

Filamentous Phage Infection of E. Coli: A Mechanistic Model

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Table of Contents

I.	Abstract	2
II.	Introduction	2
III.	Full Model	5
IV.	Model Development	8
V.	Computational Program	13
VI.	Results and Discussion	15

Abstract

The filamentous phage are a family of bacterial viruses infecting Gram negative bacteria. Their use has been instrumental in the development of modern molecular biology and biotechnology. As molecular machines, they have found application in the field of nanotechnology and may soon extend their utility within a number of other areas. Characterizing the process of infection and the impact on their microbial hosts provide important insights into realizing their full capacities as biological tools. Here, a mechanistic model of the infection process predicts how phage concentration in a microbial system changes with respect to time and spatial characteristics in a typical control volume. The dynamic behavior of the system offers perspective on the ecological characteristics of typical cell-phage interactions for further development and analysis *in silico*.

Introduction

Residing in nearly every ecological niche, bacterial viruses (bacteriophages) are among the most prevalent and diverse non-living entities on the planet [1]. Bacteriophages occupy central roles in microbial ecosystems, where they contribute to growth, evolution, and nutrient cycling. In the context of molecular microbiology, bacteriophages revolutionized the field, providing tools for biotechnology and genetic engineering and lending themselves to much of what is known about molecular genetics [2]. Medicine looks to phage as a potential solution to the rising problem of antibiotic resistance. Modeling and understanding how these non-living molecular devices interface with living organisms and perpetuate in microbial ecosystems will prove critical for harnessing them as laboratory tools and eventually as components of complex biogeochemical systems.

The filamentous phage constitute a large grouping of these bacterial viruses that infect Gram-negative bacteria. Using pili as receptors, filamentous phage anchor themselves to bacterial cells and insert their genomes, allowing them to replicate within their hosts [3]. One of the best characterized filamentous phages, M13, infects *E. coli* hosts through the F pili. It has a circular, single-stranded DNA genome packaged within a rod-shaped protein structure. The genome consists of only eleven proteins, five of which are part of the virus particle, three for phage DNA synthesis, and three for assembly and release from the host [2]. Unlike the vast majority of bacteriophage, M13 phage do not lyse their hosts. Instead, they form adhesion

zones from which they exit the cell. Consequently, the host cell does not invest all of its resources into producing the viruses, but its growth is nonetheless stunted by the infection process.

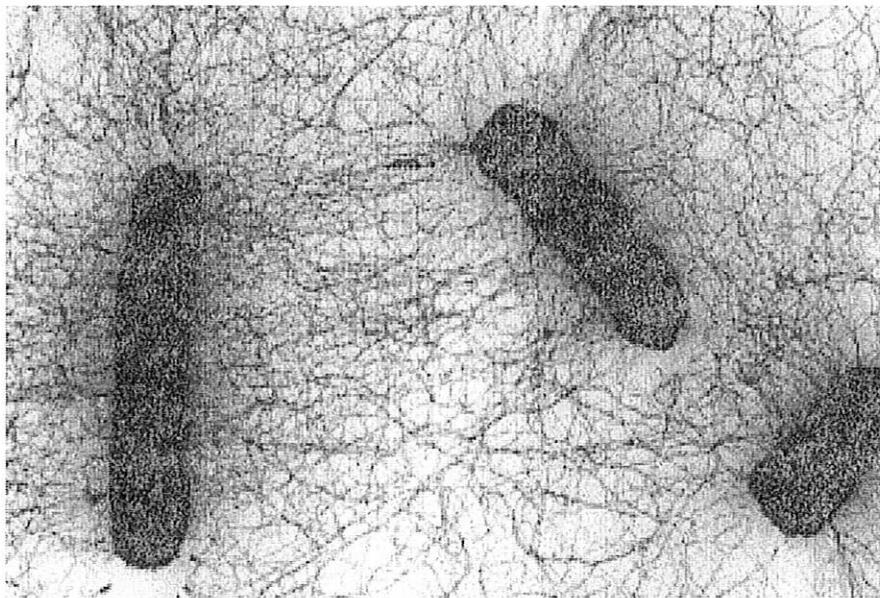
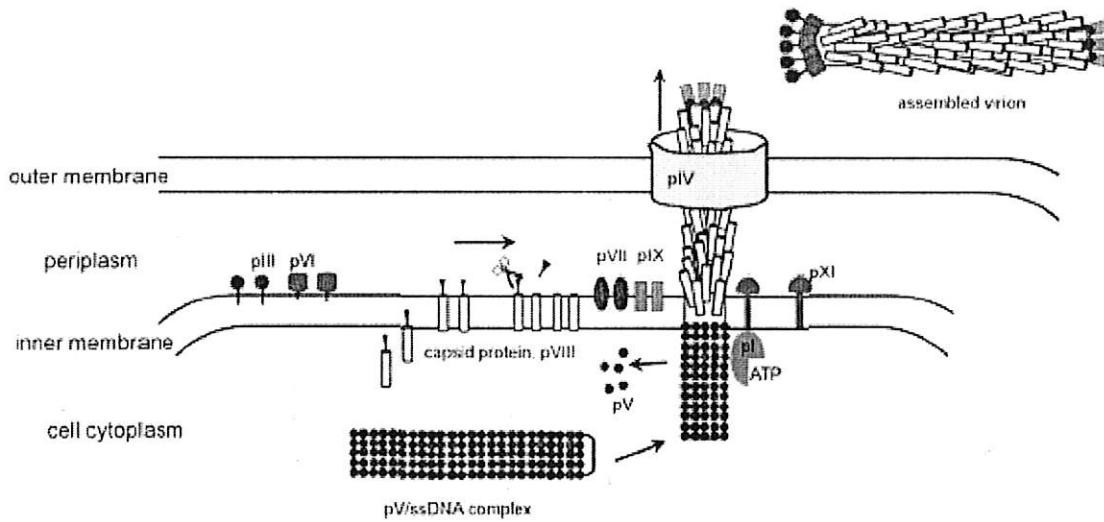


