Biomathematics Project

Bacteria-Phage mathematical model applied to the cheese industry

Source: Bacteriophage Ecology group

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INTRODUCTION

Milk fermentation in the cheesemaking process relies on the growth and acid-producing ability of bacteria (e.g. Lactococcus lactis cremoris) to obtain the desired consistency, quality and flavour of a cheese. Despite the efforts and precautions taken to prepare and choose starter cultures (bacteria), the milk fermentation cannot be protected from bacteriophage contamination that induces failure due to phage-lysis of the starter culture. Consequently, understanding and modelling bacteria-phage interactions are of enormous practical significance to the dairy industries.

In this project, we were interested in selecting and setting up a mathematical model applied to one particular bacterium species and its associated phage. To obtain biological coefficients necessary for the model, some experiments on bacteria and phage were held at Fonterra Research Centre, Palmerston North, New Zealand.

In a first part we will give a succinct description of bacteria and bacteriophage, and see the basic interactions between bacteria and bacteriophage.

In a second part, we will first describe several mathematical models and then focus on a specific kinetic model of phage interaction.

In a third part, we will present the experiments and results to determine coefficients for the mathematical model.

Finally, we will solve numerically the mathematical model for some initial values.
I) Bacteria-phage interactions

A basic activity in the cheese process consists of the fermentation of carbohydrates and nitrogenous compounds in milk, due to the presence of bacteria. Cheesemakers are not only concerned with the metabolism and activities of bacteria (living cells), but also with the release of cell contents on their death, e.g. enzymes.

A bacteriophage (phage) can be considered as a virus or a parasite that attacks bacteria (host), multiplies in the host cell cytoplasm, and is released causing the bacteria’s death (lysis).

Phage are often specific for just one or two strains of a bacterial species, so in a multiple-strain culture of organisms, a phage may decimate just one of the strains of bacteria present. We used a lactococcus lactis cremoris strain 2356 for the bacteria and a small isometric headed phage Siphoviridae for the bacteriophage in this study.

1) The Structure of Bacteria:

Most bacteria appear in variations of three different shapes: the rod (Bacillus), the sphere (Coccus), and the spiral (Spiral). Coccii are approximately 0.5µm to 1.0µm in diameter. They are usually round, but they may also be oval, elongated, or indented on one side. Some species are harmless enough to be used for producing dairy products such as yogourt, e.g. lactococcus lactis cremoris strain 2356.

Most bacteria reproduce by an asexual process called “binary fission”. The chromosome duplicates, the cell elongates and when the nuclear material has been evenly distributed the cell divides in two identical bacterial cell. Once the division is complete, bacteria grow and develop the features that make each species unique. The interval of time until the completion of the next division is called the generation time. In some bacteria, the generation time is very short and for others it is quite long.

The bacterial growth curve:

A growth curve for a population of bacteria illustrates some of the dynamics that affect the cells over time.

Four distinct phases are recognized:

- The lag phase:

  The curve remains at a plateau. During this time, bacteria adapt to their new environment, store nutrients and prepare for binary fission

- The logarithmic phase:

  This phase is also called the exponential growth phase: the population of bacteria enters an active stage of growth, the mass of each cell increases rapidly, and the number of bacteria doubles.
• **The stationary phase:**

At this stage reproductive and death rates equalize, the population enters another plateau.

• **The decline phase:**

If the conditions of the stationary phase continue, the decline phase will ensue: the number of dying cells exceeds the number of new cells produced.

The length of the phases of the growth curve can be slightly different for different values of temperature conditions and for different types of bacteria.

---

![](image.png)

Source [18]

---

2) **Phage Morphology:**

Lactococcal phages are small particles consisting of protein and nucleic acid, they have a head (prolate or isometric) containing the supercoiled DNA and a tail. Most lactococcal strains are lysogenic for one or more phages. Generally, there are different categories of phages: lytic, chronic, lysogenic, temperate, virulent. The following paragraph explains the basic differences and definitions of bacteriophage classification based on phage-host interaction.
Different categories of phage:

**Lytic:** In order to release progeny into the extracellular environment, a lytic phage must terminate its infection and breach its host's cell envelope. "Lytic phage" and "Virulent phage" are used synonymously (Lwoff, 1953. Lysogeny. *Bacteriological Reviews* 17:269-337).

**Chronic (Continuous):** A lytic infection contrasts with a chronic (or continuous) infection. A chronically infecting phage (or virus) can release progeny into the extracellular environment without terminating its infection. That is, phages are extruded across the host cell envelope.

**Lysogenic:** Also contrasting lytic (as well as chronic) is lysogenic. "A lysogenic bacterium is a bacterium possessing and transmitting the power to produce bacteriophage" (p. 271, Lwoff, 1953). During the lysogenic cycle an infected bacterium does not produce phage progeny nor release phage progeny into the extracellular environment.
Temperate: A temperate phage is one that is capable of displaying a lysogenic infection. Note that temperate phages typically display a lytic cycle as their vegetative (i.e., non-lysogenic) phase. Nevertheless, one does not refer to temperate phages as lytic phages.

Virulent: Unfortunately, the standard term used to describe a lytic but not temperate phage is virulent. A virulent phage is one that does not display a lysogenic cycle.

Common practice has been to differentiate phages into at least two types, temperate versus virulent or lytic, to which a third type, chronic or continuous, should be included. Temperate phages can produce reductive (lysogenic), productive, or abortive infections while non-temperate phages can give rise only to productive or abortive infections. Chronically infecting phages extrude their progeny from infected cells without lysing their hosts, while both temperate and lytic (or virulent) phages lyse their hosts to release progeny phages. Unfortunately, this contrast between chronic release and lytic release gives rise to an ambiguity: Temperate phages, in practice, are lytic, but, strictly speaking, are not "Lytic phages." Less ambiguous, temperate phages are not virulent phages.

The term "Virulence" dates from early phage characterization in which it was noted that some phages more readily lyse cultures of bacteria than others. The question of virulence has been mentioned and emphasis placed upon the necessity of utilizing a race of maximum virulence. By this is meant a race of bacteriophage which will cause a complete and permanent dissolution of the organisms actually present in the infectious process. Only subsequently did the term "Virulent" come to designate those phages that fail to display lysogeny in the modern sense of that term.

Phages have been classified in two categories, temperate and virulent according to the presence or absence of the power to lysogenize.

Thus, there is no such thing as a "lysogenic phage" and temperate phages typically are perfectly capable of lysing their bacteria hosts. The following figure summarizes the different definitions of bacteriophage classification based on phage-host interaction.
Life cycle of lytic lactococcal phage:

The first step is the adsorption of the phage to specific receptor sites on the bacterium cell surface. Then the phage “injects” its DNA into the cell which can be expressed and replicated. DNA replication carries on during the infection cycle with production of heads, tails and DNA molecules.
Once phage are assembled, cell lysis occurs causing a release of new phages. The number of released phages of one infected cell is the burst size and the time spent between adsorption and release of phages is called the latent period.

