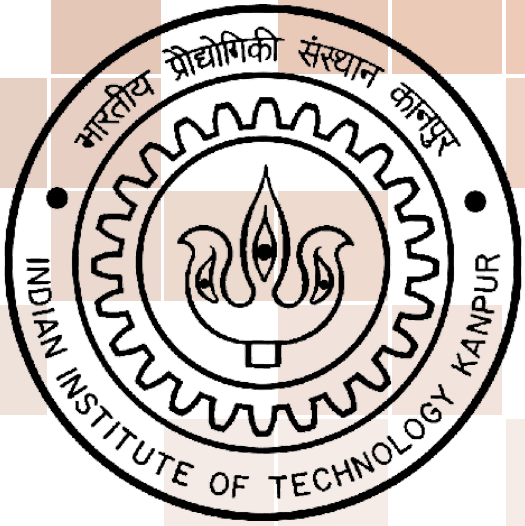

RED PLANET ODDYESY

PROJECT REPORT

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Contents

Part One : Introduction to Martian Conditions

1. Introduction

1.1 Background and Motivation

The quest to understand Mars, our neighboring planet, has long intrigued scientists and space enthusiasts alike. Mars, with its potential for past or present life, offers a tantalizing glimpse into the possibilities of life beyond Earth. The harsh and unique environmental conditions on Mars, including its thin atmosphere, low temperatures, and high radiation levels, pose significant challenges for sustaining life as we know it. However, recent discoveries, such as the presence of water-ice and organic molecules, have fueled interest in exploring the planet's habitability.

1.2 Objective of the Project

The primary objective of this project is to simulate Martian environmental conditions within a controlled laboratory setting and to study the growth and survival of microbes and plants under these conditions. This project aims to contribute to the field of astrobiology by providing valuable insights into the potential for life on Mars and informing future Mars exploration missions.

1.3 Significance of the Study

By replicating Martian conditions, we can gain a better understanding of how life, particularly microbial life, might survive and adapt on Mars. Additionally, studying plant growth under these conditions can provide crucial information for future manned missions that might rely on in-situ resource utilization for food production. This project not only advances our knowledge of astrobiology but also has practical implications for the design and planning of future Mars missions.

1.4 Project Scope

This project involves several key phases, each contributing to the overall goal of simulating Martian conditions and studying biological responses:

- **Research and Literature Review:** Conducting thorough research on Martian environmental conditions based on scientific literature and recent discoveries.

- **Simulation Development:** Developing a Python-based simulation to model the growth of bacteria under Martian conditions.
- **Chamber Design and Construction:** Designing and constructing a physical chamber to replicate Martian conditions, including temperature, pressure, and atmospheric composition.
- **Experimental Studies:** Conducting experiments to study the growth and survival of microbes and plants within the simulated Martian environment.
- **Data Analysis and Reporting:** Analyzing the collected data to draw meaningful conclusions and preparing detailed reports to document the findings.

1.5 Expected Outcomes

The expected outcomes of this project include:

- A comprehensive understanding of microbial and plant responses to Martian environmental conditions.
- A detailed analysis of the viability and adaptability of life forms under simulated Martian conditions.
- Practical insights and recommendations for future Mars exploration missions, particularly those involving in-situ resource utilization and life support systems.

By achieving these outcomes, this project aims to make a significant contribution to the ongoing exploration of Mars and the broader field of astrobiology.

1.6 Citation

Cockell, C. S., Lee, P. (2002). "The biology of impact craters—a review." *Biological Reviews*, 77(2), 279-310. doi:10.1017/S1464793101005887.

Schulze-Makuch, D., Irwin, L. N. (2006). "The prospect of alien life in exotic forms on other worlds." *Naturwissenschaften*, 93(4), 155-172. doi:10.1007/s00114-006-0089-1.

Nicholson, W. L., Schuerger, A. C., Race, M. S. (2009). "Migrating microbes and their impact on planetary protection." *Trends in Microbiology*, 17(8), 389-392. doi:10.1016/j.tim.2009.05.007.

Smith, H. D., McKay, C. P. (2005). "Drilling in ancient permafrost on Mars for evidence of a second genesis of life." *Planetary and Space Science*, 53(13), 1302-1308. doi:10.1016/j.pss.2005.08.002.

