Control Theory Analysis of the antithetic integral controller

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1 What is control theory and why are we using it?

Control theory is a branch of engineering that deals with the control of dynamical systems with the aim of designing systems with good stability, that can compensate for uncertainty, reject disturbances and attenuate noise, leading to a system with a good performance. Control theory is applied to a wide range of Engineering disciplines. For example, in electrical engineering where capacitors, resistors, operational amplifiers, etc. are being utilised to create an electrical circuit that gives a desired response or in mechanical engineering where movement of systems are described with defined systems of differential equations. Despite its use in various other Engineering areas, application of control theory in synthetic biology to create genetic circuits have been difficult due to its intrinsic non-linearity and unpredictability in biological systems. Here we will bring you onto a journey of the system analysis of the antithetic integral controller with control theory and we hope you will appreciate the usefulness and limitations of applying control theory in synthetic biology.

2 Understanding the system in a control sense

The aim of the antithetic integral controller is to allow X to be expressed at the same level even in response to uncertainties in parameters, or perturbation. The antithetic integral controller contains Z1 and Z2 as the controller species. Z1 acts as an actuator where it directly controls the amount of X being produced while Z2 acts as a sensor which its production depends on the amount of X present. Together Z1 and Z2 annihilate each other at the same rate which serve as the error signal of the system.

The equations of our system is as follows:

For activation,

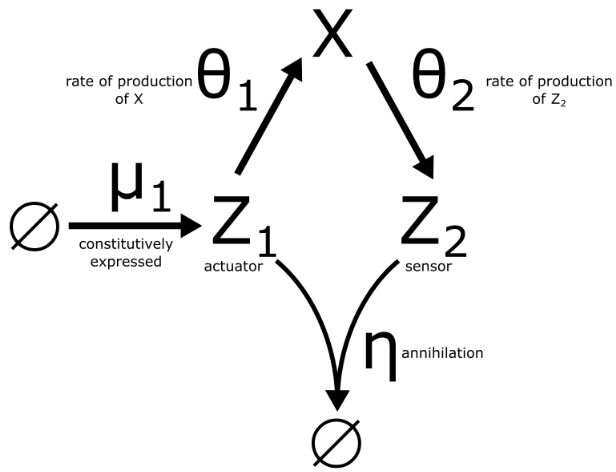

Figure 1: Chemical Reaction Network of the activation system

$$\begin{cases} \dot{x} &= \theta_1 z_1 - \gamma_p x \\ \dot{z}_1 &= \mu_1 - \eta z_1 z_2 - \gamma_c z_1 \\ \dot{z}_2 &= \theta_2 x - \eta z_1 z_2 - \gamma_c z_2 \end{cases} \tag{1}$$

For repression,

$$\begin{cases} \dot{x} &= \dfrac{\alpha}{\theta_1 z_1 + 1} - \gamma x \\ \dot{z}_1 &= \mu_1 - \eta z_1 z_2 \\ \dot{z}_2 &= \dfrac{\mu_2}{\theta_2 x + 1} - \eta z_1 z_2 \end{cases} \tag{2}$$

The following images (Figure 1 and Figure 2) shows the Chemical Reaction Network (CRN) of the activation and the repression system respectively.

In control theory, block diagrams are often utilised to represent a system. Our system can be summarised with the following block diagram (Figure 3)

What are block diagrams? (dropdown)

In control systems, the goal is to produce a controlled output Y(s) from the input U(s). The Plant/Process (P(s)) is the system we are trying to control. In order to control a system, a controller (C(s)) is designed. The transfer function in this open loop system is G(s) = C(s)P(s). Figure 4 shows the block diagram of an open loop system. The definition and the importance of transfer functions will be discussed further below.

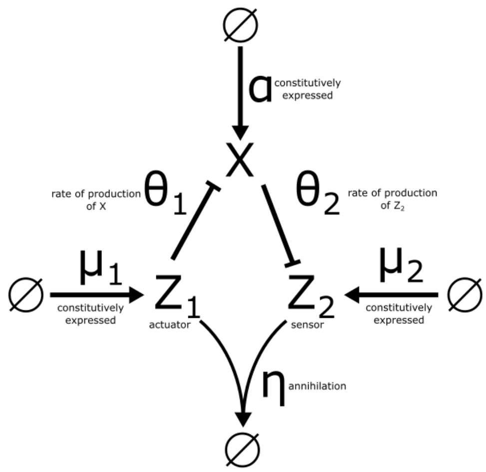

Figure 2: Chemical Reaction Network of the repression system

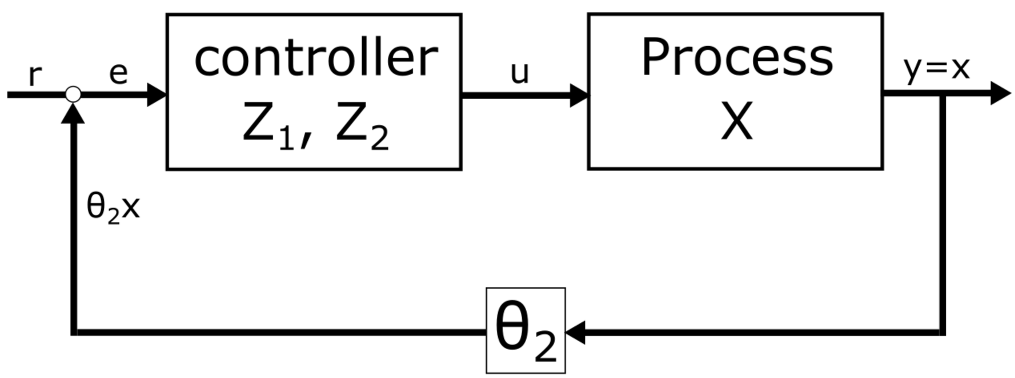

Figure 3: Block diagram of the system

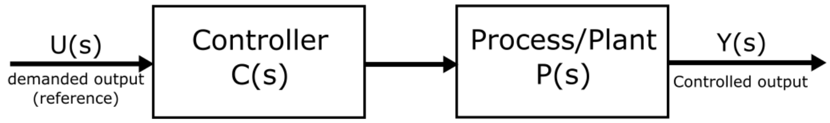

Figure 4: Block diagram of a general control system

3 How to set the system up for control theory analysis?

In this section, we will explain the reasoning behind the steps we take to get closer to analysing the system with the control theory toolbox, as well as the maths on how to do it with our system.

3.1 Equilibrium

Equilibrium point of an ODE is the value which the ODE converges to. In a simple system, the equations can be easily solved analytically. However, in more complicated systems, numerical methods have to be used to solve the equation as there may not be a closed form solution. The equilibrium position is especially important for non-linear systems as linearisation about the equilibrium position is necessary for the analysis of the stability of the control system.

The equilibrium position of x, z_1 and z_2 with different γ_c values is being plotted in the range of $\gamma_c = 0$ to $\gamma_c = 0.1$ for both activation (Figure 5) and repression (Figure 6). When $\gamma_c = 0$, there is an analytical solution for the equilibrium positions which can be easily obtained.

For activation,

$$x_{eq} = \frac{\mu_1}{\theta_2}, z_{1eq} = \frac{\mu\gamma_p}{\theta_1\theta_2}, z_{2eq} = \frac{-\gamma_c^2 + \theta_1\theta_2}{\eta\gamma_p} \tag{3}$$

For repression,

$$x_{eq} = \frac{\mu_2 - \mu_1}{\mu_1\theta_2}, z_{1eq} = \frac{\alpha\mu_1\theta_2 - \gamma\left(\mu_2 - \mu_1\right)}{\gamma\theta_1\left(\mu_2 - \mu_1\right)}, z_{2eq} = \frac{\gamma\theta_1\left(\mu_1\mu_2 - \mu_1^2\right)}{\alpha\eta\mu_1\theta_2 - \gamma\eta\left(\mu_2 - \mu_1\right)} \tag{4}$$

With γ_c not equal to 0 however, an analytical solution is difficult to obtain. Therefore, numerical solution is obtained through odeint in Python and how the equilibrium position changes depending on the value of γ_c is plotted. This range is chosen because $\gamma_c = 0.00038$ as calculated with the dilution rate of E. coli. It is interesting to note the equilibrium position follows a monotonic trend for both activation and repression when γ_c changes in the physical ranges of values.

The following parameter values are used when analysing the activation system: $\theta_1 = 0.005$ $\gamma_p = 0.00038$ $\eta = 0.018/60$ $\mu_1 = 0.7$ $\theta_2 = 0.0005$

The following parameter values are used when analysing the repression system: $\alpha = 10/60$ $\theta_1 = 0.05$ $\gamma_p = 0.00038$ $\eta = 0.018/60$ $\mu_2 = 1.5$ $\mu_1 = 0.7$ $\theta_2 = 0.005$

3.2 Linearisation and state space representation

Systems are often characterised with ODEs and these ODEs are often non-linear thus is very difficult to solve. To utilise the control systems toolbox, the system has to be linearised about its equilibrium position using a Jacobian matrix.

