows until saturation while the nutrients slope down to zero. This is because as the excess volume is being taken out of the well, no new nutrients are added and eventually all of the nutrients are taken out while all that is left is bacteria.

Simulation with u equal to 0.5:

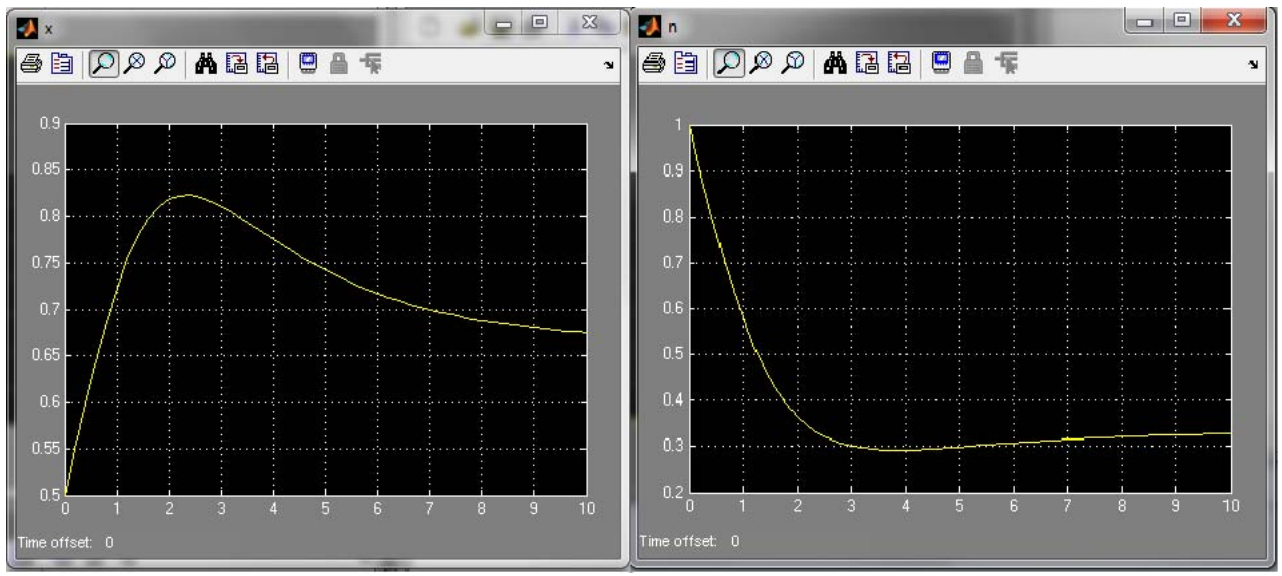


Figure 6 - Simulink Simulation with u set to 0.5

As you can see in this simulation, 0.5 grams per liter of nutrients are added periodically, so when excess volume is being sucked out, nutrients are still being added and the population of the bacteria is then limited and cannot grow to its saturation. The bacteria and the nutrients then level out of values of around 0.675 g/L and 0.33 g/L.

Simulation with u equal to 2:

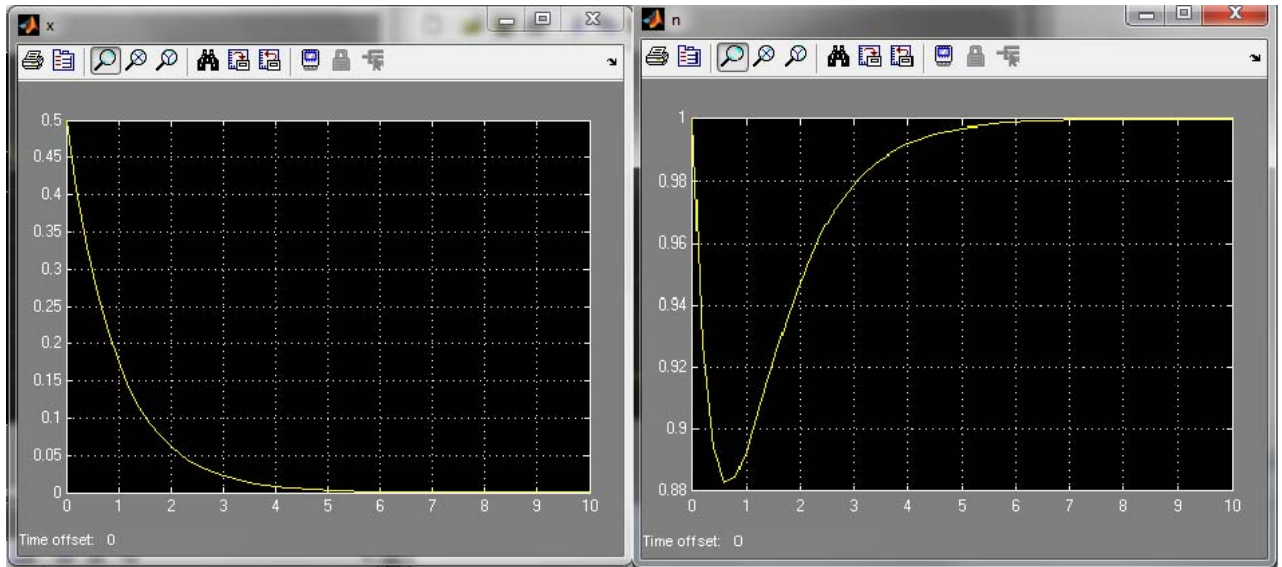


Figure 7 - Simulink Simulation with u set to 2

In this simulation, the nutrient level is too high and when excess volume is taken out, too many nutrients are being added to allow the bacteria to grow. Eventually, all of the bacteria are flushed out and all that is left is nutrients in the solution.

3. CONTROLLABLE OR OBSERVABLE

Our system is observable since we are able to observe through the outputs measurements the initial states of the system. Parameter v , the maximum growth rate, illustrates observability since finding its initial state requires experimental data or observing how long it takes for bacteria growth to reach its maxima. The system also includes controllability since system states can be changed by changing the inputs.

4. MODEL SPECIFICS

The model seems to be running as expected in the simulations. We expected to have the population grow to its saturation point when u was equal to zero, to have the population even out with a small value of u , and to have the population fall to 0 with large values of u . We still have not tested our system with the given parameters to see how our simulations compare, but this is something we anticipate doing in the near future. We

would like to have our controller be fairly accurate to have our model be within 0.1-0.2 g/L of the actual system.

5. CONCLUSION

By using the equations found in The Controlled Evolution Project we were able to model and simulate the bacterial growth and the nutrient concentration of our system. These simulations behaved as we would expect given the initial conditions and constants that we defined. Additionally, we found that our system is controllable and observable.

The analysis and modeling of our system in this milestone gave us a better understanding of how the bacteria that we are working with will behave and how adding nutrients will affect the population. We feel like we now have established a foundation for creating our controller that will help us keep a constant population in our turbidostat.

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