Figure 1. Electron micrograph of cells and cell-associated phage, 100 minute infection.

Taken from Rakonjac and Model [4].

Infection of *E. coli* hosts by bacteriophage M13 follows a predictable sequence of events. First, the M13 phage attaches to an exposed pili and inserts its ssDNA genome, shedding its major coat protein into the inner membrane of the host. Enzymes within the host bacterium convert the circular ssDNA into a double-stranded replicative form. As in normal conjugative transfer, the progeny DNA replicates through a rolling circle mechanism, at which point it forms a complex with a viral replication-assembly protein [5]. This complex protects the viral DNA within the cell. Eventually, it is replaced by coat proteins to cover and protect the DNA outside of the cell. Replacement of the replication-assembly protein sheath with the coat protein occurs as the virion extrudes itself through the membrane with the help of an assortment of viral and host proteins. Here, a procoat protein is synthesized as a precursor to the major coat protein, enabling its insertion into the membrane. Mature coat protein remains in the membrane until assembly with the phage genome, after which the fully assembled phage particle leaves the cell through adhesion zones formed in the membrane. Now, it is ready to attack and infect a new host cell and renew its replicative cycle in a new environment [1].

Figure 2. M13 Filamentous phage assembly model, taken from Aksyuk and Rossman [2].



References

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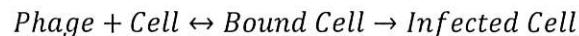
Full Model

The model assumes a cylindrical control volume (such as a petri dish) with radial diffusion and reactions occurring in the system. First, we outline the parameters in Table 1.

Table 1. Model Parameters and Definitions.

Parameter Notation	Description	Dimensions
C_u	Uninfected cell concentration	# cells/length^3
C_i	Infected cell concentration	# cells/length^3
C_r	Resistant cell concentration	# cells/length^3
C_p	Phage concentration	# phage/length^3
r_i	Infected cell growth rate	1/time
r_u	Uninfected cell growth rate	1/time
K_{max}	Cell population carrying capacity	# cells/length^3
k_{Ph}	Phage infection rate	
k_G	Phage production rate	1/time
k_f	Forward binding rate of phage to pili	1/(concentration*time)
k_r	Reverse binding rate of phage to pili	1/time
k_d	Phage degradation rate	1/time
k_{Ch}	Resistant cell conversion rate	1/time
D	Diffusivity	length^2/time

The model employs a number of assumptions. Starting with a lumped parameter system, the relationships between species within the system control volume (geometry neglected) were determined using mass action kinetics. The primary assumption with this model is that the species diffuse freely within the system and interact via collision. Upon collision, the species (cell, phage particle) initiates the reaction event (binding, genome insertion, phage production). The major reaction occurring in the system is detailed below:



The forward binding rate in this scenario is k_f , the reverse is k_r , and the transformation of bound cell into infected cell happens at a rate k_{Ph} .

