CHEME 7770:Advanced Principles of Biomolecular Engineering

Prelim1

Due by 1:25PM, May 12, 2020

Submitted by Suthara Ramachandran (sr2264)

1.a Sample Volume (V): 1mL

Avogadro number: $6.022 * 10^{23}$

Number of cells per mL $< N_c >$: $1 * 10^8$

Average mass per E.coli cell $< m_c >$: $2.8 * 10^{-13}$ (Bionumbers ID: 103905)

In the figure below, the mols of mRNA per cell is obtained by dividing it by the avogadro number.

$$\text{mols of mRNA per cell} = \frac{\text{mRNA per cell}}{\text{Avogadro number}}$$

$$\text{mols of mRNA/gDW cells} = \frac{\text{mols of mRNA per cell}_{*N_c*V}}{<m_c>}$$

Note: Please refer sheet 1a of excel file Problem1a1c1d.xlsx

IPTG (mM)	<n> (mRNA/cell)	low (mRNA/cell)	high (mRNA/cell)	<nmol> (in mols of mRNA/cell)	<nmol>*Nc*V/<mc>
0	19	18	20	3.15502E-23	0.011267944
0.0005	21	17	26	3.48713E-23	0.012454043
0.005	41	37	44	6.80821E-23	0.024315036
0.012	67	65	69	1.11256E-22	0.039734328
0.053	86	84	88	1.42806E-22	0.051002271
0.216	93	91	95	1.5443E-22	0.055153619
1	93	92	94	1.5443E-22	0.055153619

1.b mRNA balance developed in class in given by the equation

$$\dot{m} = r_{x,i}\bar{u}_i - (\mu - \theta_{m,i})m_i \tag{1}$$

At pseudo steady state, $\dot{m} = 0$

$$\implies r_x \bar{u} = (\mu - \theta_m) m^* \tag{2}$$

Rearranging the above equation, we get

$$m^* = \frac{r_x \bar{u}}{(\mu - \theta_m)} \tag{3}$$

Which is of the form,

$$m^* = K_X(G, \theta)\bar{u}(I, \kappa)$$

where $K_x(G, \theta) = r_X$ and $\bar{u}(I, k) = \bar{u}$
From the notes, the values for r_X and u are obtained as follows

$$r_X = k_E^X R_X \left(\frac{G}{\tau_X K_X + (\tau_X + 1)G} \right) \tag{4}$$

$$\bar{u}(I) = \frac{W_1 + W_2 f_I}{1 + W_1 + W_2 f_I} \tag{5}$$

where,
k_E^X: Elongation rate constant
R_X: Free RNAP concentration
G: Lac Z Gene Concentration
τ_x: Time constant for lac Z gene
K_X: Saturation constant for lac Z gene
$f_I = \frac{I^n}{K^n + I^n}$
I: Inducer concentration

Putting (4) and (5) in (3), we get,

$$m^* = \frac{k_E^X R_{X,T}}{(\mu - \theta_m)} \left(\frac{G}{\tau_X K_X + (\tau_X + 1)G} \right) \left(\frac{W_1 + W_2 f_I}{1 + W_1 + W_2 f_I} \right) \tag{6}$$

1.c **Note**: Please refer sheet 1b of excel file Problem1a1c1d.xlsx for calculations

The different variables obtained from various sources is listed below:

(1) **Doubling time τ_d: 0.667** (Given as 40 mins in problem statement)

(2) **mRNA Half life: 0.0833 hr** (Given as 5 mins in problem statement)

(3) **Elongation rate of RNA polymerase e_X: 90 nt/s** (Obtained from BioNumbers ID: 109084)

(4) **K_X: 0.0136 mM** (Obtained from PS2 solutions)

(5) **Characteristic length (L): 1000 nt** (Given in problem statement)

(6) **Average Gene Length: 924 nt** (obtained from Bionumbers ID: 111922 for prokaryotes)

(7) **Initiation rate constant (K_I): 0.041** (Obtained from McClure paper)

(8) **RNAP in cell $R_{X,T}$: 1500** (Obtained from BioNumbers ID: 101440)

(9) **Average mass per cell $< m_c >$:** $2.8 * 10^{-13}$ **gDW/cell** (Obtained from BioNumbers ID: 103905)

