Mathematically Modeling the Interactions Between Phages, Bacteria, and the Environment

MSc Thesis *(Afstudeerscriptie)*

written by

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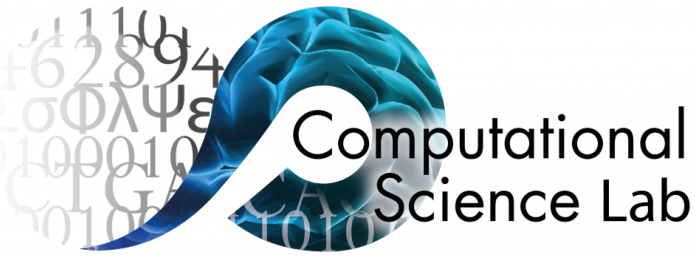

Abstract

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Abbreviations of terms

Abbreviations	Full Word
ODE/s	Ordinary Differential Equation/s
DDE/s	Delay Differential Equation/s
PDE/s	Partial Differential Equation/s
BVP	Boundary Value Problem
ABM/s	Agent Based Modelling/Models
FSD	Arms Race Dynamic
ARD	Fluctuating Selection Dynamics
SNP	Single Nucleotide Polymorphism
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Contents

1 Introduction

Phages, small viruses that infect, replicate, and kill bacteria, are nature's natural anti-microbial defense. Researchers are trying to determine phage applications in controlling bacterial infections and spread. Phages have applications in human and animal health. Phage cocktails are a medicine for sick patients with bacterial diseases, such as *E. coli*. A patient can intake a pill filled with specific phages that target *E. coli*. The phages will target the specific *E. coli* bacteria, but it will not affect the other bacteria and will not have any side effects on the body. There are 100 trillion microbes across 5,000 different types of bacteria strains in the human gut. Using medicine such as antibiotics can disrupt the intricate ecosystem of the gut microbiome, acting as a scorched-earth mechanism. Phages on the other hand specifically target a specific bacterial strain, acting as a sniper, with minimal to no effects to other bacteria. This can be used to control bacterial infections and cure people, or to prevent the spread of common bacteria in livestock. Farmers often raise livestock in tight spaces with a lack of sanitation facilities, increasing the risk of a disease spreading.

Phages have many uses in an industrial setting. Phages can be used to control the growth of bacteria like *Salmonella* while producing food in a factory [17, 12]. Due to the specificity of phages, they can be used to fight bacterial infections without affecting the gut microbiome, unlike antibiotics which destroys the gut biome and creates antibiotic resistant bacteria [15, 21]. There is however hope that phage resistant bacteria become more susceptible to antibiotics [13, 24]. Finally, phages can potentially be used to control cyanobacterial (blue-green algae) blooms in the environment and affect other agents such as plankton in the environment [4]. With this, there is hope that water quality can be engineered without using harsh chemical processes [19].

In an ecosystem like the ocean, the gut, or in soil, there are thousands of different microbes all interacting with one another or the surrounding environment. The interactions are complex, with many factors affecting the growth of bacteria, fungi, phages, and more. Not every interaction can be identified, and if an interaction has been identified, the associated parameter values are unknown and need to be experimentally derived. External factors, such as flooding, droughts, chemical spills, or introduction of new agents have a massive impact on the ecosystem. These events can add or remove nutrients from the system, change environmental parameters such as the surrounding temperature, introduce competition, or create an imbalance in the population by killing agents. These effects can affect the larger ecosystem and food chain as a whole.

Not much is known about phages in large and complex communities between other phages, bacteria, resources, and the environment. There have been previous attempts to model the complex dynamics of the populations between phages, bacteria, and resources, with the environment using Ordinary Differential Equations (ODE) and Delay Differential Equations (DDE). However, these

methods have mainly stayed with 1-to-1-to-1 models, meaning 1 phage, 1 bacteria, and 1 resource. Other methods such as Partial Differential Equations (PDE) or cellular models have been created in an attempt to model these types of dynamics. There are two main ways to model phage-bacteria dynamics: a spatial model or a non-spatial one. A spatial model means that phages and bacteria can move through space, whereas in non-spatial models, the bacteria and phages are assumed to be in a well-mixed solution. Special considerations have to be accounted for with spatial models. Bacteria and phages can only interact when they are in proximity to each other. Only a percentage p of bacteria and phages interact with one another at time step t. Spatial models can potentially lead to more interesting and complex results but are limited to smaller populations and harder to develop, while non-spatial models are easier to develop and are more effective in modeling large populations. PDE and cellular models are types of spatial models, while ODEs and DDEs are types of non-spatial models.

