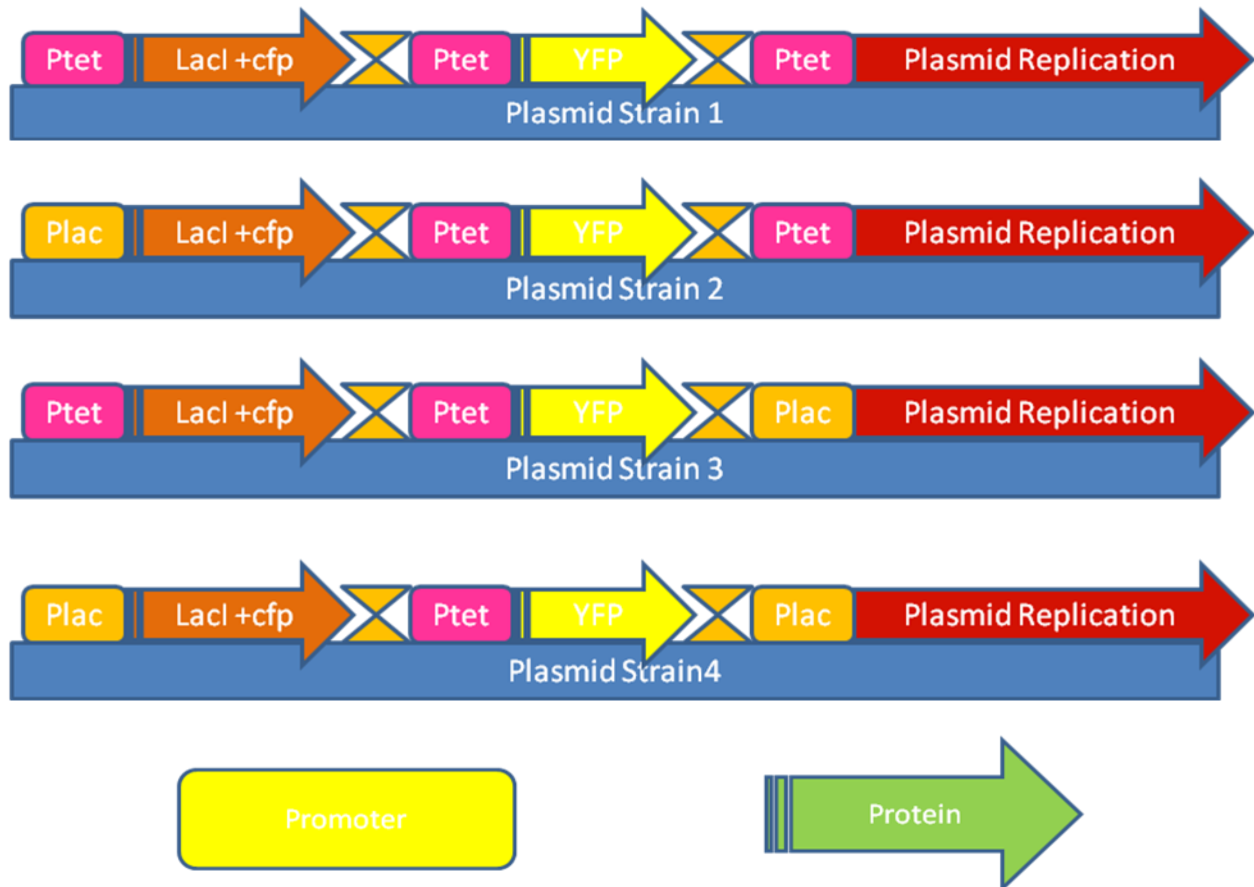
Detailed Molecular Model

Here we wish to show how the dynamics of the cellular material (proteins and plasmids) changes with time and IPTG and also how the specific growth rate of the four constructs on lactose is controlled and maximized by use of multiple feedbacks. In this model quantification by simulation was done and later results were verified by experimental data. A concept of burden on cells and normalized growth rate is introduced to show that in multiple feedback loops helps in optimizing growth rate.

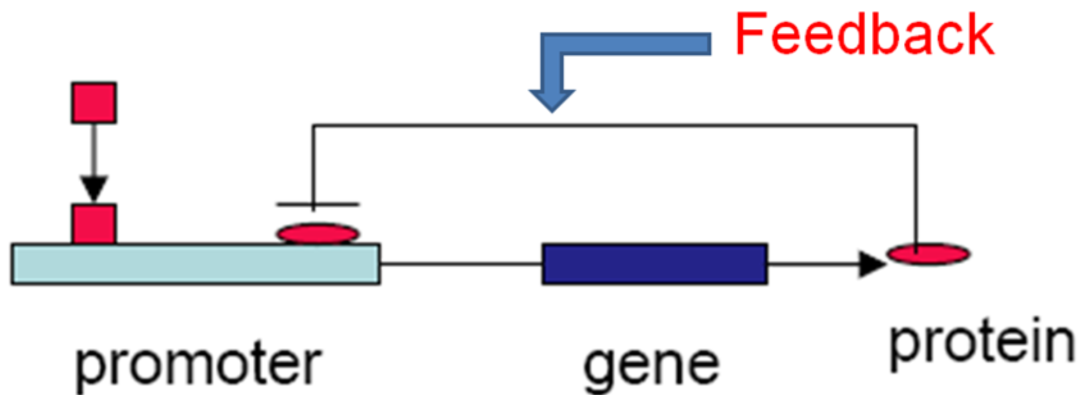
Our Constructs

Using a combination of two promoters ptet and plac, we designed 4 plasmids. The plasmid consists of fusion LacI - cyan fluorescent protein and yellow fluorescent protein, and a site for starting plasmid regulation. Yellow fluorescent protein was always associated with ptet promoter and was added to help quantify plasmid copy number. The generation of the fusion protein in strain 2 and 4 was self inhibited.

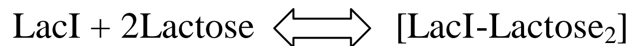


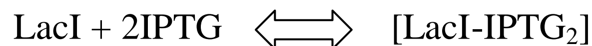
Primary Kinetics and Equations:

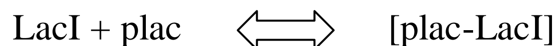
In a biological system, genes are transcribed into mRNA which then are translated into protein. Genes contain promoter sequences responsible for enhancing or reducing gene expression resulting in different protein concentration. Promoters interact with a wide series of proteins (known as activators or repressors) to maintain protein at optimized levels. LacI is one such protein that interacts with plac promoter inhibiting the transcription of DNA to form mRNA. This is typically how a feedback works in a biological system.



In our system we have the key components being plasmid copy number, fusion protein, yfp, lactose, IPTG and growth associated enzyme β-galactosidase. The E. coli genome inherently consists of β gal gene which has plac promoter. LacI interacts with lactose and IPTG and also with plac promoter.







Assuming these 3 equilibrium reactions, we can now write differential equations for the components relating their concentrations with time. Assume K_L, K_{IL} and K_{pl} to be equilibrium constants for the three reactions respectively. We have developed 4 strains, with only distinction of the plac promoter instead of ptet. For strain 1, plac promoter is present only in E. coli genome; hence the strain has no

control over the other plasmid function. In Strain 2, 3 and 4, the plasmid has plac promoter which has some feedbacks on the plasmid's function. As plasmid copy number increases the total plac promoter concentration also increase. The total amount of plac promoter present in any strain could be given by the equation:

$$Total\ Plac\ promoter\ (Ct) = Cit + a * C$$

Where '***a***' is an integer which depends on the strain for which differential equation has been used to describe.(Refer Table)

Total plac promoter, is the sum of concentration of free plac (fp) promoter and plac-LacI complex.

$$Ct = fp + Kp * fp * LacI$$

LacI total equals cfp (because they are a fusion protein). LacI refers to unbounded free LacI in the medium.

$$cfp = LacIt = LacI + plac - LacI + LacI - I_2 + LacI - L_2$$

$$cfp = LacI + Kpl * fp * LacI + Kil * LacI * I^2 + Kl * LacI * L^2$$

$$q = \frac{(cfp - Kpl * fp * LacI)}{LacI} = 1 + K_{Il} * I^2 + K_l L^2$$

$$b = \frac{q}{Kpl} + Cfp - Ct$$

$$free\ plac\ (fp) = plac1 + plac2 + plac3 = \frac{-b + \sqrt{b^2 + 4 * q * Ct/K_{pl}}}{2}$$

Note: here plac1, plac2, plac3 are the free plac associated with β-gal production, plasmid number and cfp-LacI protein.

Now we have the free plac associated with components depending on the strain.