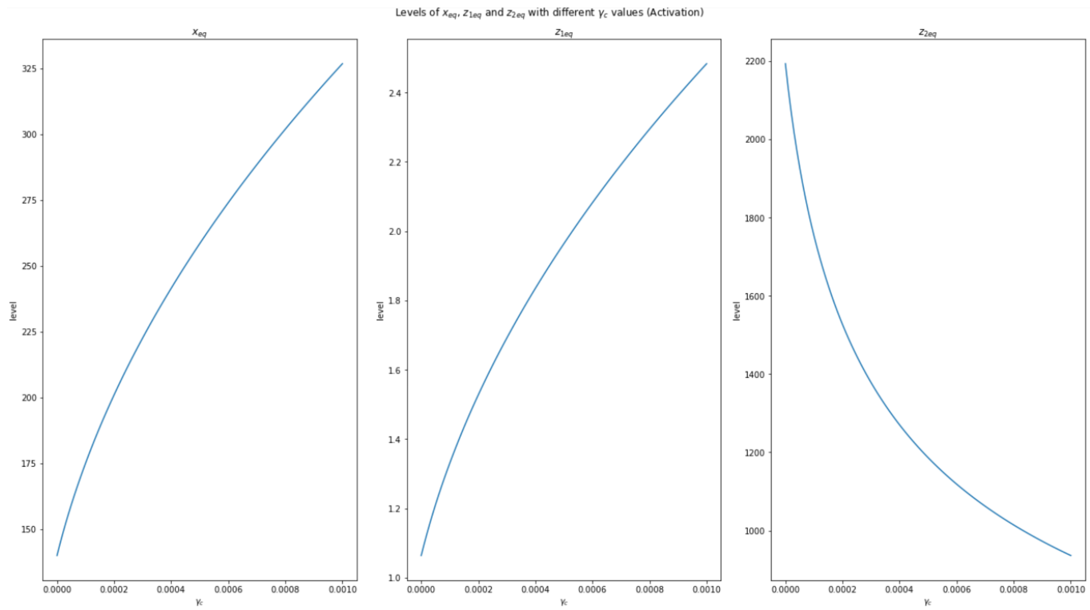

Figure 5: Equilibrium position of x, z_1 and z_2 at different γ_c for activation

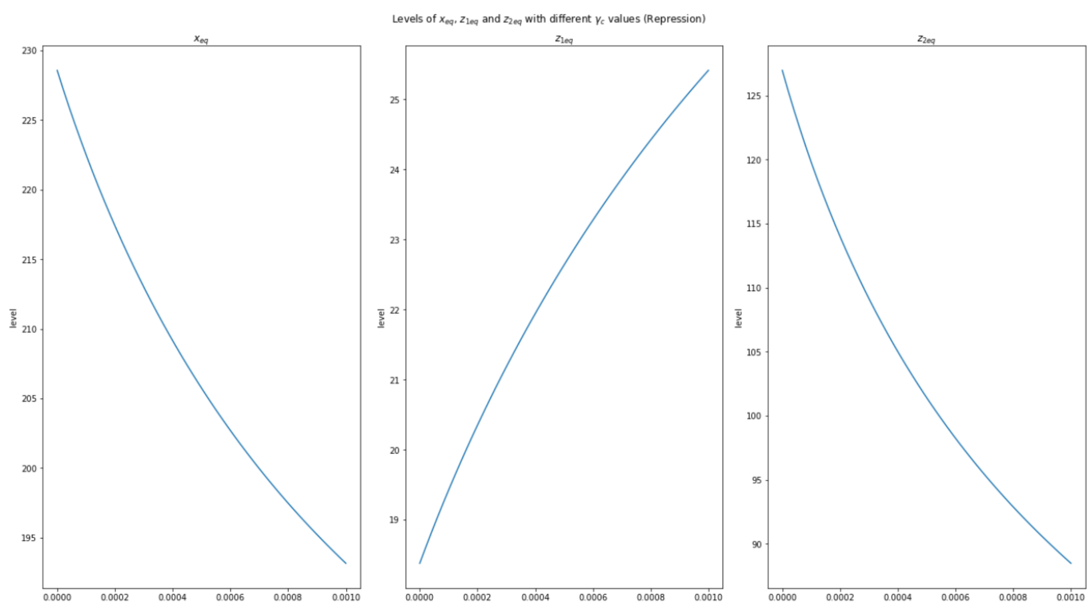

Figure 6: Equilibrium position of x, z_1 and z_2 at different γ_c for repression

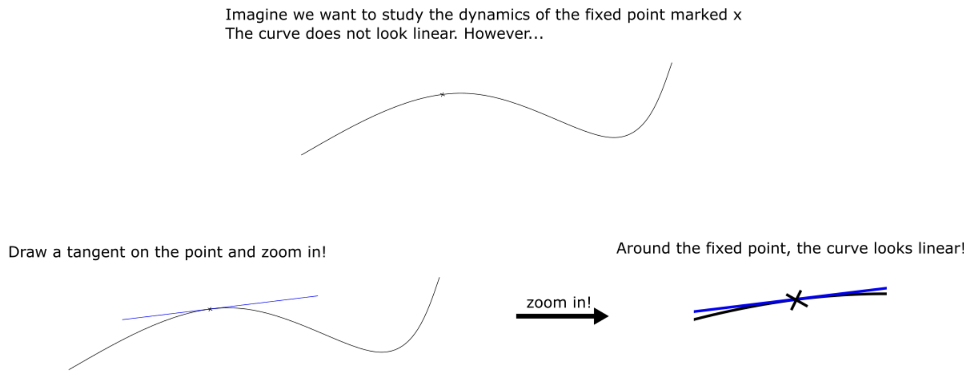

Figure 7: Schematic of how linearisation works

Linearisation is similar to zooming in into the dynamics of the system at the fixed point, assuming that just around this fixed point, the dynamics is linear. To understand how linearisation works graphically, look at Figure 7. As we can see, further from the fixed point however, linearisation of the system does not hold. Therefore, the system has to be working closed to around the fixed point for linearisation to be justified. After the ODEs are linearised, the system can be written in a state space form i.e. a sytem of linear ODEs arranged in a specific format as below.

$$\begin{cases} \dot{x} &= Ax + Bu \\ y &= Cx + Du \end{cases} \tag{5}$$

where x is the states of the system, u is the input of the system and y is the output of the system. A, B, C and D are appropriate matrix describing the system of ODEs. By taking Laplace transform, the transfer function of the system can be found.

3.2.1 What are states? (dropdown)

In a dynamical system, its memory/present output $y(t_1)$ depends not only the present input $u(t_1)$ but also on past inputs $u(t)$ where $t \leq t_1$. The states of the system x(t) are a set of variables of the system chosen to represent this memory and the choice of a state is not unique. Essentially, $x(t_0)$ summarises the effect on the future of input and states prior to $t = t_0$

There are certain methods to choose the correct states for the system. One can choose states intuitively, considering independent energy elements or energy elements of the system. For instance, in electrical circuits, the voltage across capacitors or the current in inductors are reasonable choices. In mechanics, positions and velocities of masses can often also be chosen as states. In our system, intuitively, the number of species X, Z1 and Z2 gives the memory elements of the system and thus intuitively are chosen as the state of the system.

3.2.2 Setting up the input

In order to see whether the system can robustly adapt, the input of the system is the disturbance to x. Let the disturbance be d. Here $d = 0.75\theta_1$ where θ_1 is the production rate of x for each z_1 molecule. It will not be realistic if d is set too high and the dynamics of the system will deviate to very far away from the equilibrium point, so looking at the system response and the stability of the linearised system will not be justified.

3.2.3 Activation

After linearisation about its equilibrium position,

$$\begin{bmatrix} \dot{\delta x} \\ \dot{\delta z_1} \\ \dot{\delta z_2} \end{bmatrix} = \begin{bmatrix} -\gamma_p & \theta_1 & 0 \\ 0 & -\eta z_2 - \gamma_c & -\eta z_1 \\ \theta_2 & -\eta z_2 & -\eta z_1 - \gamma_c \end{bmatrix} \begin{bmatrix} \delta x \\ \delta z_1 \\ \delta z_2 \end{bmatrix} + \begin{bmatrix} 1 \\ 0 \\ 0 \end{bmatrix} d$$

$$y = \begin{bmatrix} 1 & 0 & 0 \end{bmatrix} \begin{bmatrix} \delta x \\ \delta z_1 \\ \delta z_2 \end{bmatrix}$$

where $\text{A} = \begin{bmatrix} -\gamma_p & \theta_1 & 0 \\ 0 & -\eta z_2 - \gamma_c & -\eta z_1 \\ \theta_2 & -\eta z_2 & -\eta z_1 - \gamma_c \end{bmatrix}$ $\text{B} = \begin{bmatrix} 1 \\ 0 \\ 0 \end{bmatrix}$ $\text{C} = \begin{bmatrix} 1 & 0 & 0 \end{bmatrix}$

3.2.4 Repression

After linearisation about its equilibrium position,

$$\begin{bmatrix} \dot{\delta x} \\ \dot{\delta z_1} \\ \dot{\delta z_2} \end{bmatrix} = \begin{bmatrix} -\gamma_p & -\frac{\alpha\theta_1}{(\theta_1 z_{1eq}+1)^2} & 0 \\ 0 & -\eta z_2 - \gamma_c & -\eta z_1 \\ -\frac{\mu_2\theta_2}{(\theta_2 x_{eq}+1)^2} & -\eta z_2 & -\eta z_1 - \gamma_c \end{bmatrix} \begin{bmatrix} \delta x \\ \delta z_1 \\ \delta z_2 \end{bmatrix} + \begin{bmatrix} 1 \\ 0 \\ 0 \end{bmatrix} d$$

$$y = \begin{bmatrix} 1 & 0 & 0 \end{bmatrix} \begin{bmatrix} \delta x \\ \delta z_1 \\ \delta z_2 \end{bmatrix}$$

where

$$\text{A} = \begin{bmatrix} -\gamma_p & -\frac{\alpha\theta_1}{(\theta_1 z_{1eq}+1)^2} & 0 \\ 0 & -\eta z_2 - \gamma_c & -\eta z_1 \\ -\frac{\mu_2\theta_2}{(\theta_2 x_{eq}+1)^2} & -\eta z_2 & -\eta z_1 - \gamma_c \end{bmatrix} \text{B} = \begin{bmatrix} 1 \\ 0 \\ 0 \end{bmatrix} \text{C} = \begin{bmatrix} 1 & 0 & 0 \end{bmatrix}$$

3.3 Laplace transform (dropdown)

Systems described in ODEs can be solved and visualised in the time domain. However, while the time domain shows us its actual response, we do not have a lot of tools to analyse the system in the time domain. In the Laplace domain (s domain) however, a lot of tools from the control theory toolbox can be used to analyse the system in an simpler way. In the Laplace domain, instead of using time as the variable, s (any complex number) is being used as the variable.

Laplace transform is defined as the following:

$$L\{f(t)\} = \int_{t=0}^{\infty} f(t)e^{-st}dt \tag{6}$$

Although the Laplace transform expression looks complicated, Laplace transform of ODEs are very easy where X(s) is the Laplace transform of X.

$$L\{\dot{x}\} = sX(s) - x_0 \tag{7}$$

where X(s) is the Laplace transform of x(t) and x_0 is the initial condition of the differential.

After Laplace transform of the ODE, we obtain a transfer function that links the input of the system (U(s)) to the output of the system (Y(s)). A transfer function is the Laplace transform of the impulse response of the system. The impulse response of the system refers to how the system respond to an impulse in the time domain. Looking at the expression of transfer function and manipulating it with the control systems toolbox, one can determine its stability, amplitude and phase with inputs at different frequencies.

Let the transfer function be G(s), U(s) be the input of the Laplace domain and Y(s) be the output in the Laplace domain, U(s)G(s) = Y(s). In a single-input single-output system, Laplace transform can be taken on the single differential equation to find the transfer function.

For a multi-input multi-output system, a state space representation of a system is required. The transfer function can be obtained through state space representation through G(s) = C(sI-A)$^{-1}B + D$

4 What are the different types of systems? (dropdown)

4.1 Open loop

An open loop system is one that does not have any feedback i.e. the output will not influence the input. Let the open loop transfer function be G(s) directly linking the input (U(s)) and output (Y(s)). In principle, it is possible to choose a desired transfer function G(s) and use $C(s) = \frac{G(s)}{P(s)}$ to obtain Y(s) = G(s)U(s). In practice, this will not work because it requires an exact model of

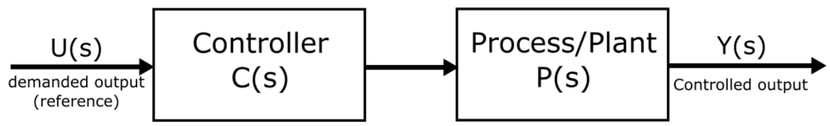

Figure 8: Block diagram of an open loop system

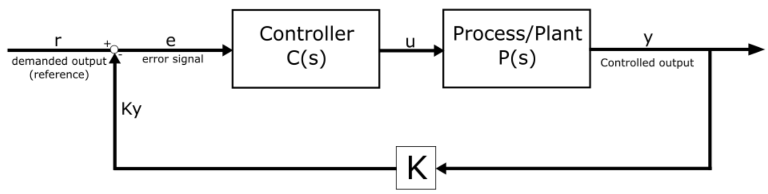

Figure 9: Block diagram of a negative feedback loop

the plant/process without any disturbances or uncertainties. Therefore, a feedback system is needed. The block diagram of an opened loop system is shown in Figure 8.