1.1 Biological Background

Phages are small viruses on the order of 27-190 nm that infect and lysis (kill) specific bacteria. The phage cycle process starts with a phage coming into contact with a bacterium. Once it has identified an injection site, the phage can inject a strain of DNA into the bacteria. The DNA strand has two options: it can either merge into the bacterial DNA, allowing the phage's DNA strand to replicate alongside the bacteria as they reproduce. This process defines the Lysogenic Cycle. After a set amount of time, the DNA of the phage can unmerge and hijack the DNA replicating mechanism, creating multiple copies of itself, using the transcription, translation, and replication process to create multiple copies of itself. The phages begin to self-assemble inside the bacteria until the bacteria is full of phages and explodes, the lysis stage, releasing the phages into the environment, ready to repeat the process again.

This process can be visualized in Figure 1 [2].

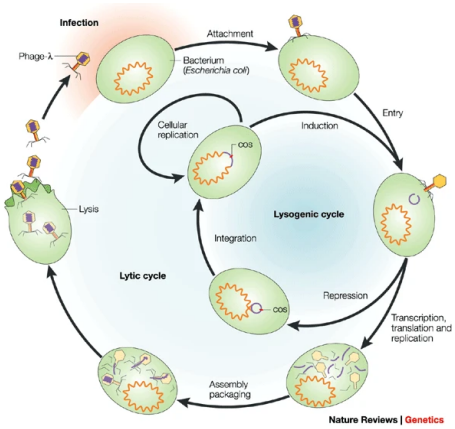

Figure 1: Life cycle of a phage, inside and outside a bacteria cell.

2 Literary Review

2.1 Methods of Modelling Phages and Bacteria

There are numerous ways to model the interactions between phages and bacteria. Models can be built at a molecular level, where the model simulates the mechanical and chemical behavior of a phage as it interacts with the surface of a bacterium using computational chemistry methods. On the other end of the spectrum, a different type of model can be built where populations of phages, bacteria, and resources can be modeled using Ordinary Differential Equations (ODEs) or Delay Differential Equations (DDEs). DDEs are similar to ODEs, except where when ODEs are calculating the values of the equations at time t using time $t-1$, DDEs can, but don't have to, use the value of the equation at time $t-\tau$, where $1 \leq \tau \leq t$.

Each type of system has its pros and cons. With the molecular level model, the model is more complex and needs significantly more startup time, simulation time, and is in general much more complex. However, more information can be gained from the simulations and can guide research in creating phages for a certain type of bacteria. The ODE method is simpler and easier to set up, however it can only capture large population dynamics. Certain assumptions about the community interactions have to be made. For example, ω percent of the bacteria population is washed out. The model can be made more complicated, by modelling each stage of the phage replication and lysis process, or instead of assuming exponential growth, there is a maximum carrying capacity of the population. The model can be further altered by using a normally distributed variable $\mathbf{N}(\mu = \omega, \sigma = 1)$ to account for noise when measuring the data. Ensuring the use of a seed value will ensure that each run of the model results in the same output.

2.1.1 Generalized Lotka-Voltera Model

The Lotka-Voltera model, a first-order non-linear differential model, is a model that captures the dynamics between predators and prey, with phages being the predator and bacteria being the prey. Any population can be modelled as such:

$$\frac{dB_i}{dt} = B_i \left(\left(r_i + \sum_j^N \alpha_{ij} B_j \right) - m_i \right)$$

where ...

