Modeling Bacterial Growth Dynamics

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Abstract

Bacteria are microscopic, single-celled organisms that are ubiquitous in nature. The study of bacteria, known as bacteriology, is an applied science that plays a crucial role in agriculture, healthcare, environmental maintenance, and the biotechnology industry. This project aims to study and understand bacterial growth and limiting criteria. To achieve this, we introduce several differential equations aimed at modeling and understanding the dynamics of bacterial populations using experimental data.

1 Introduction

It is possible to explain the various growth phenomena with mathematical model, some of them are simple and some are complicated. The most famous example is the familiar Malthusian or exponential growth model which is classified as a linear constant coefficient differential equation. However, more sophisticated models, like the logistic differential growth model, take into consideration factors such as limited resources, carrying capacity resulting in a sigmoidal growth curve.
Since this work is motivated to study bacterial growth we also attempt to incorporate Monod's [3] growth parameter to our model. It differs from classical growth models because it introduces the concept of a limiting nutrient.

We considered two distinct bacterial species: Escherichia coli and Lactobacillus delbrueckii. Escherichia coli (abbreviated as E. coli) are a large and diverse group of bacteria found in the environment, foods, and intestines of people and animals. Although most strains of E. coli are harmless. Some kinds of E. coli can cause diarrhea, urinary tract infections, respiratory illness and pneumonia, and other illnesses. It has been extensively used in both basic molecular biology and biotechnology. E. coli was the first cell host to produce recombinant proteins, for instance insulin, and it is still used for

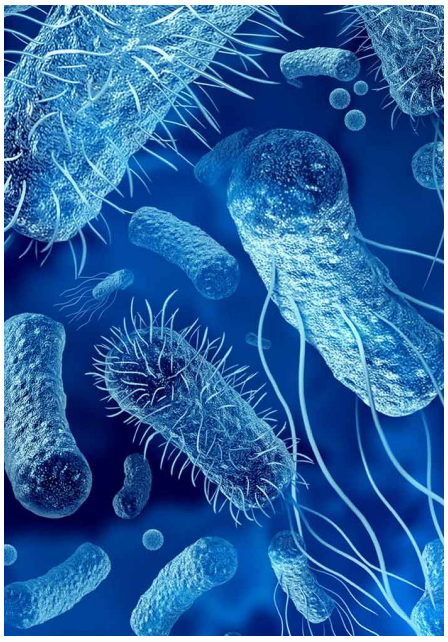
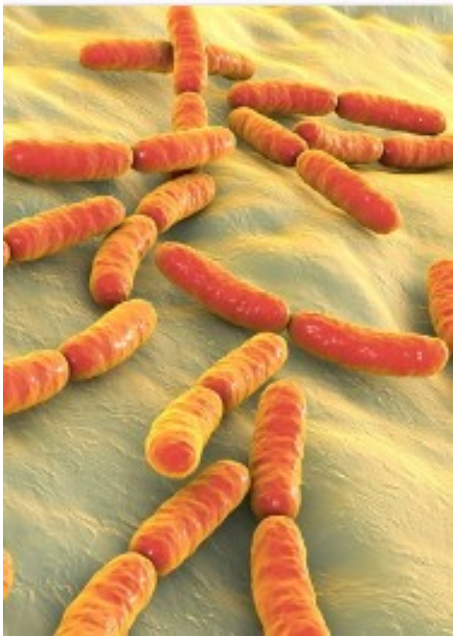

Figure 1: Left: E.coli bacteria; Right: Lactobacillus delbrueckii

the production of biopharmaceuticals. On the other hand, Lactobacillus delbrueckii also known as Lactobacillus bulgaricus is the main bacterium used for the production of yogurt . It also plays a crucial role in the ripening of some cheeses, as well as in other processes involving naturally fermented products.

1.1 Preliminary

Biomass
Biomass generally refers to the total mass of living biological material within a given volume or sample.
Cell concentration
It is the number of individual cells per unit volume of a culture.
Bacterial density
It is the Dry weight of cells per unit volume of a culture.