4.2 Negative feedback

Negative feedback is important in this case to deal with the effects of uncertainty. The closed loop transfer function for a negative feedback loop is $H(s) = \frac{G(s)}{1+KG(s)}$ where K is the feedback gain of the system. In this case, we define the return ratio L(s) = KG(s). According to the block diagram, negative feedback allows the output at that moment in time to be compared to the reference value and the error signal at that time is being fed back into the system. Looking at the denominator of the closed loop transfer function, we can see that the closed loop characteristic equation is 1+KG(s) = 0 thus the stability and characteristics of the closed loop system compared to the open loop system can be very different (Further discussed below). Essentially the sensitivity function is in the form of 1/(1+KG(s)) which means that a higher K leads to a lower sensitivity function value, thus rejecting disturbances. Although negative feedback allows disturbance rejection and can further reduce steady state error if the gain is very high, in biological systems, gain of a system is limited and that the steady state error can never be 0.

The block diagram of a negative feedback system is shown in Figure 9

Although negative feedback allows disturbance rejection and can further reduce steady state error if the gain is very high, in biological systems, gain of a system is limited and that the steady state error can never be 0.

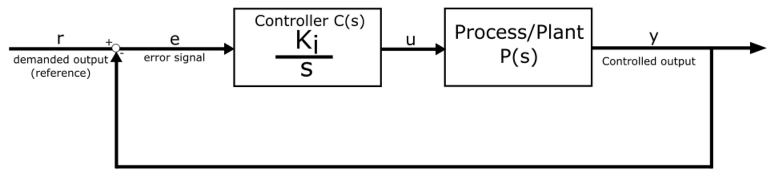

Figure 10: Block diagram of an integral controller

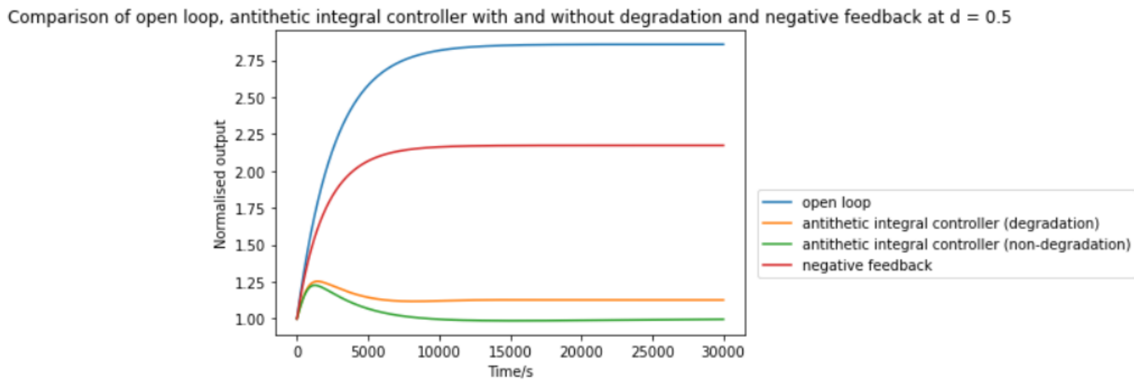

Figure 11: Simulation of the open loop, negative feedback and the antithetic integral controller (With and without degradation)

4.3 Integral controller

In Laplace domain, an integrator is $1/s$ in the transfer function. The presence of an integrator inside the controller ensures that there will be no steady state error assuming that the closed loop system is asymptotically stable, even in the presence of constant disturbances and demands. An integrator essentially is the memory of error signal through time.

To look at the integrator of the system, an additional state can be added to the state space representation which represents the error signal of the system. The detailed steps of doing this is discussed below.

The block diagram of an integrator is shown in Figure 10

4.4 Comparison

After understanding the different types of systems, it is time to simulate them to see how they respond to perturbation and whether they robustly adapt. Looking at Figure 11, we can see that the antithetic integral controller adapts robustly when there is no degradation of controller species. With degradation of controller species, although the system is not robustly adapting, it still performs a lot better than the open loop and the negative feedback loop. Now we can see how the antithetic integral controller motif can minimise steady state error!

5 Analysing systems of ODE with the control theory toolbox

In this section, control theory toolbox will be utilised to characterise the system, the most important concepts behind is equilibrium and stability.

5.1 Stability

Stability refers to whether the system will converge to a particular value. An unstable system will lead to an unbound output even when given a bound input (images!). There are 3 ways to check the stability of a closed loop system - checking the eigenvalue of the A matrix in the state space system, checking the poles of the transfer function and through drawing a Nyquist diagram. To analyse the stability of a non-linear system, linearisation about the equilibrium point is required.

What are poles of the transfer function? (drop down)
By setting the denominator equal to zero, we have the characteristic equation. The roots of the characteristic equation is called the poles.

For an unstable system, some species (states) of the system will blow up to infinity as time goes on. If the real part of any of the poles/eigenvalues are positive, the system will be unstable.The system will only be stable if all the poles/eigenvalues are non-positive. If the real part of the pole is 0, the system is marginally stable where constant amplitude and frequency oscillations will occur. If the poles are complex numbers, the imaginary part dictates the oscillation frequency of the system. The response time of a system is determined by the magnitude of the real part of the least negative pole.

Here we analyse the stability of the fixed point through analysing the eigenvalues of A with Routh-Hurwitz criterion

5.1.1 Activation - without degradation

The equation of finding the eigenvalues of A is the following
$\eta\gamma_p s z_{1eq} + \eta\gamma_p s z_{2eq} + \eta s^2 z_{1eq} + \eta s^2 z_{2eq} + \eta\theta_1\theta_2 z_{1eq} + \gamma_p s^2 + s^3$
$= s^3 + (\gamma_p + \eta(z_{1eq} + z_{2eq})s^2 + \eta\gamma_p(z_{1eq} + z_{2eq})s + \eta\theta_1\theta_2 z_{1eq}$

The Routh-Hurwitz criterion states that for the roots of the characteristic equation to be negative real part, $a_1a_2 > a_0a_3$ where $a_1a_2 = (\gamma_p + \eta(z_{1eq} + z_{2eq}))(\eta\gamma_p(z_{1eq} + z_{2eq}))$ and $a_0a_3 = \eta\theta_1\theta_2 z_{1eq}$

Substituting $z_{1eq} = \frac{\mu\gamma_p}{\theta_1\theta_2}$, $a_0a_3 = \eta\mu\gamma_p$

$a_1a_2 = z_{1eq}z_{2eq}\eta^2\gamma_p + ... = \mu\gamma_p\eta + ...$

where +... are non-negative values as all the parameters are non-negative

This proves that $a_1a_2 > a_0a_3$ and that the system is stable linearised about the equilibrium point.

Looking at the system we have used for simulation, the poles of the system are

$$-0.0386 + 0.0000i - 0.0002 + 0.0014i - 0.0002 - 0.0014i$$

5.1.2 Activation - with degradation

With $\gamma_c > 0$, there's no close form analytical solution to the steady state value of the ODE. However, given that γ_c is very small and that without degradation $a_1 a_2 >> a_0 a_3$. It is reasonable to deduce that for the range of value of γ_c we have, the equilibrium point will be stable.

For the system we have used for simulation, we can analyse its stability through looking at the poles.

The poles of the system are

$$-0.0389 + 0.0000i - 0.0004 + 0.0014i - 0.0004 - 0.0014i$$

As all the poles have a negative real part, the equilibrium point of the system is stable.

5.1.3 Repression - without degradation

The eigenvalue of the repression system leads to a very complicated expression. Here, we analyse the stability of the system through looking at the poles of the system we are simulating to show that for realistic values, the equilibrium point is stable.

The poles of the system are

$$-0.0436 + 0.0000i - 0.0002 + 0.0007i - 0.0002 - 0.0007i$$

As all the poles have a negative real part, the equilibrium point of the system is stable.

5.1.4 Repression - with degradation

Similarly, the eigenvalue of the repression system does not have a closed form solution. Therefore, like above, we analyse the stability of the system through looking at the numerical values of the poles.

The poles of the system are

$$-0.0387 + 0.0000i - 0.0004 + 0.0008i - 0.0004 - 0.0008i$$

As all the poles have a negative real part, the equilibrium point of the system is stable.

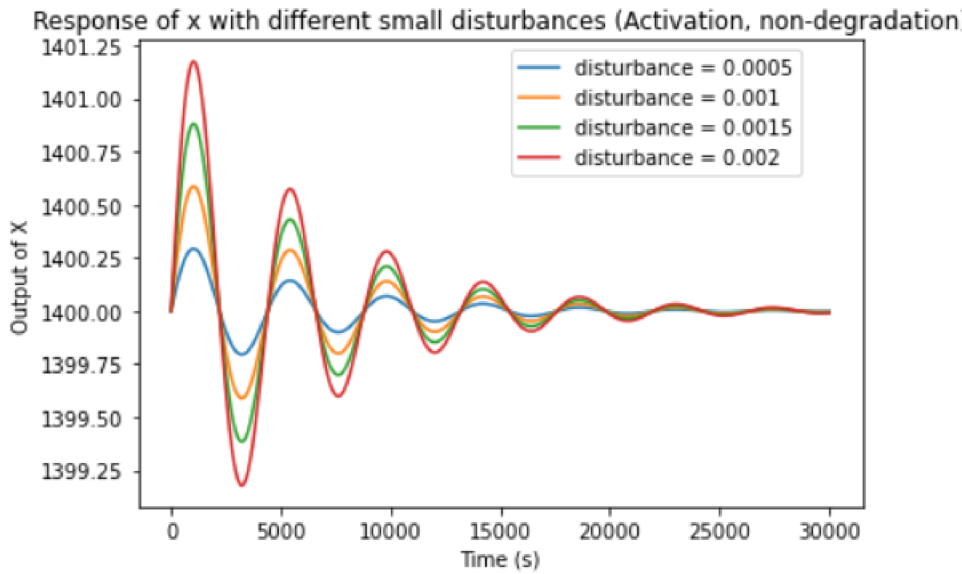

Figure 12: Change of System Response with varying values of small disturbance (Activation, non-degradation)

5.2 System response

In this section, we will simulate the systems of non-linear ODEs with Python odeint and see how the system respond to perturbations. We will then vary the magnitude of perturbation to show how the system behaves.

The graphs below shows the response of the system in response to small perturbation with varying magnitudes for both the Activation and Repression system, with and without degradation.

In order to understand the system better, we visualise the system in response to perturbation with larger magnitude difference. As we can see, for all the cases, as the disturbance increases, the system has a higher peak value but become less oscillatory.

Looking at the system with and without degradation, one can see that for the non-degradation case, the system is able to robustly adapt, return back to its original steady state value. However, for the degradation case, it reaches the new steady state faster given the same parameter value and the same disturbance. This suggests that the system with degradation is more stable - a concept known as Leaky Integrator.