2.1.2 Generalized Consumer-Resource Model

The generalized Consumer-Resource Model models the growth of a population and resource dynamics between a population of bacteria B_i and a resource R_i.

$$\frac{dB_i}{dt} = r_i B_i \left(\sum_\alpha \Delta w_{i\alpha} C_{i\alpha} R_\alpha \right) - m_i B_i \tag{1}$$

$$\frac{R_\beta}{dt} = -\sum_i C_{i\beta} R_\beta B_i + \sum_{\alpha,i} D^i_{\beta\alpha} C_{i\alpha} R_\beta B_i \tag{2}$$

$$\Delta w_{i\alpha} = \sum_\beta D^i_{\beta\alpha} w_\beta \tag{3}$$

2.1.3 Trait-Based Model

The Trait-Based Model is a model that takes into account external factors such as the temperature or pH of the system and can be modeled as follows:

$$\frac{dB_i}{dt} = (r_i - m_i) B_i \tag{4}$$

$$r_i = \frac{r^{max}_{i\alpha} R_\alpha}{R_\alpha + K_{i\alpha}} e^{S_i(T - T_{ref})} \tag{5}$$

where S_i is the sensitivity to B_i to factor T, and with trade off if $r_i^{max} >$ mean r^{max} then $S_i >$ mean S.

2.1.4 Agent-Based Models

Agent-based Models (ABM) model the system through space and time. An $x \times y \times z$ grid (often z is left out for a 2D system) is created and split into smaller subcells containing resources and microbes. Each cell acts as its own tiny environment, where resources and microbes interact within the environment, but not with the neighboring cells. Resources are diffused through the system using a PDE solver for a Boundary Value Problem (BVP). Agents are allowed to move into neighboring grids with a probability p, where p can depend on any number of parameters such as nutrient density, microbe density,

or stochastic chance.
ABMs are useful when simulating many individual elements interacting in a system. Chaotic or emergent behavior can arise from these interactions. Chaotic behavior refers to the irregular and unpredictable evolution of a system's behavior due to nonlinear equations, exhibiting sensitive dependence on initial conditions [16].
Emergent behavior is behavior that arises from the interactions of various agents in a system, that was not explicitly programmed into the system. The behavior can be beneficial, neutral, or harmful, but it can not be predicted until it arises, *if* it arises. Agents can have simple rules, but when interacting with other agents, behavior that hasn't been programmed can arise. Sometimes, people consider systems with emergent behaviors more complex than the sum of their parts.

$$\frac{\delta R_\alpha(r,t)}{\delta t} = \nabla \left[D\left(R_\alpha, r\right) \nabla R_\alpha\left(r,t\right) \right], r = (x,y) \tag{6}$$

, where r is a function of cell position (x, y), and t represents time. The cellular agents rules are as follows:

$$\frac{di}{dt} = r_i \left(\sum_\alpha \Delta w_{i\alpha} C_{i\alpha} R_\alpha \right) \tag{7}$$

, where if $i >$ threshold, $\frac{i}{2}$ expands into the neighboring grid cell with a probability p. The system consumes resources and converts them into new subresource types with the following equation:

$$\frac{dR_\alpha}{dt} = -\sum_i C_{i\alpha} R_\alpha I \tag{8}$$

$$\frac{dR_\beta}{dt} = \sum_i C_{i\beta} R_\beta I + \sum_{\alpha,i} D^i_{\beta\alpha} C_{i\alpha} R_\alpha i \tag{9}$$

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2.2 Biology of Phages

3 Methods

3.1 Network Topography of Interactions

In a microbial environment, there are numerous interactions between agents, but not every agent can and will interact with one another. Based on which agents interact with which agents, a network topography can be created, capturing the dynamics of the interactions. Each agent can be represented as a node. If an agent interacts with another agent, an edge can be linked between the agents. Each node can contain attributes and properties related to that agent, for example starting population or concentration, washout rate, or birth rate. Each edge likewise also contains attributes to capture the dynamic interactions between the agents, for example, resource usage, burst size, or affinity to infect. Adding the attributes to the nodes and edges allow for various The parameters can change between agents. For example, the initial population of phage 1 can be 300, while for phage 2 it is 150. Likewise, the attributes can be different between different agents. For example, bacteria 1 might use up resource 1 with rate constant 0.05, while bacteria 1 might use up resource 2 with rate constant 0.07. Bacteria 2 might not need resource 1 to survive, but bacteria 2 requires a lot of resource 2 to grow, with usage rate constant of 0.4. Using a graph network, these interactions between agents can be visualized, tracked and edited.