Growth Phases: In general a typical bacterial growth curve shows five distinct phases of growth [4]:

- Lag phase- the delay before the start of exponential growth;

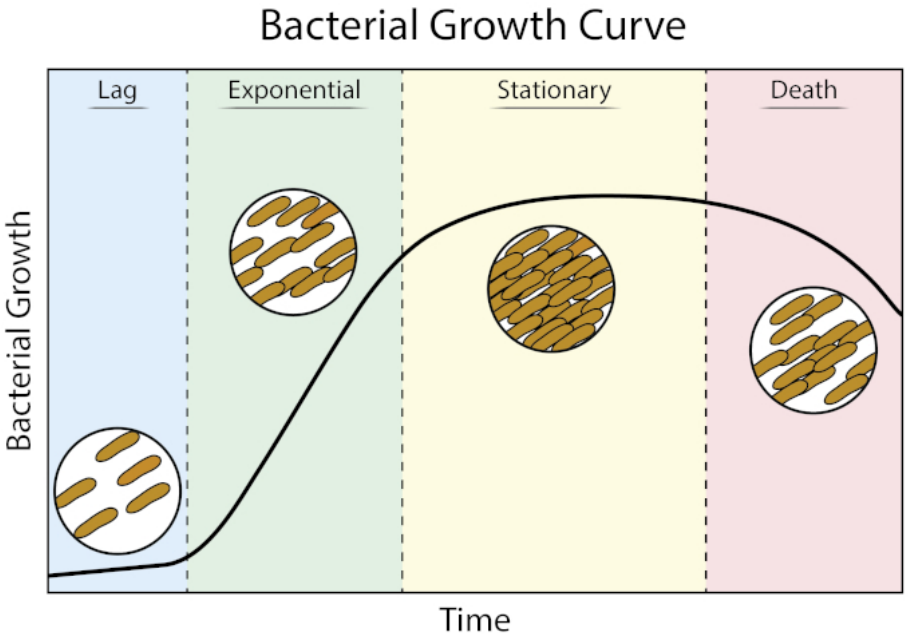

Figure 2: Bacterial Growth phases overtime

- Exponential phase-where cell division proceeds at a constant rate;
- Stationary phase- when conditions become unfavorable for growth and bacteria stop replicating ;
- death phase- when cells lose viability;

This project primarily concentrates on comprehending three key phases of bacterial growth: the lag phase, exponential phase, and stationary phase.

2 Mathematical Model

2.1 Model 1

Assumptions:

- In presence of rich and unlimited nutient source and optimal 37^oC temperature Bacteria are able to reproduce at impressive rates.[1] We consider that the bacteria growing in presence optimal condition.
- The bacteria can double its population every 20 minutes.
- The growth of bacterial population depends only on initial number of population and grows exponentially.

In our first model below we consider optimal condition to determine analytical and numerical trend of bacterial growth.This means that very large population sizes can be achieved within a few hours.

Let N(t) is the total biomass at time t. Assuming optimal growth condition the rate of change of bacterial concentration $\frac{\partial N(t)}{\partial t}$ is directly proportional to total cell concentration N(t) at time t. Mathematically,

$$\frac{\partial N(t)}{\partial t} \propto N(t)$$
$$\frac{\partial N(t)}{\partial t} = rN(t) \tag{1}$$

where,
N(t)-is the number of bacteria at time t.
r-is the per-bacterium replication rate.
Eq. (1) predicts that the population grows exponentially.

Now Integrating equ.(1)-

$$N(t) = N_0 e^{rt} \tag{2}$$

With highest replication rate if the cell concentration become twice every 20 minutes, then for t= 20 min, N(t) = $2N_0$. We can calculate the maximum per-bacterium replication rate r using this measure-

$$N_0 e^{20r} = 2N_0$$
$$\implies r = \frac{\ln 2}{20}$$
$$\implies r = 0.035 min^{-1}$$

This rate can be different for different bacterial species.

2.2 Validation:

Strength:

- Equation (1) provides a good model for the lag and exponential phase of growth of a bacterial population (Fig-2).
- Since the only constant used in this model is r which is well defined it is easy to calculate and compare the results using the model.