3) **Host resistance mechanisms:**

Resistance mechanisms are not widely spread out in bacteria but occur at some point. There are four main mechanisms:
• **Adsorption inhibition**

The phage cannot adsorb to a cell, which may be due to the absence of, or a defect in, the receptor. It is a particularly effective resistance mechanism.

• **Prevention of DNA injection**

The phage adsorbs to the cell but the phage DNA does not reach the cytoplasm of the bacteria and therefore does not replicate.

• **Restriction and modification systems**

A particular molecule (restriction endonuclease component) recognizes DNA phage and cuts it at a specific base sequence, so that DNA phage does not replicate. This mechanism allows the cell to adapt rapidly and acquire new phage resistance specificities.

• **Abortive infection**

Host defences act on phage development and interfere with DNA replication, and assembly of phage particles. An abortive mechanism usually results in the host’s death, but because phage infection is inhibited infection of other cells in the culture is preserved.

These phenomena are due to transfer of plasmids between strains or loss of plasmids from strains during the phage cycle. They are important mechanisms in changing the phage sensitivity of a strain. As bacteria acquire new defence mechanisms, phages also adapt to overcome new obstacles.
II) Mathematical model

Different possible approaches with several mathematical models have been considered. We will discuss briefly each of the models before presenting the final model we will deal with.

The first model is fully described in the following reference:
This model examines effects of the vertebrate immune system on the evolution and maintenance of virulence of microparasites (viruses, bacteria).

The rates of changes in the densities of parasite and immunity are given by:

\[
\begin{align*}
\frac{dP}{dt} &= rP - kPI \quad P < D \\
\frac{dI}{dt} &= \rho I \left( \frac{P}{P + \phi} \right)
\end{align*}
\]

- $P$ is the rate of change in the density of a parasite population
- $r$ is the net rate at which virus replicates in the host and at which it is cleared by the host’s defence
- $I$ is the immune response
- $D$ is a threshold density of parasites beyond which the host dies and the parasite density falls to zero
- $k$ is the rate constant for elimination of the parasite population by the immune response
- $\rho$ is the maximum rate of proliferation of immune cells
- $\phi$ is the parasite density at which the immune cells proliferate at half the maximum rate

Experimental data are not easy to obtain in this particular model, and this system does not give concentration of bacteriophage (parasite) and bacteria (host).

The second model is fully described in the following paper:
Productivity can influence the significance of predation and competition in determining ecological patterns in abundance, diversity and community structure.
A simple community consists of two prey species that share a common resource and a same predator.
The mathematical model is a system of four differential equations:
\[
\begin{align*}
\frac{dR}{dt} &= (R_0 - R)\omega - \frac{\varepsilon N_A \psi_A R}{K_A + R} - \frac{\varepsilon N_B \psi_B R}{K_B + R} \\
\frac{dN_A}{dt} &= \frac{N_A \psi_A R}{K_A + R} - \alpha_A N_A P - \omega N_A \\
\frac{dN_B}{dt} &= \frac{N_B \psi_B R}{K_B + R} - \alpha_B N_B P - \omega N_B \\
\frac{dP}{dt} &= \beta e^{-\tau_0} \alpha_A N_A' P' + \beta e^{-\tau_0} \alpha_A N_A' P' - \alpha_A N_A P - \alpha_B N_B P - \omega P
\end{align*}
\]

- \( R \) is the concentration of glucose in the chemostat
- \( N_A \) and \( N_B \) are the population densities of more vulnerable and less vulnerable bacteria
- \( P \) is the population density of the bacteriophage
- \( R_0 \) is the concentration of glucose in the reservoir
- \( \omega \) is the flow rate
- \( \varepsilon \) is the growth efficiency
- \( \psi_A \) is the maximum specific growth rate of more vulnerable bacteria
- \( \psi_B \) is the maximum specific growth rate of less vulnerable bacteria
- \( K_A \) is the resource concentration at which the more vulnerable bacteria grow at one-half \( \psi_A \)
- \( K_B \) is the resource concentration at which the less vulnerable bacteria grow at one-half \( \psi_B \)
- \( \alpha_A \) is the attack rate (adsorption) of bacteriophage on more vulnerable bacteria
- \( \alpha_B \) is the attack rate (adsorption) of bacteriophage on less vulnerable bacteria
- \( \beta \) is the burst size of bacteriophage
- \( \tau \) is the latent period of bacteriophage
- \( e^{-\tau_0} \) is the fraction of bacteria infected at time \( t - \tau \) that has not washed out before lysing
- \( N_A' \) is the population density of more vulnerable bacteria at time \( t - \tau \)
- \( N_B' \) is the population density of less vulnerable bacteria at time \( t - \tau \)
- \( P' \) is the population density of bacteriophage at time \( t - \tau \)

Concerning this model, the rate of decline in some replicates was slower than predicted, and extinctions were not observed contrary to theoretical predictions. For our problem we do not wish to use a chemostat to model the problem.

In this paper an analytical model is developed to consider the variation and the persistence of stochastic metapopulations. The “natural-enemy model” refers to the study of epidemics and highlight spatial heterogeneity on the persistence of a population.
This model is another alternative of considering a continuous time predator-prey or host-parasite model. The model presented in this paper has a stable attracting fixed point whereas the Lotka-Volterra model has no “deterministic force” acting against extinctions. The following system models the state of a subpopulation $i$:

\[
\begin{align*}
\dot{S}_i &= B - \beta S_i [(1 - \sigma)I_i + \sigma \bar{T}] \\
\dot{I}_i &= \beta S_i [(1 - \sigma)I_i + \sigma \bar{T}] - g I_i + \varepsilon 
\end{align*}
\]

- $S_i$ represents non-infected population
- $I_i$ represents the infected population
- $B$ is the birth rate
- $\sigma$ is a coupling term measuring the amount of interaction between communities
- $\varepsilon$ is included to prevent permanent extinctions

These equations involve terms including the variances and covariances. This model is a good approximation for many childhood diseases where the disease is rarely fatal. The use of moment closure approximations means that this model is most suited to situations where global extinctions are rare.