Let's look at the activation system first - To explain the faster settling time of the degradation case, we look at the poles of the system. With this set of parameters, the poles of the non-degradation case are

$$-0.0386 + 0.0000i - 0.0002 + 0.0014i - 0.0002 - 0.0014i$$

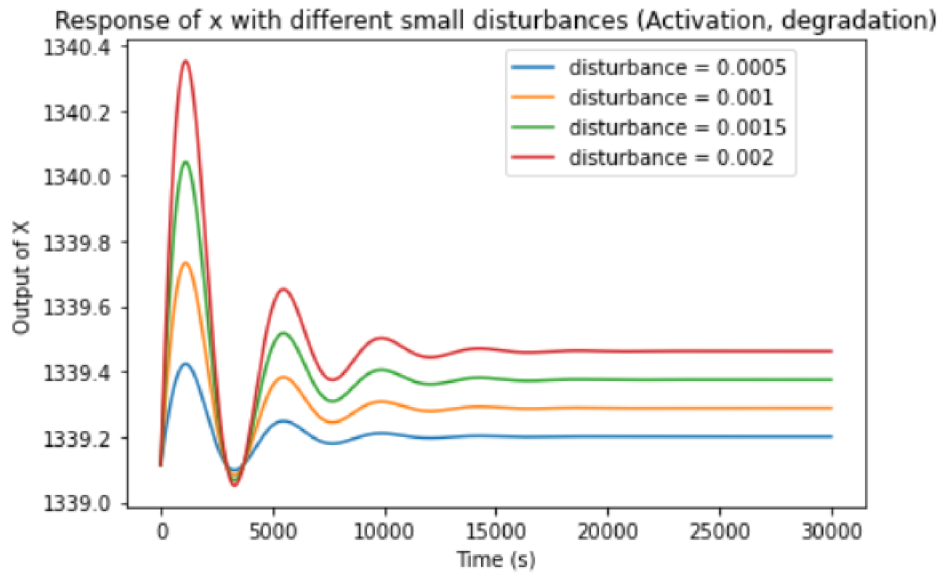

Figure 13: Change of System Response with different values of disturbance (Activation, degradation)

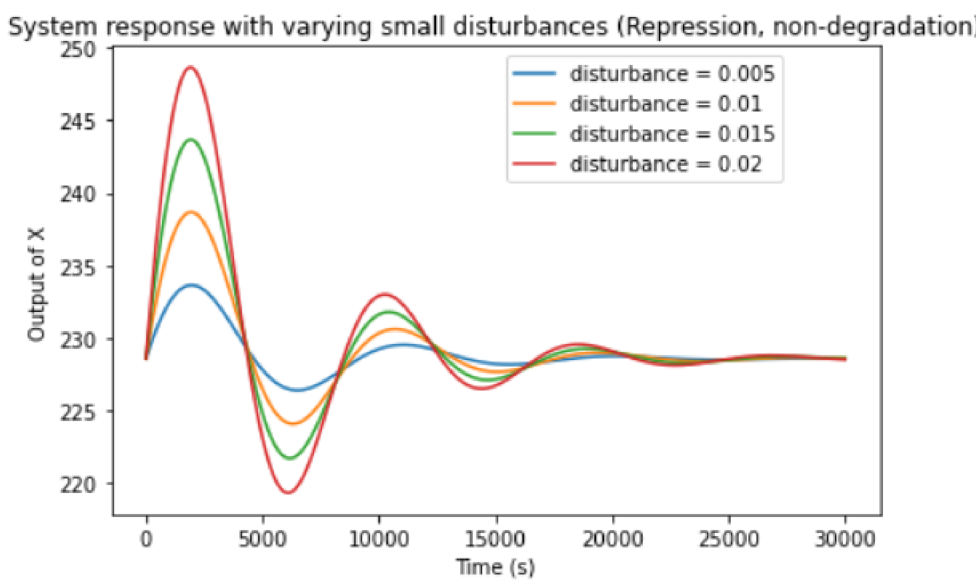

Figure 14: Change of System Response with different values of small disturbances (Repression, non-degradation)

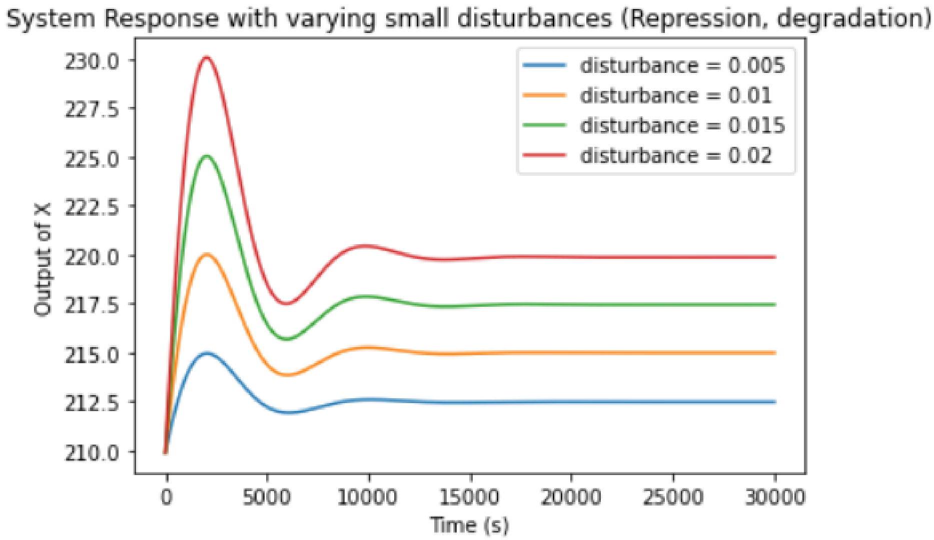

Figure 15: Change of System Response with different values of small disturbances (Repression, degradation)

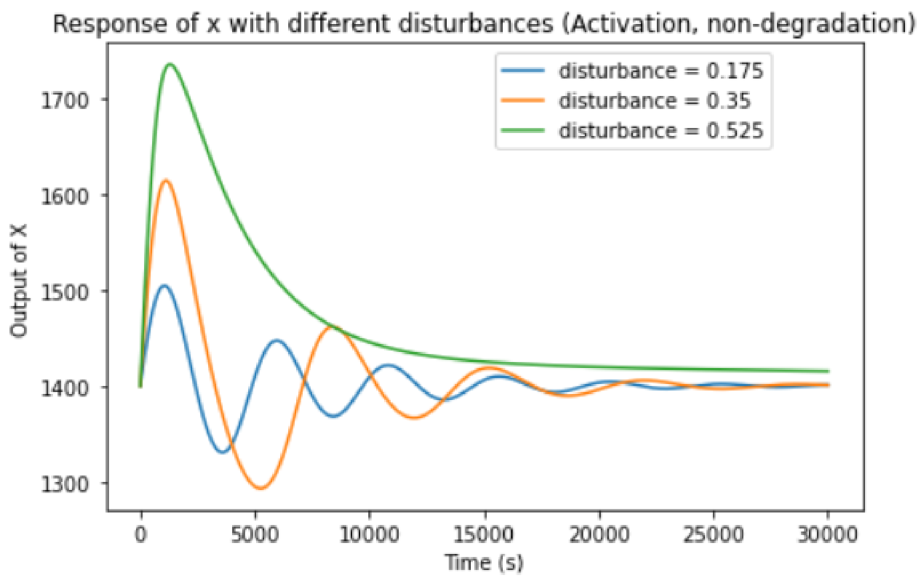

Figure 16: Change of System Response with different values of disturbance (Activation, non-degradation)

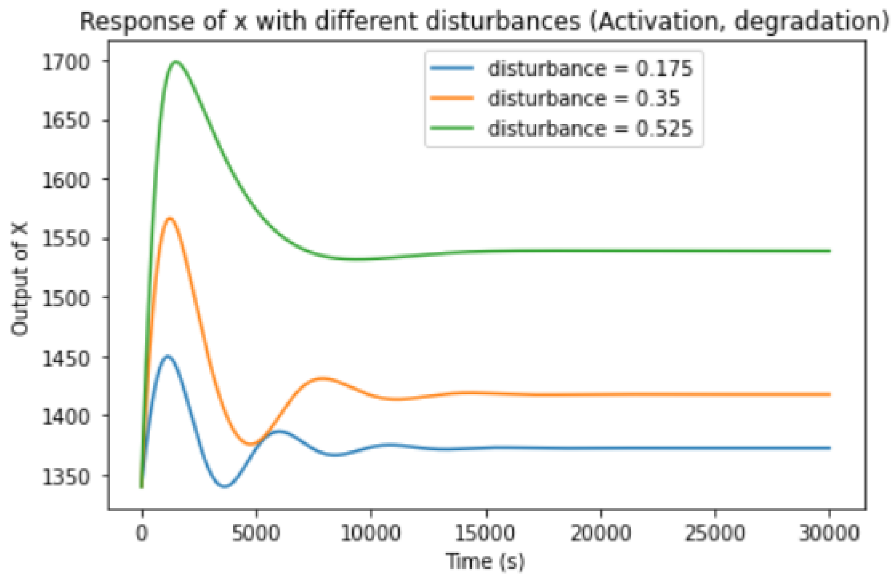

Figure 17: Change of System Response with different values of disturbance (Activation, degradation)

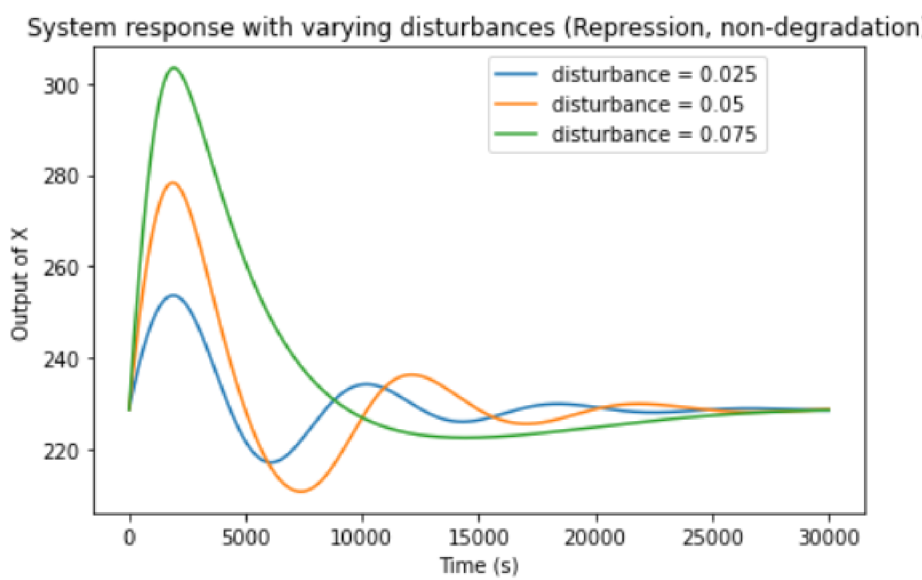

Figure 18: Change of System Response with different values of disturbance (Repression, non-degradation)

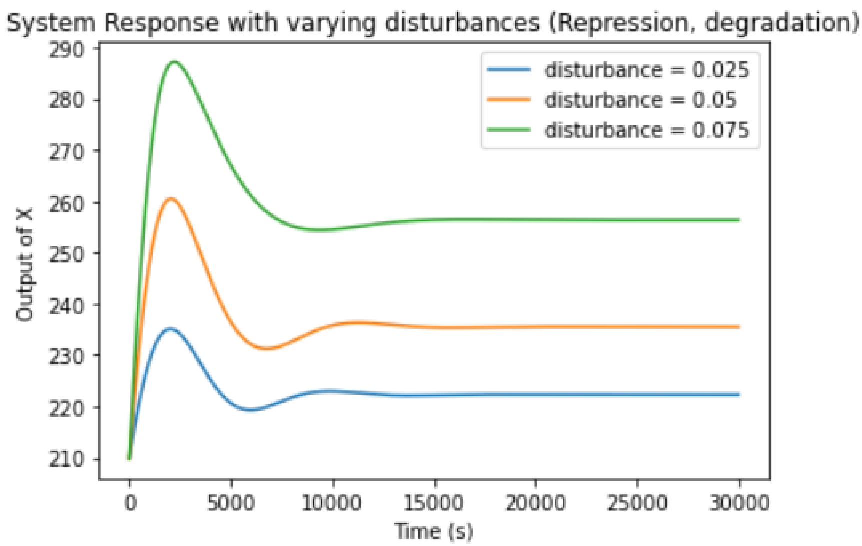

Figure 19: Change of System Response with different values of disturbance (Repression, degradation)

and the poles of the degradation case are

$$-0.0389 + 0.0000i - 0.0004 + 0.0014i - 0.0004 - 0.0014i$$

As we can see, the real part of the least negative pole is more negative in the degradation case. This explains why the system reaches the steady state quicker with degradation.