The differential equations are solved for two different conditions. Equations were first solved for 24 hours on other medium with different IPTG and no lactose.

After 24 hours the equations were solved for the same value of IPTG but on different values of lactose.

Equations for growth on no Lactose:

$$\frac{\partial C}{\partial t} = \frac{\eta 1 * p2 * C * Km * a1}{Km + C + \frac{C^2}{Ki}} - (\mu + \beta_C)C$$

$$\frac{\partial Cfp}{\partial t} = k_{11} * p3 * C * a2 - (\mu + \beta)Cfp$$

$$\frac{\partial Yfp}{\partial t} = k_{11} * C - (\mu + \beta)Yfp$$

Here an interesting thing to see is that though in Strain 1 and Strain 2 there is no feedback on plasmid replication, while solving these equations we have to inherently assume that cells have a limited volume and therefore have developed some unique self inhibiting system for increasing the plasmid number beyond a point.

Equations for growth on Lactose:

$$\mu = \mu^{max} * \frac{e}{e^{max}} * \frac{L}{K_l + L}$$

$$\frac{\partial C}{\partial t} = \frac{\eta 2 * p2 * C * Km * a1}{Km + C + \frac{C^2}{Ki}} - (\mu + \beta_c)C$$

$$\frac{\partial Cfp}{\partial t} = k_{12} * p3 * C * a2 - (\mu + \beta)Cfp$$

$$\frac{\partial Yfp}{\partial t} = k_{12} * C - (\mu + \beta)Yfp$$

$$\frac{\partial X}{\partial t} = \mu X$$

$$\frac{\partial \left(\frac{e}{e^{max}}\right)}{\partial t} = (\mu^{max} + \beta) * p1 * a3 - (\mu + \beta) * \left(\frac{e}{e^{max}}\right)$$

$$\frac{\partial L}{\partial t} = -\frac{\mu X}{Yield}$$

Constants\Strain	*1*	*2*	*3*	*4*
a	*0*	*1*	*1*	*2*
p1	*fp/Ct*	*fp/Ct*	*fp/Ct*	*fp/Ct*
p2	*1*	*fp/Ct*	*1*	*fp/Ct*
p3	*1*	*1*	*fp/Ct*	*fp/Ct*

Results

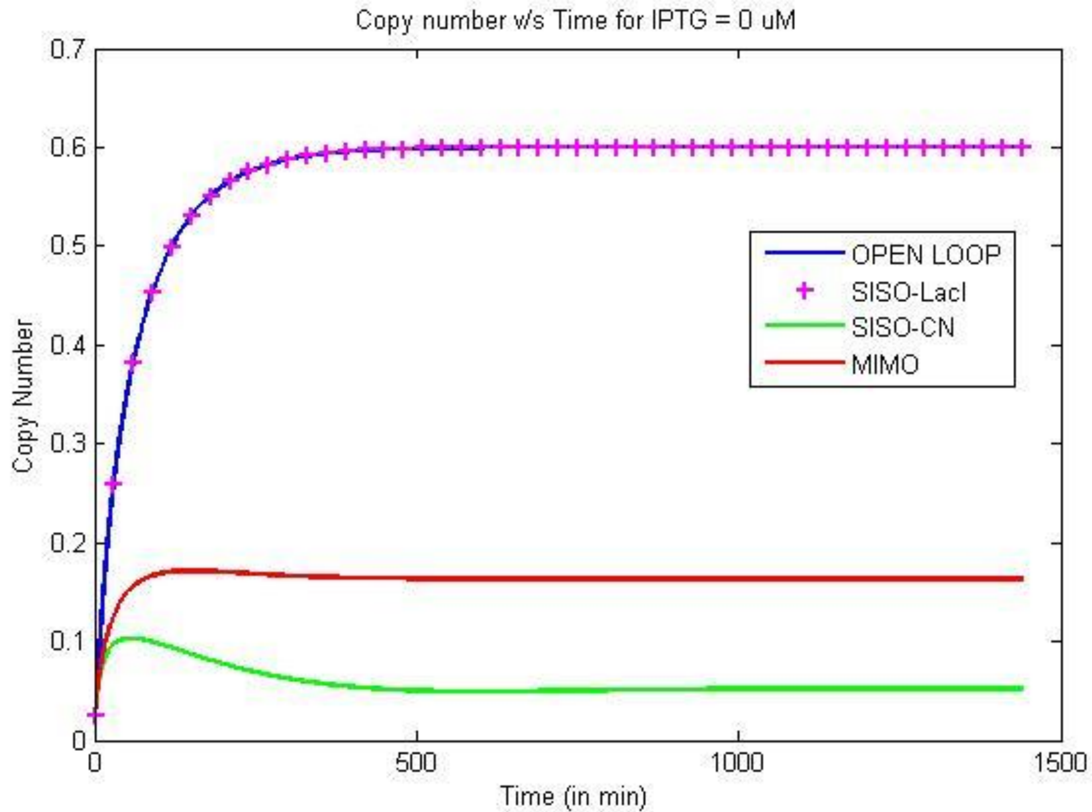
The simulated results for the 4 strains are discussed below. The results are characterized in three parts; growth on non lactose media, subsequent growth on lactose and then we show how the multiple feedback helps in increasing growth rate of cells with reduced burden for production of proteins.

Growth on non Lactose media

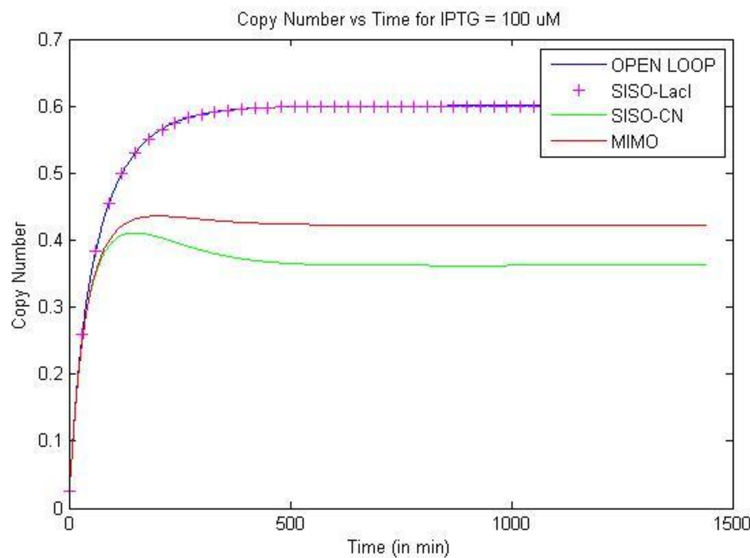
Analysis for Copy Number

Dynamic Profile

Here we see that in Strain 1 and 2 due to lack of control on plasmid number, the copy number saturates to a value as governed by the cells capacity. In strain 3 there is a feedback associated with plasmid replication and this leads to saturation at a lower value. This occurs because as copy number increases LacI also increases and therefore the amount of free plac promoter responsible for replication decreases. In Strain 4 with multiple feedbacks, copy number saturates at higher value than strain 3, mainly because amount of free plac promoter increases. This could be explained by the fact that LacI produced is lower in amount than that produced in strain3 hence lesser LacI available for binding with the promoter.