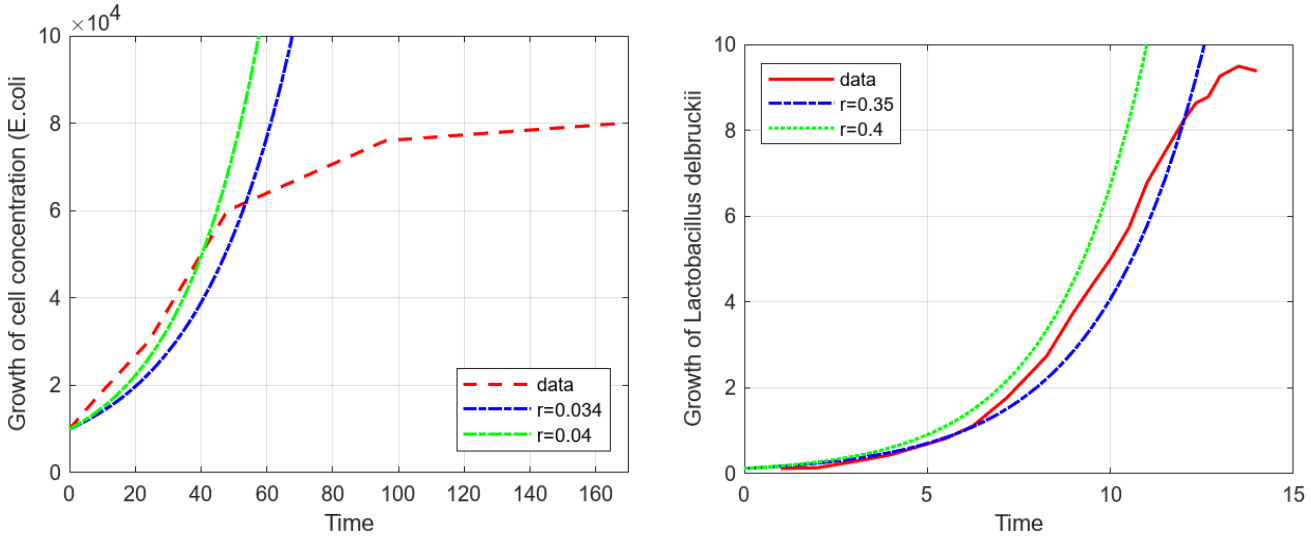

Figure 3: Growth dynamics of E.coli (left) and Lactobacillus Delbruckii (right) bacteria population using model-1 compared with experimental data.[2]

Weakness:

- The bacteria population grows in an unlimited fashion. It is quite undesirable property since it is well established that the growth rate cannot exceed a certain limit.
- Furthermore the model can only define first two growth phases and fails to explain stationary phase.(Fig-2)

2.3 Model 2

Saturating population growth can be modelled in a biologically consistent way by including the dynamics of the nutrient explicitly in the equations. We consider Monod [3] function g(s) to replace the per cell growth rate r at equation (1). In construction of a new model that takes into account the fact of limited nutrients we assume-

Assumption:

- Number of units of nutrients consumed to produce one bacterium is a constant defined by γ.
- Nutrients are limited and run out with time. All other growth conditions are same.
- Rate of change of Bacterial biomass N(t) is a function of concentration nutrient substrate s.

The classic equation for the nutrient-concentration dependent growth of a bacterial population is:

$$\frac{\partial N(t)}{\partial t} = g(s)N(t) \tag{3}$$

Where,

$$g(s) = \frac{r_m s}{K_s + s} \tag{4}$$

s = is the nutrient concentration.
r_m = is the maximal per-cell growth rate.
K_s = is the nutrient concentration at which the growth rate is half maximal.

Equation (3) depends linearly on the nutrient concentration s for low nutrient concentrations, but becomes independent of the nutrient as $s \longrightarrow \infty$. This also captures the fact that for high nutrient concentration, growth is limited by the becterium's capacity to import and use the nutrient, rather than by the availability of the nutrient in the environment.
Now equation (2) must be coupled with a dynamical equation for the nutrient concentration:

$$\frac{\partial s}{\partial t} = -\gamma g(s)N(t) \tag{5}$$

Where, γ is a constant coefficient, describe the number of units of nutrients that are consumed to produce one bacterium.