Now let's move on the repression system. With this set of parameters, the poles of the non degradation case are

$$-0.0436 + 0.0000i - 0.0002 + 0.0007i - 0.0002 - 0.0007i$$

and the poles of the degradation case are

$$-0.0387 + 0.0000i - 0.0004 + 0.0008i - 0.0004 - 0.0008i$$

As we can see, similar to the activation case, the real part of the least negative pole is more negative in the degradation case. This explains why the system reaches the steady state quicker with degradation.

5.2.1 Leaky integrator

An integrator is a controller that ensures 0 steady state error. While 0 steady state error is ideal, an integral controller have slow dynamics. A Leaky integrator is a type of integrator that allows for faster dynamics and increased stability but in the expense of having some small steady state error. Essentially, it allows

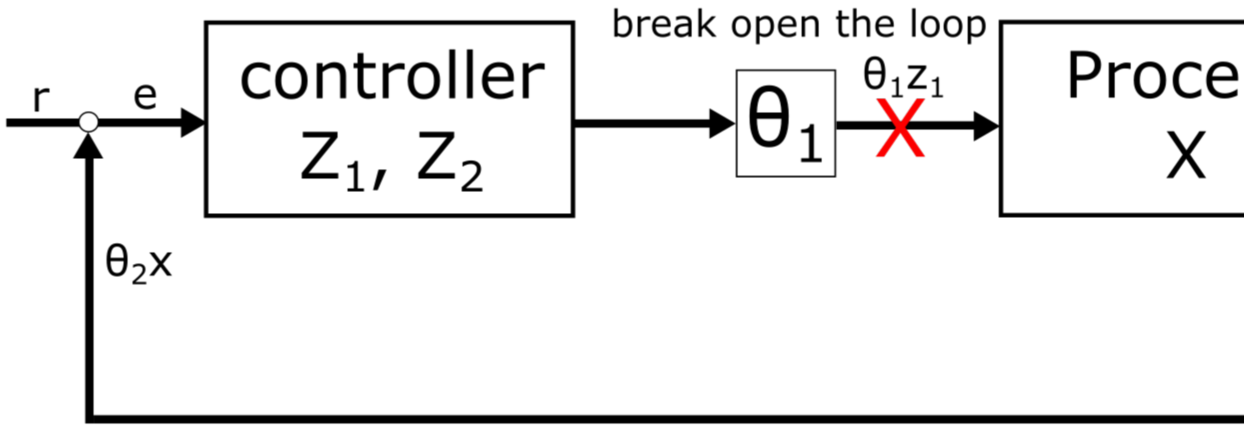

Figure 20: The process of breaking the loop open to analyse the uncertainty 1 of the system

some memory of the error to be lost in the process, just like in our system where controller species are being degraded, leading to a leaky integrator instead of a perfect integrator.

5.3 How much uncertainty can the system handle for θ_1 and θ_2?

Here, we analyse the system in the activation case only using Nyquist Diagrams and sensitivity functions to show how much variability the system can take in the value of θ_1 and θ_2. To investigate this, we first need to obtain the open loop function of the system, using the parameter θ_1 and θ_2 as the feedback gain to investigate how big θ_1 and θ_2 can be for the system to be stable.

5.3.1 Uncertainty in θ_1

To obtain the open loop system, we break open the loop in order to investigate the open loop system. The explanation of breaking the loop open is shown in Figure 20

The systems of equation characterising the open loop system is

$$\begin{cases} \dot{x} &= u - \gamma_p x \\ \dot{z}_1 &= \mu_1 - \eta z_1 z_2 - \gamma_c z_1 \\ \dot{z}_2 &= \theta_2 x - \eta z_1 z_2 - \gamma_c z_2 \end{cases} \tag{8}$$

When representing it in a state space format after linearisation,

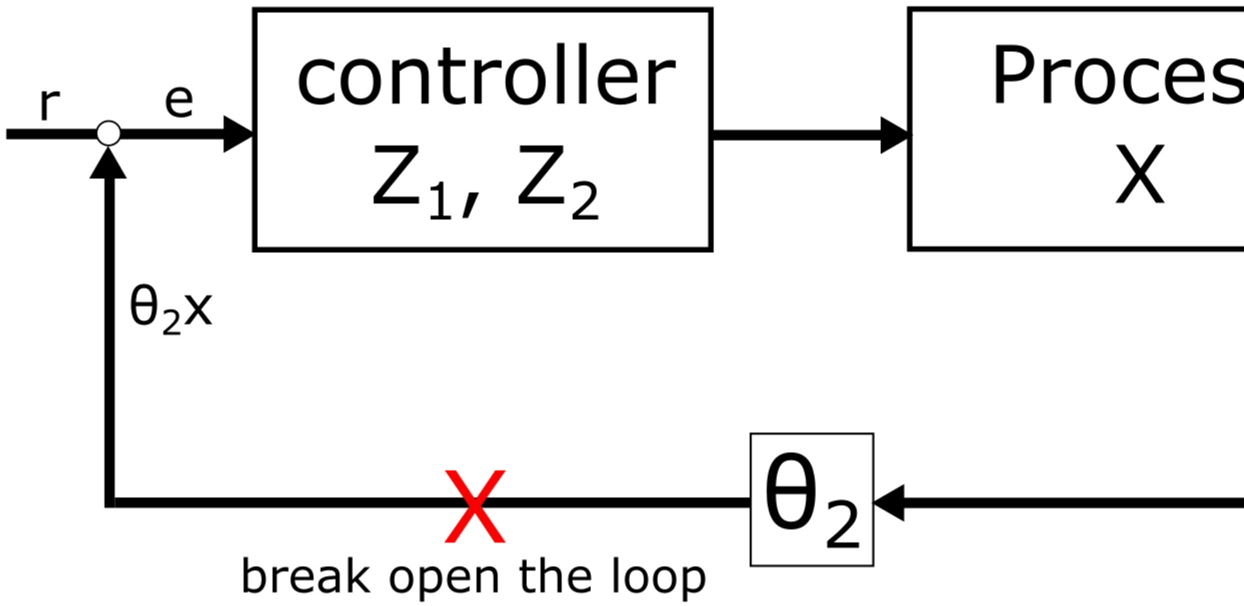

Figure 21: The process of breaking the loop open to analyse the uncertainty 2 of the system

$$\begin{bmatrix} \dot{\delta x} \\ \dot{\delta z_1} \\ \dot{\delta z_2} \end{bmatrix} = \begin{bmatrix} -\gamma_p & 0 & 0 \\ 0 & -\eta z_2 - \gamma_c & -\eta z_1 \\ \theta_2 & -\eta z_2 & -\eta z_1 - \gamma_c \end{bmatrix} \begin{bmatrix} \delta x \\ \delta z_1 \\ \delta z_2 \end{bmatrix} + \begin{bmatrix} -1 \\ 0 \\ 0 \end{bmatrix} u$$

$$y = \begin{bmatrix} 0 & 1 & 0 \end{bmatrix} \begin{bmatrix} \delta x \\ \delta z_1 \\ \delta z_2 \end{bmatrix}$$

where A = $\begin{bmatrix} -\gamma_p & 0 & 0 \\ 0 & -\eta z_2 - \gamma_c & -\eta z_1 \\ \theta_2 & -\eta z_2 & -\eta z_1 - \gamma_c \end{bmatrix}$ B = $\begin{bmatrix} -1 \\ 0 \\ 0 \end{bmatrix}$ C = $\begin{bmatrix} 0 & 1 & 0 \end{bmatrix}$

Thus the open loop transfer function of the system with θ_1 as the feedback gain is given by H(s) = C(sI-A)^{-1}B

5.3.2 Uncertainty in θ_2

To obtain the open loop system, we break open the loop in order to investigate the open loop system. The explanation of breaking the loop open is shown in Figure 21

The systems of equation characterising the open loop system is

$$\begin{cases} \dot{x} &= \theta_1 z_1 - \gamma_p x \\ \dot{z}_1 &= \mu_1 - \eta z_1 z_2 - \gamma_c z_1 \\ \dot{z}_2 &= u - \eta z_1 z_2 - \gamma_c z_2 \end{cases} \tag{9}$$

When representing it in a state space format after linearisation,

$$\begin{bmatrix} \dot{\delta x} \\ \dot{\delta z_1} \\ \dot{\delta z_2} \end{bmatrix} = \begin{bmatrix} -\gamma_p & \theta_1 & 0 \\ 0 & -\eta z_2 - \gamma_c & -\eta z_1 \\ 0 & -\eta z_2 & -\eta z_1 - \gamma_c \end{bmatrix} \begin{bmatrix} \delta x \\ \delta z_1 \\ \delta z_2 \end{bmatrix} + \begin{bmatrix} 0 \\ 0 \\ -1 \end{bmatrix} u$$

$$y = \begin{bmatrix} 1 & 0 & 0 \end{bmatrix} \begin{bmatrix} \delta x \\ \delta z_1 \\ \delta z_2 \end{bmatrix}$$

where A = $\begin{bmatrix} -\gamma_p & \theta_1 & 0 \\ 0 & -\eta z_2 - \gamma_c & -\eta z_1 \\ 0 & -\eta z_2 & -\eta z_1 - \gamma_c \end{bmatrix}$ B = $\begin{bmatrix} 0 \\ 0 \\ -1 \end{bmatrix}$ C = $\begin{bmatrix} 1 & 0 & 0 \end{bmatrix}$

Thus the open loop transfer function of the system with θ_1 as the feedback gain is given by H(s) = C(sI-A)^{-1}B

5.3.3 Sensitivity Function of the Open loop system

The sensitivity function of the parameter θ_1 and θ_2 is shown in the following graphs. As we can see from the graph, the peak of the sensitivity function of θ_1 is at 7.03 dB and 7.3dB for the non-degradation and degradation case respectively, meaning that the closed loop system can be quite sensitive to the value of θ_1. For θ_2 however, the peak of the sensitivity function are 1.53dB and 1.52dB for the non-degradation case and degradation case respectively, showing that the system will become unstable if θ_2 reaches a certain value. However, the frequency where the sensitivity function reaches to above 0dB is at the high frequency end, meaning that disturbance will very likely be rejected as disturbances usually have low frequencies.