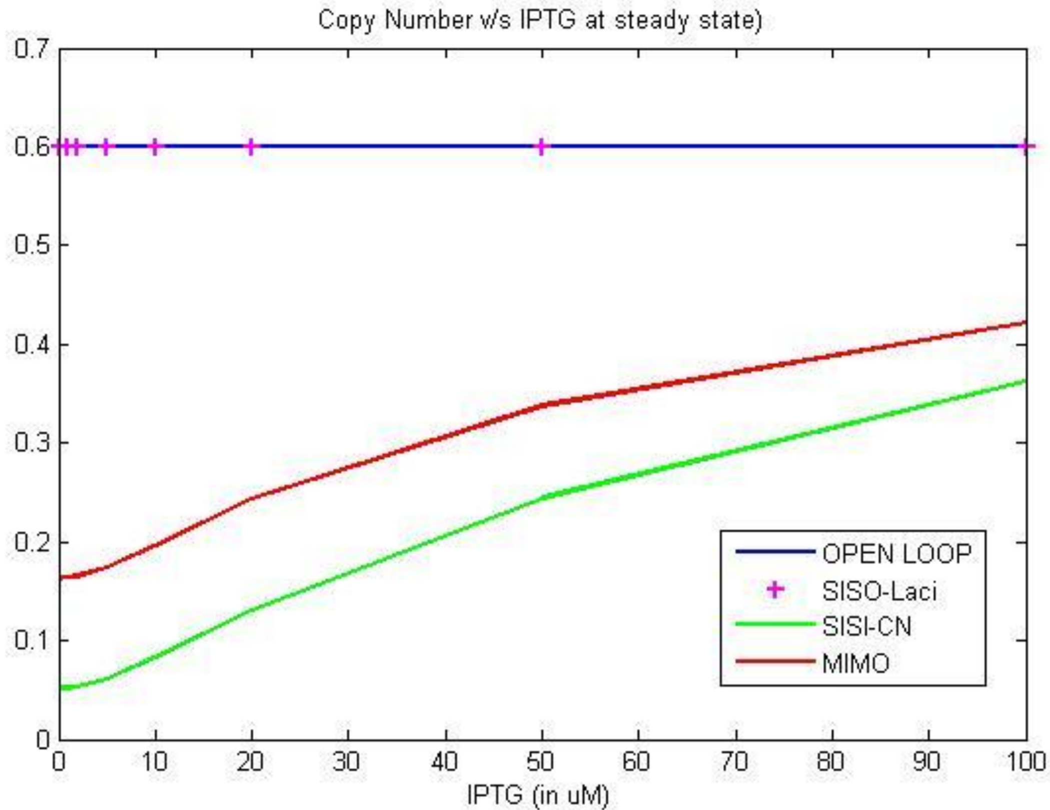


About the responses with time, all the strain starts with the same slope. As time increases the slope of the curve tends to decreases to zero. It is seen in some strain that the slope may become negative and then increase back to zero. The initial same rate is due to the fact that cells start with same initial state of zero LacI. As LacI starts to increase the replication rate of plasmid slows down and eventually goes to zero.



Here we see the results as same as above except the fact that in strain 3 and 4 copy number saturates at an higher value. Higher IPTG means amount of LacI-IPTG complex increases and hence lower concentrations of free LacI to bind to plac promoter. This implies that control of the strain starts to go away and the strain 3 and 4 start behaving like an open loop.

Steady State Copy Number v/s IPTG

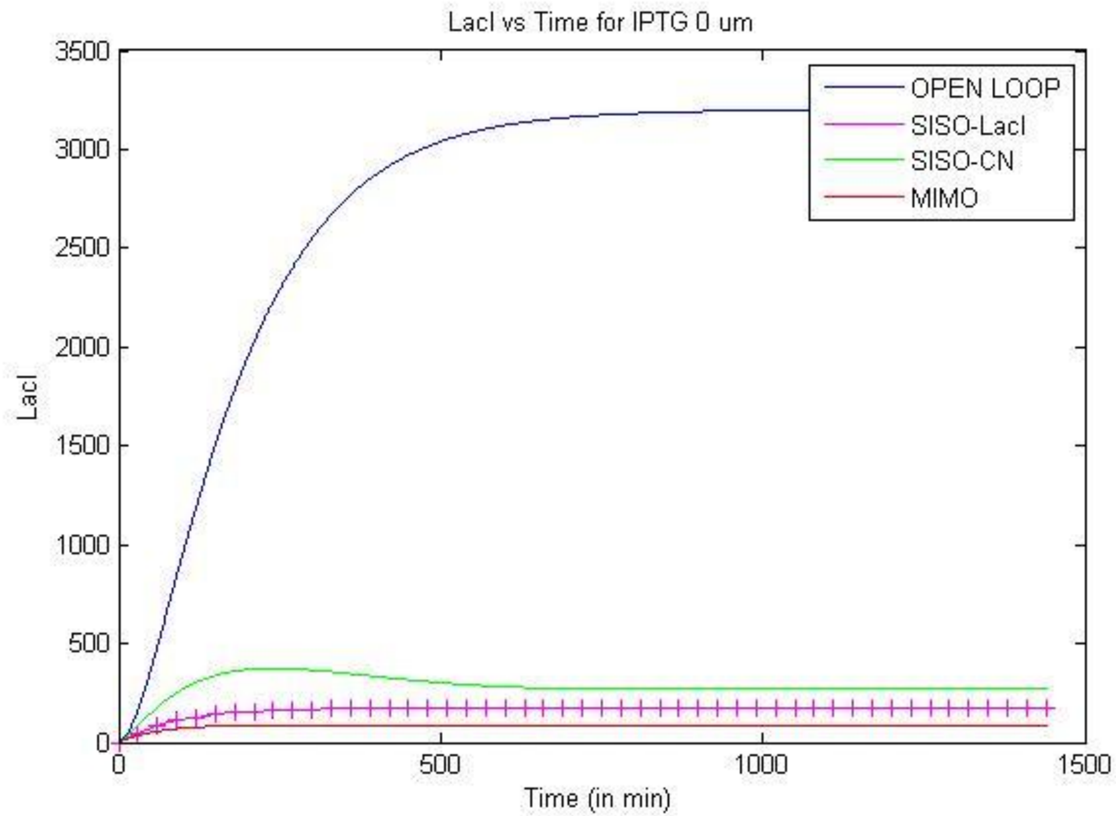


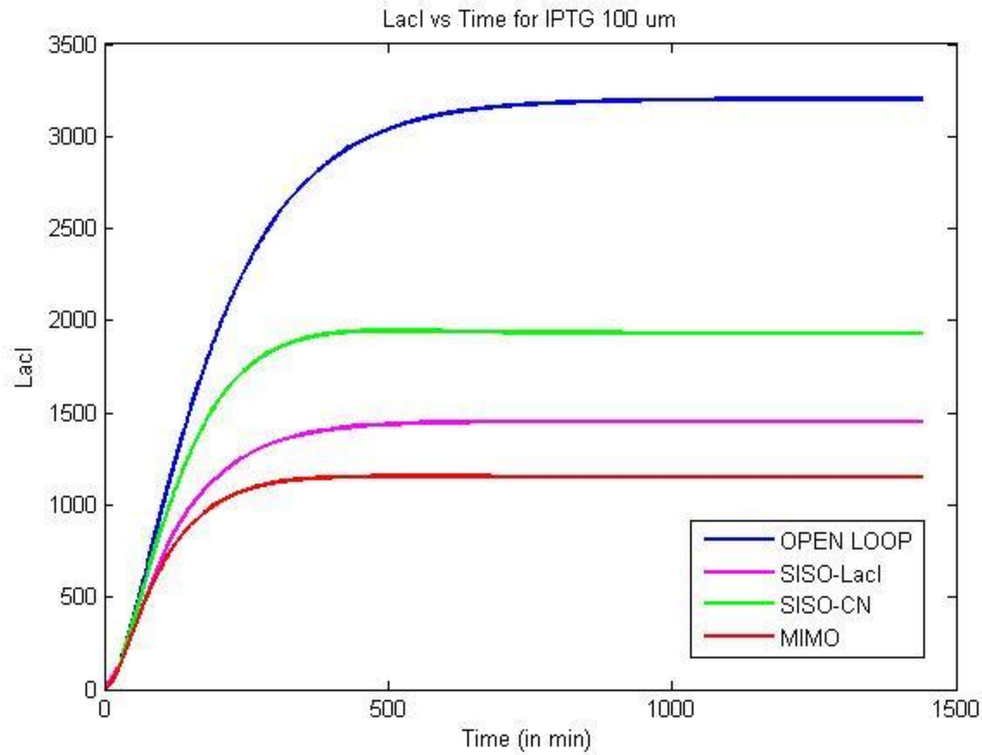
Here we see that Strain 1 and 2 are independent of IPTG concentrations. In strain 3 and 4, we see that as IPTG increases the copy number also increases. It can be inferred from the graph that as IPTG goes to a large value copy number of strain 3 and 4 also tend to behave like strain 1.