In considering the cells within the system, there is an associated growth rate. Initially, growth was assumed to be proportional to the concentration of cells, which led to an exponential function of cell concentration with respect to time. The more realistic expectation of logistic growth was employed with the introduction of a self-limiting growth rate – that is, the bacteria cannot grow past the carrying capacity of the system, a value determined by available nutrients, intrinsic death rate, and similar factors. We identify this parameter as K_{\max} . The assumption of growth was introduced into each of the differential equations for the growth rate of cells (infected, uninfected, and resistant). Each of the cell types has a separate growth rate, with uninfected cells growing at a growth rate (r_u). Infected cells are assumed to grow more slowly than uninfected cells (r_i), while resistant cells should grow at an unimpeded rate. Phage particles are assumed to degrade at some intrinsic rate k_d .

In the gradient system, we introduce an additional constant, diffusivity. Realistically, this constant should differ for each of the species within the system (cells versus particles), and it will likely be on the same order of magnitude for either of the species. For simplification and numerical solvability, the diffusive constant (D) was assumed to be the same for both the cells and the particles.

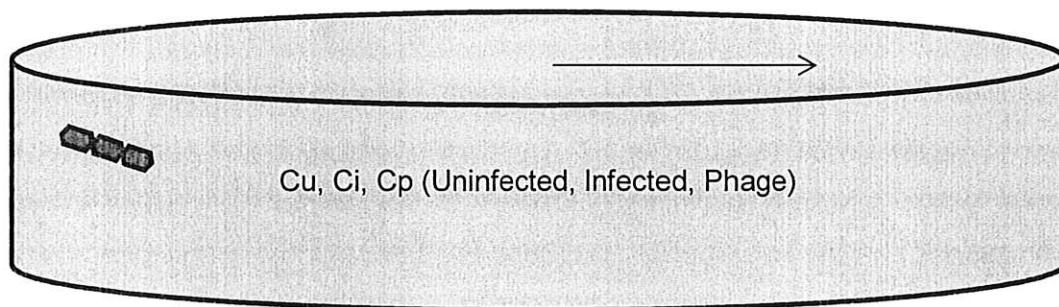


Figure 3 Reaction-diffusion system with infected and uninfected cells and phage diffusing in a cylindrical control volume (Petri dish).

Having identified the basic relationships, assumptions and principles operating in the system, the following system of partial differential equations collects these differential equations in an appropriate mathematical construct. For the reaction, the binding was assumed to be in quasi-steady state, so that the rate of change of bound cells is approximately zero.

Table 2. Partial Differential Equations and Reaction Terms for Reaction-Diffusion Network.

$\frac{\partial Cu}{\partial t} = \frac{D}{r} \frac{\partial}{\partial r} \left(r \frac{\partial Cu}{\partial r} \right) + \frac{1}{r} R(Cu)$	(1)
$\frac{\partial Ci}{\partial t} = \frac{D}{r} \frac{\partial}{\partial r} \left(r \frac{\partial Ci}{\partial r} \right) + \frac{1}{r} R(Ci)$	(2)
$\frac{\partial Cr}{\partial t} = \frac{D}{r} \frac{\partial}{\partial r} \left(r \frac{\partial Cr}{\partial r} \right) + \frac{1}{r} R(Cr)$	(3)
$\frac{\partial Cp}{\partial t} = \frac{DP}{r} \frac{\partial}{\partial r} \left(r \frac{\partial Cu}{\partial r} \right) + \frac{1}{r} R(Cp)$	(4)
$R(Cu) = ru * Cu * \left(1 - \frac{Ci + Cu + Cr}{K_{max}} \right) - kPh * \frac{kf * Cu * Cp}{kr + kPh} - kCh * Cu$	(5)
$R(Ci) = ru * Ci * \left(1 - \frac{Ci + Cu + Cr}{K_{max}} \right) + kPh * \frac{kf * Cu * Cp}{kr + kPh}$	(6)
$R(Cr) = ru * Cr * \left(1 - \frac{Ci + Cu + Cr}{K_{max}} \right) + kCh * Cu$	(7)
$R(Cp) = kg * Ci - Cp * (kd + kf * (Cu + Cr + Ci)) + kr * \frac{kf * Cp * (Cu + Ci + Cr)}{kr + kPh}$	(8)