A tool has been developed to help aid in the development of this network topography. With this tool, a network topography can be created by adding any number of agents of varying type, such as bacteria, phages, or resources. There is an optional environment node that can capture global environment data, for example the length of the simulation, number of time steps, temperature, pH, etc. The attributes of the agents, interactions, and environment can easily be edited.

Once a network topography capturing the interactions between any number of agents has been created, it would be useful to see how the population count or concentration value changes through time. A Python package has been created that allows for uploading a network topography, and with a small script that the user needs to provide, with the setting up of initial parameters and provided equations, runs a numerical solver using SciPy's solve_ivp() function.

4 Experiments and Results

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5 Discussion

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6 Conclusion and Future Work

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A Parameters

Parameters used in equations

Parameter	Parameter Full Name	Description	Default Value	Alternatives	Notes
P	Phage Parameter	Phage population count			
U	Unifected Parameter	Uninfected bacteria population count			
I	Infected Parameter	Infected bacteria population count			
R	Resource Parameter	Resource concentration			
B	Bacteria Parameter	Bacteria population			Some mo
ω	Washout Rate	Rate of parameter washing or flowing out of the system			
β	Burst Size	Number of phages created when bacteria cell bursts			
t	Time	Time step during simulation			
μ	Mean	Mean			
σ	Standard Deviation	Standard deviation			
T_{min}	Minimum Temperature	Minimum operating temperature for a microbe			
T_{opt}	Optimal Temperature	Optimal operating temperature for a microbe			
T_{max}	Maximum Temepra-ture	Maximum operating temperature for a microbe			
pH_{min}	Minimum pH	Minimum operating pH for a microbe			
pH_{opt}	Optimal pH	Optimal operating pH for a microbe			
pH_{max}	Maximum pH	Maximum operating pH for a microbe			

B Appendix 1: Real World Applications of Phages

Due to the nature of killing bacteria, there are numerous applications where a researcher or an organization might be interested in controlling bacterial populations.

A Food Safety Specialist might be interested in introducing a solution containing a high concentration of phages during food production to prevent the spread and growth of *Salmonella* or *E. coli* in the pet food. Alternatively, the Food Safety Specialist might want to promote beneficial bacteria like *Streptococcus thermophilus* used in the production of Emmental cheese, which heat would kill when the milk undergoes the pasteurization process.

A doctor might be interested in providing swallowable pills, more commonly known as phage cocktails, to a patient with a bacterial infection. There is evidence that phage-resistant bacteria are more susceptible to antibiotics, so the doctor might prescribe both medicines to effectively deal with the infection.

An Environmental Protection Officer might be interested to see how they can use phages to stop the spread of *Cyanobacteria* blooms in waterways, more commonly known as blue-green algae, a photosynthetic microscopic organism that is technically a type of bacteria. This would keep waterways safe for boating and swimming activity, aquatic life, and water consumption in farms, factories, and homes.

When there are a few known bacterial strains, a targeted concoction of phages can be used to control the bacterial population growth in any setting, either be it food, healthcare, or environmental. Phages offer properties of microbial control that other methods do not, making them an ideal candidate for some applications.

B.1 Controlling Foodborne Bacteria

Foodborne diseases are one of the primary ways for bacteria to spread to humans and animals. Some bacteria use the food as a vector to infect hosts, while some bacteria will deposit toxins on the food that is then ingested. If consumed in large enough quantities, or further produced in the host, the toxins can be fatal to the host.

Methods exist to control bacterial growth, for example by storing food below 5°C or above 60°C. Bacteria need moisture to grow, so starches like rice will have minimal bacterial growth. Bacteria prefer to live in slightly acidic to neutral pH environments, so having an environment that is extremely acidic like vinegar will prevent bacterial growth. The use of chemical antibacterial agents such as bleach is not desirable due to leaving chemicals on the food, which can be fatal if ingested. Physical agents like heat or radiation can kill bacteria, but at the cost of altering the food quality [6].

For example, *Streptococcus thermophilus* is one of three different bacteria strains used to create Emmental cheese. However, Emmental cheese does not use pasteurized milk, increasing the risk of *E. coli*. Emmental cheese producers

can add phages that target *E. coli* to the milk during the production stage, while not affecting the bacteria used to produce the cheese.