Analysis for LacI

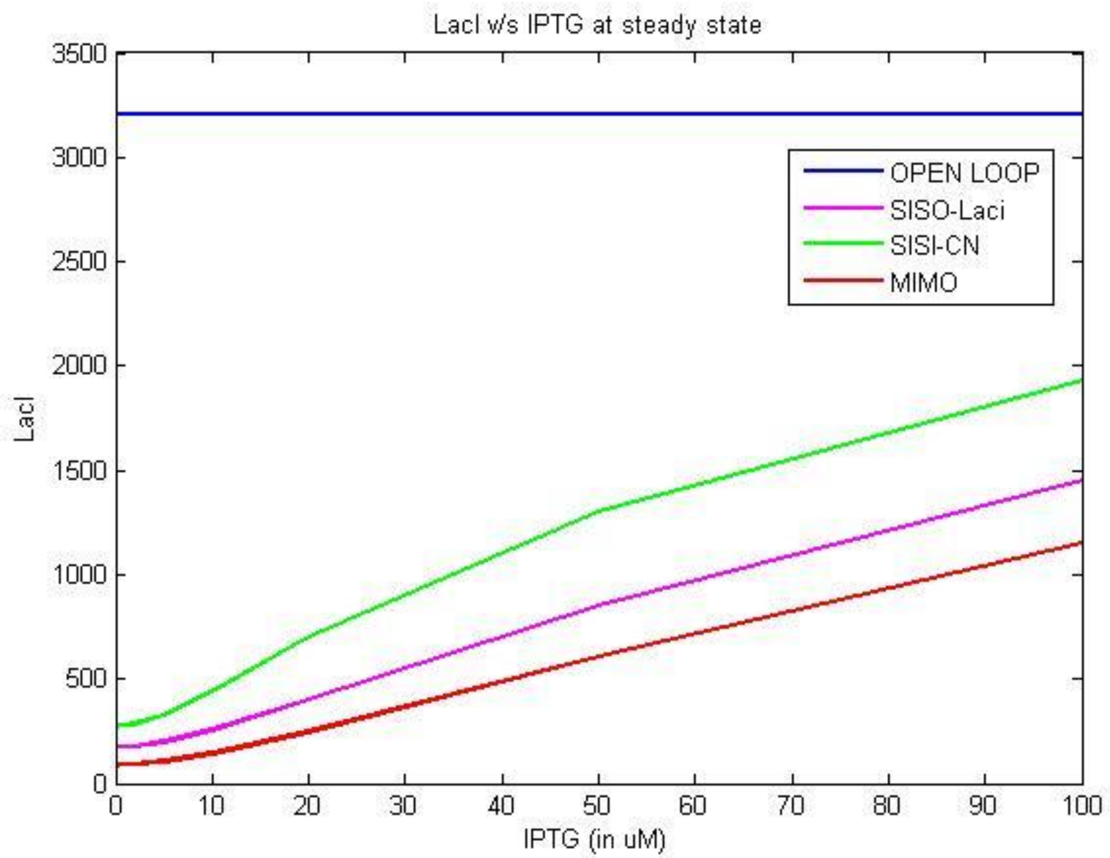
Dynamic Profile

Strain 1has the highest concentration of LacI at the steady state. Strain 4 has the lowest concentration of LacI as expected. Strain 3 has lower concentration of LacI than strain 1; because copy number in strain 3 is less than that in strain1. Also as IPTG is increased, LacI concentration of all the strains except strain 1 increases. Same as copy number, the LacI-expression rate is same initially and gradually decreases to zero for all strains. The slope changes to zero first for strain 4 and last for strain 1. This implies that LacI response is faster in strain 4. The graphs are plotted on the next page.


LacI vs Time for IPTG 0 um
LacI
Time (in min)
OPEN LOOP
SISO-LacI
SISO-CN
MIMO
3500
3000
2500
2000
1500
1000
500
0
0
500
1000
1500

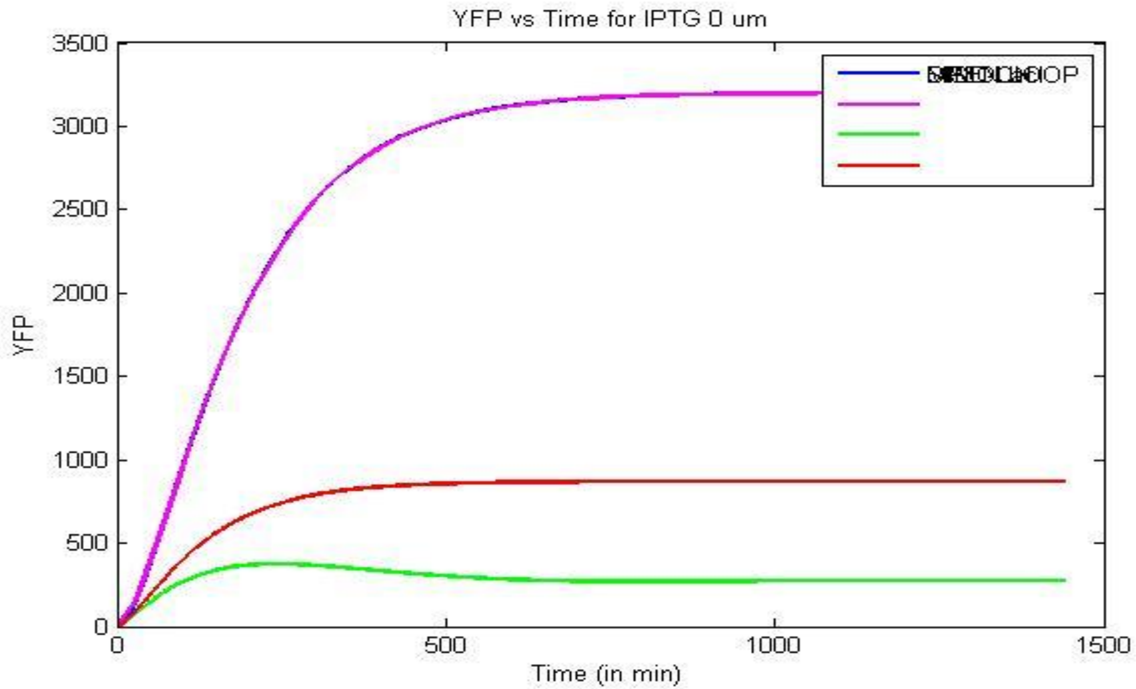

LacI vs Time for IPTG 100 um
LacI
Time (in min)
OPEN LOOP
SISO-LacI
SISO-CN
MIMO
3500
3000
2500
2000
1500
1000
500
0
0
500
1000
1500

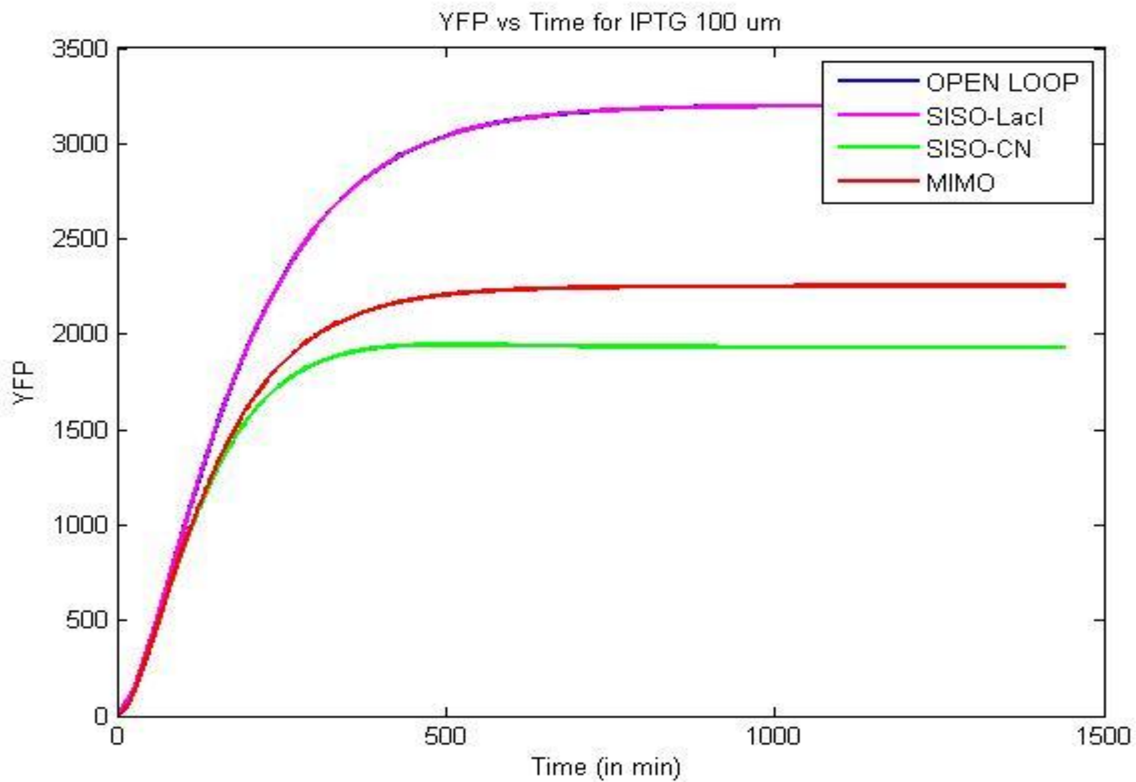
Steady State LacI Concentration vs IPTG



LacI expression increases for strain 2, 3 and 4 with increase in IPTG as expected. Strain 1 shows no dependence on IPTG concentration.

Analysis for YFP





YFP profile exactly states what said in for copy number. This is because of the amount of yfp produced is directly proportional to copy number. This is also the fact we have yfp as it would help quantify the plasmid copy number.