5.3.4 Sensitivity function and Nyquist Diagram (dropdown)

Nyquist diagram provides an easy way to visualise the stability of a feedback system and to access the maximum feedback gain obtained before the system becomes unstable. It allows us to deduce the properties of the closed loop system based on the properties of the open loop system. It essentially plots the value of the open loop transfer function transfer function (G(s)) in the complex plane at all frequencies from $\omega = 0$ to $\omega = \infty$. Assuming that the open loop system is stable, the closed loop system will be stable if the Nyquist diagram doesn't have a net encirclement of the -1/K point. In the analysis of our system, K = 1 so if there's no encirclement of the -1 point. The closed loop system is stable.

Why is the -1 point significant? (dropdown)
Remember that the denominator of a closed loop transfer function is 1+L(s).

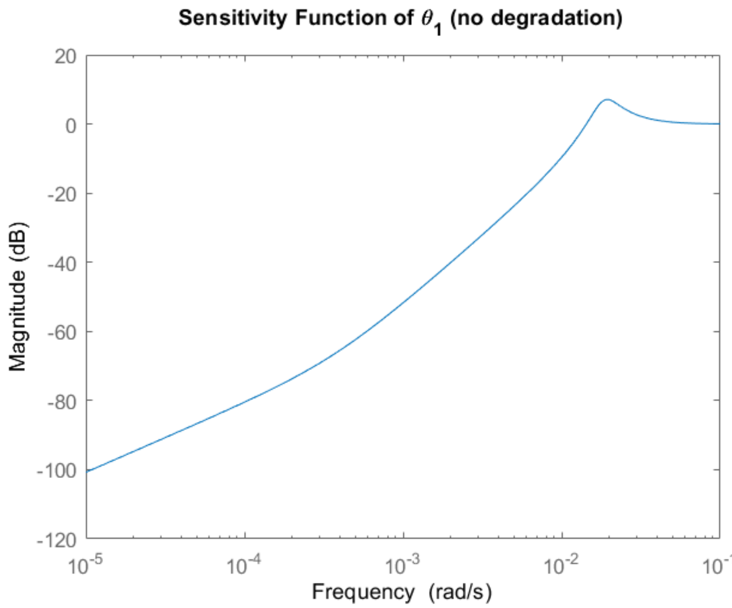

Figure 22: Sensitivity Function of the open loop system with θ_1 as the feedback gain (Activation, non-degradation)

If L(s) = -1, the denominator of the system will be 0 and the system will go to infinity at the frequency and thus is unstable at that specific frequency.

Apart from whether the Nyquist diagram encircles the -1 point, there are other properties of the Nyquist diagram that is important for the analysis of the robustness of the system - gain margin, phase margin and the peak of sensitivity function. The gain margin is the amount of change in the open-loop gain required to make a closed-loop system unstable i.e. the distance between the -1 point to the point where the Nyquist diagram crosses the real axis. The phase margin is the amount of change in open-loop phase required to make a closed-loop system unstable where phase is strongly related to the time delay of the system.

In control theory, sensitivity function is a very important concept and is given by $S = (1 + L(s))^{-1}L(s)$. The property of the sensitivity function can be obtained through looking at the Nyquist diagram of the system. Essentially, the closer the Nyquist plot of L(j ω) is to the point -1, the less robust the system will be when the loop is closed. If there is uncertainty in parameters such that the process or the controller is larger than what we model it to be, the Nyquist diagram will magnify. Therefore, the closer the Nyquist diagram is to the -1 point, the less it can magnify before it crosses over the -1 point, leading to instability. Moreover, the Nyquist diagram also contains phase information where time delay causes the Nyquist diagram to rotate, which can bring some

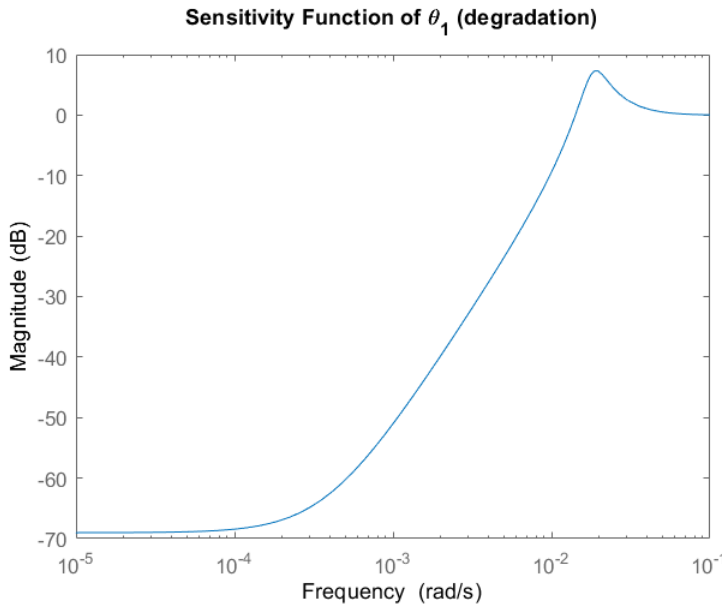

Figure 23: Sensitivity Function of the open loop system with θ_1 as the feedback gain (Activation, degradation)

part of the Nyquist diagram closer to the -1 point.

The peak of the sensitivity function is the minimum distance from the -1 point to the Nyquist plot. Therefore, when looking at the sensitivity function, the magnitude of the peak gives us information on the robustness. Remember that $S = (1 + L(s))^{-1}L(s)$ so the higher S is, the closer L is to -1 at that particular frequency and the more likely the system will be unstable. Apart from the peak of the sensitivity function, it is also good practice to look at the frequencies of the sensitivity function. Ideally we want the sensitivity function to have a lower magnitude at lower frequency as it is closely associated with reference tracking and disturbance rejection.

The interpretation of gain margin, phase margin and the peak of the sensitivity function is shown in the following diagram

5.4 Looking at the Integrator

As the antithetic circuit acts as an integral controller, looking at the integrator of the system is important. An integrator is the error signal of the system which in this case, is governed by the difference of z_1 and z_2. In order to look at the integrator, new states has to be created to visualise the integrator.

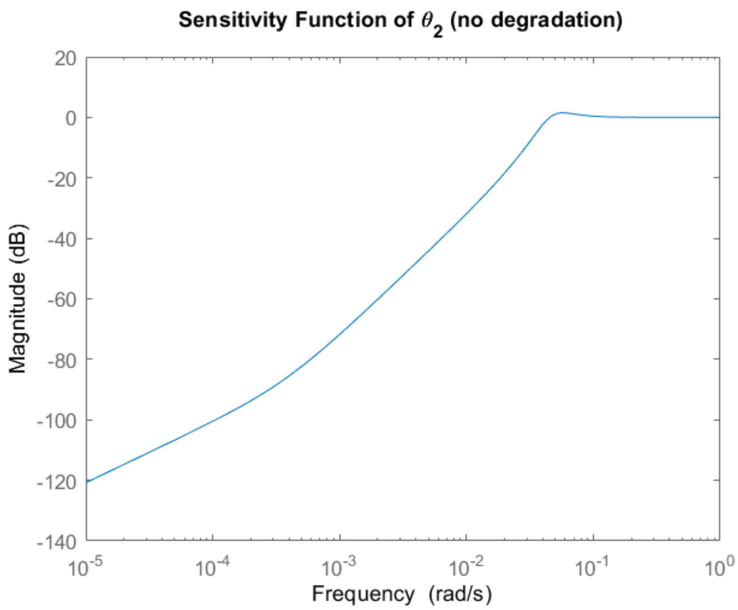

Figure 24: Sensitivity Function of the open loop system with θ_2 as the feedback gain (Activation, non-degradation)

Therefore, we set

$$\begin{cases} \dot{q}_1 &= \dot{z}_1 - \dot{z}_2 \\ \dot{q}_2 &= \dot{z}_1 + \dot{z}_2 \end{cases} \tag{10}$$

in order to visualise the integrator (q_1).

5.4.1 Activation

Including integrator as states, we get

$$\begin{cases} \dot{x} &= \theta_1 \dfrac{q_1 + q_2}{2} - \gamma_p x \\ \dot{q}_1 &= \mu_1 - \theta_2 x + \gamma_c (z_2 - z_1) \\ \dot{q}_2 &= \mu_1 + \theta_2 x - 2\eta z_1 z_2 - \gamma_c (z_1 + z_2) \end{cases} \tag{11}$$

Substituting

$$\begin{cases} z_1 &= \dfrac{q_1 + q_2}{2} \\ z_2 &= \dfrac{q_2 - q_1}{2} \end{cases} \tag{12}$$

We obtain

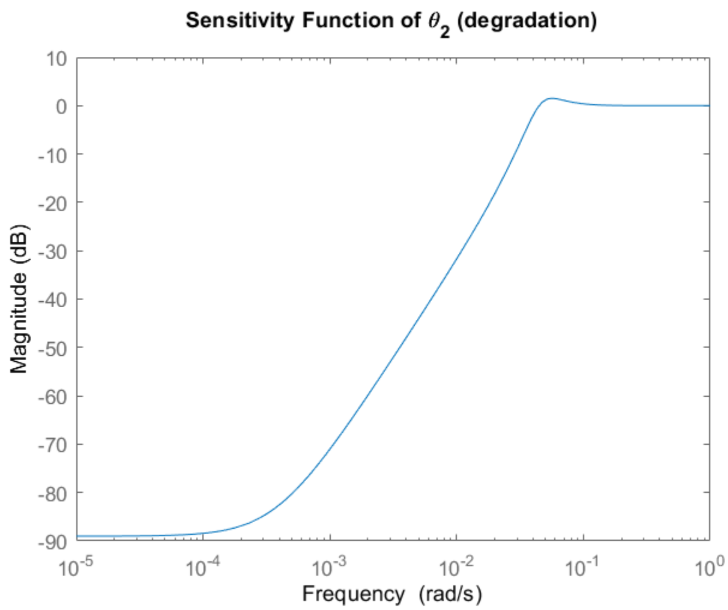

Figure 25: Sensitivity Function of the open loop system with θ_2 as the feedback gain (Activation, degradation)

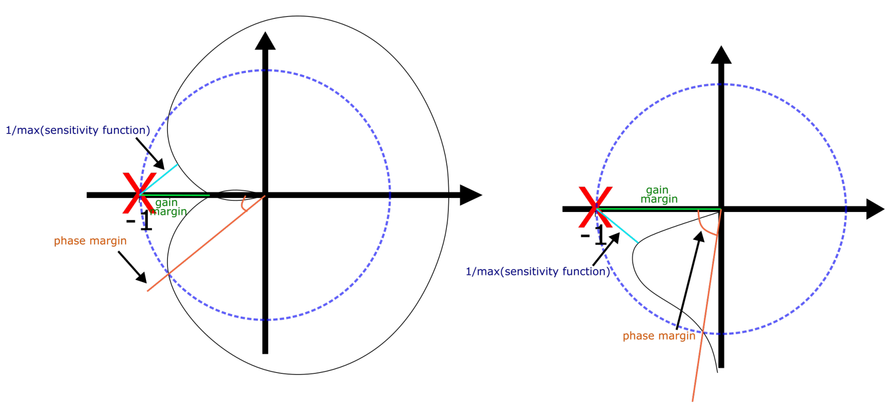

Figure 26: The relationship between Nyquist Diagram, phase margin, gain margin and sensitivity function