B.1.1 Current Applications

Phage cocktails like SalmoFresh^{TM} have been proven to safely reduce *Salmonella* contamination in pet food and raw pet food ingredients [17], as well as in romaine lettuce and bean sprouts [23]. Pet food contains meat and vegetables, where vegetables grown in or on the ground are at risk of *Salmonella* due to contact with soil, manure, compost, and other agricultural runoff from neighboring farms [12]. Figure 2 [17] and Figure 3 [23] show how applications of phages have reduced the count of *Salmonella* in ingredients used in pet food as well as romaine lettuce and bean sprouts. In Figure 2, each food group noticed at least a 68% reduction in CFU/g compared to the control when the 9×10^6 phage treatment was applied. There was at least an 80% reduction in CFU/g across all food groups when treated with a 9×10^6 or stronger phage solution. In Figure 3, the lettuce and bean sprouts noticed a reduction of at least 0.6 log CFU/mL in *Salmonella* count across all temperature ranges. The smallest reduction in bacteria count in lettuce was noticed at 1 hour at 2°C with an absolute reduction in 62.0% between the control and treatment, while the largest reduction in bacteria of 90.0% was found at 72 hours at 2°C. For the bean sprouts, the lowest reduction in phages was found at 1 hour at 2°C with a reduction of 78.1%, and the largest reduction was 90.0% at 25°C after 48 hours. Although these values are still high above food safe, the ability to reduce the *Salmonella* population by at least 62% and up to 90% at different temperatures and incubation periods is impressive and can prolong shelf life, especially for foods that do not have long shelf lives before spoiling due to bacteria. As such, phages can be shown to control the spread of *Salmonella* in food sources and extend the potential shelf life of certain foods.

B.2 Phage Therapy and Antibiotics

Antibiotics are a common way to treat bacterial infections. However, antibiotics are not selective in the bacteria they kill, killing both harmful and beneficial bacteria. This can lead to the development of antibiotic-resistant bacteria, which makes it harder to combat that bacteria in the future. It has also been shown that antibiotics have a negative effect on the gut microbiome and brain development in mice. Phages are an alternative to antibiotics, as they are selective in the bacteria they kill and do not interact with cells or other important biological functions. The rise in antibiotic resistant bacteria can be attributed to the overuse and over-prescription of antibiotics and incorrect usage of antibiotics (for example prematurely stopping) [15]. These actions provide an evolutionary pressure on bacteria to mutate and gain resistance to the antibiotics. The phage therapy can contain any number of different phages that can target specific bacterial infections such as *Streptococcus pneumoniae* with minimal risk of side effects.

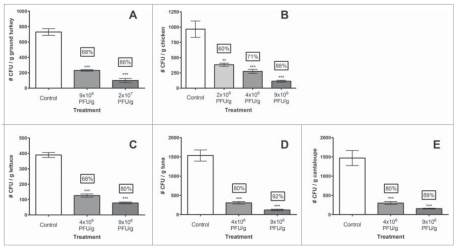

Figure 2: SalmoLyse® reduces Salmonella contamination on various food surfaces: Mean and standard error bars shown. Statistical analyses were carried out for each food group independently. Asterisks denote significant reduction from corresponding controls based on one-way ANOVA with Tukey's post-hoc tests for multiple corrections: ** denotes $p < 0.01$, while *** denotes $p < 0.001$ compared to the corresponding controls. There was significant reduction in Salmonella on all food surfaces with the addition of SalmoLyse® compared to the controls; the mean percent reductions from the control are noted in the boxes above treatment bars. CFU/g D colony forming units per gram. Each letter denotes a food group that was tested with SalmoLyse® and compared to a control: A= chicken; B= lettuce; C= tuna; D= cantaloupe; E= ground turkey [17].