2.4 Validation:

Strength:

- The model can successfully explain the lag phase, exponential phase and the stationary phase. It also explain the rate of changes in substrate concentration.
- The model fit quite well with the experimental data. (Fig-3).

Weakness:

- Due to its non-linear nature and the necessity for multiple parameter estimations, this model presents challenges in terms of convenience and applicability across various dynamical scenarios.

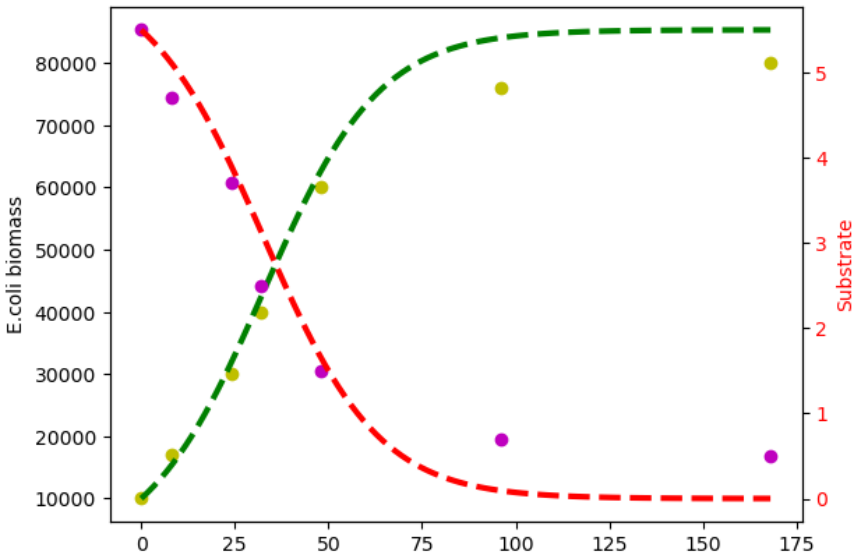

Figure 4: Dynamics of E.coli bacteria considering effect of limiting nutrient (substrate) according to model-2.

- The model is not considering other influencing factors for bacterial growth such as maintenance metabolism, temperature change, dissolved oxygen or pH effects.

2.5 Model-3

Taking into account the limitations of previous models, we are introducing a simple logistic growth model that addresses these concerns. This model is straightforward to handle and effectively explains the stationary phases of bacterial growth.

Assumptions:

- Growth of bacterial biomass is not unlimited whether in bacterial culture or human body. Rather it is limited by maximum carrying capacity K.
- The nutrient source is fixed. When it is depleted, the bacteria cease replication and enter the stationary phase.

Considering the assumption above the model defining bacterial growth can be given by-

$$\frac{\partial N}{\partial t} = rN(1 - \frac{N}{K}) \tag{6}$$
$$t = 0, N(t) = N_0$$

Where,
r= Growth rate of bacteria population any given situation.
K = maximum carrying capacity.
The term $(1-N/K)$ in equation (6) accounts for the reduction in the effective growth rate as N increases.
Separating the variables from equation (6) we get-

$$\frac{K}{N}\frac{\partial N}{\partial t} = r(K - N)$$
$$\implies \frac{K\partial N}{N(K-N)} = r\partial t$$
$$\implies (\frac{1}{N} - \frac{1}{K-N})\partial N = r\partial t$$

Now integrating both sides-

$$\ln(\frac{N}{K-N}) = rt + c \tag{7}$$

c is the constant of integration.
When t= 0, N= N_0-

$$c = \ln(\frac{N_0}{K-N_0})$$

Substituting value of c from equation (7) we get-

$$\implies \ln(\frac{N}{K-N}) = rt + \ln(\frac{N_0}{K-N_0})$$
$$\implies \ln\frac{N(K-N_0)}{N_0(K-N)} = rt$$
$$\implies N(K-N_0) = N_0(K-N)e^{rt}$$
$$\implies N = \frac{N_0Ke^{rt}}{K + N_0(e^{rt}-1)}$$

Finally we have,

$$N(t) = \frac{N_0 e^{rt}}{1 + \frac{N_0}{K}(e^{rt} - 1)} \tag{8}$$

2.6 Validation:

Strength:

- The model can successfully explain the lag phase, exponential phase and the stationary phase. (Fig-4)
- The parameters possess biological significance and are readily identifiable.
- The model's flexibility in parameter estimation makes it applicable to various experimental datasets.