This paper develops the study of phage therapy and antibiotic therapy as a population biological phenomenon using the dynamic interaction of bacteria with a predator (phage) inside a host (mouse). The mathematical model is a system of five equations:

\[
\begin{align*}
\dot{N} &= \psi_N N - k_N IN - \delta NP - k_a [A/(K + A)]N \\
\dot{R} &= \psi_R R - k_R IR - k_a [A/(K + A)]R \\
\dot{I} &= \rho I[(N + R)/(N + R + \theta)] \\
\dot{P} &= \delta NP\beta \\
\dot{A} &= -Ad_A
\end{align*}
\]

- $N$ is the density of phage-sensitive bacteria
- $R$ is the density of phage-resistant bacteria
- $I$ is the intensity of the mouse’s immune response against the bacterium
- $P$ is the density of bacteriophage
- $A$ is the concentration of antibiotic
- $\psi_N$ is the phage-sensitive bacteria growth rate in the absence of an immune response, phage, and antibiotics
- $\psi_R$ is the phage-resistant bacteria growth rate in the absence of an immune response, phage, and antibiotics
- $k_N$ is the inhibition parameter for the phage-sensitive populations
- $k_R$ is the inhibition parameter for the phage-resistant populations
- $k_a$ is the inhibition constant of the antibiotic
\[ K \] is the concentration of antibiotic when the inhibitory effect is half its maximum value
\[ d_A \] is the decay rate of antibiotic
\[ \delta \] is the adsorption parameter of phage
\[ \beta \] is the burst size
\[ \rho \] is a constant rate at which \( I \) increases with the combined density of bacterial antigens \((N + R)\) relative to a normalizing quantity \((N + R + q)\)

The goal of this paper was to identify hypotheses consistent with their results and to motivate further empirical tests of those hypotheses.

[6] Levin Bruce R., Stewart Franck M., Chao Lin, Resource-limited growth, competition, and predation: a model and experimental studies with bacteria and bacteriophage
This paper presents models of a bacteriophage-bacteria interaction which are based on specific assumptions about the habitat, the use of primary resources, the population growth, and the nature of the interaction between predator and prey.
The following system includes interactions with \( K \) bacteriophage species, \( J \) different kinds of resource, and \( I \) bacteria species.

\[
\begin{align*}
\dot{r}_j &= \rho (C_j - r_j) - \sum_{i=1}^{J} \phi_{ij} (n_i + \sum_{k=1}^{K} m_{ik})
\dot{n}_i &= n_i \sum_{j=1}^{J} \phi_{ij} e_{ij} / e_{ij} - \rho n_i - \sum_{k=1}^{K} \gamma_{ik} n_i p_k
\dot{m}_{ik} &= \gamma_{ik} n_i p_k - \rho m_{ik} - e^{-\gamma_{ik}} \gamma_{ik} n_i (t - l_{ik}) p_k (t - l_{ik})
\dot{p}_k &= \sum_{i=1}^{J} b_{ik} e^{-\gamma_{ik}} \gamma_{ik} n_i (t - l_{ik}) p_k (t - l_{ik}) - \rho p_k - \sum_{k=1}^{K} \gamma_{ik} n_i p_k
\end{align*}
\]

The \( j^{th} \) resource has a concentration \( r_j \text{ mg/ml} \) within the habitat and a concentration \( C_j \text{ mg/ml} \) in the reservoir from which it is flowing.

Within the habitat there is a concentration of \( n_i \) individuals per millilitre of the \( i^{th} \) prey species and a concentration of the \( k^{th} \) predator population is \( p_k \) individuals per millilitre.

\( l_{ik} \) is the latent period
\( m_{ik} \) is the concentration of prey of the \( i^{th} \) type which have been attacked by a predator of the \( k^{th} \) type and are in the process that results in the birth of new predators.

Each individual in the \( i^{th} \) prey population takes up the \( j^{th} \) at a rate \( \phi_{ij} (r_i - r_j) \)

To produce a new member the \( i^{th} \) prey population must consume \( e_{ij} \text{ mg} \) of the \( j^{th} \) resource

\( \gamma_{ik} \) is the adsorption coefficient

\( b_{ik} \) is the burst size

\( \rho \) is the rate of flow through the habitat
Stable equilibria with all populations coexisting are possible when the number of distinct predator and the number of prey is not more than the sum of the number of resources and the number of predator populations. With some experiments equilibrium concentrations were similar to those predicted by the model, however, with the estimated values of the parameters, the experimental system fell in the range where the model predicted that the oscillations would increase to the point where the populations would eventually become extinct.

This paper concerns studies of bacteriophage as therapeutic agents and uses a mathematical model to delineate different categories of outcomes.
The change in concentrations over time are described by the differential equations:

\[
\begin{align*}
\frac{dx}{dt} &= ax - bvx - H(t)x \\
\frac{dy}{dt} &= ay + bvx - ky - H(t)y \\
\frac{dv}{dt} &= kLy - bvx - mv - h(t)v
\end{align*}
\]

- \( x(t) \) represents the concentration of uninfected bacteria
- \( y(t) \) the lytic bacteria
- \( v(t) \) the free phage
- \( a \) is the replication coefficient of the bacteria
- \( b \) is the transmission coefficient
- \( k \) is the lysis rate coefficient
- \( L \) is the burst size
- \( m \) is the decay rate of free phage
- \( H(t) \) is the host response against the bacteria
- \( h(t) \) is the host response against the phage

This model illustrates that many of the apparently paradoxical aspects of phage therapy can arise from nonlinear density-dependent phenomena.

This paper concerns the possible use of bacteriophage to control bacterial infections. Treatment outcome depends on various density-dependent thresholds, and the ability to predict these thresholds and associated critical time points are discussed. The mathematical model used describes the kinetics of a generalized phage-bacteria system of the form:
\[
\begin{align*}
\frac{dx}{dt} &= ax - bvx \\
\frac{dy}{dt} &= ay + bvx - ky \\
\frac{dv}{dt} &= kLy - bvx - mv
\end{align*}
\]

- \( x(t) \) represents the concentration of uninfected bacteria
- \( y(t) \) the lytic bacteria
- \( v(t) \) the free phage
- \( a \) is the replication coefficient of the bacteria
- \( b \) is the transmission coefficient
- \( k \) is the lysis rate coefficient
- \( L \) is the burst size
- \( m \) is the decay rate of free phage

This model is a more simple approach than the model studied in reference [9] and turns out to be the best model fitting our initial problem.


This paper manipulates a bacteria-phage model system to investigate empirical and theoretical conditions allowing for the coexistence of a specific bacteria species host with each of two virulent phage species.

The rate of change in the component populations are given by the following equations:

\[
\begin{align*}
\frac{dC}{dt} &= \sigma[C_0 - C] - \psi(C)\varepsilon[N + M] - \psi_R(C)\varepsilon R \\
\frac{dN}{dt} &= \psi(C)N - \delta NV - \omega N \\
\frac{dR}{dt} &= \psi_R(C)R - \omega R \\
\frac{dM}{dt} &= \delta NV - \delta N\varepsilon e^{-\omega t} - \omega M \\
\frac{dV}{dt} &= \beta \delta N\varepsilon e^{-\omega t} - \delta NV - \delta MV - \delta_R RV - \omega V - d_V V
\end{align*}
\]

- \( C \) represents the concentration of resource
- \( N \) represents the densities of sensitive bacteria
- \( M \) represents the densities of infected bacteria
- \( R \) represents the densities of phage-resistant bacteria
- \( V \) represents the densities of phage
- \( \psi_R(C) \) is the growth rate of resistant bacteria
- \( \varepsilon \) is the resource conversion efficiency parameter
- \( \delta \) is the adsorption rate parameter
• $\tau$ is the latent period
• $\beta$ is the burst size
• $d_{\nu}$ is the mortality rate of free phage

This article suggests that glass surfaces act as a spatial refuge for sensitive bacteria: this hypothesis predicts that bacteria-phage stability will increase when opportunities for wall growth increase. This model accurately predicted persistence and extinction in most cases when it was adapted to describe bacteria-phage interactions in serial transfer in a liquid environment. However, the bacteria-phage dynamics in serial culture often differed from the model’s predictions.

