University of Groningen
Modelling and Simulation
Simulation of Escherichia coli (E. Coli) Growth in Presence of Various Carbon Sources
Group 19

Satyanarayan Nayak, Swastik
s.nayak.1@student.rug.nl
S4151968

Baskaran, Siddharth
s.baskaran.1@student.rug.nl
S3922782

November 8, 2019

Contents

1 Introduction

The *E. Coli* is one of the most common anaerobic bacteria found in the large intestines of warm-blooded animals and the bacteria plays an important role in digesting food. Everyday around 10billion *E. Coli* bacteria is ejected out of the animal body through organic wastes.

They predominantly feed on the Glucose and other sugars like Galactose, lactose etc. Other sugar molecules are only metabolized once the Glucose reserve is completely exhausted. The bacteria tends to divide rapidly when under the influence of Glucose but slows down while consuming other carbon sources[1].

The *E. Coli* adapts to its environment, and when there is an absence of Glucose or Galactose. The *E. Coli* bacteria with a specific enzyme beta-galactosidase will convert available lactose into Glucose and Galactose. But over a period of time if the bacteria colony is provided with rich Glucose supply the number of enzyme produced by the *E. Coli* cell diminishes drastically. A lack of Glucose will cause the bacterial growth to slow down and possible death of few cells due to starvation. The *E. Coli* colony will start synthesizing the beta-galactosidase enzyme under the presence of lactose molecules, and the *E. Coli* adapts to the new environment[2].

2 Literature Survey

Metabolism of Ecoli bacteria under glucose environment: Ecoli is able to metabolize a variety of sugars and convert them into energy, but only under the abundance of glucose exhibits a faster growth rate in comparison to other sugars. When the glucose supply is exhausted the Ecoli will slowly shift towards a consuming lactose or other sugar sources. under the influence of lactose the growth rate of the Ecoli is significantly decreased when compared to the growth rate in a glucose rich environment[1][2].
The Ecoli requires a special enzyme beta-galctosidase or lactase to break down the lactose molecules into glucose and galactose molecules, which is later consumed for it sustenance. When the Ecoli is under the influence of Glucose, the amoung of lactase enzyme it produces will significantly decrease, this enzyme is required in thousands to break down the lactose into glucose and galactose molecules. When the Ecoli is metabolising the lactose rather than glucose, it will start generating the lactase enzyme. There is also a slight lag phase before the Ecoli switches from metabolising the glucose molecules to lactose molecules [2].

3 Project Goals

- Simulate the division of the *E. Coli* bacteria under the presence and absence of carbon sources and observe the impact. The simulation will take into consideration that the bacteria will divide at 20min intervals (relative or absolute scaling can be used).
- Simulate the *E. Coli* cells when the carbon sources have depleted and lactose is the only energy source available. To process Lactose as its unique source of energy in the absence of Glucose, the *E. Coli* needs several thousand copies of the beta-galactosidase or lactase enzyme in-order to break down the lactose into glucose and galactose molecules. In the presence of Glucose the enzyme beta-galactosidase in the *E. Coli* cell will diminish to a very low count of approximately 10. The beta-glactosidase is an inducible enzyme, it is only synthesized by the *E. Coli* in the presence of lactose molecules[2].
- Simulate stable cells that does not go extinct.
- Simulate the cells only under the presence of lactose source
- simulate the cells under only glucose and galactose as the food sources.
- Simulate the cells where constant supply of glucose and galactose is provided.

4 Equation modelling

The concept and the equations to simulate this project are currently in an early stage and will be subject to change as the research on the topic progresses.

The differential equations to simulate the cells under the influence of Glucose and Galactose are relatively straight forward as shown below, The equations described here and the actual implementation might appear different, but they are conceptually the same.

Calculate the resource available after the *E. Coli* cells consumes the resource

$$R = R - Ec * Rc \tag{1}$$

```
where, R - Resource/Available Resource
       Ec - \textit{E. coli} Count
       Rc - Resource Consumption
```

Equation to check for shortage of Resources, when RS $\neq 0$

$$Rs = min(0, R - (Ec * Rc)) \tag{2}$$

```
where, Rs - Resource Shortage
       Rc - Resource Consumption
```

Equation to calculate partial starvation and partial division of the cells under shortage of food, but not Zero

$$Ps = Rs/Rc \tag{3}$$

```
where, Ps - Partial starvation/starvation at abs(Rs) = Ec * DR
```

Partial cell division equation, when there is resource shortage

$$Pd = (Ec - Ps) * Dr \tag{4}$$

```
where, Pd - Partial division
```

Complete starvation equation where there is zero resources available,

$$Cs = Ec * Df \tag{5}$$

```
where, Df - Death factor (percentage)
       Cs - Complete starvation
```

Ecoli cells cell division equation

$$Ec = (Ec * Dr) - Ps - Cs \tag{6}$$

```
where, Ec - Ecoli Count
       Dr - Division Rate
       Ps - Partial Starvation
       Cs - Complete Starvation
```

Total Resources

$$R = Gl + Ga \tag{7}$$

```
where, Gl - Glucose count
       Ga - Galactose count
```

