Tryptophan Synthesis in Various Organisms

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Abstract - The tryptophan biosystem is a prime example of the body's ability to adapt and regulate resources. In E. Coli, the tryptophan synthesis system is controlled by a negative feedback loop to conserve tryptophan. This system operates on control put in place biologically, where a high concentration of tryptophan will prevent the synthesis of tryptophan. Using differential equations, Jacobian, and the Laplace transform to describe this system, a linearized equation was able to derive the transfer function to prove a stable system. Other equations are the control mechanisms of the system and modeled in Simulink to show its effects.

Keywords - Tryptophan, Laplace transform, Transfer function, Simulink, E.coli

I. Introduction:

The alpha-amino acid Tryptophan is essential for humans for neurotransmitters, hormones, and vitamins. It is used in biosynthesis of proteins. There is a disorder known as fructose malabsorption in which tryptophan concentration levels in the blood are significantly low. Low levels of tryptophan can result in depression due to the fact that tryptophan is responsible for the synthesis of neurotransmitters such as serotonin and melatonin, as well as vitamin B3, which is responsible for energy metabolism and DNA production.

For certain plants and bacteria, there exists a negative feedback loop in which high levels of tryptophan alter the transcription of DNA that results in the production of tryptophan. In these organisms, the production of tryptophan relies heavily on the concentrations of anthranilate synthase and the mRNA responsible for encoding this synthase. Our closed-loop system model will be based on these material balances in order to regulate the rate of production of tryptophan.

Tryptophan is an alpha-amino acid that acts as the precursor to other substances such as hormones, vitamins, or neurotransmitters like serotonin or melatonin. The relevant components to the control system include the enzyme Anthranilate Synthase, mRNA, an aporepressor molecule R, e.coli, and free operators O.

The metabolic mechanism is a negative control system in which Tryptophan molecules bind to an aporepressor molecule R, which then bonds to a free operator O. This unit becomes the trp operon, and begins the encoding process in the absence of a repressor.

The presence or absence of a repressor depends on the concentration of active tryptophan in the system, and assumes there is some initial amount of tryptophan in the system, along with a couple of other assumptions.

II. Assumptions

In order to be able to study the system that is about to be presented, there are several assumptions that are made beforehand.

One assumption that must be made regarding tryptophan is that it's not present anywhere else in the system other than the cell in question. The system's integrity only holds if there are no extracellular tryptophan present.

Another assumption that can be made is that transcription is a linear process that varies with the mRNA concentration. The system proposed below is designed with that in consideration.

With these assumptions in mind, the simulated system is simplified enough to a point where we are able to analyze it with the proceeding equations and block diagrams.

III. The System

A. Differential Equations

The system in which tryptophan is made is dependent on transcription, translation, and tryptophan synthesis. As mentioned before, it should be noted that we disregard any extracellular tryptophan in the loops.

$$\frac{d}{dt}[O_R] = k_1[O_t]C_1[T] - k_{d1}[O_R] - \mu[O_R] \quad [1]$$

$$\frac{d}{dt}[mRNA] = k_2[O_R]C_2[T] - k_{d2}[mRNA] - \mu[mRNA] \quad [2]$$

$$\frac{d}{dt}[E] = k_3[mRNA] - \mu[E] \quad [3]$$

$$\frac{d}{dt}[T] = k_4C_3[T][E] - k_{d2}[mRNA] - \mu[mRNA] \quad [4]$$

B. Controller Equations

$$C_1(T) = \frac{K_{i,1}^{\eta H}}{K_{i,1}^{\eta H} + T^{\eta H}} \quad [5]$$

$$C_2(T) = \frac{K_{i,2}^{1.72}}{K_{i,2}^{1.72} + T^{1.72}} \quad [6]$$

$$C_3(T) = \frac{K_{i,3}^{1.2}}{K_{i,3}^{1.2} + T^{1.2}} \quad [7]$$

$C_1(T)$,$C_2(T)$, and $C_3(T)$ are the controllers to each process. $C_1(T)$ is the regulation on the free operator during the transcription process, $C_2(T)$ is the attenuation on the mRNA transcription process, $C_3(T)$ is the inhibition on the Tryptophan synthesis.