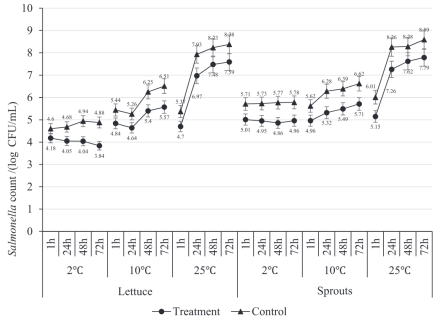

Figure 3: *Salmonella* count in a mixture of 5 *Salmonella* strains spot-inoculated (CFU/g) onto a) lettuce and b) sprouts after spraying with a mixture of bacteriophage (SalmoFresh™) relative to positive controls at 2, 10 and 25C and stored for 1, 24, 48 and 72 h. [23]

B.2.1 Current Applications: Bacterial Infection Control

One active area of research is the use of phages to control bacterial infections. Due to the specificity of phages, they can be used to target specific bacteria strains without affecting other beneficial bacteria. When sick with a bacterial infection, patients swallow antibiotic pills to help the body fight the infection. Antibiotics work by either interrupting intercellular processes like the synthesis of RNA [7], by disrupting the structural integrity of the cell wall [18], or by inhibiting protein synthesis [20].

However, antibiotics are not strain specific and indiscriminately kill gut and

other bacteria. Common side effects of antibiotics, although usually not serious, include diarrhea, nausea, and headaches. It has also been shown that the effects of early-stage penicillin exposure in mice has found to have a long-lasting effect on the gut microbiome, frontal cortex gene expression, and amygdala gene expression [21]. Penicillin increases cytokine expression (small proteins used in cell signaling) in the frontal cortex of the brain, modifies the blood-brain barrier integrity, and alters behavior. The mice exhibited an increase in aggression and anxiety-like behavior [14]. Phages can be used as an alternative to antibiotics without the side effects and without affecting the gut biome.

With an increase in antibiotic usage, there has been an increase in antibiotic-resistant bacteria. The World Health Organization has stated that antibiotic resistance threatens the modern medicine and the sustainability of an effective, global public health response to the enduring threat from infectious diseases. Common infections, that previously would have been easy to treat, are harder to treat, and can increase the risk of disease spread, severe illness, and death [8].

One area of research is exploring how bacteria can exchange traits such as phage resistance and antibiotic resistance. Some bacteria are multi-drug resistant, and don't react with the medicine anymore.

Nana Nguefang Laure et al. showed evidence that *Salmonella Typhimurium* is more susceptible to ampicillin in the presence of phages, and phage-resistance can lead to reduced virulence and decreased antibiotic resistance [13].

Yuanyang Zhao et al. showed that there exists an antagonist coevolution between the bacteria and phages, where the dynamics changed from an arms race dynamic (ARD) to a fluctuating selection dynamics (FSD). Due to phage selection and bacterial competition pressure, when the bacteria gained phage resistance, it lost antibiotic resistance. A genome analysis revealed mutations in the btuB gene of *Salmonella anatum*, with q higher mutation frequency in the ARD stage. A knockout experiment confirmed that the btuB gene is a receptor for the JNwz02 phage and resulted in reduced bacterial competitiveness. Further analysis detected multiple single nucleotide polymorphism (SNP) mutations in the phage-resistant strains. The SNPs potentially affected the membrane components, partially weakening the cell defense against antibiotics. These findings help advance our understanding of phage-host-antibiotics interactions and the impact of adaptations to antibiotic resistance. The research shows how phages can be used to re-introduce antibiotic susceptibility to previous insusceptible bacteria, preventing costly and lengthy research in new antibiotics [24].

Phage research is facing challenges due to bacterial strains evolving resistance to phages. Understanding the interplay between antibiotics and phages is essential for shaping future research [24].

B.3 Environmental Protection

Algae blooms, also called red tides, is the rapid spread of bacterial or algae organisms. Blooms are a growing environmental concern impacting water quality,

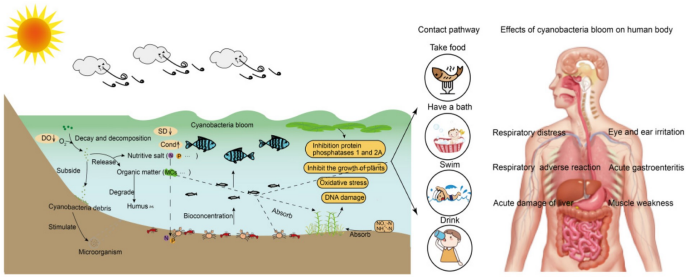

Figure 4: Cyanobacteria degradation cycle, main hazards of cyanobacteria bloom to water bodies, aquatic organisms, and the human body. (DO: dissolved oxygen; SD: water transparency; Cond: conductivity; N: nitrogen; P: phosphorus; MCs: microcystins). [22]

aquatic ecosystems, and human health. These rapid increases in algae populations, often fueled by excess nutrients like nitrogen and phosphorus, can occur in freshwater, coastal, and marine environment.