Glucose consumption equation

$$Gl = Gl - Ec * Rc + LMGL \tag{8}$$

```
where, Rc - Resource consumption
```

Galactose consumption equation

$$Ga = Ga + min(Gl, 0) + LMGA \tag{9}$$

Lactose Metabolism Find the maximum Lactose metabolism possible,

$$LM = (LAC/RLAC) \tag{10}$$

```
Where, LM -> Lactose metabolism possible
       LAC -> Lactase or Beat-galactosidase enzyme available
       RLCA -> Minimum Lactase enzyme required to metabolise a single molecule of Lactose
```

Metabolise the Lactose,

$$LMT = \begin{cases} LM, & \text{if } LACT \geq LM \\ LACT, & \text{otherwise} \end{cases} \tag{11}$$

```
Where, LMT -> Lactose that can be metabolised
```

$$LACT = LACT - LMT \tag{12}$$

$$LMGL = LMT * GMF \tag{13}$$

$$LMGA = LMT * GMA \tag{14}$$

```
where, LACT -> Lactose count, in our algorithm we use it only to track the lactose count.
LMGL -> Lactose metabolised to Glucose
GMF -> Factor at which Lactose metabolised to Glucose
LMGA -> Lactose metabolised to Galactose
GMA -> Factor at which Lactose metabolised to Galactose
```

Lactase enzyme is expended after metabolism

$$LAC = LAC - LMT * RLAC \tag{15}$$

```
Where, LAC -> Lactase enzyme
```

Equation to produce the Lactase/beta-galactosidase enzyme under lactose influence,

$$LAC = LAC + Ec * Gf \tag{16}$$

```
Where, Gf -> Generation factor, in our simulation we have set this at 50.
```

The beta-galactosidase is a enzyme that diminishes in a Glucose rich environment and will be abundantly present in a Glucose deficient environment but the presence of lactose. The probability of the enzyme existing in the cells/*E. Coli* bacteria will increase or decrease based on the current environment. A differential equation has to be crafted to simulate this scenario. A probability factor will also be introduced to the equations to simulate the randomness of the death of the *E. Coli*, especially during the absence of desired carbon source (Glucose).

5 Experimentation

Platform used for simulation: Python
GitHub: https://github.com/Swastik-RUG/Modelling-Simulation
Instructions to setup the project and configure the environment can be found on the GitHub. The repository also consists of demonstration of the various results mentioned here.

5.1 Assumptions

The assumptions we made while modelling this system are as shown below,

- **Ecoli Resource Consumption:** Each ecoli cell consumes 0.12 molecules or sugar per iteration.
- **Ecoli Starvation:** The Ecoli colony can survive 2 iterations without food under starvation period, after which 30% of the population will die.
- **Ecoli multiplication time:** Ecoli is able to multiply by a factor of 2 every 20minutes. In the model we have down-scaled the 20min to 2 iterations (i.e each iteration = 10min).
- **Ecoli Growth rate:** Under Glucose the Ecoli multiplies to twice (2x) its count every 20minutes, and when under the influence of only Lactose the growth is dropped to 1.8 times (figure 1) the count every 20 minutes [1]. The Ecoli has a 2 iteration lag period before swapping to metabolise lactose, this is to simulate the lag phase of the biological Ecoli.
- **Lactase enzyme generation:** The lactase is an enzyme that is produced by the E.Coli only under the influence of a Lactose environment, we have assumed that each E.Coli under the influence of Lactose will generate 50 Lactase enzyme per iteration. And 100 Lactase enzyme is required to break down a single molecule of Lactose.

- **Lactose Metabolism:** We have assumed that each Lactose molecule will be metabolised into Glucose and Galactose molecule at a 1:1 ratio.

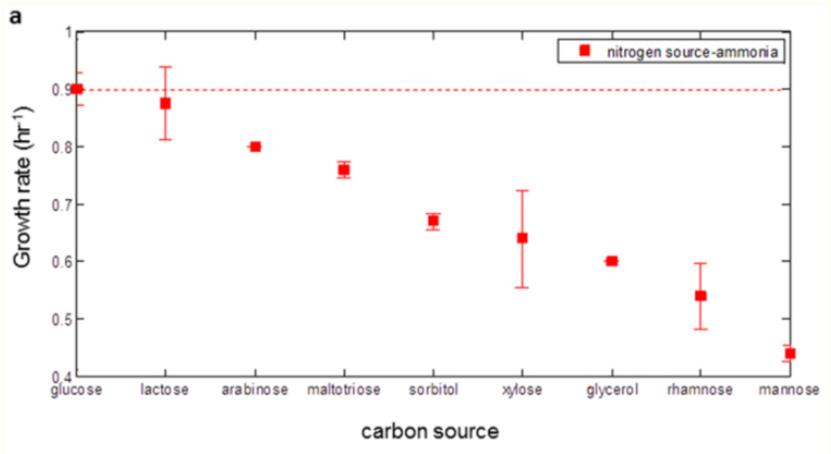

Figure 1: Plot of Ecoli growth under different carbon sources, from [1].

5.2 Independent Variables

Independent variables are the parameters where the experimenter can manipulate to simulate the system. The following are the independent variables provided in our experiment for further experimentation.