C. Parameters

The constants for the equations are as follows:

	Parameter	Value
Specific growth rate of E. Coli	μ	0.01 min^{-1}
Kinetic Constant	g	25 $\mu M\ min^{-1}$
Half-saturation constant for uptake of tryp. for protein synthesis	K_g	0.2 μM
Half-saturation constants	$K_{i,1}$	3.35 μM
	$K_{i,2}$	0.04 μM
	$K_{i,3}$	810 μM
Sensitivity of genetic regulation	η_H	1.92
Kinetic rate constants for synthesis of free operator, mRNA transcription, translation, and tryptophan	k_1	50 min^{-1}
	k_2	15 min^{-1}
	k_3	90 min^{-1}
	k_4	59 min^{-1}
Total concentration of free operator	O_t	3.32 nM
Degradation rate constants of free operator	k_{d1}	0.5 min^{-1}
	k_{d2}	15 min^{-1}

D. Linearization of Nonlinear Equations

In the system, equation [4] is nonlinear and needs to be linearized. The equation itself describes the change in the concentration of tryptophan using the concentration of the enzyme anthranilate synthase(the precursor to Tryptophan).

This is done by finding the Jacobian first through calculating the partial derivatives of dT with respect to T and E. Then, the equation is linearized at a zero steady state, meaning both T and E are equal to zero. Finally, we multiply by the signals V-tilde and E-tilde to retrieve the linearized equation.

Thus, the linearized equation takes the form:

$$\frac{d}{dt}\tilde{T} \approx -125\tilde{T} + 59\tilde{E} \quad [8]$$

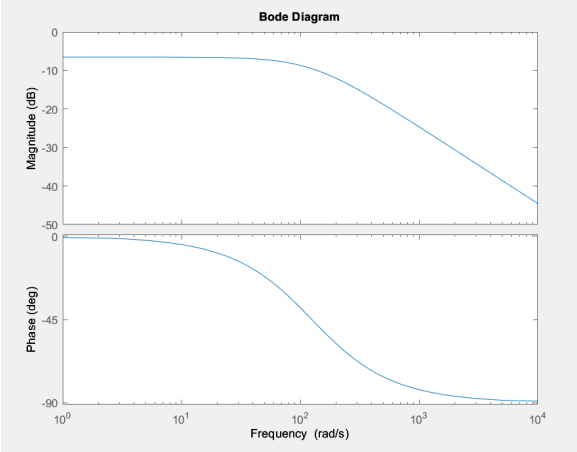
E. Laplace Transform and Transfer Function

To find the stability of the system, the Laplace transform of the linearized equation, [8], must be taken. This will allow for finding the output over the input in our case $\frac{T(s)}{E(s)}$. It is recognized that the fourth equation has T(s) and E(s). Taking the Laplace transform of the linearized fourth differential equation, we can derive the transfer function:

$$\frac{\tilde{T}(s)}{\tilde{E}(s)} = \frac{59}{s+125} \quad [9]$$

Fortunately, the transfer function has one negative real pole at 125, which means the system is stable.

F. Bode Plot



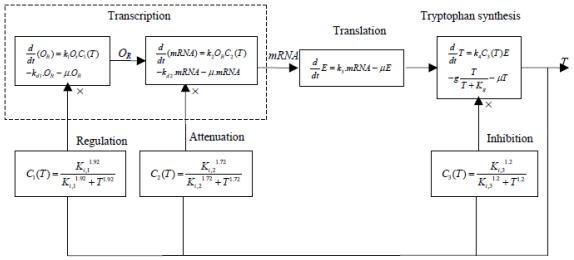
(Fig. 1 Bode Diagram)

From the transfer function, the bode plot can be found. To provide more insurance that the system is stable, the phase margin can also be checked. From the phase plot, it can be seen that the phase never reaches ±180°. The bode plot proves that the system is stable.

IV. Block Diagrams

A. Logic Flow

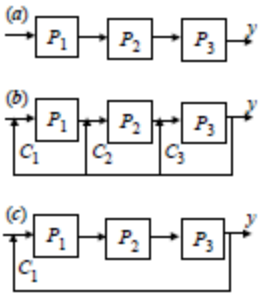
The following is the block diagram of the model used in the simulation of distributed feedback structures in a tryptophan system:



(Fig. 2 Flow of Design [1])

The concentration of tryptophan (T) is independently distributed to the three-processes-in-series structure, which are Transcription, Translation, and Tryptophan synthesis, respectively. The constants stated on the diagram are explained above with a list.