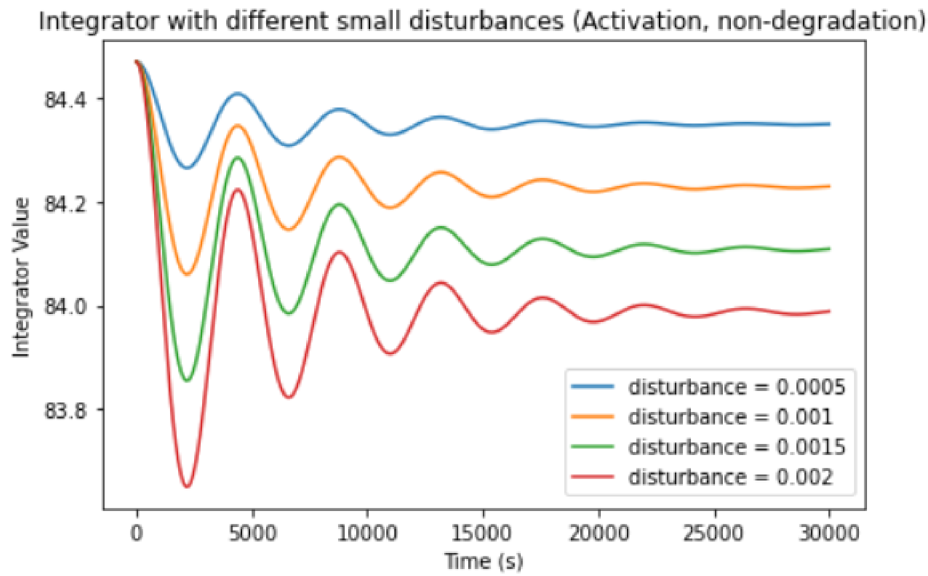

Figure 27: Change of Integrator Response with different values of small disturbance (Activation, non-degradation)

$$\begin{cases} \dot{x} & = \theta_1 \dfrac{q_1 + q_2}{2} - \gamma_p x \\ \dot{q}_1 & = \mu_1 - \theta_2 x - \gamma_c q_1 \\ \dot{q}_2 & = \mu_1 + \theta_2 x - \dfrac{\eta}{2}(q_2^2 - q_1^2) - \gamma_c q_2 \end{cases} \tag{13}$$

The response of the integrator in response to small varying perturbation is shown in Figure 27 for the non-degradation case and Figure 28 for the degradation case.

Like above, to understand the system better, we give the system a larger perturbation to study the response of the integrator. Figure 29 shows the integrator response for the system without degradation while Figure 30 shows the integrator response for the system with degradation.

Just like what we have seen in the system response, the integrator reaches steady state a lot faster in the degradation case compared to the non-degradation case.

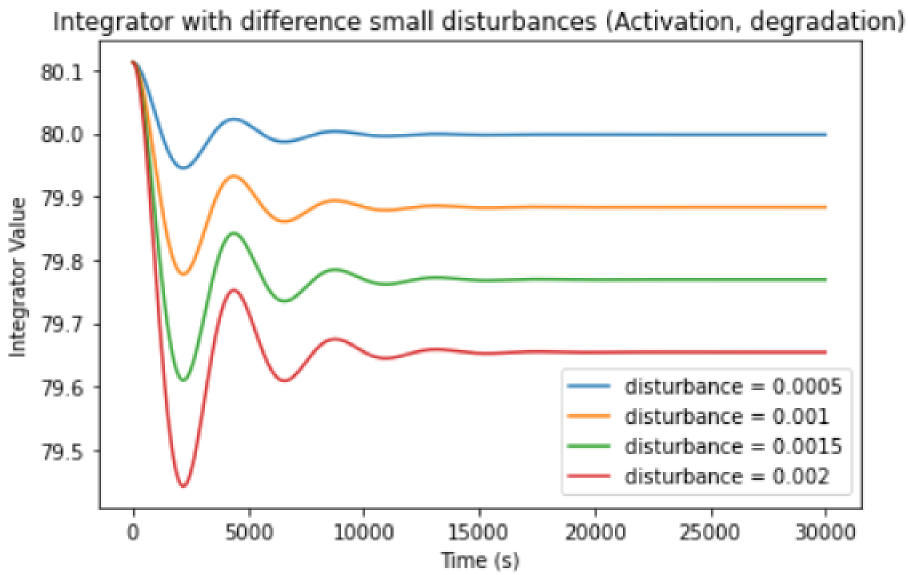

Figure 28: Change of Integrator Response with different values of small disturbance (Activation, degradation)

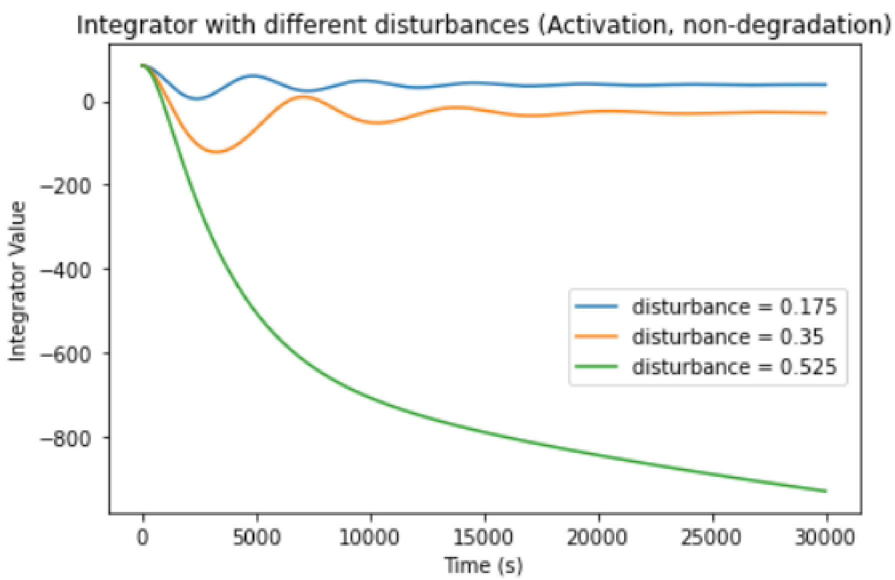

Figure 29: Change of Integrator Response with different values of disturbance (Activation, non-degradation)

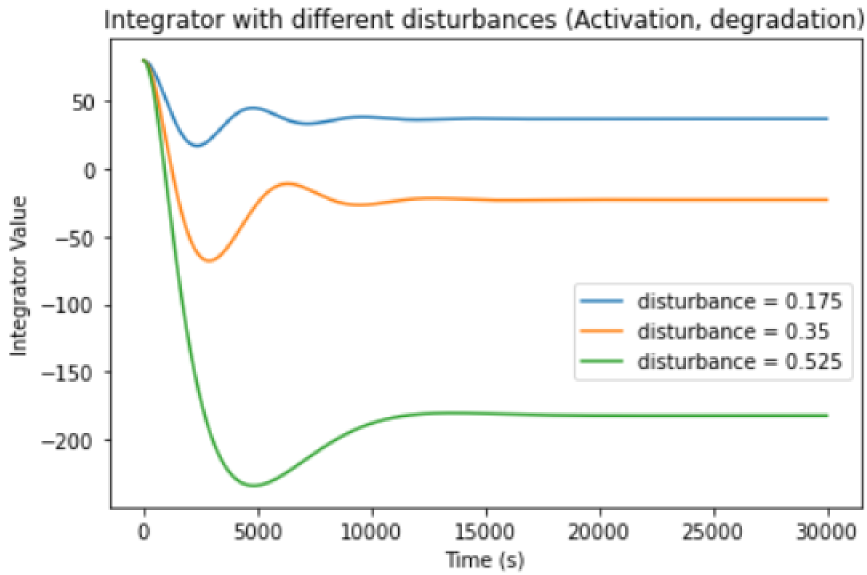

Figure 30: Change of Integrator Response with different values of disturbance (Activation, degradation)

5.4.2 Repression

For the repression case, the systems of ODEs visualising the integrator becomes

$$\begin{cases} \dot{x} &= \dfrac{\alpha}{\theta_1 \frac{q_1+q_2}{2} + 1} - \gamma_p x \\ \dot{q}_1 &= \mu_1 - \dfrac{\mu_2}{\theta_2 x + 1} - \gamma_c q_1 \\ \dot{q}_2 &= \mu_1 + \dfrac{\mu_2}{\theta_2 x + 1} - \dfrac{\eta}{2}(q_2^2 - q_1^2) - \gamma_c q_2 \end{cases} \tag{14}$$

where q_1 is the integrator

Writing it as the state space representation, after linearisation, we obtain

$$\begin{bmatrix} \dot{\delta x} \\ \dot{\delta q_1} \\ \dot{\delta q_2} \end{bmatrix} = \begin{bmatrix} -\gamma_p & -\frac{\theta_1 \alpha}{2(\frac{\theta_1}{2}(q_1+q_2)+1)^2} & -\frac{\theta_1 \alpha}{2(\frac{\theta_1}{2}(q_1+q_2)+1)^2} \\ \frac{\theta_2 \mu_2}{(\theta_2 x_{eq}+1)^2} & -\gamma_c & 0 \\ -\frac{\theta_2 \mu_2}{(\theta_2 x_{eq}+1)^2} & \eta q_1 & -\eta q_2 - \gamma_c \end{bmatrix} \begin{bmatrix} \delta x \\ \delta q_1 \\ \delta q_2 \end{bmatrix} + \begin{bmatrix} 1 \\ 0 \\ 0 \end{bmatrix} d$$

$$y = \begin{bmatrix} 0 & 1 & 0 \end{bmatrix} \begin{bmatrix} \delta x \\ \delta q_1 \\ \delta q_2 \end{bmatrix}$$

The response of the integrator in response to small varying perturbation is shown in Figure 31 for the non-degradation case and Figure 32 for the degradation case.