- **lactose_count:** An integer value, which can be used to set the initial lactose molecule count in the system.

- **glucose_count:** An integer value, which can be used to set the initial glucose molecule count in the system.

- **galactose_count:** An integer value, which can be used to set the initial galactose molecule count in the system.

- **lactase:** An integer value, which can be used to set the initial glucose lactase or beta-galactosidase count in the system.

- **ecoli:** An integer value, which can be used to set the initial ecoli count in the system.

- **iterations:** Number of iterations the simulation has to be performed.

- **replenish_interval:** If the experimenter wants to simulate an environment where the carbon sources are replenished for specific time-frames. If this value is set to 0, the simulation will not replenish any resources, if it is set to a R, where R ¿ 0; the simulation will replenish the carbon sources in the intervals R,2R,3R.....

- **replenish_glucose:** An integer value, which can be set to indicate the glucose count that has to be replenished at replenish_intervals.

- **replenish_galactose:** An integer value, which can be set to indicate the galactose count that has to be replenished at replenish_intervals.

- **replenish_lactose:** An integer value, which can be set to indicate the lactose count that has to be replenished at replenish_intervals.

- **Sample frame:** An assumption has been made to divide the E.Coli into N buckets so as to simulate an environment where atleast one bucket of E.Coli will multiply, this was performed to simulate a biological E.Coli colony, where cells replicate at every second.

- **Lactase depletion rate:** Lactase enezyme will deplete at a rate of 10% for every Glucose and Glactose cycle.

The independent variables can be set in the **conf.ini**; a sample snapshot of the configuration is as shown below,

```
[custom]
lactose_count = 500
glucose_count = 500
galactose_count = 100
lactase = 200
ecoli = 1
iterations = 100
replenish_glucose=100
replenish_galactose=100
replenish_lactose=100
replenish_interval=2
```

5.3 Observed Parameters

We tested the Ecoli's metabolism under variety of conditions by tweaking the independent variables. Our observations include

- The count of Ecoli cells increasing or decreasing in the system
- The Glucose, Glactose, Lactose consumption by the Ecoli colony.
- The Lactase/beta-galactosidase production or consumption by the Ecoli colony.

6 Results

6.1 Normal Scenario

Configuration used;

```
[Normal]
lactose_count = 150
glucose_count = 300
galactose_count = 200
lactase = 100
ecoli = 1
iterations = 45
```

In this experimentation we are simulating a scenario where the Ecoli colony starts with 1 cell, and has sufficient resources to upkeep a small colony. We are not replenishing any carbon resources in this scenario. From the figure 2 we can observe that at there is a exponential raise in the number of Ecoli cells in the colony as there is sufficient resources available in its immediate vicinity to grow but not sustain the growth of the colony.

The resources are gradually depleting as where glucose is first consumed, followed by galactose.At around 22 iterations the colony reaches its peak, at which there is insufficient resources and the colony starts to starve. After iteration 22 we can observe a steep decline of the ecoli cells, declining at a rate of 30% (death by starvation ratio). And around iteration 23 the Colony starts metabolising the lactose molecules.

We can observe that at iteration 40 the colony starts to gradually increase its numbers by consuming lactose as its new food source. The colony goes extinct due to insufficient resources. The goal of this experiment was to see how colony prioritizing the consumption of its resources.

In this scenario we are trying to simulate an environment with insufficient starting resources and the resources are not replenished at regular intervals. We are keeping the lactase enzyme at a high concentration to eliminate it as a factor for the colony extinction.