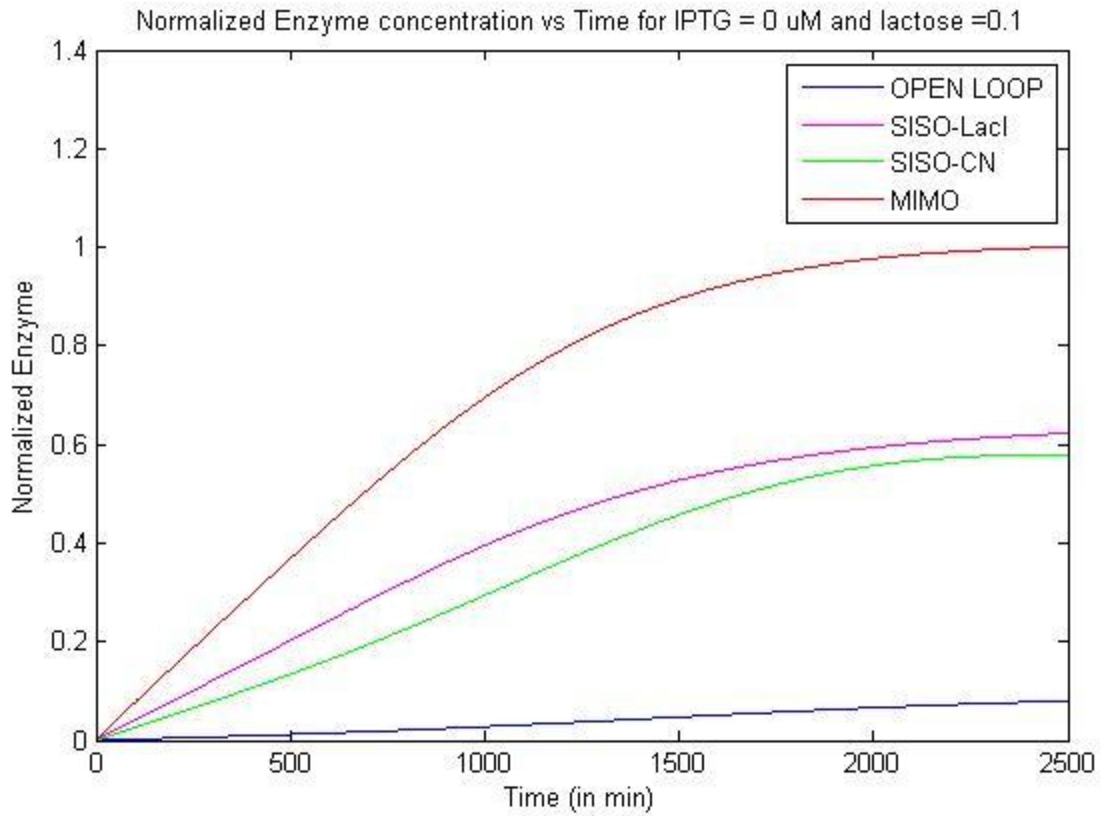
Growth on Lactose Media

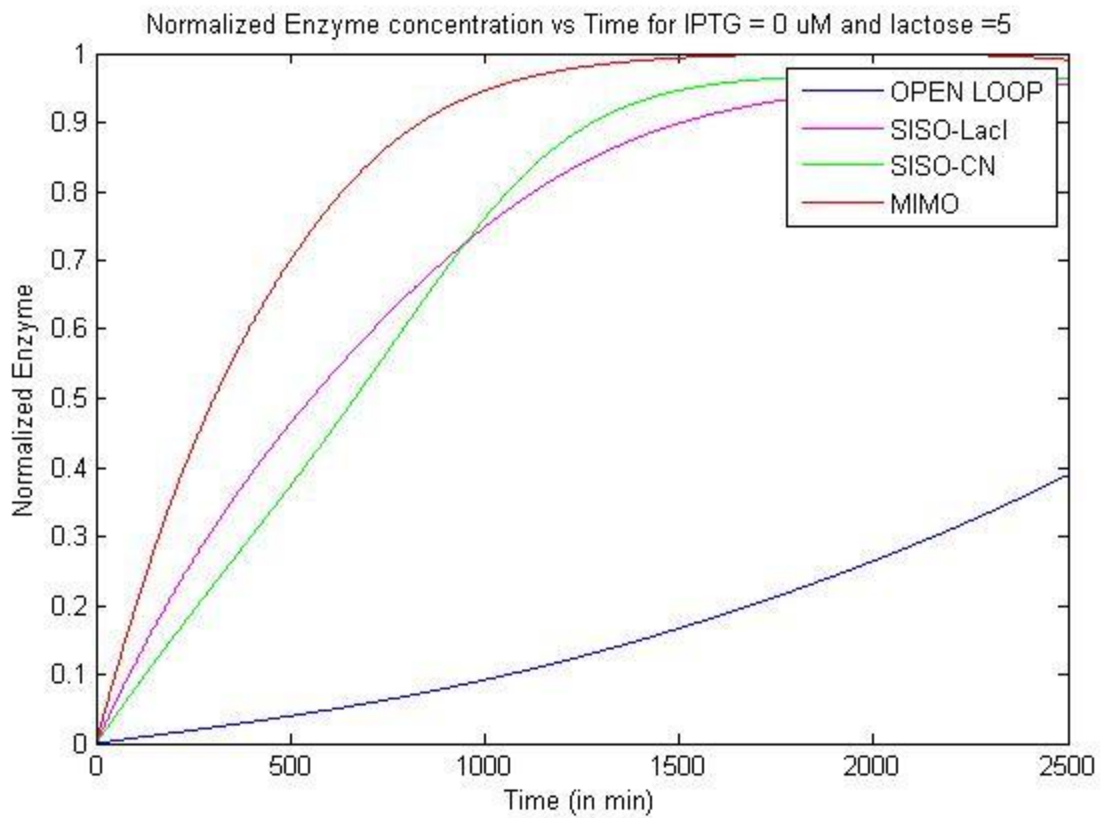
Now we study how the strains grown for 24 hours on other media and different values of IPTG were now transferred to different lactose amount with same IPTG concentration. This was used to study how β-gal varied with time and how growth rate changes with Lactose and IPTG for the four strain.

Analysis for β-gal/β-gal ^{max}

Dynamic profile

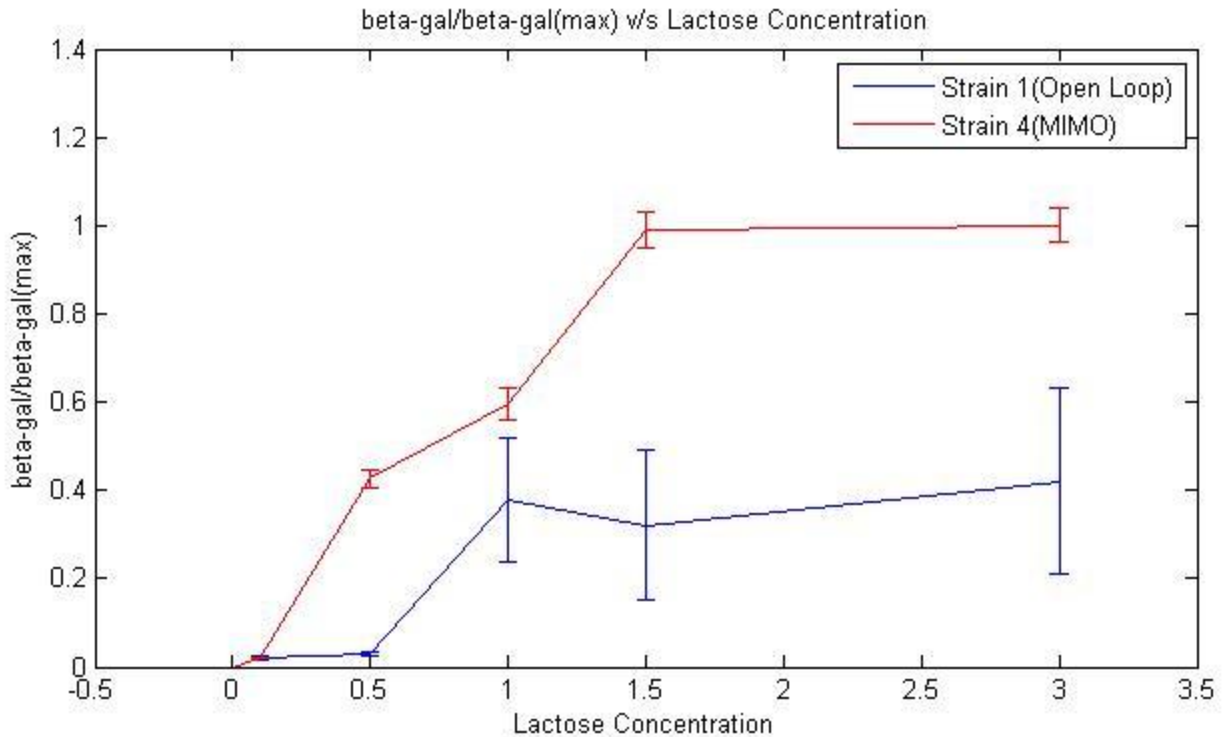
Strain 1 has high concentration of LacI and therefore it has a very low β-gal expression. Strain 4 has the highest β-gal concentration. As amount of lactose is increased the profile of strain1, 2 and 3 tend to the values of strain 4. Similar results were obtained for IPTG.