Based on a minimal set of the essential biological bacteria-bacteriophage interactions, we use a model based on a kinetic model of phage interaction described in the papers referred to in [8], [9].

$x(t)$ represents the concentration of uninfected bacteria, $y(t)$ the lytic bacteria, and $v(t)$ the free phage. We can model the changes in concentrations over time by the differential equations:

\[
\begin{align*}
\frac{dx}{dt} &= ax - bv
\\
\frac{dy}{dt} &= ay + bv - ky
\\
\frac{dv}{dt} &= kLy - bv - mv
\end{align*}
\]

• $a$ is the replication coefficient of the bacteria
• $b$ is the transmission coefficient
• $k$ is the lysis rate coefficient
• $L$ is the burst size
• $m$ is the decay rate of free phage

Values of the different coefficients vary considerably from one bacteria-phage system to another.

This mathematical model is built under certain assumptions described in the following paragraph:

**ASSUMPTIONS:**

This model is appropriate for lytic phage but not for temperate phage.

Here any host responses against bacteria or against phages are neglected.
This model does not include the possibility of bacterial growth constrained by target cell limitation.

The basic reproductive number of phage depends on the density of bacteria and the density of phage. This means that when the number of sensitive bacteria is very low, the number of phages will also decline which allows sensitive bacteria to increase again. Therefore, we use the hypothesis of “numerical refuge” [13] that enables the mathematical model to predict that bacteria and phage can coexist in theory under a set of conditions.

We assume that adsorption to an already infected cell adds nothing to the cell population so any such adsorption contributes to the death rate of phage.

**DISCUSSIONS:**

Some hypotheses are not taken into account in this minimal model, but we need to be aware of their existence to bring improvements to the model at some point.

Bacteria and bacteriophage continually evolve with mutation and selection. As bacteria evolve to resist the dominant phage population, phages evolve by mutation and can infect the dominant resistant bacteria population.

During specific stages of bacterial life cycles, sensitive bacteria may become transitorily resistant to infection. This “physiological refuge hypothesis” [13] is illustrated by the following example: during periods of rapid bacterial growth the number of receptors permitting phage adsorption might be altered and so consequently the number of released phages might be altered as well. These evolutions would be hard to estimate and to be implemented in the mathematical model.

Another interesting fact concerns the “spatial refuge hypothesis” [13], that predicts that physical heterogeneity in the environment may protect some bacteria from phage infection. For example, bacteria are capable of adhering to glass and therefore such “wall populations” play an important role in the non-extinction of bacteria population. This hypothesis shows that bacteria-phage stability may also depend on the possibility of “wall populations” to grow.
III) Determining experimental values

As no data were reported concerning lactococcus lactis cremoris strain 2356 for the bacteria and small isometric headed phage Siphoviridae for the bacteriophage, we decided to get values of the coefficients $a$ (replication coefficient of the bacteria), $b$ (transmission coefficient), $L$ (the burst size), and $m$ (decay rate of free phage), in a laboratory at Fronterra Research Centre, Palmerston North.

In this part we will first describe the methods used to obtain values for the model coefficients, and then we will talk about the results and how to derive values for those coefficients.

1) Procedures:

a) Phage titre:

The first procedure consists of obtaining a phage titre that gives an estimation of the number of phage present.

To obtain a count per ml or titre of the phage, an agar plate overlay method is used. The overlay consist of a mixture of the phage and the bacterial host.

Method:

i) Take the built up, filtered phage sample and make dilutions in 5 ml of 1/10 M17 diluent as follows:

<table>
<thead>
<tr>
<th>Phage stock ((\phi))</th>
<th>-2</th>
<th>-4</th>
<th>-5</th>
<th>-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05 ml</td>
<td>→ 0.05 ml</td>
<td>→ 0.55 ml</td>
<td>→ 0.55 ml</td>
<td>→ 0.55 ml</td>
</tr>
</tbody>
</table>

with 5 ml of 1/10 M17 diluent. Vortex each tube as dilutions are made.

ii) Label M17 agar plates with phage number and dilution.

iii) To sterile tubes held in the 50°C heating block, add:

- 2.5 ml of molten M17 soft agar
- 1 drop of \(CaCl_2\)
- 0.1 ml of phage\(\phi\) dilution (sample size used in calculation)

Add 0.2 ml of the appropriate (host) M17 broth culture last, mix (vortex) and pour immediately onto labelled M17 agar plates.

iv) Allow plates to set 10 minutes.

v) Incubate at 30°C for 24 hours.

vi) Count.

\[
\text{Titre} = \text{Number of plaques} \times \text{dilution} \times \text{sample size}
\]

The titre is given in pfu/ml = plaque forming units per ml

The following figure illustrates this phage titre method using agar plates.
The next photo shows how a plate looks like after incubation, by counting the number of phage plaques we have an estimation of the initial phage concentration.
b) **Replication coefficient of bacteria:**

This method uses a spectrophotometer that takes readings of a tube of 1 ml of freshly grown overnight bacterial culture with 10 ml of M17 broth every 15 minutes over 5 hours.

c) **Bacteriophage one step growth curve:**

This procedure gives the burst size, the latent period and the burst time specific to the bacteriophage.

i) Take 1 ml of a 3.5 hour culture. Centrifuge 1 minute in a sterile Eppendorf tube.

ii) Resuspend pellet in 1mL M17 plus 50µl of Calcium chloride.

iii) Add 100µl phage \((10^8 - 10^9 \text{ pfu/mL})\)

iv) Stand 5 minutes at temperature T for adsorption

v) Centrifuge 2 minutes at 10 000 tours/min. Discard supernatant and resuspend cells in 1 ml M17 plus 50µl of Calcium chloride.

vi) Dilute to \(10^4\) in M17 plus calcium (0.1 ml to 10 ml then 1 ml to 100 ml)

vii) Incubate 30°C, remove 1ml samples at appropriate intervals to Eppendorf tubes containing 100µl chloroform (or add immediately).

viii) Centrifuge 3 minutes at 10 000 tours/min.

ix) Titre supernatant.