Using the finite difference method to discretize the differential equation, the reaction diffusion equations take the form below:

$$C_m^{p+1} = \frac{D\Delta t}{r} \left(\frac{r(C_{m+1}^p - 2C_m^p + C_{m-1}^p)}{\Delta r^2} + \frac{C_{m+1}^p - C_m^p}{\Delta r} \right) + \frac{\Delta t}{r} R(C_m^p) + C_m^p$$

These reaction equations were subsequently implemented in Excel using the finite difference method.

Model Development

Model development started with a number of assumptions, primarily a closed, lumped parameter system involving internal reactions and relationships governed by the principles of mass action kinetics. Refer to figure 4 for the system set-up. The initial parameters and relationships presumed to be necessary are listed in the following table.

Table 3. Initial Model Parameters and Relationships.

Parameters/Variables	Relationships
Volume of cell, V_{cell}	Total Volume = $V_{tot} = V_{sys} + n_{cell} * V_{cell}$
Volume of System, V_{sys}	$n_{cell} = n_{inf} + n_{uninf}$
Total number of cells, n_{cell}	Concentration = # (cells or particles) / V_{sys}
Number of infected cells, n_{inf}	$V_{cell} = 4/3 * \pi * R_{cell}^3$ (spherical cell)
Number of uninfected cells, n_{uninf}	
Number of phage particles, n_{phage}	

Iteration I

Assumptions

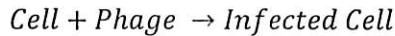
- 1) Assume $V_{tot} = V_{sys} \gg n_{cell} * V_{cell}$ for all time t, and that the system is at constant pressure, volume and temperature.
- 2) Assume that phage particles and cells move randomly as they diffuse within the system.
- 3) Cells are represented at the population level – attributes of cells (volume, size, mass, etc.) are averaged across the cell population to describe the population characteristic.
- 4) Assume there is a growth rate of the cell population that is dependent on characteristics of the cells (ie, a doubling time) and the availability of nutrients.
- 5) Assume that cells grow at a constant rate that does not depend on whether the cells have been infected or not.
- 6) Assume that mass action kinetics (reaction networks) can represent interactions among species within the system.
- 7) There are three species involved – infected cells, uninfected cells, and phage particles.

Using the above outlined relationships and assumptions, a population balance on the system yields the following information. Asking the question, "How does the concentration of cells change over time?", the simplest answer is that the rate of cell growth depends on the number

of cells currently in the system. Deriving a proportional model based on the assumption that cell growth is defined as cell division, we arrive at the following relationship:

$$\frac{dC_{cell}}{dt} = \alpha C_{cell}, \quad C_{cell}(t) = C_{cell,0} e^{\alpha t}$$

There is also a reaction of fundamental importance occurring within the system – phage infection of the cells. We model this reaction as follows:



Here, the reaction occurs with a rate constant k_{Ph} , with dimensions of time⁻¹. The new population balance takes the following form:

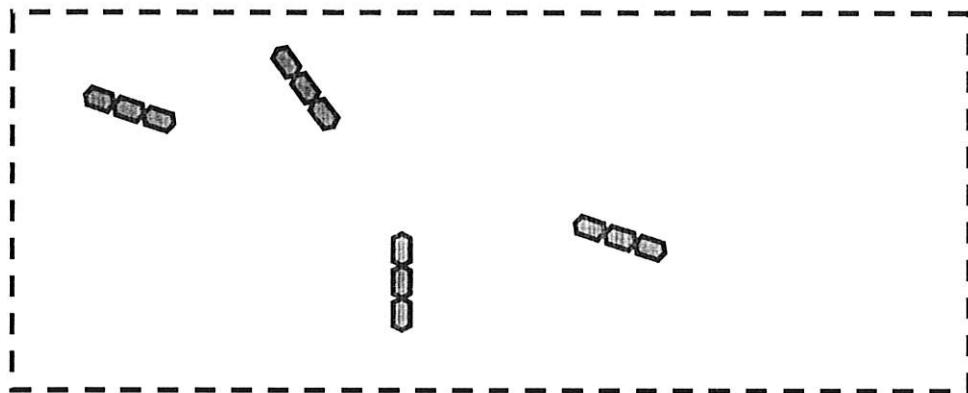
$$\begin{aligned}\frac{dCu}{dt} &= \alpha * Cu - kPh * Cu * Cp \\ \frac{dCi}{dt} &= \alpha * Ci + kPh * Cu * Cp\end{aligned}$$

The notation in this case uses Cu as uninfected cells, Cp as the phage concentration, and Ci as the concentration of infected cells. To obtain the differential equation for the concentration of phage as a function of time, we assume that the concentration of phage is proportional to the concentration of infected cells and obtain the following relationship (after incorporating the reaction term):

$$\frac{dCp}{dt} = \beta * Ci - kPh * (Ci + Cu)$$

The parameter β represents the phage production rate by infected cells. Each of these parameters was roughly estimated initially to determine model behavior, using the following general guidelines: $\beta > \alpha > 0$ because phage production will proceed more rapidly than cell division, $kPh > 0$ with binding and infection of the cell occurring relatively slowly on the time-scale of the system.

Figure 4. System set-up with phage particles, infected cells, and uninfected cells.



Iteration 2

Here, we delete the assumption that cell growth follows an exponential growth curve. We employ the more reasonable assumption of logistic growth, implying that there is some carrying capacity on the amount of bacteria that the system can sustain. Now, growth rate depends on cell concentration. To obtain this behavior, we desire the growth rate to decrease as the concentration of cells increase, and to have nearly uninhibited growth when the cell population is low. As the population rises, it should begin to plateau at some critical cell concentration, or carrying capacity. To introduce such a function we start with the following relationship:

$$Growth(C_{cell}) = \alpha - \delta C_{cell}$$

We introduce this term into the previous differential equation for cell growth to replace the former α term. Thus, we obtain the following differential equation:

$$\frac{dC_{cell}}{dt} = (\alpha - \delta C_{cell}) * C_{cell}$$

After factoring out the alpha term, the equation takes the form:

$$\frac{dC_{cell}}{dt} = \alpha \left(1 - \frac{\delta}{\alpha} C_{cell}\right) * C_{cell}$$

The population should plateau at the value δ/α , which will be the carrying capacity or threshold growth of the system. For now, we will replace δ/α with K_{max} into the equations from iteration 1, and we have:

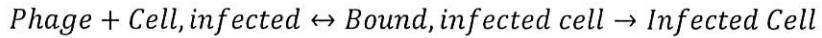
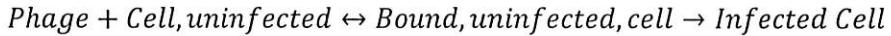
$$\begin{aligned}\frac{dCu}{dt} &= \alpha * \left(1 - \frac{Cu + Ci}{K_{max}}\right) * Cu - kPh * Cu * Cp \\ \frac{dCi}{dt} &= \alpha * \left(1 - \frac{Cu + Ci}{K_{max}}\right) * Ci + kPh * Cu * Cp\end{aligned}$$

The equation for the concentration of phage remains unchanged, and the diagram is as before. K_{max} will temporarily be estimated as some multiple, $O(10^3)$, of the initial cell concentration.

Iteration 3

Two assumptions are changed for this iteration of the model:

- 1) The assumption of the interactions causing infection of the cell are revised as follows:



These reactions assume reversible binding of phage to the pili of the cell. There are two new kinetic rate constants, k_f and k_r , the forward and reverse binding rates, respectively.

- 2) The growth rate of infected and uninfected cells should differ. Two growth rate constants r_i and r_u reflect the growth of infected and uninfected cells, with $r_i < r_u$.