The following is the three strategies for the processes involved:

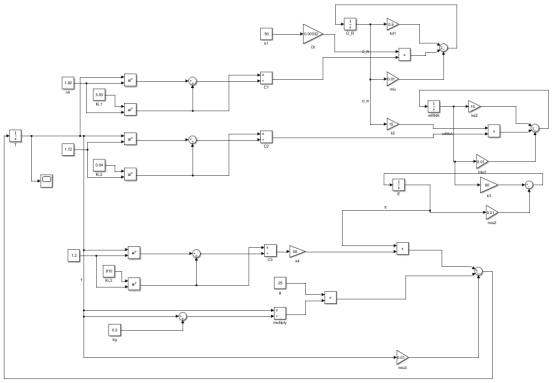


(Fig. 3 (a) Schematic of three processes (Pi) in series. (b) Schematic of the multiple feedback loop strategy for a three-process-in-series system. (c) Schematic of the single feedback loop strategy [2].)

B. Simulink Models

The project seeks to showcase the ability of the negative feedback loops which are genetic repression, mRNA attenuation, and enzyme inhibition, to still provide homeostasis of Tryptophan concentration despite variations present in the environment.
One way to measure the performance of the control system is by rise and settling time. A fast rise time would indicate a quick adapting system while a fast settling time would mean a large overshoot.

The block diagram looks like the following:



(Fig.4 Block Diagram)

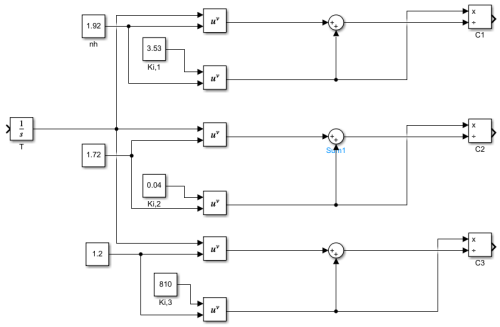
Figure 4 is the block diagram produced by the differential equation system. The process of the whole system:

1. Transcription: including the transcription of mRNA denoted as mRNA, and the transcription of free operators denoted as OR.
2. Translation: the translation process includes the concentration of the enzyme anthranilate synthase, which is an enzyme important to Tryptophan production, denoted as [E].
3. Tryptophan synthesis: denoted by T.

The other parameters has its own meaning:

1. k1, k2, k3, k4 are the kinetic rate constants for the synthesis of the free operator, mRNA, transcription, translation, and Tryptophan synthesis.
2. O_t is the total operator site concentrations
3. μ is the specific growth rate of E.coli.
4. k_{d1} and k_{d2} are the degradation rate of the free operator.
5. K_g is the half saturation constant
6. g is the kinetic constant for the uptake of tryptophan for protein synthesis in the cell.

The controllers $C_1(T)$,$C_2(T)$, and $C_3(T)$ are modeled as such:



(Fig.5 Design of the Controller)

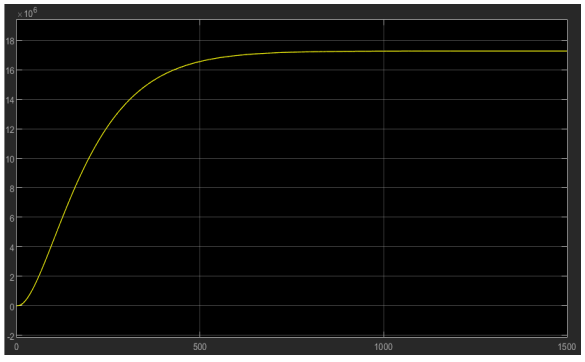
C1, C2, and C3 are modeled by the Michaelis-Menten kinetics equation which is an equation modeling the enzyme activity. The equation is shown as:

$$v = \frac{\mathrm{d}[\mathrm{P}]}{\mathrm{d}t} = V_{\max}\frac{[\mathrm{S}]}{K_{\mathrm{M}} + [\mathrm{S}]}$$

The constants $K_{i,1}$, $K_{i,2}$, $K_{i,3}$ represent the half-saturation constants.