2. Methodology

2.1 Research papers and lectures

2.1.1 Sustainability of plant growth on Lunar and Martian regolith

1. **Bioregenerative Life Support Systems (BLSS):**
   - Developed to provide food sources for crewed Moon and Mars missions.
   - Combined with In Situ Resource Utilization (ISRU), they can make sustainable food production feasible on these celestial bodies.
2. **Lunar and Martian Regolith Simulants:**
   - Physicochemical properties and mineralogical composition of simulants are essential for understanding their potential as plant growth substrates.
   - Simulants can be modified with composted organic wastes to improve their similarity to terrestrial soil.
3. **Importance of Self-Sustaining Systems:**
   - Long-term space missions and settlements need to be economically and resourcefully self-sustaining, reducing reliance on terrestrial resources.
4. **In Situ Resource Utilization (ISRU):**
   - Critical for purifying water, revitalizing the atmosphere, and producing food in a closed-loop system.
   - Potential for space farming using local resources, facilitating water recycling, organic waste composting, and oxygen production.
5. **Characteristics of Regolith:**
   - Regolith is a fine mineral material lacking living matter, found on planetary surfaces.
   - Lunar regolith mainly comprises low-Ti basalt and high-Ca anorthosite, while Martian regolith is composed of basaltic sand with plagioclases, mafic minerals, and iron oxides.
6. **Agricultural Potential of Regolith Simulants:**
   - Evaluating the feasibility of using regolith simulants for plant growth is critical

for developing BLSS.
    - Adding organic amendments enhances the bioavailability of essential nutrients in regolith simulants.
7. **Historical and Recent Studies:**
    - Early experiments showed that Lunar material could support a wide range of plant species.
    - Recent studies have tested various crops on Lunar and Martian simulants, with promising results when combined with organic matter.
8. **Challenges and Solutions:**
    - Regolith simulants lack organic matter and key nutrients, making them insufficient for sustainable crop production without amendments.
    - Strategies include adding organic matter, using pioneer plants, and composting to enhance regolith fertility.
9. **Future Research Directions:**
    - More research is needed to assess the true potential of extra-terrestrial farming.
    - Investigating the role of biota and bio-stimulants in extra-terrestrial soils to improve environmental functions and sustainability.

2.1.2 Limits of life on planets and Extremophiles

1. **Importance and Scope of Extremophiles Research:**
    - **Dominance of Prokaryotic Life:** Prokaryotic life has dominated Earth's evolutionary history, with extremophiles representing key research areas.
    - **Polyextremophiles:** Organisms adapted to survive in multiple extreme conditions (temperature, pH, pressure, radiation, salinity, nutrient limitation).
    - **Advances in Understanding:** Research on extremophiles has advanced molecular biology, medicine, and our understanding of life's origins and evolution.
2. **Parameters Limiting Life:**
    - **Main Factors:** Water activity and pH are crucial, influencing cellular energy generation and survival.
    - **Extreme Conditions:** Life can survive extreme temperatures, pressures, pH levels, and radiation.
3. **Adaptations and Survival Strategies:**
    - **pH Adaptations:** Microorganisms maintain near-neutral cytoplasmic pH and can alter environmental pH.
    - **Temperature Adaptations:** Psychrophiles and thermophiles adapt to cold and heat, respectively, often in saline or high-pressure environments.
    - **Pressure Adaptations:** Piezophiles survive high pressures through membrane fluidity adjustments and other strategies.
    - **Radiation Resistance:** Some microorganisms can survive extreme radiation through DNA repair, genome redundancy, and protective pigments.
4. **Microbial Communities and Ecosystem Influence:**
    - **Community Composition:** Temperature, pH, salinity, and pressure influence microbial community structure and abundance.
    - **Environmental Influence:** Extreme environments like mining sites and hypersaline basins host unique microbial communities.
5. **Implications for Extraterrestrial Life:**
    - **Potential for Life:** Understanding extremophiles helps map possible conditions for life on other planetary bodies.

- **Planetary Analogs:** Conditions on Mars, Europa, and other bodies might support life, especially in subsurface environments.
- **Water as a Key Factor:** Presence of liquid water is critical for considering potential life on other planets.

6. **Future Research Directions:**
   - **Interaction of Multiple Parameters:** More focus needed on how multiple extreme conditions interact to influence life.
   - **Unexplored Limits:** Current research suggests we have yet to find the true limits of life on Earth and possibly elsewhere.