From the first assumption, we make the intuitive conclusion that the cell will remain in its bound state for very little time compared to the infected or uninfected state. Mathematically, this is a statement of quasi-steady state for these species such that the following relationship should hold:

$$\frac{dC_{cell,bound}}{dt} = 0 = kf * Cp * C_{cell} - kr * C_{cell,bound} - kPh * C_{cell,bound}$$

After algebraic rearrangement, we can replace the bound cell term with:

$$C_{cell,bound} = \frac{kf * Cp * C_{cell}}{kr + kPh}$$

The system of differential equations becomes the following after introducing the new term:

$$\begin{aligned}\frac{dCu}{dt} &= ru * \left(1 - \frac{Cu + Ci}{Kmax}\right) * Cu - kPh * \frac{kf * Cp * Cu}{kr + kPh} \\ \frac{dCi}{dt} &= ri * \left(1 - \frac{Cu + Ci}{Kmax}\right) * Ci + kPh * \frac{kf * Cp * Cu}{kr + kPh} \\ \frac{dCp}{dt} &= \beta * Ci - kf * Cp * (Cu + Ci) + kr * \frac{kf * Cp * (Cu + Ci)}{kr + kPh}\end{aligned}$$

Iteration 4

This iteration removes the assumption that the volume of the system stays constant – the volume of cells is increasing, so logically the volume of the system should decrease according to the initially stated relationship, $V_{tot} = V_{sys} + n_{cell} * V_{cell}$ or $V_{sys} = V_{tot} - n_{cell} * V_{cell}$. In differential form, this relationship makes the statement:

$$\frac{dV_{sys}}{dt} = -V_{cell} \frac{dn_{cell}}{dt}$$

The volume of the cells, as stated initially, is assumed to be a population average, and thus remains constant. The number of cells, however, changes with time, and we must now change the former system of differential equations to reflect this change. Instead of using bulk concentrations, the concentrations will be replaced with number of cells per volume of the system. Using the product rule, we obtain this relationship:

$$\frac{dC_{cell}}{dt} = \frac{d(n_{cell}/V_{sys})}{dt} = \frac{1}{V_{sys}} \frac{dn_{cell}}{dt} - \frac{n_{cell}}{V_{sys}^2} \frac{dV_{sys}}{dt}$$

We apply this relationship to the system of differential equations to recast the equations with changing volume taken into account.

Iteration 5

After computationally invoking the previous model iteration, we found that in cases in which the control volume is much greater than the maximum volume occupied by cells in the system (determined by the Kmax value), there is little effect on the behavior of the system. Removing the assumption of a constant volume system may be of value in other situations, but for now, this revision to the model will be omitted.

For iteration five, we make the following two assumptions to increase the reality of the model:

- 1) The phage particles have an intrinsic rate of degradation, denoted k_d .
- 2) A fourth species may arise in the system as a result of evolution, resistant cells, which are incapable of producing phage after infection.

The phage degradation rate introduces a term into the differential equation for phage production by once again assuming a proportional relationship (k_d) between the amount of phage present and the rate of degradation. The resistant cell population arises from the uninfected cell population, and will grow at the same rate as uninfected cells. The model for this behavior will require a proportional term (k_{Ch}) relating the concentration of uninfected cells to the generation of resistant cells. The new system of differential equations appears below:

$$\begin{aligned}\frac{dCu}{dt} &= ru * \left(1 - \frac{Cu + Ci + Cr}{K_{max}}\right) * Cu - kPh * \frac{kf * Cp * Cu}{kr + kPh} - k_{Ch} * Cu \\ \frac{dCi}{dt} &= ri * \left(1 - \frac{Cu + Ci + Cr}{K_{max}}\right) * Ci + kPh * \frac{kf * Cp * Cu}{kr + kPh} \\ \frac{dCr}{dt} &= ru * \left(1 - \frac{Cu + Ci + Cr}{K_{max}}\right) * Cr + k_{Ch} * Cu \\ \frac{dCp}{dt} &= \beta * Ci - Cp * (k_d + kf * (Cu + Ci + Cr)) + kr * \frac{kf * Cp * (Cu + Ci + Cr)}{kr + kPh}\end{aligned}$$

Why are these different
from final model (pg 8)?
eqns 1-4

Computational Program

The computational program has been implemented in two software packages: Mathcad and Microsoft Excel. In Mathcad, the reaction-diffusion of the system is not taken into account, but the output is more amenable to interpretation and rapid usage. Each of the programs has particular advantages; in the Mathcad option, output appears using a graphical representation of time-course data for concentration of the four species in the model. Here, we focus on the use of the Excel spreadsheet as a tool for interpreting model output.