(10) **G:** $\frac{2}{<m_c>*\textbf{avogadro number}*10^6} = 1.18652 * 10^{-5}$ **mols/gDW** (Given 2 copies per cell in problem statement)

(11) n = 1.5 (Obtained from Class notes)

(12) K = 0.3 (Obtained from Class notes)

(13) W_1=0.26 (Obtained from Class notes)

(14) W_2=300 (Obtained from Class notes)

Calculations

$\mu = \frac{log(2)}{\tau_d} = 1.039720771\ hr^{-1}$

$\theta_m = \frac{log(2)}{\text{mRNA half life}} = 8.317766167\ hr^{-1}$

Characteristic elongation rate constant $< k_E^X >= e_X L^{-1} = 0.09\ s^{-1}$

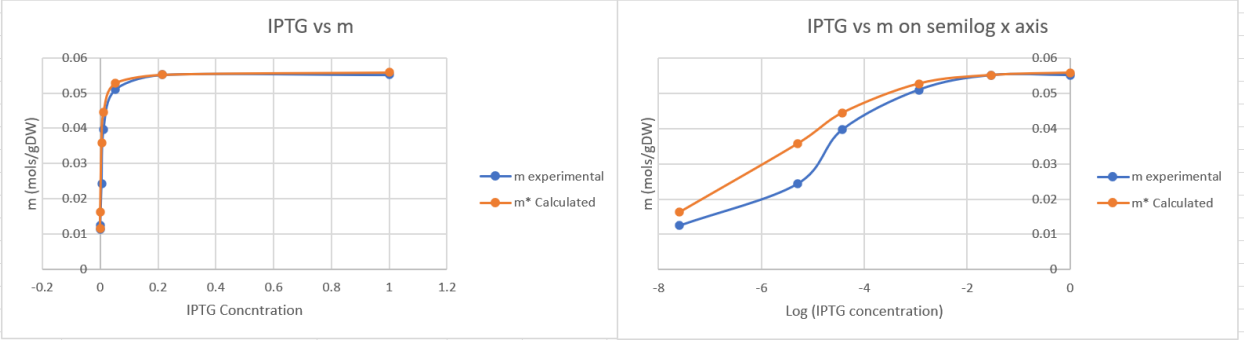
$k_E^X =< k_E^X > * \frac{L}{\text{Average Gene Length}} * 3600 = 350.6493506\ hr^{-1}$

$\tau_X = \frac{k_E^X}{k_I} = 8552.423187$

The values of m^* was calculated using Eqn (6) from 1b.

IPTG (mM)	m* experimental	m* calculated
0	0.011267944	0.011566497
0.0005	0.012454043	0.016299106
0.005	0.024315036	0.035743957
0.012	0.039734328	0.044519479
0.053	0.051002271	0.05278653
0.216	0.055153619	0.055204506
1	0.055153619	0.055866961

1.d Using the above data, the lacZ concentration as a function of IPTG is shown.



2.a Dynamic system of equations for the network diagram shown in Figure 1B that includes the action of signal, S is as follows:

$$\frac{d\tilde{X}}{d\tilde{t}} = \frac{\tilde{\alpha_X} + \tilde{\beta_X} S}{1 + S + (\tilde{Z}/\tilde{Z_X})^{n_{ZX}}} - \tilde{\delta_X}\tilde{X} \tag{1}$$

$$\frac{d\tilde{Z}}{d\tilde{t}} = \frac{\tilde{\alpha_Z}}{1 + (\tilde{X}/\tilde{X_Z})^{n_{XZ}}} - \tilde{\delta_Z}\tilde{Z} \tag{2}$$

2.b The variables for non dimentionalizing the above equations are,

$\delta_X = \frac{\tilde{\delta_X}}{\tilde{\delta_X}}$ and $\delta_Z = \frac{\tilde{\delta_Z}}{\tilde{\delta_X}}$

$\alpha_X = \frac{\tilde{\alpha_X}}{\tilde{\alpha_Z}}$ and $\alpha_Z = \frac{\tilde{\alpha_Z}}{\tilde{\alpha_Z}}$