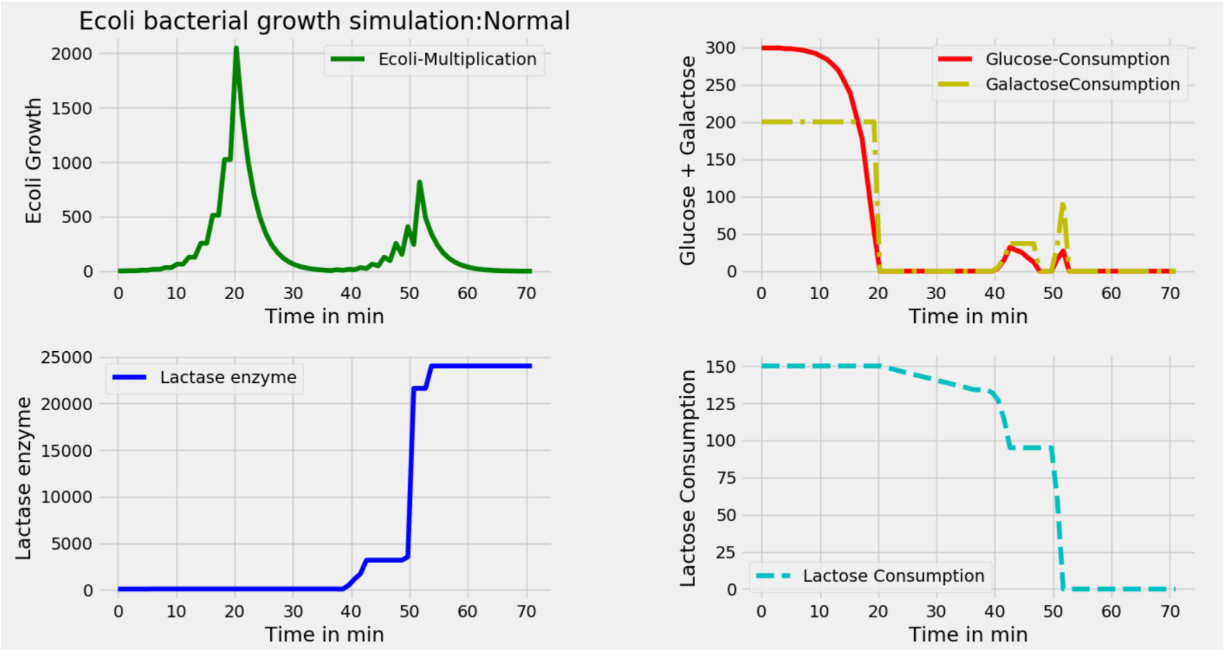

Figure 2: Ecoli colony with sufficient resources, without resource replenishment

6.2 Insufficient Resources

```
[Starvation]
lactose_count = 100
glucose_count = 50
galactose_count = 10
lactase = 2000
ecoli = 1
iterations = 50
```

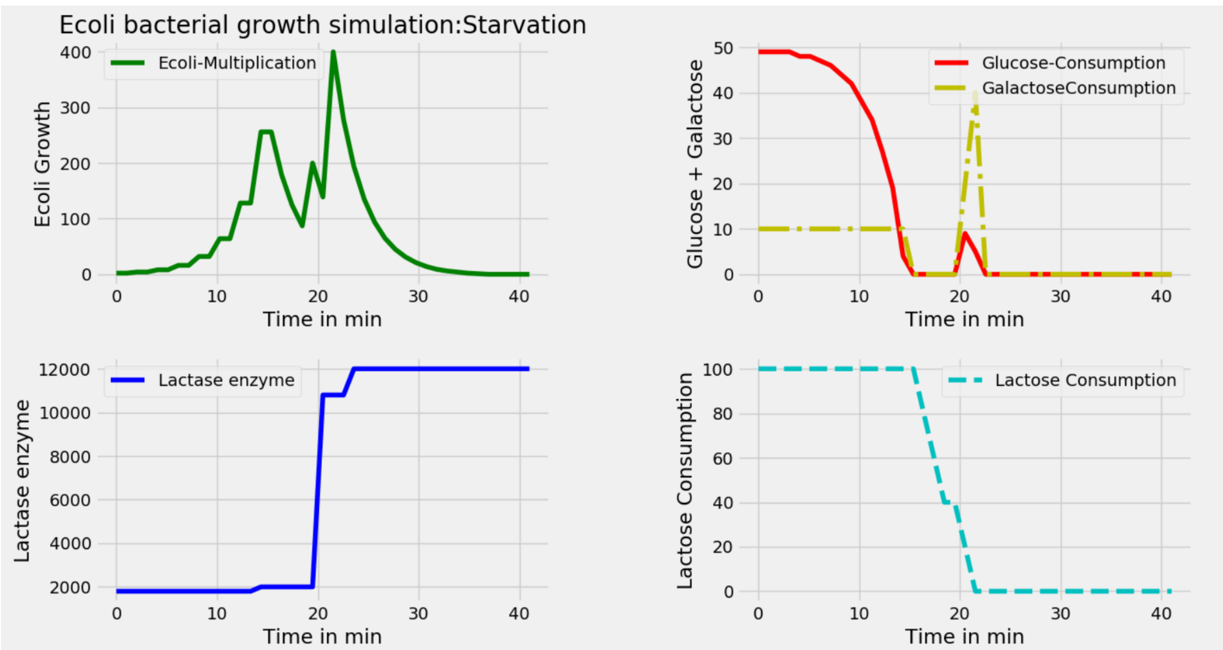

Figure 3: Ecoli colony with insufficient resources and without resource replenishment

At iteration 20 we can observe a spike in the colony as it has adjusted to the new food source (Lactose). The lactose plot shows a steep decline at 17-20, as this is when the Ecoli colony adapted to the lactose environment, from iteration 15-17 the colony was under a lag phase adapting to the Lactose environment. But due to insufficient lactose, the colony goes extinct at iteration 33. The goal of this simulation was to observe if there is any change in the colonies replication when there is insufficient resources. We can clearly see that the ecoli growth subplots from the figure 2 and figure 3 look similar. But the number of Ecoli cells in the colony has a significant difference.
We arrive at a conclusion that the colony will behave similarly regardless of the amount of food resources in its vicinity, the consumption pattern does not vary.

6.3 Lactose Only Environment

In this scenario, we are trying to simulate the behaviour of the Ecoli colony under the absence of Glucose and Galactose. Lactose is the only source available,

```
[OnlyLactose]
lactose_count = 300
lactase = 1000
ecoli = 100
iterations = 50
glucose_count = 0
galactose_count = 0
```

From the below figure 4 we can see that in comparison to the Glucose environment, the Ecoli multiplication here has steep rise and drops and the population growth is low, where the saturation point for the colony was observed at 1000 cell count, and under a glucose rich environment this was realised at 2000 cell count. But the interesting plot of this experiment is the Lactase plot.