By inputting values, users can specify the parameters of their system to determine the dynamic behavior through the graphical output. Specifying different values of each of the parameters, using the definitions outlined in the table in *Full Model*, the user can obtain output in the form of concentrations of phage at specific radial values and time points. There are two sheets of interest to the user of this program: Concentration vs Time and Model Input. The Model Input section prompts the user for the parameters outlined in Table 4.

Table 4. Model Input Parameters.

delt (time step)	0.1	Kmax	4	kr	1E-05	ru	0.7	kCh	0.001
delr (radial step)	0.5	kPh	0.5	kG	5	ri	0.6		
D (cell diffusivity)	0.08	kf	1	kd	0.5	DP (phage diffusivity)	0.3		

For full model utility, units on each of the parameter values must be consistent (that is, all in CGS, molar concentrations, or other connected unit systems). Specifying modest changes to the values in the current table will modify the model output only slightly. For complete clarity in model utilization, the parameters to be specified are the time step (delt), position step (delr), diffusivity (D), carrying capacity (Kmax), phage infection rate (kPh), phage forward binding rate (kf), phage reverse binding rate (kr), phage production rate (kG), degradation rate (kd), uninfected growth rate (ru), infected growth rate (ri), phage diffusivity (DP), and resistant cell conversion rate (kCh). After specifying these parameters, the user also has the option to choose the initial conditions for the phage concentrations. Boundary conditions for this system are Neumann, and initial conditions are assumed to be uniform throughout the plate and constant at the center of the plate ($r = 0$).

After modifying the parameters and initial conditions as desired, output will be available directly below the input module. Graphical representation of system behavior appears in the

sheet labeled Concentration vs Time. Screen captures of sample model input and expected output follow in figure 5.