Summary

Extremophiles demonstrate life's adaptability to extreme conditions, pushing the boundaries of what is considered habitable. This research not only enhances our understanding of life's resilience on Earth but also informs the search for extraterrestrial life by highlighting the possible conditions under which life might thrive elsewhere in the universe.

2.1.3 Shewanella oneidensis MR-1 and its applications in electro-biotechnology

1. **Introduction to Shewanella Genus:** Describes the genus Shewanella, highlighting its diversity and ecological roles, including its ability to respire a wide range of electron acceptors.
2. **Bioelectrochemical Applications:** Discusses S. oneidensis MR-1 as an electrochemically active bacterium capable of interacting with electrodes in bioelectrochemical systems (BESs). It mentions applications such as microbial fuel cells (MFCs), microbial electrolysis cells (MECs), and microbial electrosynthesis (MES).
3. **Metabolic Characteristics:** Details MR-1's metabolic pathways, emphasizing its preference for organic compounds like lactate and pyruvate over sugars due to deficiencies in glucose utilization pathways.
4. **Electrochemical Characteristics:** Explores MR-1's electrochemical behavior, including its ability to generate anodic and cathodic currents in BESs, influenced by electrode potentials and electron transfer pathways.
5. **Biofilm Formation:** Highlights the importance of biofilm formation in MR-1 for efficient electrochemical interactions with electrodes, influenced by growth conditions and genetic factors regulating biofilm formation.
6. **Applications in Electro-Fermentation (EF) and Electro-Genetics (EG):** Discusses the potential of MR-1 in EF for producing chemicals and EG for controlling gene expression using electrode potentials, suggesting future directions for research and development in these areas.

Overall, the article provides a comprehensive overview of the biological, electrochemical, and biotechnological aspects of Shewanella oneidensis MR-1, highlighting its potential as a model organism for studying bioelectrochemical processes and applications.

2.1.4 Ustilago maydis and radiation resistance

1. **Organism and Radiation Resistance:**
   - Ustilago maydis, a phytopathogenic fungus, shows extreme resistance to UV and ionizing radiation.
   - This resistance may be due to continual genotoxic stress in its environment.

2. **Genome and Molecular Systems:**
    - Genome size: 20.5 megabases, with approximately 7000 genes.
    - Similarities to Saccharomyces cerevisiae, which has 6600 genes.
    - Genome sequence provides insights into molecular systems contributing to radiation resistance.
3. **Mechanisms of Radiation Resistance:**
    - Mechanisms include recombinational repair systems.
    - Presence of Rad51 and Rad51 paralog, as well as Rad52 and Brh2 mediators.
    - Involvement of homologous recombination and extended synthesis-dependent strand annealing (ESDSA) for repair.
4. **Comparative Studies:**
    - Comparative studies with Deinococcus radiodurans highlight similarities and differences in radiation resistance mechanisms.
    - D. radiodurans is known for high intracellular Mn2+ concentration and unique RecA protein function.
5. **Evolutionary and Environmental Context:**
    - Adaptations to environmental stressors like UV, ionizing radiation, and DNA-damaging toxins.
    - Insights into how fungi like U. maydis and others adapt and survive under extreme conditions.
6. **Research Implications:**
    - Importance of understanding these mechanisms for broader implications in biology, genetics, and potentially biotechnology.

    These points summarize the key aspects of Ustilago maydis's radiation resistance and its implications in the broader context of microbial biology and environmental adaptation.

2.2 Martian Test Chamber

From the study of the given review articles, it has been shown that there are chances of plant and microbial growth on Martian environment. In order to test this survivability, a sealed chamber with controlled conditions to replicate the Martian environment has been prepared. By simulating the chamber's temperature, pressure, atmosphere, etc. – the conditions that could possibly affect plant survival and growth on the planet- we can expand our knowledge about plants' resistance to such extreme conditions beyond the theoretical aspect.
Such experiments have been conducted (or still under progress) by organizations like NASA (the SAM instrument on the Curiosity Rover), etc. with future prospects including the inhabitation of Mars.
Further details about the designing, working and making of the chamber are described below.

II Part Two : Computational Simulations

3. Python and its Libraries

3.1 Introduction

Python is one of the easiest languages to grasp and it has a ton of libraries designed for specific tasks which makes it suitable for simulating microbial growth.
We will be using PyLab as a convenience module that bulk imports **matplotlib.pyplot** (for plotting) and **NumPy** (for Mathematics and working with arrays) in a single name space.We just have to write the following code to import all modules from PyLab :

```
from pylab import *
```

Firstly, you should have a basic knowledge of Control Flow, Numpy Arrays, Lists, Functions and basic curve plotting in Python as usual.
Python Tutorial : Click here
PyLab : Click here
Below are some basic topics we'll be using...