From figure 4 sub plot of Lactase, we can observe that until iteration 5 the ecoli were not producing the Lactase enzyme at a significant rate. But since the colony has to survive under only lactose, lactase enzyme is produce at a rapid rate. The Colony went extinct due to the shortage of lactose supply. This is a adaptation nature of the Ecoli which is depicted in the book Molecules, Dynamics Life: An Introduction To Self-Organization of Matter[2].

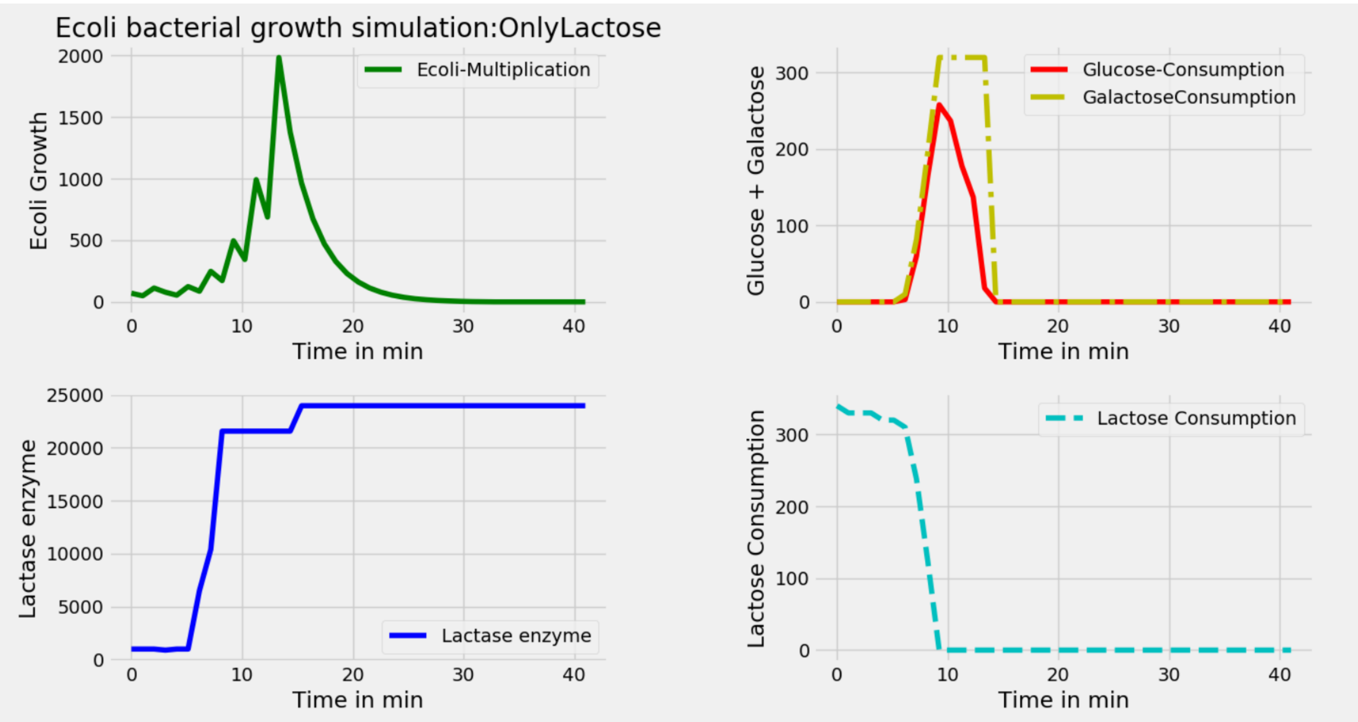

Figure 4: Ecoli colony with only Lactose as resources and without resource replenishment

6.4 Stable Colony

From the previous simulation, we arrived at a conclusion that the colony cannot stabilize until we supply carbon sources at constant intervals or provide the colony with unlimited carbon source. The former is a much realistic approach to solve our problem. We introduced the replenishment cycle to the model, where the food sources are replenished by a certain amount at a certain intervals.
This simulation shows simulates a Ecoli colony that stabilizes over a period of time, carbon sources are constantly fed into the system at every 2 iterations. The configuration used to simulate this is,

```
[stable_colony]
lactose_count = 500
glucose_count = 500
galactose_count = 100
lactase = 200
ecoli = 1
iterations = 60
replenish_glucose=100
replenish_galactose=100
replenish_lactose=100
replenish_interval=2
```

From the above configuration we can see that we are replenishing 100 units of Glucose, Galactose and Lactose at replenish_interval 2.

From the figure 5 Ecoli growth plot, we can see that at iteration 35 the colony reached its peak saturation point, where the colony experienced shortage of food and starved until it reached a stable point at iteration 37.
From the figure 5 Glucose and Galactose plot we can observe spikes at iteration 35, this is when the colony was at its peak and consumed a majority of the resources depleting it to approximately 0. The initial rise in from iteration 0 to 20, is where the replenishment of glucose and galactose was higher than the consumption rate of the Ecoli colony. At iteration 40 the colony stabilizes and does not grow even under the abundance of lactose due to insufficient lactase enzyme. The colony has a lag period of 2 and a replenishing interval of 2, the colony is unable to adapt towards producing lactase enzyme as a constant supply of Glucose is hindering the process.