d) **Transmission coefficient:**

This procedure consists of determining the concentrations of sensitive bacteria, lytic bacteria and free phage at different times to obtain an estimate value of the transmission coefficient.

i) Add 2 ml of freshly grown overnight bacterial culture to 100 ml M17 plus 0.5 ml calcium chloride.

ii) Grow at 30°C for 2 hours (or as required).

iii) Add 1 ml high titre phage \((10^7 - 10^9 \text{ pfu/ml})\)

iv) At \(t = 0\)

\[\text{a. Mix well and take 1.5 ml to 100 ml diluent}\]

\[\text{b. Centrifuge immediately in 2 Falcon tubes of 50 ml.}\]

\[\text{c. Transfer the supernatant to a bijou for later tittering (this titre will be } v(t), \text{ the concentration of free phage).}\]

\[\text{d. Immediately resuspend pellet in 20 ml diluent per tube.}\]

\[\text{e. Transfer to one tube and spin again.}\]

\[\text{f. Resuspend in 1.5 ml diluent. Stand on ice.}\]

\[\text{g. Immediately titre as for phage (using sensitive lawn). (This will be } y(t), \text{ the concentration of lytic bacteria)}\]

\[\text{h. From the material in step f, spread 0.1 ml on an M17 plate.}\]

\[\text{i. Dilute the material from step f to } 10^4 - 10^4 \text{ and spread 0.1 ml of each dilution on an M17 plate. The count from step h or i will be } x(t), \text{ the concentration of uninfected bacteria.}\]

v) Repeat step iv) at other time points as required.
e) **Decay rate coefficient:**

This method gives an estimate value of the decay rate coefficient of free phage.

i) Take 1 ml of high titre phage \((10^7 \text{ to } 10^9 \text{ pfu/ml})\) in 100 ml M17 broth.

ii) Titre immediately.

iii) Titre at another time \(t\) (one week later for example).

2) **Results:**

a) **Replication coefficient of bacteria: \(a\)**

The following chart gives the values of three data replicates:

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Replicate 1 (spectrophotometer)</th>
<th>Replicate 2 (spectrophotometer)</th>
<th>Replicate 3 (spectrophotometer)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0,138</td>
<td>0,131</td>
<td>0,12</td>
</tr>
<tr>
<td>15</td>
<td>0,145</td>
<td>0,128</td>
<td>0,146</td>
</tr>
<tr>
<td>30</td>
<td>0,145</td>
<td>0,14</td>
<td>0,143</td>
</tr>
<tr>
<td>45</td>
<td>0,14</td>
<td>0,145</td>
<td>0,159</td>
</tr>
<tr>
<td>60</td>
<td>0,143</td>
<td>0,15</td>
<td>0,16</td>
</tr>
<tr>
<td>75</td>
<td>0,149</td>
<td>0,145</td>
<td>0,141</td>
</tr>
<tr>
<td>90</td>
<td>0,137</td>
<td>0,136</td>
<td>0,176</td>
</tr>
<tr>
<td>105</td>
<td>0,16</td>
<td>0,156</td>
<td>0,171</td>
</tr>
<tr>
<td>120</td>
<td>0,168</td>
<td>0,162</td>
<td>0,185</td>
</tr>
<tr>
<td>135</td>
<td>0,177</td>
<td>0,188</td>
<td>0,209</td>
</tr>
<tr>
<td>150</td>
<td>0,2</td>
<td>0,188</td>
<td>0,202</td>
</tr>
<tr>
<td>165</td>
<td>0,218</td>
<td>0,218</td>
<td>0,223</td>
</tr>
<tr>
<td>180</td>
<td>0,227</td>
<td>0,23</td>
<td>0,25</td>
</tr>
<tr>
<td>195</td>
<td>0,269</td>
<td>0,251</td>
<td>0,266</td>
</tr>
<tr>
<td>210</td>
<td>0,295</td>
<td>0,289</td>
<td>0,3</td>
</tr>
<tr>
<td>225</td>
<td>0,315</td>
<td>0,303</td>
<td>0,311</td>
</tr>
<tr>
<td>240</td>
<td>0,348</td>
<td>0,323</td>
<td>0,33</td>
</tr>
<tr>
<td>255</td>
<td>0,36</td>
<td>0,348</td>
<td>0,348</td>
</tr>
<tr>
<td>270</td>
<td>0,367</td>
<td>0,35</td>
<td>0,4</td>
</tr>
<tr>
<td>285</td>
<td>0,427</td>
<td>0,391</td>
<td>0,43</td>
</tr>
<tr>
<td>300</td>
<td>0,439</td>
<td>0,419</td>
<td>0,44</td>
</tr>
</tbody>
</table>
Replication measurements for the uninfected bacteria population

We can see on the previous figures that the growth curve of bacteria follows the lag phase, the exponential phase and the beginning of the stationary phase over five hours. If we use data fitting to approximate the growth curve we find that the exponential functions for the three replicates are very similar:

Replicate 1: \( y = 0.1066e^{0.0647x} \)
Replicate 2: \( y = 0.1052e^{0.0633x} \)
Replicate 3: \( y = 0.1121e^{0.0634x} \)

We can therefore pick one of the exponential approximate functions to estimate values of the replication coefficient of bacteria.
We choose to use the function of the third replicate that is:
\[ y = 0.1121e^{0.0634x} \text{ for } t \leq 5 \text{ hours} \]

To determine more accurate values more experiments over longer periods of time should be held.
b) **Decay rate coefficient of free phage:** \( m \)

Two replicates were titred at an interval of one week.

\[
\begin{align*}
    t = 0 & \quad t = 168 \text{ hours} = 1.008 \times 10^4 \text{ minutes} \\
    \text{Replicate 1:} & \quad 3.2 \times 10^6 \text{ pfu/ml} \quad 1.0 \times 10^5 \text{ pfu/ml} \\
    \text{Replicate 2:} & \quad 1.07 \times 10^7 \text{ pfu/ml} \quad 2.7 \times 10^5 \text{ pfu/ml}
\end{align*}
\]

In this case \( x = 0, y = 0 \), therefore in the differential equation

\[
\frac{dv}{dt} = kLy - bvx - mv
\]

becomes \( \frac{dv}{dt} = -mv \).

Subsequently we find for the first replicate: \( m = 31 \)
And for the second replicate: \( m = 38.6 \)
On average, we will take \( m = 34.8 \).

c) **Burst size:** \( L \)

To determine the burst size we use the bacteriophage one step growth curve.