3.2 Numpy

NumPy is the fundamental package for scientific computing in Python.We will mostly use numpy arrays in our growth simulations.
NumPy arrays have a fixed size at creation, unlike Python lists (which can grow dynamically). The elements in a NumPy array are all required to be of the same data type, and thus will be the same size in memory. (except one can have arrays of objects, thereby allowing for arrays of different sized elements)

Example of slicing

```
a = array([1, 2, 3, 4, 5, 6])
print(a[2:-1]) #[2:-1] picks out all elements except 1st two and last
```

[3 4 5]

Zeros and Ones arrays

```
    print(zeros(7))
    print(ones(5) * 7)
```

[0 0 0 0 0 0 0]
[7 7 7 7 7]

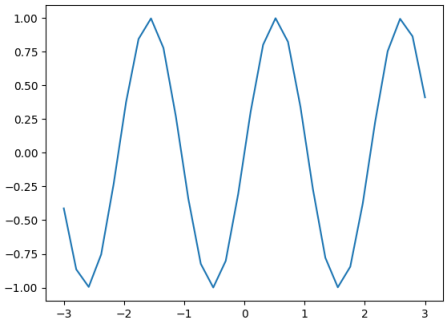
3.3 Plotting curves in PyLab

```
from pylab import *
x = linspace(-3, 3, 30)
# It will create an array of uniformly separated values between -3 & 3
y = sin(3*x)
plot(x, y)
show()
```

Symbols:

-, –, -., ,, ., o, ,, v, <, >, s, +, x, D, d, 1, 2, 3, 4, h, H, p, |, _

They specify the style of curve. Eg- '.'(point marker), '-'(solid line), '–'(dashed line), 'o'(circle marker), 'x'(cross marker) etc.

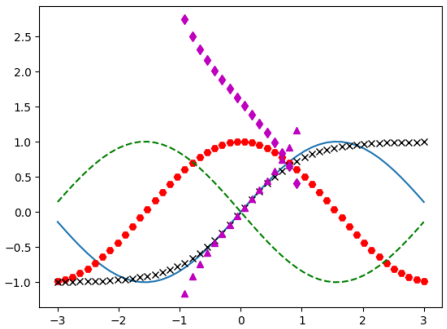


Colors:

b, **g**, **r**, **c**, **m**, **y**, **k**, **w**(white)

Example:

```
from pylab import *
x = linspace(-3, 3, 50)
plot(x, sin(x))
plot(x, cos(x), 'rH')
plot(x, -sin(x), 'g--')
plot(x,tanh(x),'kx')
plot(x, arccos(x),'md')
plot(x,arcsin(x),'m^')
show()
```



4. Microbial Growth Simulation

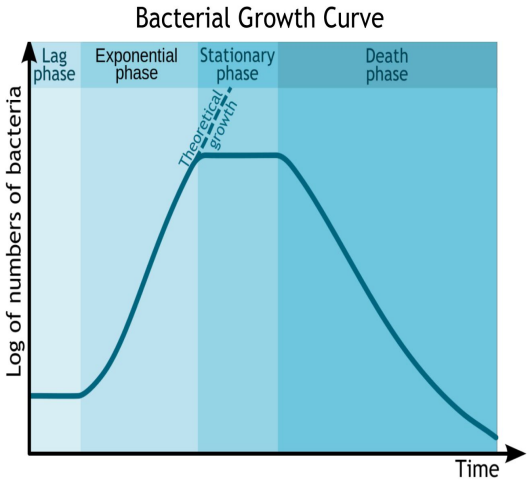
4.1 Introduction to bacterial growth curve

Bacterial growth is a type of population dynamics, which is the study of population size (number of individuals) over time. Bacterial growth can be studied both by analyzing data from a real experiment or by creating a virtual experiment. So,we will simulate bacterial growth on the computer via using Python and its libraries.
The link to the reference Jupyter notebook is here.