Normalized Enzyme concentration vs Time for IPTG = 0 uM and lactose =0.1
Normalized Enzyme
Time (in min)
OPEN LOOP
SISO-LacI
SISO-CN
MIMO
0
0.2
0.4
0.6
0.8
1
1.2
1.4
500
1000
1500
2000
2500


Normalized Enzyme concentration vs Time for IPTG = 0 uM and lactose =5
Normalized Enzyme
Time (in min)
OPEN LOOP
SISO-LacI
SISO-CN
MIMO
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
500
1000
1500
2000
2500

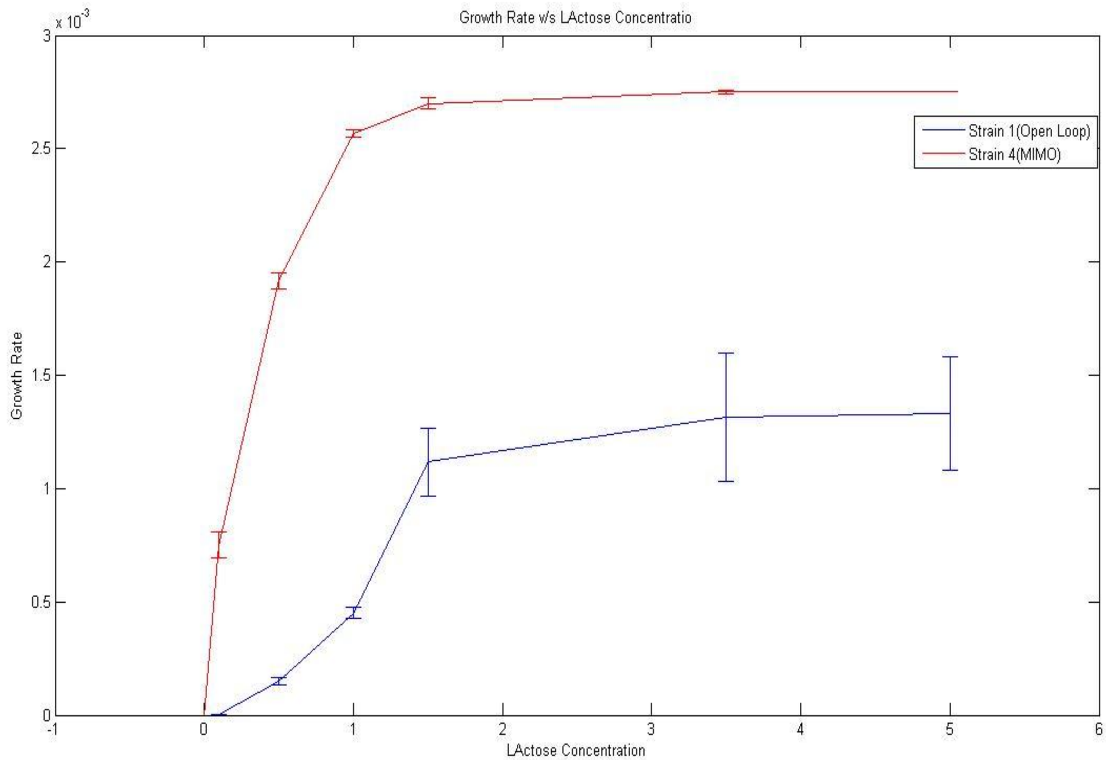
Normalized βgal vs Lactose

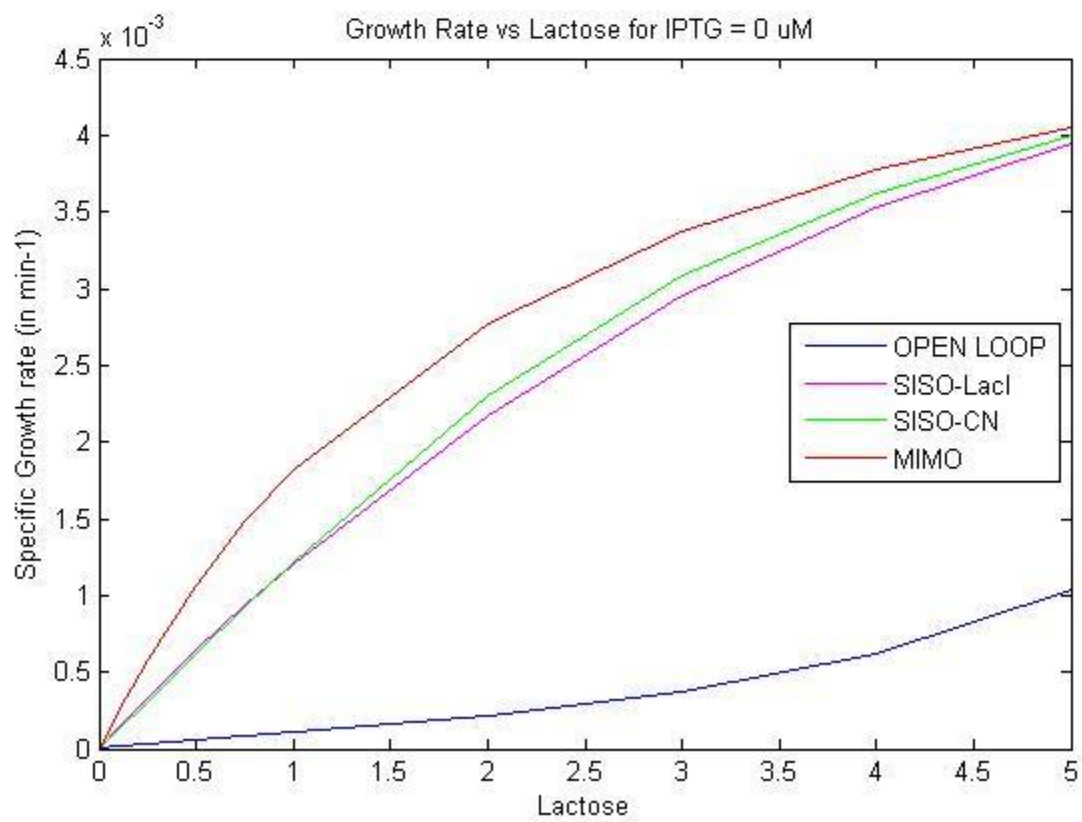
For various values of Lactose concentrations, steady state values of normalized enzyme concentrations are plotted with Lactose. Experiments done with strain 1 and strain2 show similar profiles. The experiments showed high variance for the data in strain1 but a comparatively lower value in strain 4. The shapes of the expression profiles match the simulated profile but due to unavailability of exact values of parameters used in the differential equations, it is hard to correlate them accurately to experimental data.