$\beta_X = \frac{\tilde{\beta_X}}{\tilde{\alpha_Z}}$ and $\beta_Z = \frac{\tilde{\beta_Z}}{\tilde{\alpha_Z}}$

$Z = \frac{\tilde{Z}\tilde{\delta_X}}{\tilde{\alpha_Z}}$ and $X = \frac{\tilde{X}\tilde{\delta_X}}{\tilde{\alpha_Z}}$

$Z_X = \frac{\tilde{Z_X}\tilde{\delta_X}}{\tilde{\alpha_Z}}$ and $X_Z = \frac{\tilde{X_Z}\tilde{\delta_X}}{\tilde{\alpha_Z}}$

Nondimentionalization of Eqn (1) and Eqn(2) is done by inserting these non dimentional variables.

$$\frac{d\tilde{X}}{d\tilde{t}} = \frac{\tilde{\alpha_X} + \tilde{\beta_X} S}{1 + S + (\tilde{Z}/\tilde{Z_X})^{n_{ZX}}} - \tilde{\delta_X}\tilde{X}$$

becomes,

$$\frac{\cancel{\tilde{\alpha_Z}}\cancel{\tilde{\delta_X}}}{\cancel{\tilde{\delta_X}}}\frac{dX}{dt} = \frac{\cancel{\tilde{\alpha_Z}}\alpha_X + \cancel{\tilde{\alpha_Z}}\beta_X S}{1 + S + (\frac{\cancel{\tilde{\alpha_Z}}Z}{\cancel{\tilde{\delta_x}}}/\frac{\cancel{\tilde{\alpha_z}}X_Z}{\cancel{\tilde{\delta_x}}})^{n_{ZX}}} - \frac{\cancel{\tilde{\alpha_Z}}\cancel{\tilde{\delta_X}}X}{\cancel{\tilde{\delta_X}}}$$

$$\frac{dX}{dt} = \frac{\alpha_X + \beta_X S}{1 + S + (Z/Z_X)^{n_{ZX}}} - X \tag{3}$$

$$\frac{d\tilde{Z}}{d\tilde{t}} = \frac{\tilde{\alpha_Z}}{1 + (\tilde{X}/\tilde{X_Z})^{n_{XZ}}}\tilde{\delta_Z}\tilde{Z}$$

becomes

$$\frac{\cancel{\tilde{\alpha_Z}}dZ}{\cancel{\tilde{\delta_x}}}\frac{\cancel{\tilde{\delta_x}}}{d\tilde{t}} = \frac{\cancel{\tilde{\alpha_Z}}}{1 + (\frac{\cancel{\tilde{\alpha_Z}}X}{\cancel{\tilde{\delta_x}}}/\frac{\cancel{\tilde{\alpha_z}}X_Z}{\cancel{\tilde{\delta_x}}})^{n_{XZ}}} - \frac{\tilde{\delta_z}\cancel{\tilde{\alpha_Z}}Z}{\tilde{\delta_x}}$$

$$\frac{dZ}{dt} = \frac{1}{1 + (X/X_Z)^{n_{XZ}}} - \delta_Z Z \tag{4}$$

2.c The values of the parameters provided are
α_x= 1.5
β_x=5.0
Z_X=0.4
n_{ZX}=2.7
X_z=1.5
n_{XZ}=2.7
δ_z= 1.0 Eqn(3) and Eqn(4) is

$$\frac{dX}{dt} = \frac{\alpha_X + \beta_X S}{1 + S + (Z/Z_X)^{n_{ZX}}} - X$$

and

$$\frac{dZ}{dt} = \frac{1}{1 + (X/X_Z)^{n_{XZ}}} - \delta_Z Z$$

At steady state, dX/dt and dZ/dt = 0
The above equations become

$$X = \frac{\alpha_X + \beta_X S}{1 + S + (Z/Z_X)^{n_{ZX}}}$$

and

$$\delta_Z Z = \frac{1}{1 + (X/X_Z)^{n_{XZ}}}$$

Inserting the parameter values

$$X = \frac{1.5 + 5S}{1 + S + (Z/0.4)^{2.7}}$$

and

$$Z = \frac{1}{1 + (X/1.5)^{2.7}}$$

2.d The three equations used to plot the graphs shown below are as follows

$$\frac{dX}{dt} = \frac{\alpha_X + \beta_X S}{1 + S + (Z/Z_X)^{n_{ZX}}} - X \tag{1}$$