Phases of bacterial growth

Bacteria are unicellular organisms that tend to reproduce asexually by the means of binary fission. The time taken for a bacterial cell to double is called **generation time**. In a closed system with enough nutrients, a bacteria shows a predictable growth pattern that is the bacterial growth curve :-

1. **Lag Phase** : The bacteria upon introduction into the nutrient medium take some time to adapt to the new environment. In this phase, the bacteria does not reproduce but prepares itself for reproduction.
2. **Log Phase** : During this phase, there is an exponential increase in the number indicated by a section of the growth curve. This is reproduction phase. Also called exponential phase.
3. **Stationary Phase** : In the stationary phase, the rate of growth of the cells becomes equal to its rate of death. The bacterial population remain constant.
4. **Death Phase** : This is the last phase of the bacterial growth. At this stage, the rate of death is greater than the rate of formation of new cells.



We will first create a Mathematical model of bacterial growth and then create a computational model of it via programming !!
We will study **Exponential** & **Logistic growth** models with some assumptions and will also study bacterial phases, effects of temperature, death rate and analyse them.

4.2 EXPONENTIAL GROWTH

We are all familiar with the basic exponential curves since JEE. We'll encounter 2^x curve in the exponential phase where due to binary fission, a bacteria divides into two off springs and the cycle continues. We can write it mathematically as :

$$\mathbf{E_n = E_{n-1} + \Delta E = 2\Delta E}$$

```
from pylab import *
N=5
E =zeros(N)
E[0]=10000

for n in range(1,N):
    E[n]=2*E[n-1]
print("Number of Bacteria",E)

plot(E, 'o-')
xlabel('Generation')
ylabel('Population size, E')
title("Modeled bacterial growth
    ")
show()
```

Number of bacteria: [10000, 20000, 40000, 80000, 160000]
The Population doubles after each generation time due to binary fission.

> Let E_n be the number of bacteria at a given time step 'n'.

> t_n be the time after n states.

> Δt be the generation time.

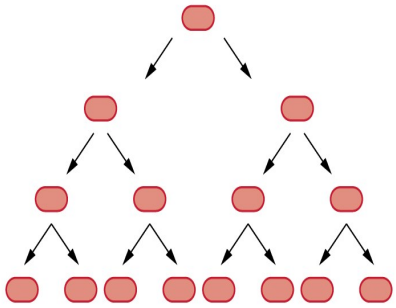
> E_0 be the initial population.

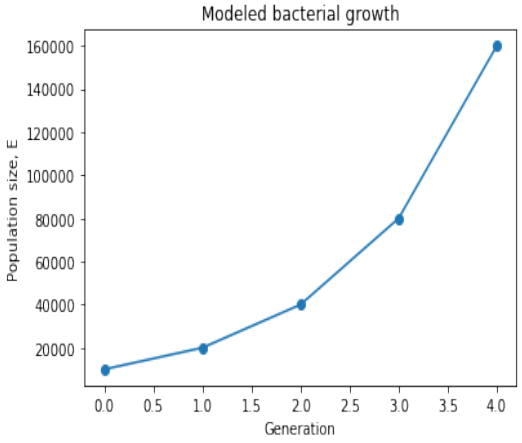
> ΔE is the number of bacteria per timestep.

Assumptions
> no factors limiting growth

> no death

> constant generation time

Number of generations (n)	Number of cells	Each division adds two new cells
0	1	
1	2	
2	4	
3	8	

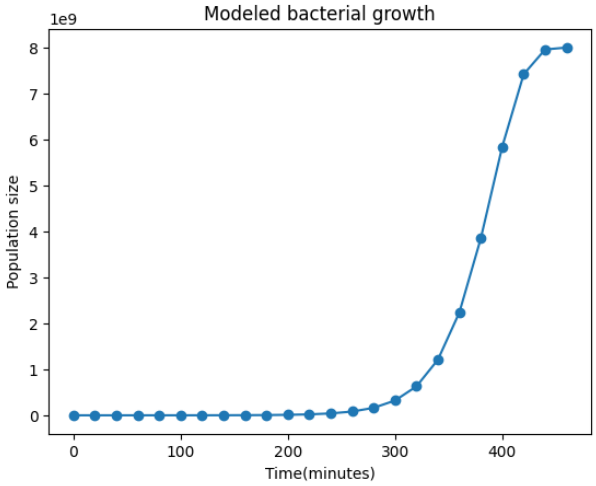


4.3 LOGISTIC GROWTH

Now, the bacterial population must not be exponentially growing in real conditions due to nutritional and other barriers. So, we use logistic growth to slow down the growth after some time. We can write it as :