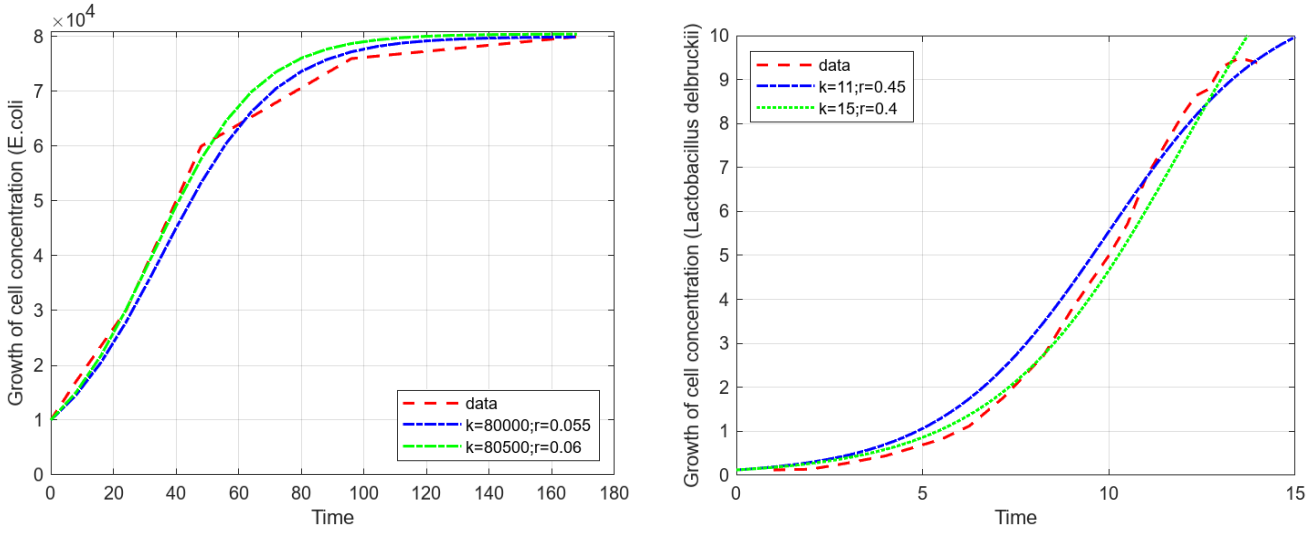

Figure 5: Left: Growth of E.coli bacteria; Right: Growth of Lactobacillus Delbruckii bacteria using based on experimental data.[2]

weakness:

- The model is not considering other influencing factors for bacterial growth such as maintenance metabolism, limiting substrates, temperature change, dis-solved oxygen or pH effects

3 Results:

Considering its performance, flexibility, adherence to general bacterial growth theories, and alignment with experimental datasets, Model-3 has emerged as the optimal mathematical model for understanding bacterial growth.

Two sets of data are utilized to compare the numerical results obtained using the Logistic growth model. The first set of experimental data pertains to E. coli, while the second set is for Lactobacillus delbrueckii, as outlined in Table-1 and Table-2 respectively.

Time (hr)	Cell conc.	Calculated (a)	Calculated(b)
0	10000	10000	10000
8	17000	14523.8	15011.9
24	30000	27875.1	30146
32	40000	36292.5	39585.4
48	60000	53350.1	57675.1
96	76000	77246.4	78750.5
168	80000	79945.7	80476.2

Table 1: Comparison of observed values (U.O.D/L) with calculated values for **E.coli bacteria**. (a) r= 0.055, k= 80000; (b) r= 0.06 , k= 80500.

The cell concentration of E. coli and Lactobacillus delbrueckii has been calculated based on the model given by equation (8). As shown in Table 1 and Table 2 (columns 2, 3, and 4), there is good agreement between experimental and calculated data. In Table 1, for E. coli bacteria, we observe a high replication rate of approximately 0.06 per minute, which is very rapid. On the other hand, the rate for the second bacterial species is below 0.5 per hour, indicating a comparatively slower rate than E. coli.