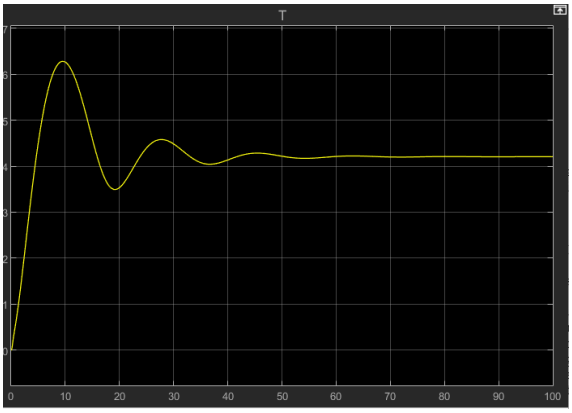
V. Results

A. Simulation Scenarios and Outcomes



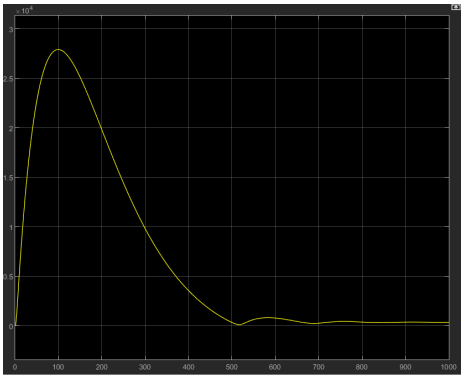
(Fig. 6 No Controllers simulation result)

Following **Figure 3(a)**, **Figure 6** shows the simulation results that no controller is controlling the system. It reaches steady state at about $1.73*10^7$ μM with a rising time of about 395 minutes. We deduce that this steady state value is the maximum tolerance of tryptophan within a single cell. The steady state concentration of Trp is also limited by other parameters in the model including k_s, k_{ds}, etc. Generally, we do not expect this huge amount of Trp production present in the body which may cause problems in hormone and neurotransmitter synthesis.



(Fig. 7 Three controllers simulation result)

Following **Figure 3(b)**, **Figure 7** shows that as three controllers C_1, C_2, and C_3 are working at the same time: regulating, attenuating, and inhibiting the processes of tryptophan synthesis, this system is stable and reaches its steady state at about 4.2μM at about 50 minutes starting from simulating. The rising time of this system is at about 6.8 minutes, and the settling time is at about 32 minutes. Besides, a small overshoot is detected. We propose that this fast response modeling system is likely to be the best simulation of the real synthesis of Tryptophan as it helps the amount of tryptophan to keep at a healthy value.



(Fig. 8 One Controller (regulation) simulation result)

Following **Figure 3(c)**, **Figure 8** shows the result of only controller C_1 is working, which means there is only the regulation control on the transcription of the free operator. It shows a very large overshoot at the beginning and decayed to its steady state concentration at about 0 μM. The rising time is about 62 minutes and the settling time is about 750 minutes.

B. Errors Associated with Results

A source of potential error inherent to our simulation is the margin of error that comes with the initial figures and constants we pulled from the literature to begin with.

Another source of error could be from the infinitesimal amount of error associated with the discretization of the system in Matlab, as it would be pseudo-continuous.

These sources of error become relevant as the organism's average tryptophan levels become lower and lower since the measured error from both of the aforementioned sources would be of a constant magnitude, which would become relatively larger as the magnitude of the organism's tryptophan control system decreases.

V. Conclusion

A. Simulation Results and Physiological Observation

The simulations make sense due to the way this biosystem works. It tightly regulates tryptophan, meaning it has to have quick control over it. Equations (5), (6), and (7) act as the controls. Using these three control parameters, the simulation in **Figure 7** has the fastest rise time and a quick settling time of 6.8 minutes and 32 minutes respectively. The biosystem has adapted to have its own control that ensures rapid regulation with a small overshoot.

In **Figure 6**, there are no controllers present. Although the system is stable, the rise time is extremely long. Furthermore, it settles at a large concentration of tryptophan that isn't considered healthy. This proves why the control equations are needed.

In **Figure 8**, only the regulation controller is present. The system is stable, but the longer rising and settling times as compared to **Figure 7** should be noted. This proves that the 3 controllers in the biosystem are necessary for healthy regulation of tryptophan.

B. Clinical Applications of the System

Two clinical syndromes that can apply to our system include Hypertryptophanemia and Hartnup disease in which the gut's E. Coli produces tryptophan, but the body is unable to metabolize amino acids. As a result, there is an excess of tryptophan that begins to accumulate, causing the body's lack of many important proteins or hormones. Here, the high concentration of tryptophan controls its own synthesis through the negative feedback loop, and the E. Coli will eventually cease the production of tryptophan as needed.

For this system, using a simulation is beneficial in that it saves money because it doesn't require in vivo testing. It allows for tighter control over the system by having the ability to change the C1, C2, and C3 controllers at any time by altering the concentration of Tryptophan. A few disadvantages entails the lack of experimental data and the overall lack of control over any unforeseen external factors.

By describing the tryptophan biosystem of E. Coli in the human gut, the control systems inside the human body can be better understood. The human body has multiple negative feedback loops such as those regulating temperature, blood pressure, and glucose levels. With biosystem regulation understood, pathologies can be treated with a working knowledge of how to control the affected system.

VI. References

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