$\mathbf{E_n} = \mathbf{E_{n-1}} + (1 - \mathbf{E_{n-1}}/\mathbf{K})\mathbf{E_{n-1}}$ where, $\Delta E = (1 - E_{n-1}/K)E_{n-1}$

```
from pylab import *

N=24
dt=20 #in min
K=8e9

E=zeros(N)
t=zeros(N)
E[0]=10000

for n in range(1,N):
    E[n]=E[n-1]+(1-E[n-1]/K)*E[n-1]
    t[n]=t[n-1]+dt
plot(t,E,'o-')
xlabel('Time(minutes)')
ylabel('Population size')
title('Modeled bacterial growth')
show()
```



> K is the maximum sustainable population (K= 8 X 10^9) means when population reaches K then growth stops.

> when $E_{n-1} <<< K$ then it behaves like exponential growth as E_{n-1}/K tends to zero.

Assumptions

> If there is no limitation in resources available, the population approximately doubles each generation.

> There is a finite and constant supply of resources available each generation.

> constant generation time

4.4 DEATH RATE

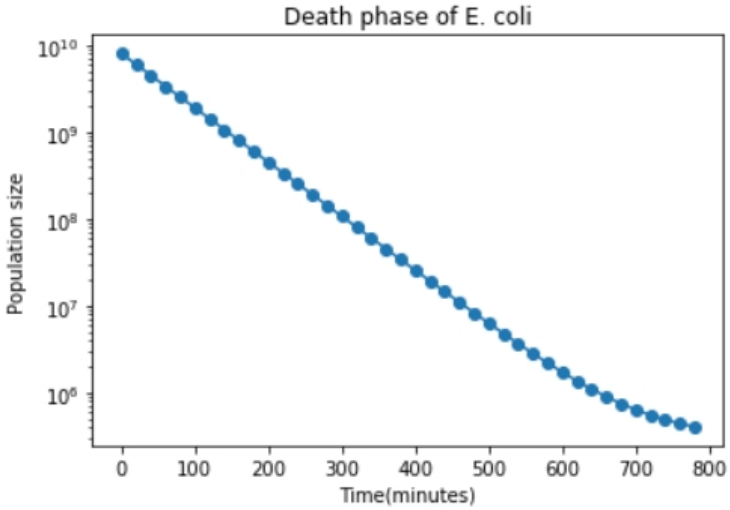
Logistic growth approximately models 3 phases of a bacteria's life (lag,log and stationary phases). Now, the last phase remains is death phase which we will model via death rate.

$\mathbf{E_n = E_{n-1} + D(E_{n-1} - F)}$

> D :- the fraction by which bacteria die each generation.

> F :- equilibrium population (3e5 for E-Coli) after this number death rate drastically decreases.

```
 from pylab import *

N=24
dt=20 #in min
D=0.25
F=3e5
E=zeros(N)
t=zeros(N)
E[0]=8e9 #it starts after reaching K

for n in range(1,N):
    E[n]=E[n-1]-D*(E[n-1]-F)
    t[n]=t[n-1]+dt

plot(t,E,'o-')
xlabel('Time(minutes)')
ylabel('Population size')
title('Death phase of E. coli')
yscale('log') #so that it will be a approx line
show()
```



Assumptions

> The bacteria do not reproduce in this phase.

> The rate of bacteria that dies in each time step, is proportional to the difference between the number of bacteria and the long-term sustainable number of bacteria.

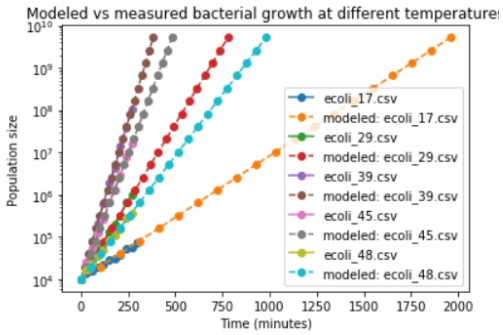
4.5 Effects of temperature

According to the reference research paper, the relationship between temperature and bacterial growth rate is typically non-linear and can vary significantly among different bacterial species. Generally, each bacterial species has an optimal temperature range where it grows most rapidly. By using the given data about E. Coli present in research paper we can see the the plots to growth rate v/s temperature & bacterial growth v/s time. Firstly, generation time and growth rate(Its just the slope of the population size v/s time graph denoted as a) are related as :