The following chart gives the results obtained for three replicates:

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Replicate 1 (pfu/ml)</th>
<th>Replicate 2 (pfu/ml)</th>
<th>Replicate 3 (pfu/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>230</td>
<td>60</td>
<td>80</td>
</tr>
<tr>
<td>15</td>
<td>150</td>
<td>80</td>
<td>90</td>
</tr>
<tr>
<td>30</td>
<td>340</td>
<td>70</td>
<td>140</td>
</tr>
<tr>
<td>40</td>
<td>230</td>
<td>100</td>
<td>80</td>
</tr>
<tr>
<td>45</td>
<td>210</td>
<td>120</td>
<td>150</td>
</tr>
<tr>
<td>50</td>
<td>400</td>
<td>140</td>
<td>120</td>
</tr>
<tr>
<td>55</td>
<td>400</td>
<td>125</td>
<td>110</td>
</tr>
<tr>
<td>60</td>
<td>400</td>
<td>150</td>
<td>110</td>
</tr>
<tr>
<td>65</td>
<td>800</td>
<td>140</td>
<td>600</td>
</tr>
<tr>
<td>70</td>
<td>800</td>
<td>750</td>
<td>900</td>
</tr>
<tr>
<td>75</td>
<td>800</td>
<td>700</td>
<td>1100</td>
</tr>
<tr>
<td>90</td>
<td>800</td>
<td>1700</td>
<td>1300</td>
</tr>
</tbody>
</table>

Remark: we have to take into account a time of 15 minutes spent for the adsorption before time \( t = 0 \) in the chart. Consequently, to calculate the burst time we have to add 15 minutes for each replicate.

The following graphs illustrate these data, the burst time corresponds to the time in the middle of the exponential curve, the burst size corresponds to the division of the average number of phage after the burst time and the average number of phage before the burst time, and the latent period corresponds to the period before the exponential curve starts.
Bacteriophage one step growth curves

<table>
<thead>
<tr>
<th>Replicate</th>
<th>Latent period (minutes)</th>
<th>Burst size (per bacteria)</th>
<th>Burst time (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replicate 1</td>
<td>80</td>
<td>3</td>
<td>82</td>
</tr>
<tr>
<td>Replicate 2</td>
<td>80</td>
<td>17</td>
<td>92</td>
</tr>
<tr>
<td>Replicate 3</td>
<td>75</td>
<td>12</td>
<td>87</td>
</tr>
</tbody>
</table>

The first replicate was not obtained with a freshly grown overnight culture which explains why the burst size is so low for this replicate.

Finally, on average we have for:
- The burst time: $\tau = 85$ minutes
- The burst size: $L = 15$
- The latent period: 77 minutes
d) **Transmission coefficient:** \( b \)

To obtain this coefficient we titre the lytic bacteria, the uninfected bacteria and the free phage at 3 time points: at \( t = 0 \), \( t = 45 \) (before the burst time), and \( t = 120 \) (after the burst time).

<table>
<thead>
<tr>
<th>Replicates</th>
<th>Time (minutes)</th>
<th>( t_0 = 0 )</th>
<th>( t_1 = 45 )</th>
<th>( t_2 = 120 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replicate 1</td>
<td>( x(t) )</td>
<td>( 1.9 \times 10^4 )</td>
<td>( 6.8 \times 10^3 )</td>
<td>( 1.02 \times 10^4 )</td>
</tr>
<tr>
<td></td>
<td>( y(t) )</td>
<td>( 5.4 \times 10^3 )</td>
<td>( 8.8 \times 10^3 )</td>
<td>( 2.7 \times 10^3 )</td>
</tr>
<tr>
<td></td>
<td>( v(t) )</td>
<td>( 7.4 \times 10^4 )</td>
<td>( 4.1 \times 10^4 )</td>
<td>( 4.1 \times 10^4 )</td>
</tr>
<tr>
<td>Replicate 2</td>
<td>( x(t) )</td>
<td>( 5.6 \times 10^3 )</td>
<td>( 1.7 \times 10^3 )</td>
<td>( 5.6 \times 10^2 )</td>
</tr>
<tr>
<td></td>
<td>( y(t) )</td>
<td>( 1.5 \times 10^3 )</td>
<td>( 1.9 \times 10^3 )</td>
<td>( 1.1 \times 10^3 )</td>
</tr>
<tr>
<td></td>
<td>( v(t) )</td>
<td>( 1.7 \times 10^4 )</td>
<td>( 2.9 \times 10^4 )</td>
<td>( 2.2 \times 10^4 )</td>
</tr>
<tr>
<td>Replicate 3</td>
<td>( x(t) )</td>
<td>( 3.3 \times 10^3 )</td>
<td>( 3.1 \times 10^3 )</td>
<td>( 1.5 \times 10^3 )</td>
</tr>
<tr>
<td></td>
<td>( y(t) )</td>
<td>( 3.8 \times 10^6 )</td>
<td>( 1.0 \times 10^6 )</td>
<td>( 2.8 \times 10^6 )</td>
</tr>
<tr>
<td></td>
<td>( v(t) )</td>
<td>( 2.5 \times 10^6 )</td>
<td>( 1.4 \times 10^6 )</td>
<td>( 1.4 \times 10^6 )</td>
</tr>
</tbody>
</table>

Remark: the concentration of uninfected bacteria, lytic bacteria and phage are given in pfu/ml.

The bacteria will be cleared if the inoculum of phage is greater than a certain threshold (see reference \( [9] \) for further details). Therefore, during such a clearance the role of secondary infection is negligible, so the behaviour of phage can be approximated by ignoring the lysis term: \( \frac{dv}{dt} = -bv - mv \).

And the behaviour of the uninfected bacteria is dominated by the transmission term, so that \( \frac{dx}{dt} = -bv \). And \( -ky \) partially cancels so the change in \( y \), \( dy \) can be approximately associated with \( b \), therefore \( \frac{dy}{dt} = bvx \), i.e. \( \frac{dx}{dt} = -dy \).

Thus, the transmission coefficient is given by: \( \frac{dt}{xy} \).

The following chart gives the approximate values of \( b \) for the three replicates:

<table>
<thead>
<tr>
<th>Replicates</th>
<th>( dt = t_1 - t_0 )</th>
<th>( dt = t_2 - t_1 )</th>
<th>( dt = t_2 - t_0 )</th>
<th>average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replicate 1</td>
<td>( b = 3.22.10^{-6} )</td>
<td>( b = 1.75.10^{-5} )</td>
<td>( b = 9.6.10^{-7} )</td>
<td>( b = 7.22.10^{-6} )</td>
</tr>
<tr>
<td>Replicate 2</td>
<td>( b = 5.6.10^{-6} )</td>
<td>( b = 1.30.10^{-5} )</td>
<td>( b = 2.1.10^{-6} )</td>
<td>( b = 6.9.10^{-6} )</td>
</tr>
<tr>
<td>Replicate 3</td>
<td>( b = 4.5.10^{-4} )</td>
<td>( b = 3.429.10^{-4} )</td>
<td>( b = 6.10^{-5} )</td>
<td>( b = 2.843.10^{-4} )</td>
</tr>
</tbody>
</table>
Remark: the time unit is taken in hours and the value of $b$ is the absolute value.