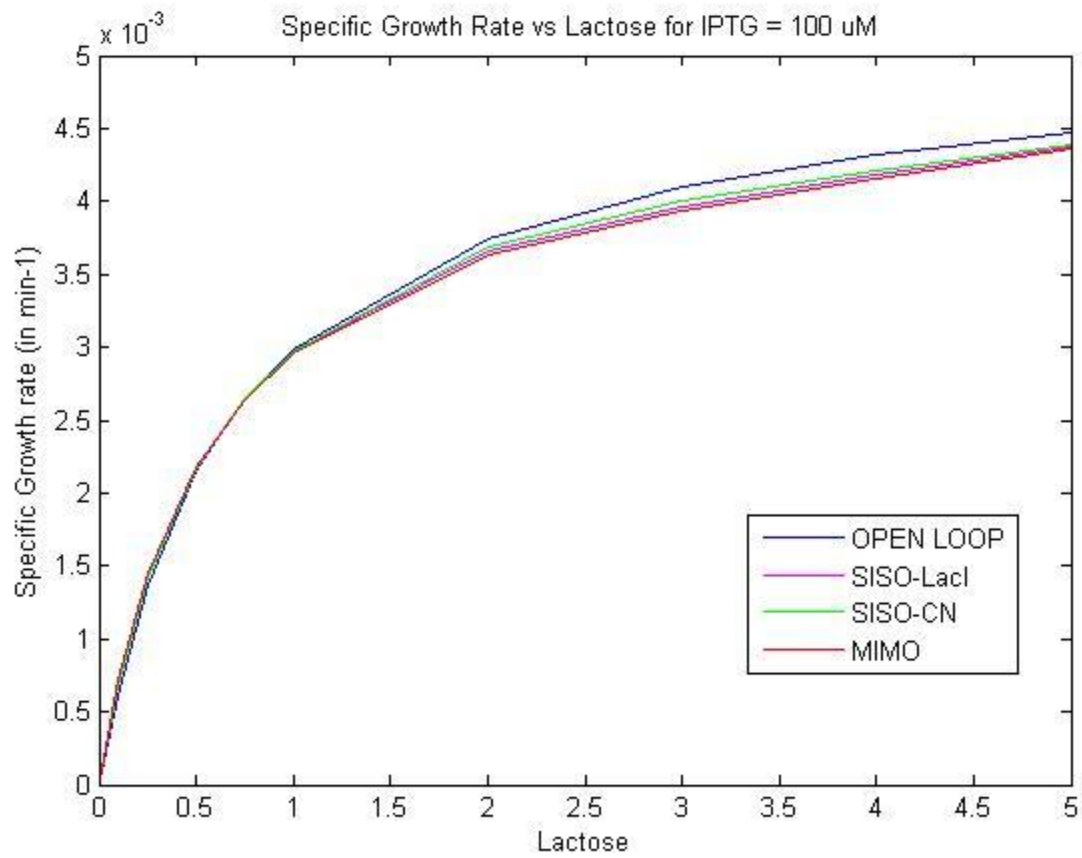
Growth rate

For various lactose concentration, specific growth rate was analysed. Again, the shape of growth rate correlate with that obtained from experiments. Growth rate of strain 1 is the least. Growth of strain 2 and 3 are nearly the same for all Lactose concentrations. Growth of strain 4 is the highest and at higher lactose strain 2 and 3 also tend to merge with strain1. At higher IPTG growth rate of all strain increases to that of strain 4, mainly because of the fact that free LacI decreases and free plac promoter increases, resulting in higher β-gal production.





Growth Rate vs Lactose for IPTG = 0 uM
x 10^-3
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Specific Growth rate (in min-1)
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
Lactose
OPEN LOOP
SISO-LacI
SISO-CN
MIMO


Specific Growth Rate vs Lactose for IPTG = 100 uM
x 10^-3
Specific Growth rate (in min-1)
Lactose
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
OPEN LOOP
SISO-LacI
SISO-CN
MIMO

Burden-Growth Rate relationship:

We now define the cost that cell has to pay for growing in the Open loop and MIMO strains. In open loop, cell overproduces plasmid, LacI, Yfp and β-gal.
In MIMO, it optimizes this load to as low as possible and is able to grow at higher specific growth rate. We define the burden on the cell by 2 different definitions:
Definition 1:

$$Burden\,(defn.1) = \frac{\beta gal + LacI + C + YFP}{\beta gal^{max} + LacI^{max} + C^{max} + YFP^{max}}$$

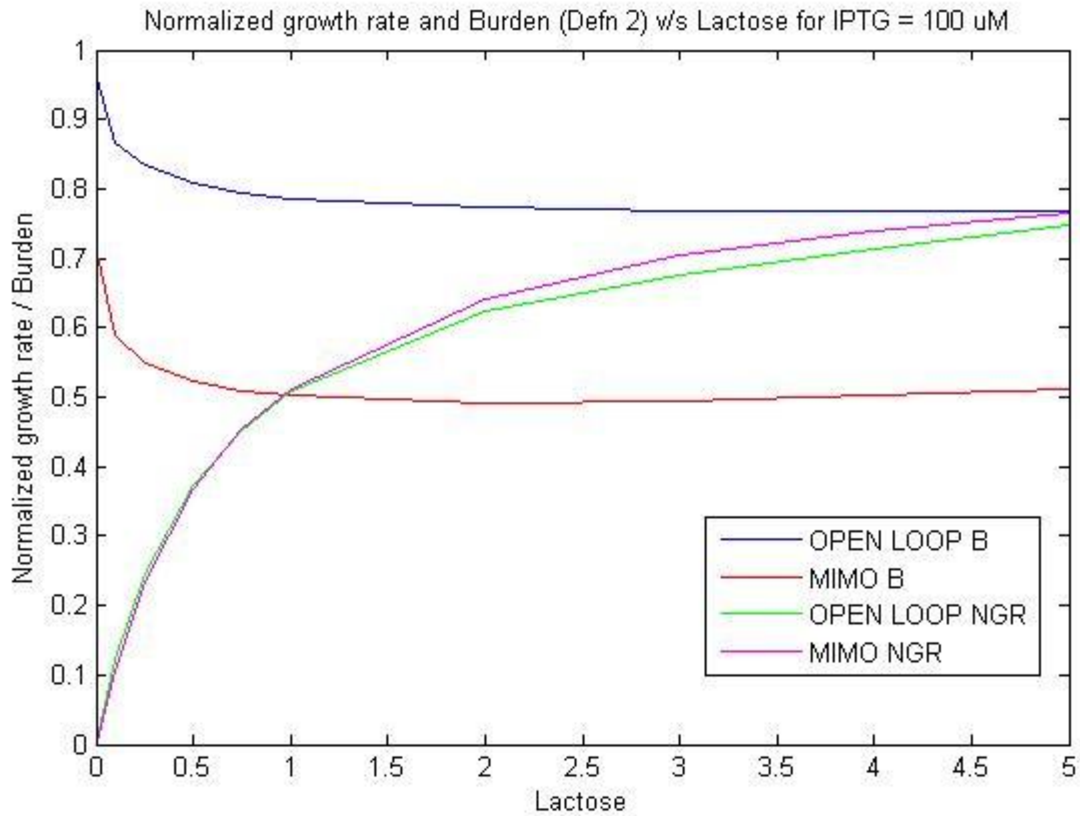
Here all maximum values are the maximum amount of the protein or plasmid produced by mutant strain. Other definition used for Burden is
Definition 2

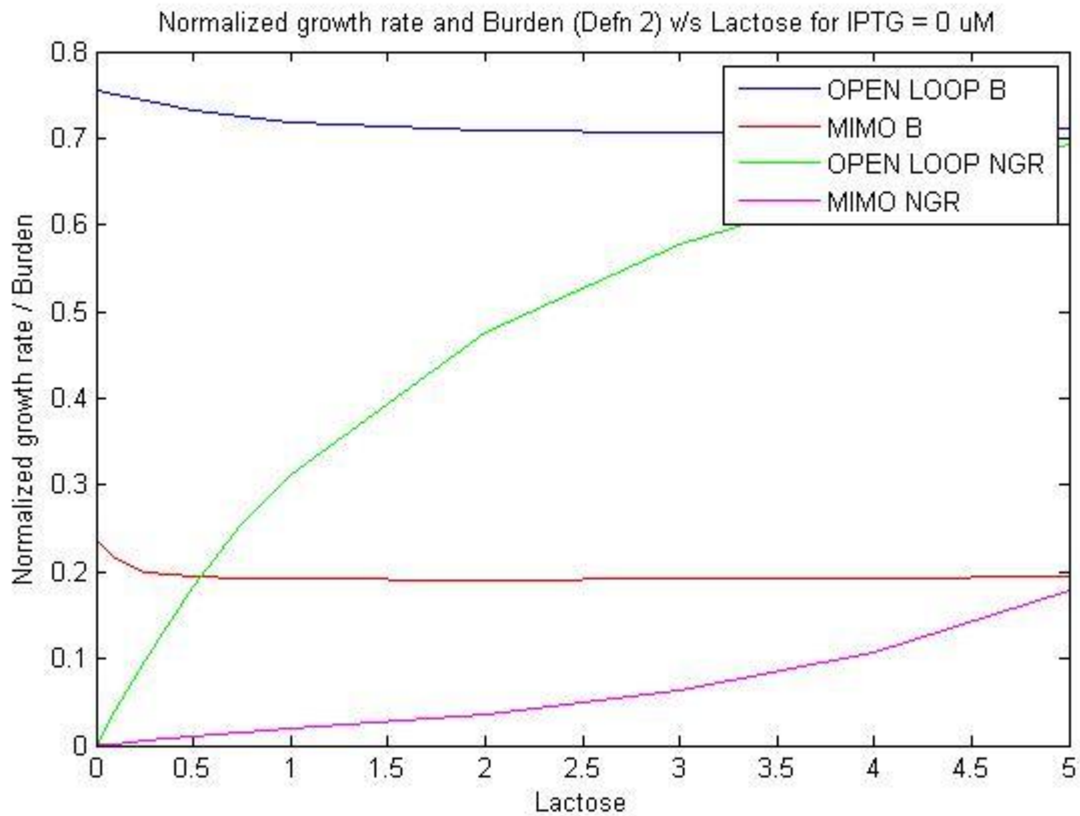
$$Burden(defn.2) = \frac{\frac{\beta gal}{\beta gal^{max}} + \frac{LacI}{LacI^{max}} + \frac{C}{C^{max}} + \frac{YFP}{YFP^{max}}}{4}$$