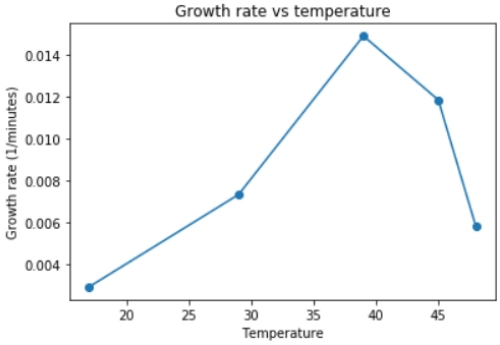
$$a = \frac{log_{10}(2)}{generation-time}$$

We can write it in discrete form.

```
    a = (log10(E[-1]) - log10(E[0])) / (t[-1] - t[0])
    generation_time = log10(2)/a
```



(a) Comparing with real data



(b) Its code is available below

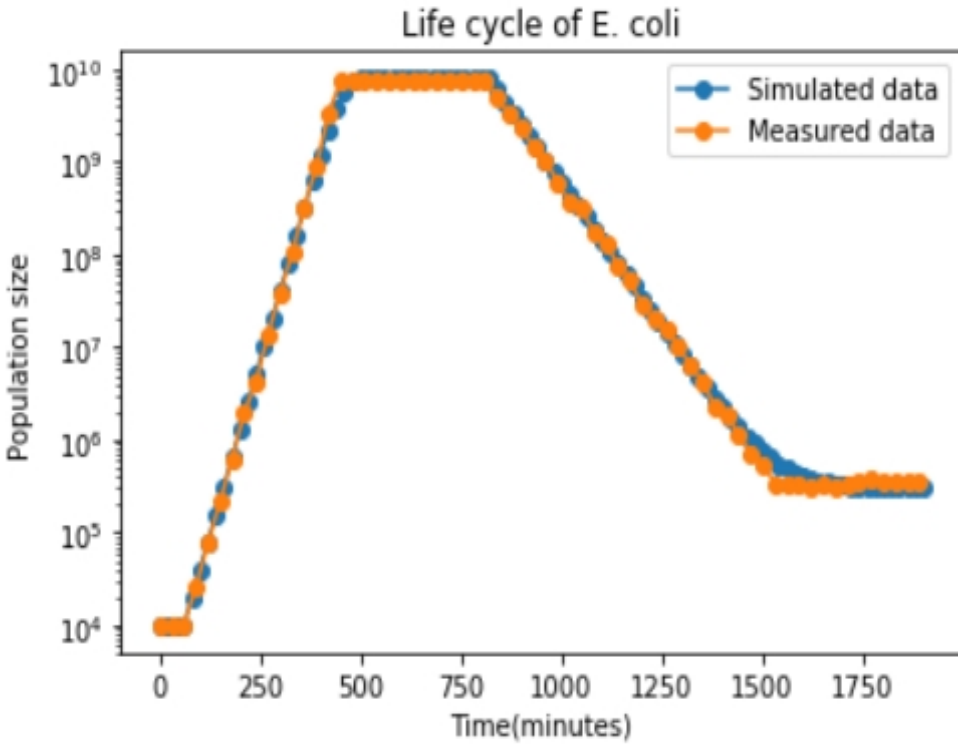
```
    from pylab import *

file_list = ['ecoli_17.csv', 'ecoli_29.csv', 'ecoli_39.csv', 'ecoli_45.csv'
    , 'ecoli_48.csv']

temperatures = [17, 29, 39, 45, 48]
rates = []

# Loop over each file
for file_name in file_list:
    # read data:
    t, E = loadtxt(file_name, delimiter=',', unpack=True)

    # find generation time:
    a = (log10(E[-1]) - log10(E[0])) / (t[-1] - t[0])

    # Append to a list
    rates.append(a)

# plot the results:
plot(temperatures, rates, 'o-')
xlabel('Temperature')
ylabel('Growth rate (1/minutes)')
title("Growth rate vs temperature")
show()
```

We can see that our model is able to accurately predict the E. coli growth at different temperatures, provided that we give it the correct generation time.