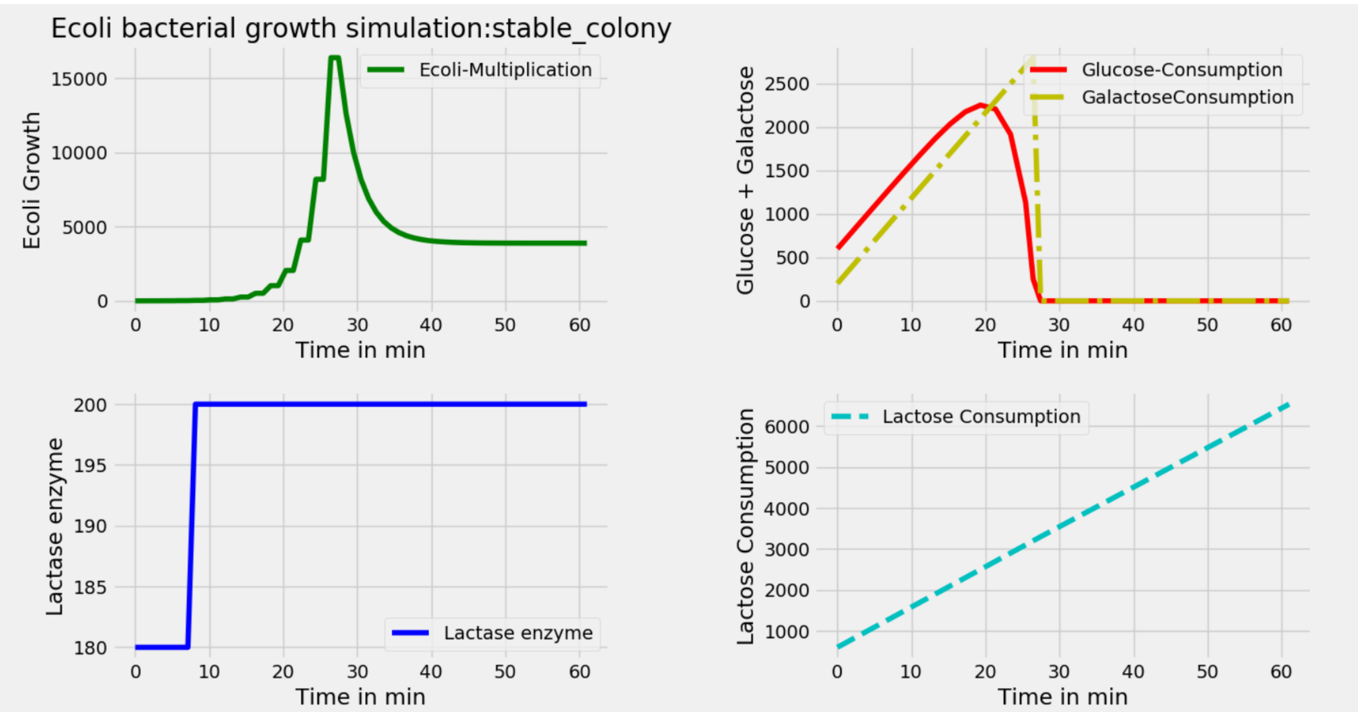

Figure 5: Ecoli colony Stable Colony with regular replenishment

6.5 Stable Colony under Glucose and Galactose

We extended our investigation to see if the Colony can survive on only Glucose and Galctose sources with minimal replenishment of the resource. We used the following configuration to simulate this scenario.

```
[stable_colony_glucose]
lactose_count = 0
glucose_count = 100
galactose_count = 100
lactase = 0
ecoli = 1
iterations = 50
replenish_glucose=100
replenish_galactose=50
replenish_lactose=0
replenish_interval=2
```

This simulation is focused to observe a colony under the absence of lactose and a constant supply of Glucose and Galactose is provided to the system at intervals of 2.
The figure 6 We can see that the ecoli colony exhibits a similar growth pattern as shown in the figure 5, where there is spike in the growth until the colony has reached a saturation point and starts to starve until the colony stabilizes at lower population.
In the figure we can see that the Ecoli colony under glucose is much stable when compared to the previous simulation. A possible reason for this behaviour could be the colony in this simulation does not depend on the Lactase enzyme for metabolism, or is not affected by Lactase. Whereas in the previous simulation, the Lactose was metabolised into Glucose and Galactose which could break the lactose cycle for few ecoli cycles and switch them to a consume only glucose. This assumption is done by observing the two Glucose and Galctose plots of both the simulations.
Note The Glucose and Glactose in this plot after iteration 30 is not zero it is a very small number and the scaling is approximating it to appear as if it is close to 0.