To have a better approximation of $b$ more replicates would be needed. On average $b = 9.947 \times 10^{-5} = 1.10^{-6}$.

e) **Lytic rate coefficient: $k$**

If $k$ represents the lytic rate coefficient it means that for a burst time $\tau = 85$ minutes, $k = 1$, therefore, if we take 1 hour as the unit time, we estimate $k = 0.706$. 
IV) Simulations

In this part we solved the system of differential equations numerically with a MATLAB code. We have three different sets of initial values obtained with the experimental procedure to calculate the transmission coefficient $b$ described in the previous part.

The coefficient values deduced from the experimental procedures are:

- Replication coefficient of bacteria: $a(t) = 0.1121e^{0.634t}$
- Decay rate coefficient of free phage: $m = 34.8$
- The burst size: $L = 15$
- Transmission coefficient: $b = 1.10^{-6}$
- The lytic coefficient: $k = 0.706$

We assume that the replication coefficient follows an exponential law for five hours and as no more data are available beyond that period of time, we will limit the simulation to a maximum period of time of five hours.

The three sets of starting values at $t = 0$ are:

- $x(t) = 1.9.10^4$, $y(t) = 5.4.10^3$, $v(t) = 7.4.10^4$
- $x(t) = 5.6.10^3$, $y(t) = 1.5.10^3$, $v(t) = 1.7.10^4$
- $x(t) = 3.3.10^3$, $y(t) = 3.8.10^6$, $v(t) = 2.5.10^6$

The MATLAB code uses a Runge-Kutta method of order four to solve the system and the code is fully written in the annexes.

1) First set of values:

The first two figures show the simulations for the first set of starting values:

$x(t) = 1.9.10^4$, $y(t) = 5.4.10^3$, $v(t) = 7.4.10^4$

![figure 1: Replicate 1 simulation 1 (5 hours) (logarithmic scale)]
The simulation shows that the uninfected bacteria population dominates and the phage population decreases during the first five hours.

The following figure shows a simulation going over 100 hours, assuming the replication rate of the bacteria follow the same exponential law over this period of time. This assumption does not rely on experimental values and further experiments should be held. This simulation just gives an idea of what could happen over a longer period of time.

We can see that the phage and lytic bacteria populations increase and the uninfected bacteria population decreases over that period of time.

The next figures show the experimental values taken at three time points: $t = 0$, $t = 0.75$, $t = 2$

We can see that the phage population dominates during this time and that the bacteria populations decrease. However, the simulation shows that bacteria population dominates and
phage population decreases during the first two hours. Therefore, for this first set of values, the simulations are not predicting what is actually happening experimentally.

![Graph](image1)

**figure 4: Replicate 1 measurements (logarithmic scale)**

![Graph](image2)

**figure 5: Replicate 1 measurements**

2) **Second set of values:**

The first two figures show the simulations for the second set of starting values:

\[ x(t) = 5.6 \times 10^3, \ y(t) = 1.5 \times 10^3, \ v(t) = 1.7 \times 10^4 \]
The following figure shows a simulation going over 100 hours, assuming the replication rate of the bacteria follow the same exponential law over this period of time. This assumption does not rely on experimental values and further experiments should be held. This simulation just gives an idea of what could happen over a longer period of time. We can see that the phage population increases and the bacteria population decreases over that period of time.
The next figures show the experimental values taken at three time points: $t = 0, t = 0.75, t = 2$

We can see that phage population dominates during this time and that bacteria population decrease. However, the simulation shows that bacteria population dominates and phage population decreases during the first two hours. Therefore, for this second set of values, the simulation are not predicting what is actually happening experimentally.
3) **Third set of values:**

The first two figures show the simulations for the third set of starting values:
\[ x(t) = 3.3 \times 10^3, \quad y(t) = 3.8 \times 10^6, \quad v(t) = 2.5 \times 10^6 \]
The following figure shows a simulation going over 100 hours, assuming the replication rate of the bacteria follow the same exponential law over this period of time. This assumption does not rely on experimental values and further experiments should be held. This simulation just gives an idea of what could happen over a longer period of time.

We can see that the phage population increases and the bacteria population decreases over that period of time.

The next figures show the experimental values taken at three time points: $t = 0$, $t = 0.75$, $t = 2$

We can see that phage population dominates during this time and that bacteria population decrease. However, the simulation shows that bacteria population dominates and phage population decreases during the first two hours. Therefore, for this third set of values, the simulation are not predicting what is actually happening experimentally.
The simulations do not show exactly the same behaviour given by experimental values for the three different sets of initial values. These differences could be due to a lack of accurate estimates of coefficients or incorrect assumptions to build the mathematical model.
Conclusions

The failure of the model can be attributed to some incorrect assumption about the interactions between bacteria and bacteriophage or to errors in the estimates of parameter values. Therefore, with a more biologically realistic version of this model and more accurate parameter estimates, it is likely that mathematical modelling could be made to work. This work was an interesting approach of biological prey-predator interactions and shows how complex understanding and modelling these systems is.
References


Appendix

**Bacteriophage Adsorption:**

Method of adsorption:
   i) Titre phage to be tested
   ii) Dilute phage to $10^5$ pfu/ml in 10% M17
   iii) Titre the diluted phage: A
   iv) Equilibrate all components (below) at the temperature adsorption is to be tested at.
      1.0 ml phage at $10^5$ pfu/ml + 1.0 ml 5 hours bacterial culture + 0.05 ml Calcium chloride.
   v) Hold 15 minutes at temperature adsorption is to be tested at.
   vi) Immediately centrifuge 6000 tours/min for 10 minutes
   vii) Titre supernatant: B

The adsorption in percentage is given by: $(A - B) / A \times 100$

The experimental results are given in the next charts:

<table>
<thead>
<tr>
<th>Replicate 1:</th>
<th>Temperature (°C)</th>
<th>23.3</th>
<th>26.7</th>
<th>30.9</th>
<th>31.9</th>
<th>37</th>
<th>44</th>
<th>50</th>
<th>62.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adsorption (%)</td>
<td></td>
<td>97.13</td>
<td>87.38</td>
<td>90.38</td>
<td>91.88</td>
<td>80.88</td>
<td>81.25</td>
<td>70</td>
<td>99.93</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Replicate 2:</th>
<th>Temperature (°C)</th>
<th>23.3</th>
<th>26.7</th>
<th>30.9</th>
<th>31.9</th>
<th>37</th>
<th>44</th>
<th>50</th>
<th>62.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adsorption (%)</td>
<td></td>
<td>47.54</td>
<td>60.25</td>
<td>38.93</td>
<td>69.26</td>
<td>56.15</td>
<td>64.34</td>
<td>79.92</td>
<td>99.88</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Replicate 3:</th>
<th>Temperature (°C)</th>
<th>23.3</th>
<th>26.7</th>
<th>30.9</th>
<th>31.9</th>
<th>37</th>
<th>44</th>
<th>50</th>
<th>62.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adsorption (%)</td>
<td></td>
<td>48.57</td>
<td>51.71</td>
<td>55.14</td>
<td>76</td>
<td>77.14</td>
<td>93.42</td>
<td>94.29</td>
<td>99.69</td>
</tr>
</tbody>
</table>

Those results are quite spread and for more accurate conclusions more experiments are needed to be able to compare those adsorption coefficients as functions of temperatures.