One important observation from both tables is that the model provides a better estimation of bacterial population when the carrying capacity is higher. However, in such cases, the replication process takes longer to reach the stationary phase compared to experimental cases. This suggests that

limiting resources such as nutrients, minerals, temperature fluctuations, etc., control the growth apart from the maximum carrying capacity.

Time (hr)	Cell conc.	Calculated (a)	Calculated(b)
1	0.123	0.15	0.18
2	0.139	0.29	0.27
3	0.283	0.45	0.4
4	0.442	0.7	0.5
5.5	0.82	1.3	1.0
6.25	1.12	1.7	1.4
7.17	1.77	2.2	1.9
7.70	2.24	2.8	2.32
8.25	2.73	3.4	2.74
9	3.77	4.3	3.48
9.53	4.43	4.9	4.05
10	5	5.5	4.7
10.50	5.72	6.1	5.33
11	6.78	6.76	6.04
11.5	7.51	7.3	6.77
12	8.23	7.86	7.5
12.33	8.63	8.34	9
12.67	8.78	8.34	8.3
13	9.27	8.96	9.0
13.50	9.49	9.14	9.7
14	9.38	9.4	10

Table 2: Observed and predicted cell concentrations (U.O.D/ml) during the growth of **Lactobacillus delbruckii**. (a) k= 11, r= 0.45; (b)k=15, r= 0.40

4 Conclusion and Individual Reflection

In Model-1, bacterial growth is assumed to occur in an environment with unlimited resources, where the growth rate is directly proportional to the current population size, leading to exponential growth over time. This simplistic model is suitable for relatively large populations. However, for smaller populations, it may be more accurate to consider that replication occurs as discrete events rather than continuously.

In such cases, it is more convenient to use dynamical variables that influence the total biomass of the population rather than just the initial number of bacteria. Mathematical functions such as the classical Monod's parameter or carrying capacity are then employed to better understand limited growth, providing a more biologically realistic model. Despite the appeal of incorporating Monod's parameter that can define limiting nutrients, the classic logistic differential equation ultimately emerged as the superior model for understanding bacterial growth dynamics. Its ability to achieve better agreement with experimental data and its flexible parameter estimation requirements solidify its position as the optimal choice, given its wide-ranging applications across various scientific disciplines.

5 Learning Task

The learning tasks for mathematical modeling of bacterial growth dynamics aims to equip students with the necessary skills to develop, analyze, and validate models that reflect real-world biological processes. Through a structured approach involving three distinct models—each building on the complexity of the previous—students will explore exponential, logistic, and Monod-based models to capture the essence of bacterial growth in varying conditions. These tasks are designed not only to enhance students' theoretical knowledge but also to sharpen their analytical and computational abilities, preparing them for further academic and professional pursuits in scientific research and application.

5.1 Learning objective:

- The students will learn about stages of bacterial growth.
- Learn to model population growth using calculus.
- Understand and apply exponential, logistic growth models along with Monod's growth model.

Materials: Programming knowledge of MATLAB, Python, or R.

5.2 Learning task-1

The growth of bacterial biomass, whether in a laboratory setting or within the human body, is contingent upon its environment, which encompasses factors such as nutrient availability, temperature suitability, species-specific replication rates, and the carrying capacity of the medium. In the presence of a rich and unlimited nutrient source, coupled with an optimal temperature of 37^0C, bacteria demonstrate impressive reproductive capabilities. Under such ideal conditions, bacteria can double their population approximately every 20 minutes.

Learning objective:

- Review the assumptions of the exponential growth model, particularly the conditions of unlimited nutrients and optimal growth temperature.
- Understand the derivation of the exponential growth equation:

$$\frac{\partial N(t)}{\partial t} = rN(t)$$

where N(t) is the population at time t and r is the growth rate.

- Plot the growth curve and use experimental data to estimate parameters.

Validation

- Discuss the limitations of the exponential growth model, particularly its inability to account for nutrient depletion and other environmental factors.
- Compare the model predictions with actual data to assess the model's accuracy.
- Suggest improvements or alternatives that could address these limitations.