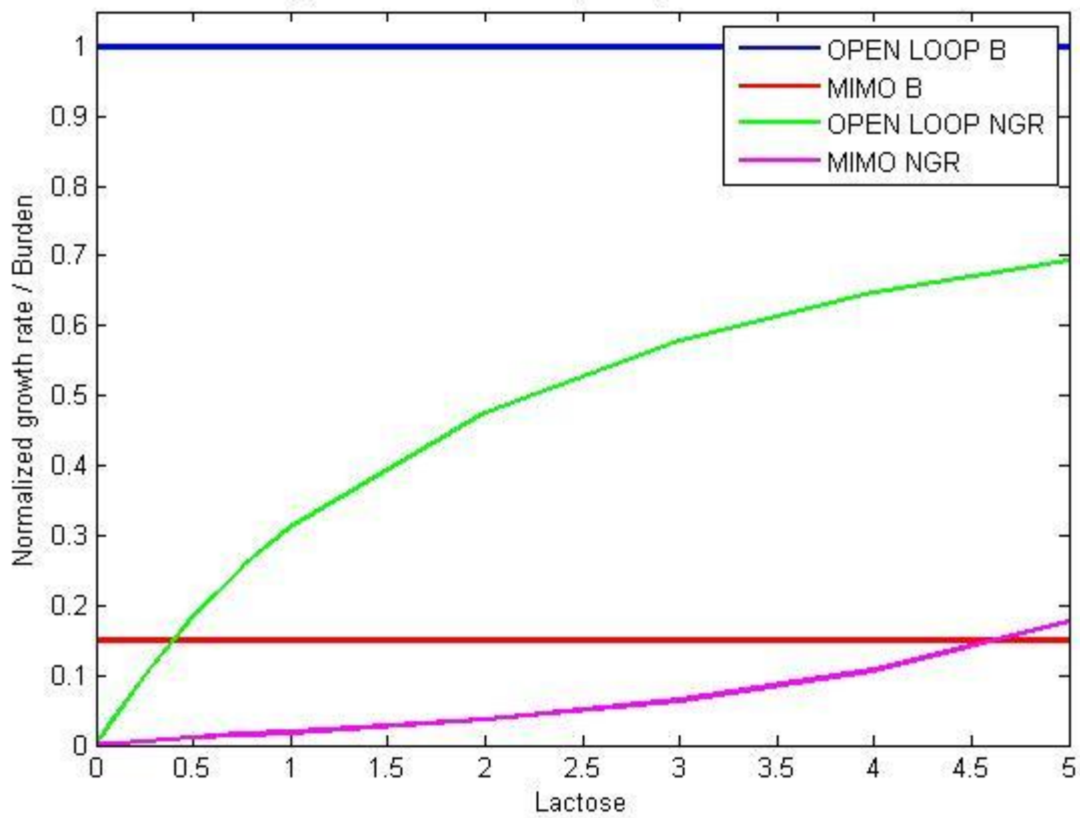
The Normalized growth rate is

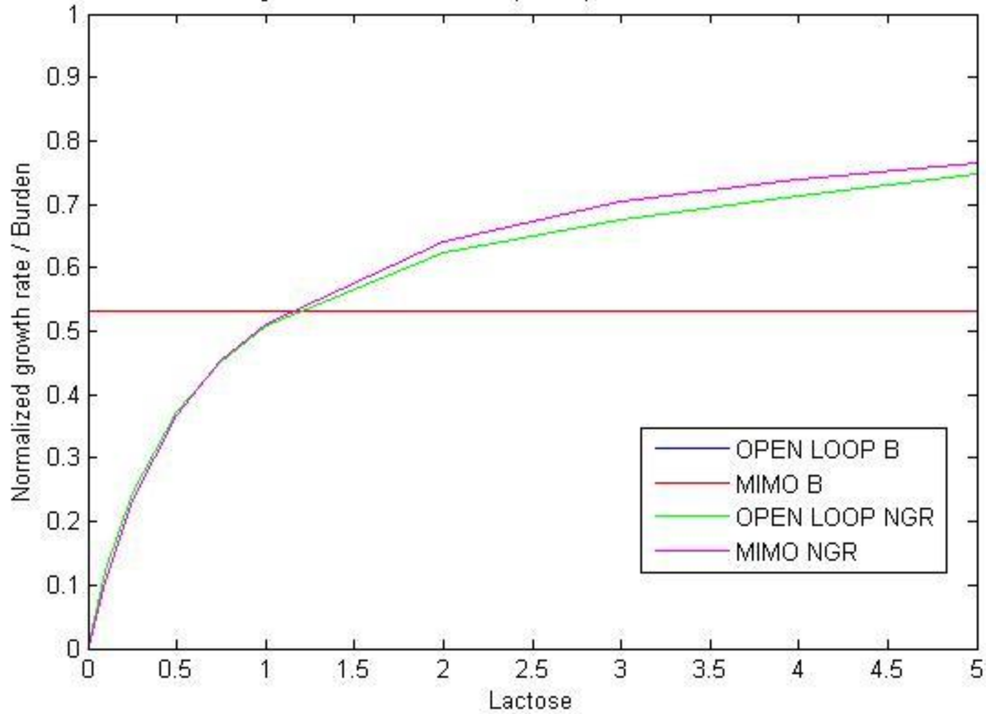
$$Normalized\ growth\ rate = \mu/\mu^{max}$$

Plots of Burden and Normalized growth rate at various Lactose show, that the strain 4 has been able to successfully reduce its burden and optimize its growth, whereas in strain 1 the overproduction occurs at the cost of reduced growth rate. At higher IPTG when MIMO strain behaves like Open loop it could be seen that burden on the cell increases.
For cells growth, cell has to produce the β-gal. In order to produce β-gal, our mutant strains have been forced to produce LacI and YFP protein. Due to this, cells now have only a part of machinery working for cell division. This is the burden that cells have to pay for growing at a particular specific growth rate.


Normalized growth rate and Burden (Defn 2) v/s Lactose for IPTG = 100 uM
Normalized growth rate / Burden
Lactose
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0.5
1.5
2
2.5
3
3.5
4
4.5
5
OPEN LOOP B
MIMO B
OPEN LOOP NGR
MIMO NGR


Normalized growth rate and Burden (Defn 2) v/s Lactose for IPTG = 0 uM
Normalized growth rate / Burden
Lactose
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
OPEN LOOP B
MIMO B
OPEN LOOP NGR
MIMO NGR


Normalized growth rate and Burden (Defn 1) v/s Lactose for IPTG = 0 uM
OPEN LOOP B
MIMO B
OPEN LOOP NGR
MIMO NGR
Normalized growth rate / Burden
Lactose
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0.5
1.5
2
2.5
3
3.5
4
4.5
5


Normalized growth rate and Burden (Defn 1) v/s Lactose for IPTG = 100 uM
OPEN LOOP B
MIMO B
OPEN LOOP NGR
MIMO NGR
Normalized growth rate / Burden
Lactose
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0.5
1.5
2
2.5
3
3.5
4
4.5
5

Conclusions

1. The detailed model was developed to generate the dynamic profiles of the plasmid copy number, LacI, Yfp, β-gal, Lactose and biomass. Using the above model, we are able to correlate the simulation results with the experimentally obtained values.
2. We also see that growth on lactose for strain 4 is highest among the 4 strains with lesser burden on the cell to produce the unnecessarily higher amount of protein for growth.
3. We observe that as lactose concentration is increased within our simulation range, burden of the cell does not change. For strain 4, as lactose concentration increases, the normalized growth rate crosses the burden, indicating that cell has now optimized its growth for the corresponding burden. For strain 1, the increase in lactose does not have any such effect and burden is always above the normalized growth rate. As IPTG increases, burden on strain 4 increases, the growth rate now crosses the burden at an higher value of lactose. Also as IPTG increases growth rate of strain 1 also increases.