Cyanobacteria blooms have major effects on the aquatic environment as well as human health. Cyanobacteria release nitrogen and phosphorous, which the bacteria use to grow with oxygen, outpacing other aquatic growth, and killing aquatic marine life. Toxins can make their way into the food and water consumed by humans, causing muscle fatigue, respiratory issues, liver damage, and gastrointestinal issues [22]. Figure 4 shows the process of how cyanobacteria degrade and are absorbed into the environment, eventually making their way into the human body via various contact points.

B.3.1 Current Applications

There is interest in using phages to control cyanobacteria blooms. Phages can offer better and safer options than chemical options when trying to control bacterial blooms. Chemical options are indiscriminate, killing cyanobacteria, while also killing other beneficial bacteria and aquatic life, and can eventually seep into groundwater. Although not used to control bacteria blooms, some chemicals like PFAS, also called "Forever Chemicals", can last a long time in the environment and don't degrade and keep on negatively affecting the environment. Due to the specificity of phages, only the cyanobacteria will be targeted, and will not affect the surrounding environemnt.

Tucker and Pollard found that an isolated phage cocktail collected from Lake Baroon in Australia could decrease the abundance of *M. aeruginosa* by 95% within 6 days in a lab setting, before recovering within 3 weeks time [19]. There is evidence that phage-resistant bacteria can influence the population dynamics of other bacteria. It has been shown that the plankton level has been experimentally affected by the frequency of the phage-resistant *Nodularia* marine bacteria. Populations with high phage resistance ($> 50\%$) dominate the

plankton communities despite a high phage count and eventually out compete other bacteria due to their slower loss in population count. Contrastingly, populations of bacteria with low phage resistance (between 0% and 5%) were lysed to extinction, releasing nutrients like nitrogen. This allows for other bacterial strains to absorb the nutrients and dominate the bacterial community. Phages and the lysis of bacterial strains can have a dramatic effect on community dynamics and composition of other agents like phages, bacteria, and resources [4]. Phages have the potential to be used as a highly specific strategy for the control of cyanobacterial blooms, with minimal effects to the environment, and offer control of bacterial blooms, with limited impact to the environment. Usage should be relatively safe, novel, efficient, and sensitive.

However, there are issues with using phages to control bacterial blooms. Bacterial blooms can cover vast areas, or be in areas that would be hard to reach like marshlands, applying phages to combat the bloom might be infeasible. If the method of choice was to spray a solution of water containing phages, the solution needs to be shipped to the site and loaded onto special boats to spray the solution into the water, or the trucks need to drive along the shore and spray the solution into the water.

The phage density in the solution will have to be relatively high to quickly combat the bloom. These problems provide major logistical problems with creating the phages in a lab or factory, transporting the phages, and finally the administration of the phages to the waterways. Phages can only diffuse through the water, and can't actively swim, so they are dependent on the rate of diffusion and water currents. This will be difficult in marshlands, where the bacteria can "hide" in the grass and crevices created by aquatic life. If the bloom is in a high current area, like in a river or a bay, the water can wash the phages away.

Scientists have not yet fully understood the phage infection mechanism, and research into the artificial engineering of phages is limited, making it challenging to conduct studies in this area [9, 5].

Algae can produce toxins that threaten wildlife, contaminate drinking water, and disrupt local economies dependent on fishing and tourism. In the state of Florida, between the years 1995 and 2000, the restaurant and hotel industry lost an estimated $6.5 million to algae blooms. This accounts for about 25% of the average total monthly sales revenue in the region from June through October, the months that are most commonly affected by red tide[1]. During a red bloom event, hospital diagnoses in the county of Sarasota for pneumonia, gastrointestinal, and respiratory illness increased by 19%, 40% and 54% respectively [3, 11], with a respiratory illness visit costing between $0.5 and $4 million [10].