4.6 Modelling all phases

Finally, we can model all phases by the knowledge we learnt earlier!
The Pseudo code is given below for that :

```
    N_lag = 4      # Generations in the lag phase
    N_log = 38     # Generations in the logarithmic growth phase
    N_death = 54   # Generations in the death phase

    # Total number of generations
    N = N_lag + N_log + N_death
    # Total number of generations
    N = N_lag + N_log + N_death

    dt = 20  # min
    K = 8e9
    D = 0.25
    F = 3e5

    for n in range(1, N_lag):
        # perform lag phase modeling

    for n in range(N_lag, N_lag + N_log):
        # perform logistic growth modeling

    for n in range(N_lag + N_log, N):
        # perform death phase modeling

    #plot the data in log scale
```



The above plot shows the accuracy of our model by comparing it with real life data available in the reference research paper. It successfully models all the phases of a bacterial growth cycle !!

III

Part Three : Chamber Construction

5. Building the Chamber

5.1 Design process

1. The setup for our chamber would be done in the Astrobiology lab , SPASE department, ESB Building. The chamber design comprises of a cube (dimensions 15cm*15cm*15cm) whose outer structure would consist of 2 layers of aluminium wall which would help to separate the inner and outer environment.
2. The cube would have a lid on top which would allow us to replenish the components after an experiment. This lid would be connected through screws or tucks which would have gaskets in between the layers to ensure no loss.
3. The walls would be ___ mm thick and would hold resin in between to hold the walls. The resin we would be using is an epoxy resin (High strength, compressive strength, low shrinkage and low cost).
4. The inner wall would be connected through pipes which would allow us to transport components like microorganisms, CO2 and N2 and also help us to maintain the pressure inside the chamber(600 Pa at max).
5. In addition, the inner wall would have temperature and pressure sensors. Inside the inner layer we will have a UV source, martian soil, nichrome wire and copper tubes.
6. The temperature regulation would be taken care of through copper tubes (liquid N2 would be circulated for cooling) and nichrome wire (for heating).

5.2 Materials Used

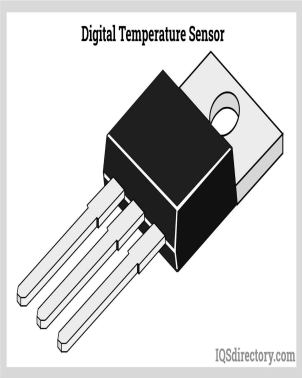
- **Resin and Acrylic material :**

- **Copper Capillaries and Nichrome wire :**

- **UV source :**

- **Pipes for gas transport :**

- **Temperature Sensor :**



- **Pressure Sensor :**

- **Gaskets :**

- **Gas Cylinders :**

- **Vaccum Pump :**

- **Martial Soil(Regolith Simulant) :**

- **Valves :**

- **Airlock :**

Conclusions and Future Work

In the coming weeks, we plan to take our research to the next stage by enhancing our simulations and diversifying the species being tested. Currently, we have only experimented with *Ustilago meydis* , in the future, we aim to conduct long-term experiments on a more diverse range of microbial samples and plant cells. We shall also try to further reduce atmospheric pressure, to achieve levels even closer to Martian atmospheric pressure.

Potential Applications

Similar experiments, where Martian conditions are simulated in a controlled chamber, are being carried out at a large scale in space agencies across the world. Investing in enhancing our approach towards this research would lead us to delving deeper into the field of astrobiology, opening up the scope for improving technologies for space agriculture and In-Situ Resource Utilization (ISRU). By studying genetic modification in extreme conditions, we may also be able to learn about how we can genetically modify plants so they can resist diseases, land infertility, and changing environmental conditions here on Earth.

Colab link to all the codes of the project

https://colab.research.google.com/drive/1C0DREo8hpsiVrVzBBQJjflMfNB4CEqei?usp=sharing

Indivisual Contributions

MENTORS:

Astitva Roy & Srisha Singh - Two of us collaborated to determine the appropriate materials for the chamber. We conducted extensive research online to identify suitable suppliers, placed orders for the necessary items, and personally visited local stores to purchase materials that were readily available. We also worked towards making a good design of the chamber along with the mentees which involved meeting with different faculty who could give us good advice over the design of the chamber.

Paaristosh Jain & Ayush Bokad - Together, we oversaw the students' theoretical work, ensuring its accuracy and completeness. We also provided hands-on assistance with simulations, addressing the challenges they faced due to their limited understanding of Python and its associated libraries. In addition to these responsibilities, we took charge of organizing and preparing all the materials and presentations required for the SnT council, ensuring that the content was well-structured and effectively communicated.