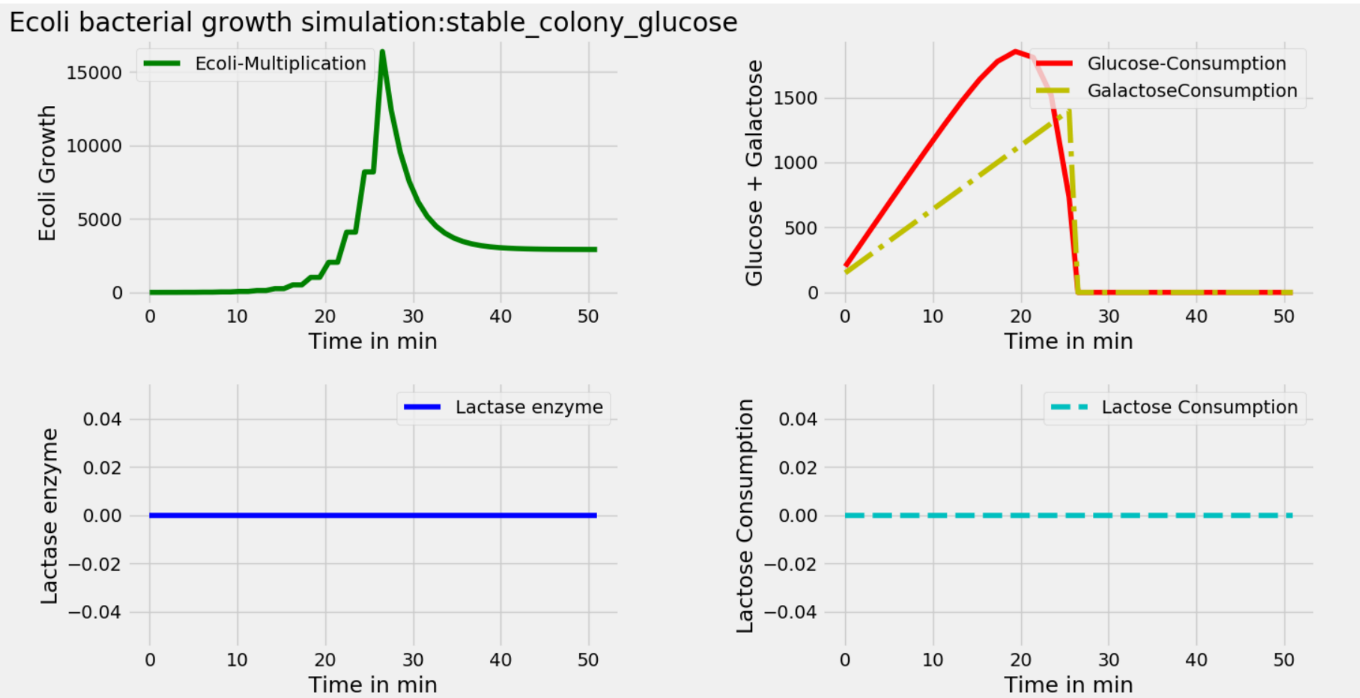

Figure 6: Ecoli colony Stable Colony with regular Glucose and Galactose replenishment

6.6 Stable Colony under Lactose

This simulation was inspired by the previous simulation, the goal of this simulation is to see if the ecoli colony can stabilize only under lactose supply. The configuration used for this simulation are as follows.

```
[stable_colony_lactose]
```

```
lactose_count = 1000
glucose_count = 0
galactose_count = 0
lactase = 1000
ecoli = 1
iterations = 100
replenish_glucose=0
replenish_galactose=0
replenish_lactose=100
replenish_interval=2
```

In this simulation figure 7 the ecoli growth curve resembles to that of figure 6 until it has reached the saturation point. The ecoli colony in figure 7 exhibits a drastic drop in numbers under starvation. At this point of time, the colony had depleted their lactose supply, and due to insufficient lactose the colony starves until it has reached a stable point at iteration 60. At iteration 60 the lactase and lactose plots show a square wave like pattern indicating the resource usage. The ecoli growth curve at iteration 60 also shows a re curing pattern of a stable ecoli colony.