5.3 Learning task-2

Explore the Monod model incorporating nutrient limitations to understand how nutrient availability affects bacterial growth.

Learning Objectives:

- Study the Monod equation and its assumptions, especially the relationship between nutrient concentration and growth rate:

$$g(s) = \frac{r_m s}{K_s + s}$$

- Understand how the Monod model modifies the exponential growth model to include nutrient dynamics.
- Use experimental data to estimate the parameters.
- Analyze how changes in nutrient levels affect the growth dynamics, particularly the transition from exponential to stationary phases.

Validation

- Evaluate the strengths and weaknesses of the Monod model in capturing realistic bacterial growth behavior.
- Validate the model by comparing its predictions with experimental data, focusing on nutrient-limited conditions.
- Discuss the biological significance of the model parameters and their impact on model predictions.

5.4 Learning task-3

Apply the logistic growth model to describe bacterial growth considering carrying capacity, which limits growth at high population densities.

Learning Objective

- Review the logistic growth equation and its derivation:

$$\frac{\partial N}{\partial t} = rN(1 - \frac{N}{K})$$

  where K is the carrying capacity.

- Fit the logistic growth model to experimental data to estimate the parameters r and K.

- Analyze the goodness of fit and how well the model captures the stationary phase of growth.

Validation:

- Discuss the applicability of the logistic model to different types of bacterial growth scenarios. Validate goodness of simulated data fit.

- Consider the limitations of the logistic model, especially in complex environments where factors other than carrying capacity might influence growth.

5.5 Compare results and make conclusion

Each model provides distinct insights into bacterial growth dynamics under particular conditions. Through the analysis of these tasks, students will report their findings and compare the effectiveness of each model in estimating the experimental results of bacterial growth. Student should also determine if the mathematical answer makes sense in terms of the experimental data used and verifies the answer is within a valid range of values. Determines if conclusions are satisfactory in all respects; if not, shows evidence of iteration of the process to improve the model.

5.6 Assessment Plan

Here are the fundamental guidelines and assessment plan for each learning task. Assessments are conducted on a scoring scale of 0 to 4, with 4 indicating the highest attainable score.

Modeling Element	Score-4	Score- 3	Score-2	Score -0/1
Understand the problem	Fully identifies the population dynamics problem, objective of the solution, and factors that affect the solution. Define relevant terminology.	Partially identifies the population dynamics problem, objective of the solution, and factors that affect the solution.	Limited in identifying the dynamics problem, objective of the solution, and factors that affect the solution.	Demonstrates little to no evidence.
Pose a simplified version of the situation	Fully determines useful information given. Makes useful, appropriate assumptions and choices. Define relevant terminology.	Partially determines useful given information Partially makes useful, appropriate assumptions and choices.	Limited in determining given information, making necessary assumptions, and making appropriate choices.	Demonstrates little to no evidence.
Model formulation	Translates the information and assumptions into mathematical form.	Partially Translates the information and assumptions into mathematical form.	Limited in translating the information and assumptions into mathematical form.	Does not translate information into mathematics.
Solution of the model	Performs calculations correctly in the model (possibly one minor error). Checks for precision.	Performs calculations correctly in the model (with few errors). Is aware of checking for precision.	Limited with calculations in the model (with multiple errors) and is not aware of needing to check for precision.	Demonstrates little to no evidence.
Results and Interpretation	Reports and interprets the mathematical solution in terms of the original situation.	Partially Reports and interprets the mathematical solution in terms of the original situation.	Limited with Reporting and interpreting the mathematical solution in terms of the original situation.	Demonstrates little to no evidence.
Validate the conclusions	Determines if the mathematical answer makes sense in terms of the experimental data used and verifies the answer is within a valid range of values. Determines if conclusions are satisfactory in all respects; if not, shows evidence of iteration of the process to improve the model.	Partially determines if the mathematical answer makes sense in terms of the experimental data used. Demonstrates awareness that the answer is within a valid range of values. Partially determines if conclusions are satisfactory.	Limited in determining if the mathematical answer makes sense in terms of the experimental data used. Shows little awareness that verification of the solution should be made.	Demonstrates little to no evidence.