$$\frac{dY}{dt} = \frac{\alpha_Y + \beta_Y S}{1 + S + (X/X_Y)^{n_{XY}}} - \delta_Y Y \tag{2}$$

$$\frac{dZ}{dt} = \frac{\alpha_Z}{1 + (X/X_Z)^{n_{XZ}} + (Y/Y_Z)^{n_{YZ}}} \delta_Z Z \tag{3}$$

The plots were made in Julia. Please refer File: Problem2d.jl
Note: This code is generates the trends at all concentrations of S in a single graph. The plots were separated into different ones to show the trends clearly. It is essentially one and the same. Commenting out the other plots should reveal the individual plots.

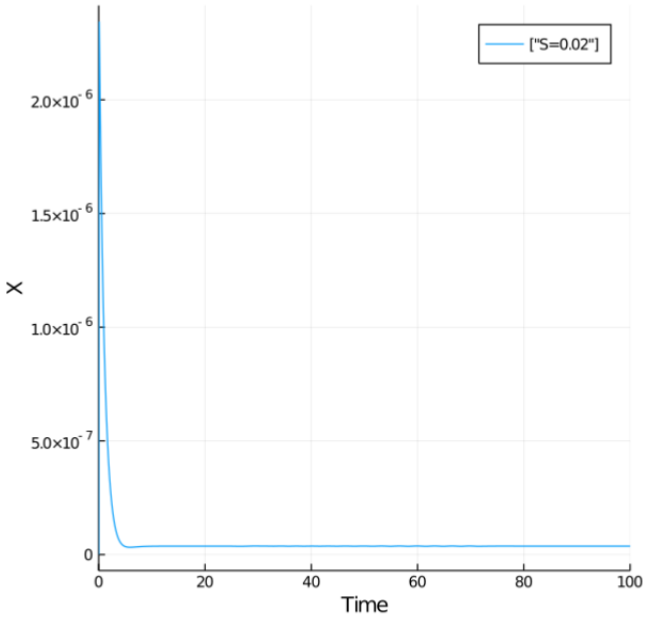


Figure 1: S=0.02

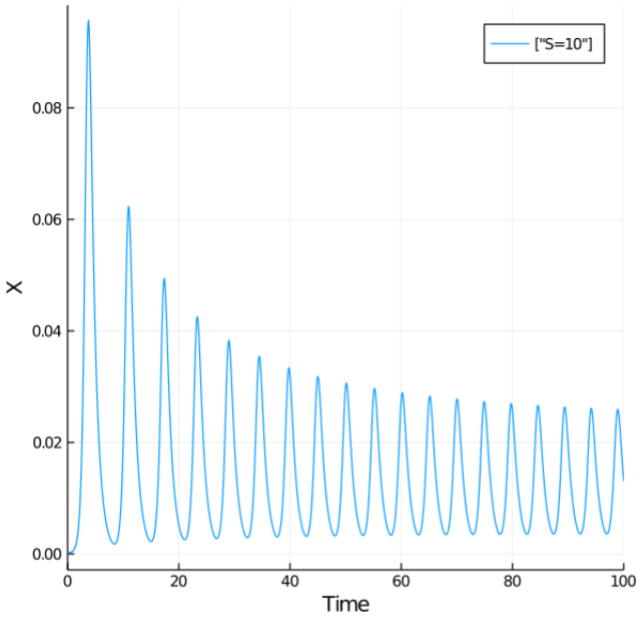


Figure 2: S=10.0

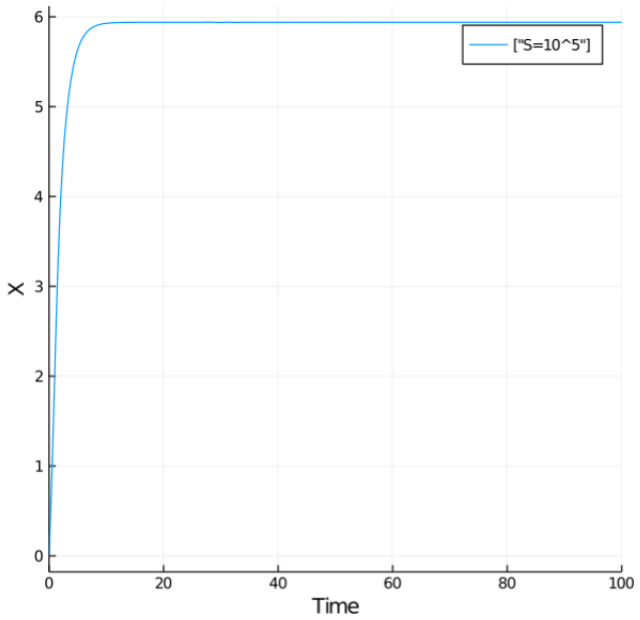


Figure 3: S=10^5

2.e The stable steady states were determined by decreasing the S value (for a point below Hopf bifurcation) and increasing the S values (for a point above Hopf bifurcation). This was done in a trial and error basis.

The stable steady states for a value of a signal below the Hopf bifurcation point was determined to be at a concentration of 0.1
Please check the Julia file named Problem2eIncoherent.jl
The steady state value of X, Y, Z was obtained from the solution as
X = 0.00013837468618962102
Y = 0.48373744984872236
Z = 0.0004969447548690912
Please check the Julia file named Incoherent.jl