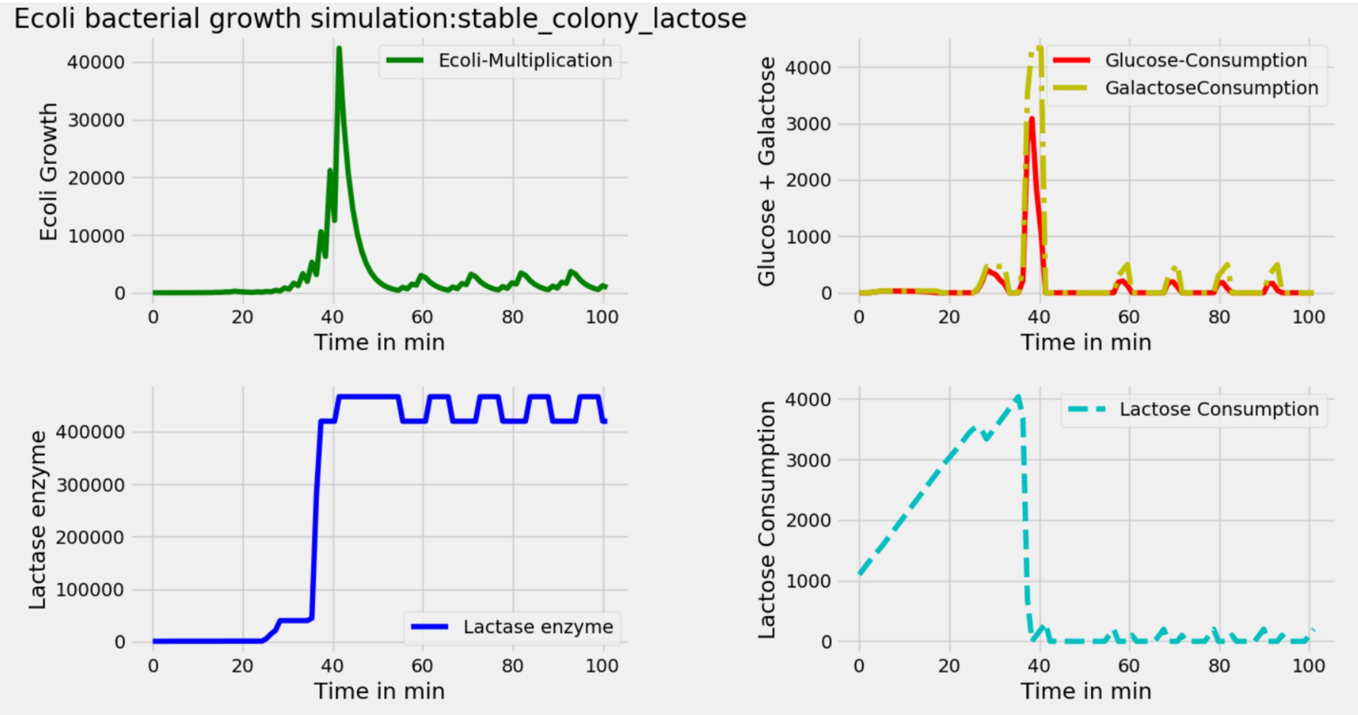

Figure 7: Ecoli colony Stable Colony with regular Lactose replenishment

6.7 Custom Simulation

We have also created a configuration called ”custom”. This can be used to trigger a simulation of your choice on our model. Requirements:

- Python environment
- Clone our repository from git: https://github.com/Swastik-RUG/Modelling-Simulation
- Change the configuration as per the requirements using the ”conf.ini” file in the project source code. ex:

```
[custom]
lactose_count = 5000
glucose_count = 0
galactose_count = 0
lactase = 1000
ecoli = 1
iterations = 60
replenish_glucose=0
```

```
replenish_galactose=0
replenish_lactose=100
replenish_interval=2
```

- Execute the "ecoli_simulation.py" file to observe the results.
- Select "custom" from the list of selections.

7 Conclusion

From the simulations we can conclude that the Ecoli adapts to his environment with ease. Its ability to multiply at a rapid rate ultimately leads to its starvation, but the colony has always stabilised itself at a certain point and has come back from being close to extinct.
The resource consumption and growth pattern of the E.Coli colony showed no significant different under the availability of food and under insufficient food, as show in figure 2 and figure 3.
The E.Coli colony that has a constant supply of Glucose and Galactose attained a impressive stable state figure 6. Whereas the E.Coli colony which was fed with a constant supply of Lactose attained a stable state where the number of E.coli in the colony fluctuated between a constant range (figure 7).
A mix of Glucose, Galactose and Lactose was constantly supplied to the simulation figure 5, we expected the E.COli colony to alternate between lactose and glucose cycles, but the E.Coli colony rather stabilised at a point where it consumed only the Glucose and Galactose, which is similar to the figure 6.

8 Future Enhancement

The variables like resource consumption rate, lactose metabolism ration, sampling frame can be externalized to provide more options to extend the simulation capabilities.
The nitrogen sources can be used as a new source of energy for the Ecoli, as demonstrated in the article "Glucose becomes one of the worst carbon sources for E. coli on poor nitrogen sources due to suboptimal levels of cAMP"[1].

9 Individual Contribution

During the course of our project we have tried our best to share equal work and responsibilities. Due to dependencies and other factors, we were unable to equally divide the work across the project. As modelling and simulation was a new domain for us to explore, we had to invest a considerable amount of time on learning. As a team we have been proactive and have strived to utilize our time and skills effectively.

	Research	**Research Plan document**	**Modelling**	**Programming**	**Final Report**
Satyanarayan Nayak, Swastik (S4151968)	60%	60%	60%	60%	50%
Bhaskaran, Siddharth (S3922782)	40%	40%	40%	40%	50%
	Siddharth was ramping up on his python skills he had to invest a considerable amount of time there.				

References

[1] A. Bren, J. O. Park, B. D. Towbin, E. Dekel, J. D. Rabinowitz, and U. Alon, "Glucose becomes one of the worst carbon sources for e. coli on poor nitrogen sources due to suboptimal levels of camp," *Scientific reports*, vol. 6, p. 24834, 2016.

[2] A.BABLOYANTZ, *Molecules, Dynamics Life: An Introduction To Self-Organization of Matter.*