6 Appendix

MODEL-1

```
%MODEL-1
clear variables;
clear;
clc;
%e.coli data
time= [0, 8, 24, 32, 48, 96, 168];
cell= [10000, 17000, 30000, 40000, 60000, 76000, 80000];

%equations
t= 0:168;
n0= 10000;
r = 0.034;
N= n0.*exp(r*t);
r1 = 0.04;
N1= n0.*exp(r1*t);
%plots
plot(time,cell,Color='r',LineStyle='--',LineWidth=1.5)
hold on
plot(t,N,Color='b',LineStyle='-.',LineWidth=1.5)
plot(t,N1,Color='g',LineStyle='-.',LineWidth=1.5)
hold off
grid on
xlabel('Time')
ylabel('Growth of cell concentration (E.coli')
axis([0 170 0 100000]);
legend('data','r=0.034','r=0.04',Location='southeast')
```

MODEL-2

```
import numpy as np
import matplotlib.pyplot as plt
from scipy.integrate import odeint
from scipy.optimize import curve_fit

"""Experimental data!"""
```

```
t_exp = np.array([0, 8, 24, 32, 48, 96, 168])
S_exp = np.array([5.5, 4.7, 3.7, 2.5, 1.5, 0.7, 0.5])
X_exp = np.array([10000, 17000, 30000, 40000, 60000,
        76000, 80000])

"Model of the microbial growth and the TOC degradation"
# SETTING MODEL
def f(t, u, K, Y):
    'Function that returns mutually dependent
    variables X and S'
    def growth(x, t):
        X = x[0]
        S = x[1]
        "Now differential equations are defined!"
        dXdt = (u * S * X )/(K + S)
        dSdt = ((-Y) * u * S * X )/(K + S)
        return [dXdt, dSdt]
    "initial Conditions"
    init = [10000, 5.5]
    results = odeint(growth, init, t)
    "Taking out desired column vectors from results array"
    return results[:,0], results[:,1]

# CURVE FITTING
"""k, kcov = curve_fit(f, t_exp, [X_exp, S_exp], p0=(1, 2, 2))
u = k[0]
K = k[1]
Y = k[2]"""

# RESULTS
t_mod = np.linspace(0, 168, 100)
compute = f(t_mod, 0.8, 75, 0.00008)
X_mod = compute[0]
S_mod = compute[1]

# PLOT-MODEL vs OBSERVED DATA
fig = plt.figure()
```

```
ax1 = fig.add_subplot(111)
ax1.plot(t_exp, X_exp, "yo")
ax1.plot(t_mod, X_mod, "g--", linewidth=3)
ax1.set_ylabel("E.coli biomass")

ax2 = ax1.twinx()
ax2.plot(t_exp, S_exp, "mo", )
ax2.plot(t_mod, S_mod, "r--", linewidth=3)
ax2.set_ylabel("Substrate", color="r")
for tl in ax2.get_yticklabels():
    tl.set_color("r")
plt.show()
```

MODEL-3

```
    clear variables;
clear;
clc;
%DATA
time= [0, 8, 24, 32, 48, 96, 168];
cell= [10000, 17000, 30000, 40000, 60000, 76000, 80000];
k=80000;
t= 0:8:168;
n0= 10000;
r = 0.055;
k1= 80500;
r1 = 0.06;
%equations
N=(n0.*exp(r.*t))./(1+(n0/k).*(exp(r.*t)-1));
N1=(n0.*exp(r1.*t))./(1+(n0/k1).*(exp(r1.*t)-1));

%PLOTS
plot(time,cell,Color='r',LineStyle='--',LineWidth=1.5)
hold on
plot(t,N,Color='b',LineStyle='-.',LineWidth=1.5)
plot(t,N1,Color='g',LineStyle='-.',LineWidth=1.5)
hold off
grid on
```

```
xlabel('Time')
ylabel('Growth of cell concentration (E.coli')
axis([0 180 0 81000]);
legend('data','k=80000;r=0.055','k=80500;r=0.06',Location='southl
```

References

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[3] Jacques Monod. The growth of bacterial cultures. *Annual Review of Microbiology*, 3(Volume 3, 1949):371–394, 1949.

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