The highest percentages of adsorption are found for a temperature of 62.5°C which is quite surprising. These high percentages are due to the fact that most of the page die at such a temperature. This hypothesis was confirmed with tittering only phage at 62.5°C. We found that 99.59% of phage were dead in the second replicate, and 98.08% of phage were dead in the third replicate.
Matlab code:

1) Principal file: phage_bacteri.m

clear all;
close all;
%% Parameters:
global a b k L m;

%%%%%%%%%%%%%%%%%%%%%%%%
%% coefficient values
%%%%%%%%%%%%%%%%%%%%%%%%
%% In this case the burst time is taken as the unit time
%% therefore k equals to 1
%% a: replication coefficient of the bacteria
%% this value is given by a function of time in the file a_rep.m
%% b: transmission coefficient
b = 1.0000e-006;
%% k: lysis rate
k = 0.706;
%% L: burst size
L = 15.0;
%% m: decay rate of free phage
m = 34.8;

%%%%%%%%%%%%%%%%%%%%%%%%
%% Data 1
%%%%%%%%%%%%%%%%%%%%%%%%
%% initial input of bacteria
%% uninfected bacteria x(t)
x0 = 1.9*10^4;
%% lytic bacteria y(t)
y0 = 5.4*10^3;
%% free phage v(t)
v0 = 7.4*10^4;
t0 = 0.0;
tfi = 5;
%% number of points used to solve the system
N = 1000;

%%%%%%%%%%%%%%%%%%%%%%%%
%% Data 2
%%%%%%%%%%%%%%%%%%%%%%%%
%% initial input of bacteria
%% uninfected bacteria x(t)
x0 = 5.6*10^3;
%% lytic bacteria y(t)
y0 = 1.5*10^3;
%% free phage v(t)
v0 = 1.7*10^4;
t0 = 0.0;
tfi = 5;
%% number of points used to solve the system
N = 1000;
%% initial input of bacteria
%% uninfected bacteria \( x(t) \)
\( x_0 = 3.3 \times 10^3; \)
%% lytic bacteria \( y(t) \)
\( y_0 = 3.8 \times 10^6; \)
%% free phage \( v(t) \)
\( v_0 = 2.5 \times 10^6; \)
\( t_0 = 0.0; \)
\( t_{fi} = 5; \)
%% number of points used to solve the system
\( N = 1000; \)

\[
\begin{align*}
\text{\texttt{runge_kutta4(x0,y0,v0,ti,tf,b,k,L,m,N);}} \\
\text{\texttt{vector time}} \\
\text{\texttt{time= [ti:(tf-ti)/(N-1):tf];}} \\
\text{\texttt{legend('uninfected bacteria','lytic bacteria','free phage');}} \\
\text{\texttt{title('Bacteria-phage concentrations in logarithmic scale');}} \\
\text{\texttt{xlabel('time in hours');}} \\
\text{\texttt{ylabel('concentration');}} \\
\text{\texttt{figure(2);}} \\
\text{\texttt{plot(time,x,time,y,time,v);}} \\
\text{\texttt{legend('uninfected bacteria','lytic bacteria','free phage');}} \\
\text{\texttt{title('Bacteria-phage concentrations');}} \\
\text{\texttt{xlabel('time in hours');}} \\
\text{\texttt{ylabel('concentration');}}
\end{align*}
\]

2) Bacteria replication function: \texttt{a_rep.m}

\texttt{function rep=a_rep(t)}
\texttt{rep=0.1121*exp(0.0634*t);}

3) First equation of the system: \texttt{f1.m}

\texttt{function z = f1(t,x,y,v,b)}
\texttt{z = a_rep(t)*x-b*v*x;}

4) Second equation of the system: \texttt{f2.m}

\texttt{function z = f2(t,x,y,v,b,k)}
\texttt{z = a_rep(t)*y+b*v*x-k*y;}

5) Third equation of the system: \texttt{f3.m}

\texttt{function z = f3(t,x,y,v,b,k,L,m)}
\texttt{z = k*L*y-b*v*x-m*v;}

6) Runge-Kutta method: \texttt{runge_kutta.m}

\texttt{function [x,y,v] = runge_kutta4(x0,y0,v0,ti,tf,b,k,L,m,N)}
\texttt{\% this function solves a system of 3 equations with a runge kutta methods}
\texttt{\% of order 4}
\texttt{\% x'=f1(x,y,v)}
\texttt{\% y'=f2(x,y,v)}
%% v'=f3(x,y,v)

t = ti;
dt = (tf-ti)/N;

x = zeros(1,N);
y = zeros(1,N);
v = zeros(1,N);

x(1) = x0;
y(1) = y0;
v(1) = v0;

for i=1:(N-1)

k11 = dt*f1(t,x(i),y(i),v(i),b);
k12 = dt*f2(t,x(i),y(i),v(i),b,k);
k13 = dt*f3(t,x(i),y(i),v(i),b,k,L,m);

k21 = dt*f1(t+dt/2,x(i)+k11/2,y(i)+k12/2,v(i)+k13/2,b);
k22 = dt*f2(t+dt/2,x(i)+k11/2,y(i)+k12/2,v(i)+k13/2,b,k);
k23 = dt*f3(t+dt/2,x(i)+k11/2,y(i)+k12/2,v(i)+k13/2,b,k,L,m);

k31 = dt*f1(t+dt/2,x(i)+k21/2,y(i)+k22/2,v(i)+k23/2,b);
k32 = dt*f2(t+dt/2,x(i)+k21/2,y(i)+k22/2,v(i)+k23/2,b,k);
k33 = dt*f3(t+dt/2,x(i)+k21/2,y(i)+k22/2,v(i)+k23/2,b,k,L,m);

k41 = dt*f1(t+dt,x(i)+k31,y(i)+k32,v(i)+k33,b);
k42 = dt*f2(t+dt,x(i)+k31,y(i)+k32,v(i)+k33,b,k);
k43 = dt*f3(t+dt,x(i)+k31,y(i)+k32,v(i)+k33,b,k,L,m);

x(i+1) = x(i)+1/6*(k11+2*k21+2*k31+k41);
y(i+1) = y(i)+1/6*(k12+2*k22+2*k32+k42);
v(i+1) = v(i)+1/6*(k13+2*k23+2*k33+k43);
end