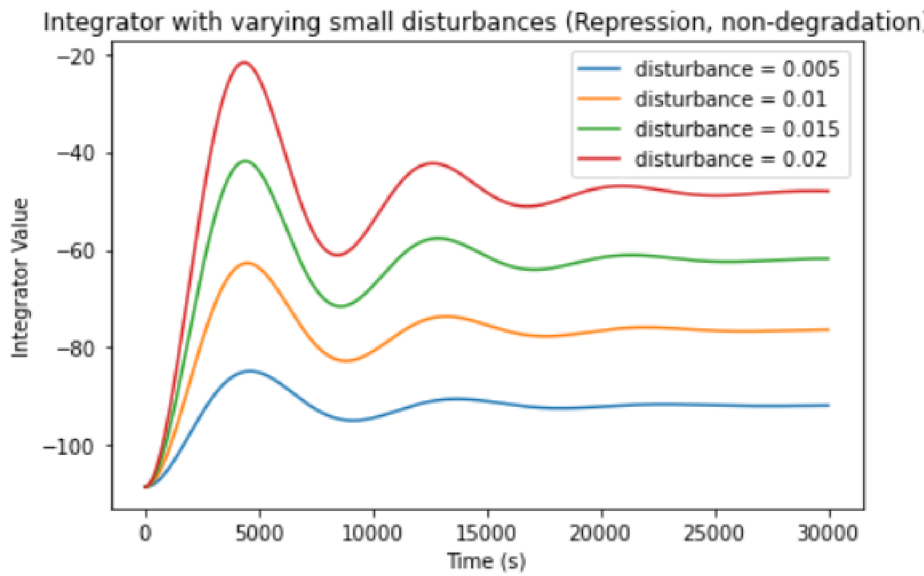

Figure 31: Change of Integrator Response with different values of small disturbance (Repression, non-degradation)

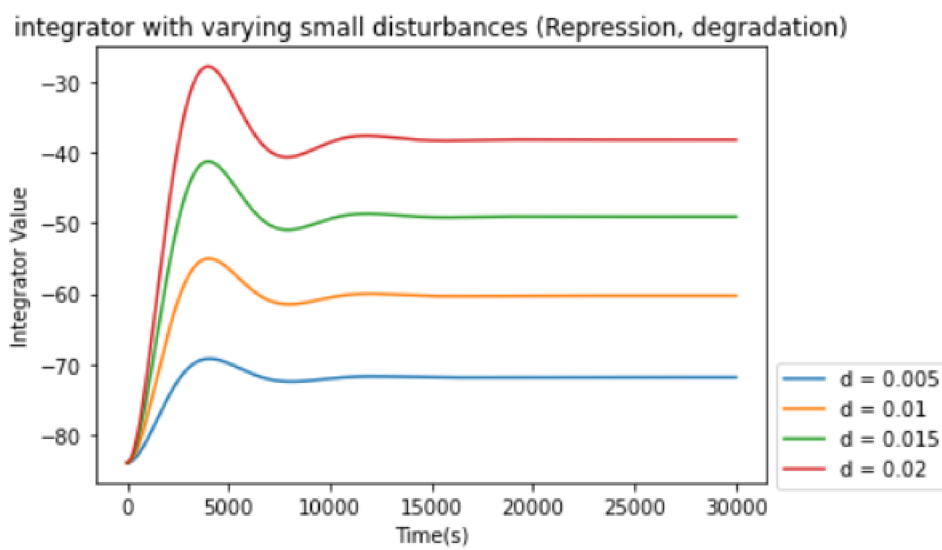

Figure 32: Change of Integrator Response with different values of small disturbance (Repression, degradation)

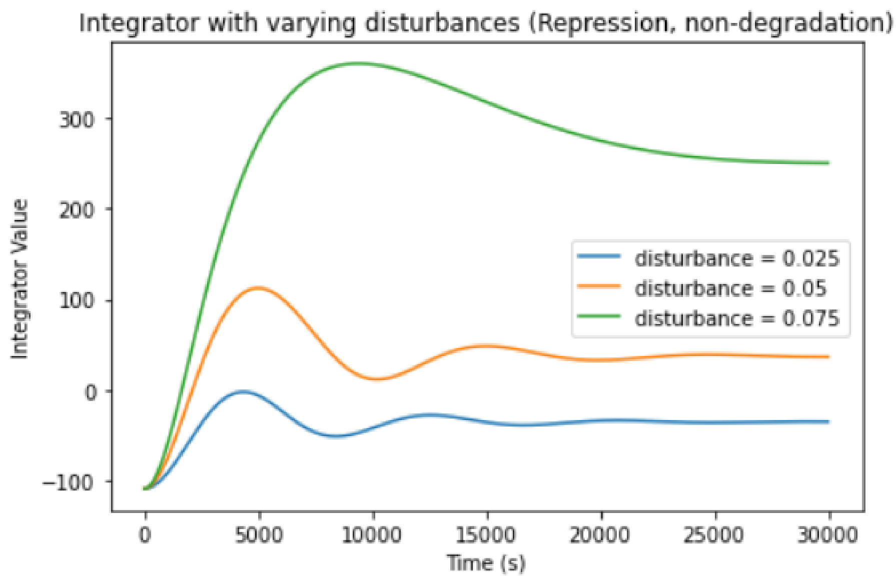

Figure 33: Change of Integrator Response with different values of disturbance (Repression, non-degradation)

Like above, to understand the system better, we give the system a larger perturbation to study the response of the integrator. Figure 33 shows the integrator response for the system without degradation while Figure 34 shows the integrator response for the system with degradation.

Just like what we have seen in the system response, the integrator reaches steady state a lot faster in the degradation case compared to the non-degradation case.

6 Comparison with non-linearised system

As we can see above, we are analysing the system through linearisation. Analysing the system response of the linearised system is only justified if the dynamics of the system is close to the equilibrium point being linearised about. Here we show how different the response of the linearised and the non-linearised system can be if the disturbance is set too high, deviating the system away from the equilibrium fixed point.

Let's first look at the response of the system when the disturbance is d=$0.75\theta_1$ for both the linearised and the non-linearised system. We can see that the system response is very similar and thus the analysis of system response with the linearised system is justified.

Let's now look at the response of the system when the disturbance is d=0.525. We can see that the system response of the linearised and the non-linearised sys-

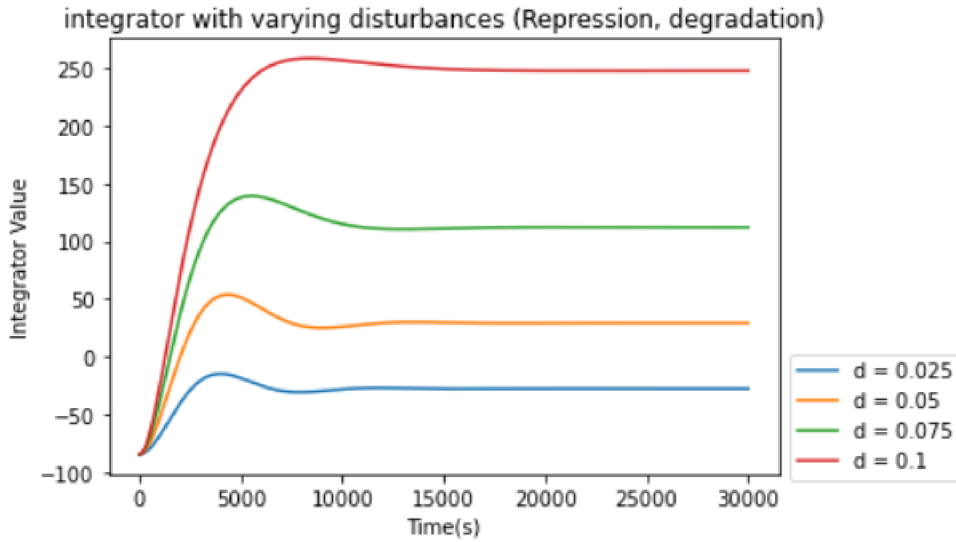

Figure 34: Change of Integrator Response with different values of disturbance (Repression, degradation)

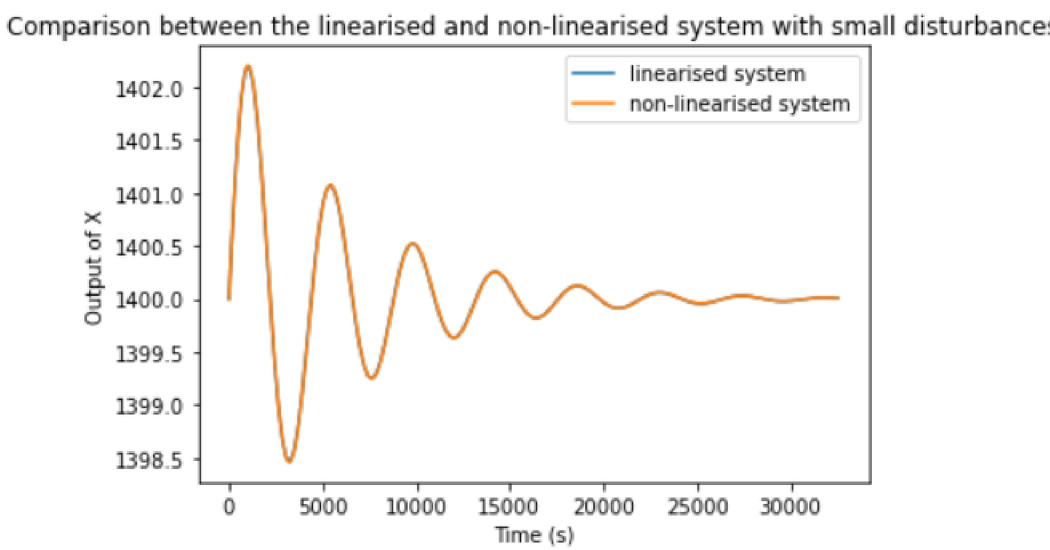

Figure 35: Comparison between the linearised and non-linearised system at small disturbances

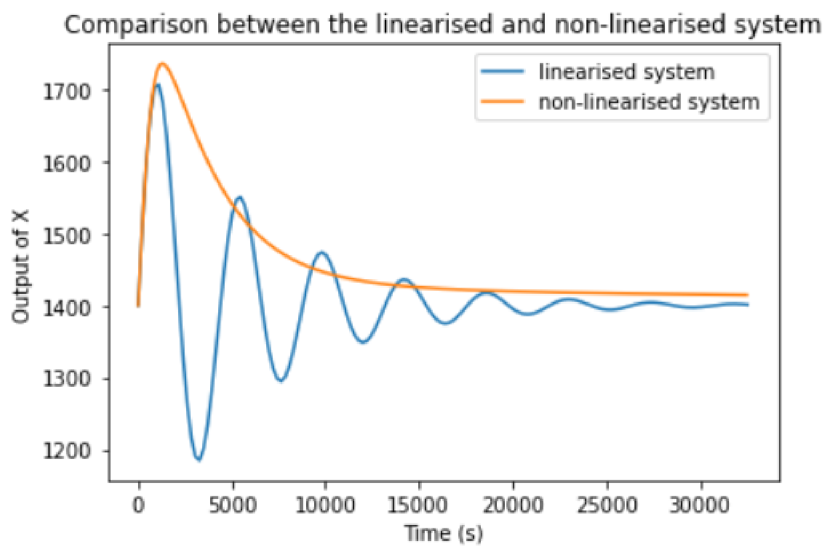

Figure 36: Comparison between the linearised and non-linearised system at high disturbances

tem is very different.

From this, we can see how linearisation of the system makes calculations easier and allows us to apply the control theory toolbox easily to analyse the stability of the system about the point we linearised at. However, we should bear in mind that if the disturbance is too big where we push the system away from the equilibrium, analysing the system response of the linear system is no longer valid as the linear system's behaviour may deviate a lot from the non-linear system's behaviour.

It is also important to note that in this journey of using control theory to analyse the system, we are only using 3 ODEs to describe the system, nesting various dynamics into single variables. In real biological systems, there are multiple steps that goes in between, for instance, the variable θ_1 represents the action of z1 on the promoter that affects the transcription rate, the strength of RBS thus the translation rate also plays a role. Therefore, to make our system more realistic, we can add in extra ODEs to describe these dynamics but this will make our model more complicated, making it more difficult to analyse.

7 Outlook of application of control theory in synthetic biology

In this part, we have discussed one of the recent advances of using an antithetic integral controller to reduce steady state error in synthetic biology. As we can see, we have applied the classical control theory toolbox to design robust genetic circuits, making them more reliable in the presence of noise and be less sensitive to to variability. Throughout the past years, genetic circuit based controller have been evolving, from single transcription unit level (e.g. autoregulatory feedback) to single cell level (e.g. incoherent feedforward loop, antithetic integral controller) to the control of single cell populations or multiple populations. Recently, in-silico methods is being investigated to build more sophisticated controllers. With such amazing evolution over the past years, we are hopeful that in the future, control theory will be applied even more in synthetic biology.