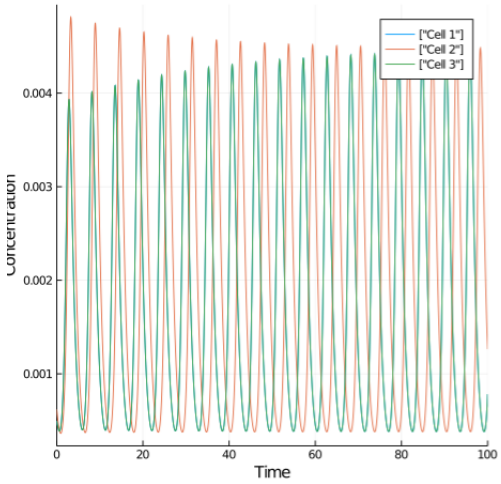


Figure 4: Incoherent Oscillations

The Oscillations are incoherent since they are out of phase.

The stable steady states for a value of a signal above the Hopf bifurcation point was determined to be at a concentration of $5 * 10^4$
Please check the Julia file named Problem2eCoherent.jl
The steady state value of X, Y, Z was obtained from the solution as
X = 5.73411473764851
Y = 0.0051471284797299
Z = 0.000420886125650721
Please check the Julia file named Coherent.jl

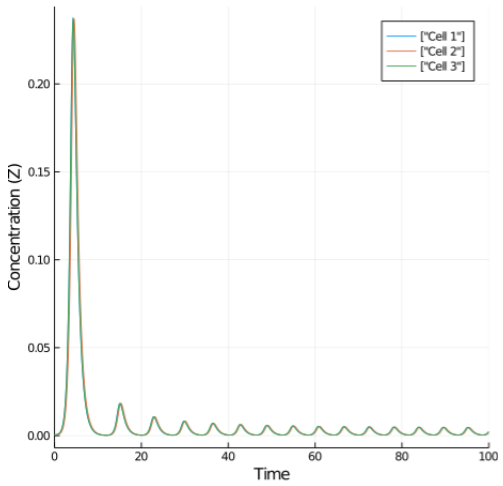


Figure 5: Coherent Oscillations

The Oscillations are coherent since they are all in phase with each other.

2.f We can believe the authors' statement that they achieved coherent oscillations when moving from higher signal S of 105 to 100. This was shown in the fig 5 wherein moving from a higher S to lower resulted in coherent oscillations.As discussed in the paper, oscillations that originate from Hopf bifurcation start the spiralling close to an unstable centre and hence small variation is initial concentrations can lead to phase difference. In contrast, when decreasing from higher to lower signal, cells passes through saddle node bifurcation instead of Hopf bifurcation. The signals tend to be coherent here since expression level are not close to oscillatory regime.