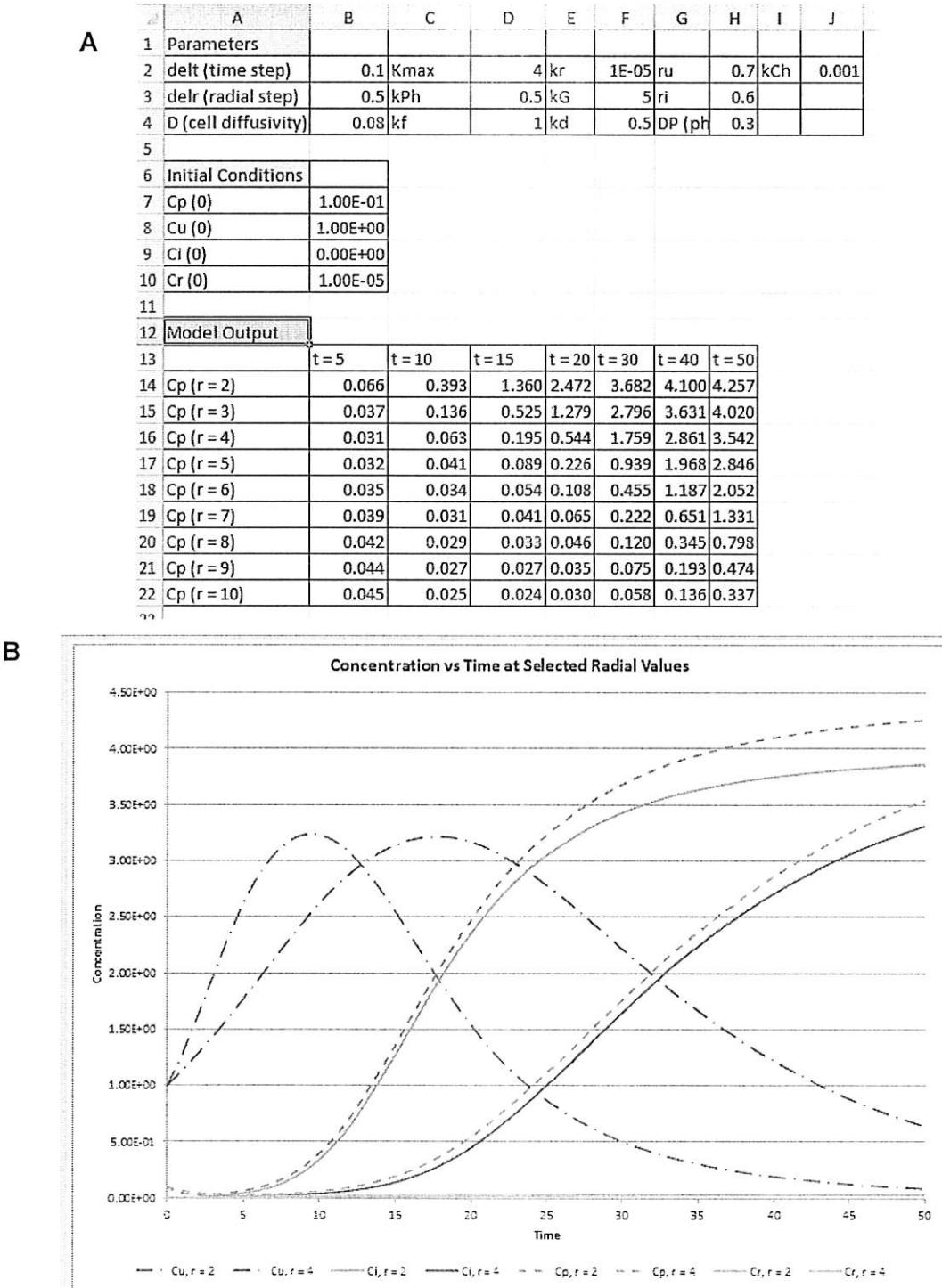


Figure 5. Sample model inputs and output. **A** Parameter and initial condition input with phage concentration output at selected time and radial values. **B** Graphical output. Radial values and corresponding concentrations appear at the bottom.

Results and Discussion

The final model produces the expected system behavior when considering non-lytic infection of bacteria by phage. As outlined in the introduction, filamentous phage bind to pili on the surface of susceptible cells, subsequently infecting and converting their hosts into viral factories. In a laboratory setting, given even a small initial amount of bacteria, binding and insertion of the viral genomes proceeds rapidly. Following this event, the bacteria begin to commit resources to the assembly and extrusion of phage particles from their surface, and within 100 minutes, nearly the entire surface of the cell is covered with adhesion zones for the rapid export of phage. The model successfully demonstrates dynamic behavior of this nature.

Starting with a low initial concentration of both bacteria and phage in the system, growth begins along with infection, so that while initially there are very few phage particles and no infected bacteria, the situation rapidly changes. Nearly all of the uninfected bacteria in the system become infected within a short time span as the system activates. The initially low population of phage particles rise as infected bacteria produce (without cessation) thousands of phage particles, which can then diffuse in the medium to infect the remaining uninfected bacteria. Thus, the infected population rises, inducing a concomitant rise in phage particles until the system approaches equilibrium in which phage production and cell growth and death begin to plateau. At this point, viruses saturate the system, and with no remaining uninfected bacteria, they are depleted (by degradation and reinfection of infected cells) at the same rate as they are produced. The system behavior described here is graphically demonstrated in figure 6, a sample model output from the computational model, which uses a combination of somewhat arbitrary values and inferred values that successfully yield the expected behavior.

An apparent weakness of this model is the ability to incorporate an entire suite of realistic parameter values for prediction of the model. While it can use growth rate parameters (r_u and r_i) determined from solving these values using an exponential approximation, many of the other parameters lead to numerical instability in the system, preventing convergence on a solution. For growth rate, many papers report doubling time, which can be decomposed into a parameter by making the following approximation $2 = e^{kt}$, where k is the growth rate. Thus, $k = \ln(2)/t$. For a doubling time of 1 hour, this gives a growth rate of approximately 0.7/hour. Assuming this value as the uninfected growth rate of the population, the infected growth rate should be slightly (or substantially) lower than the uninfected growth rate. The estimate for the scenario depicted in figure 6 is 0.6/hour.

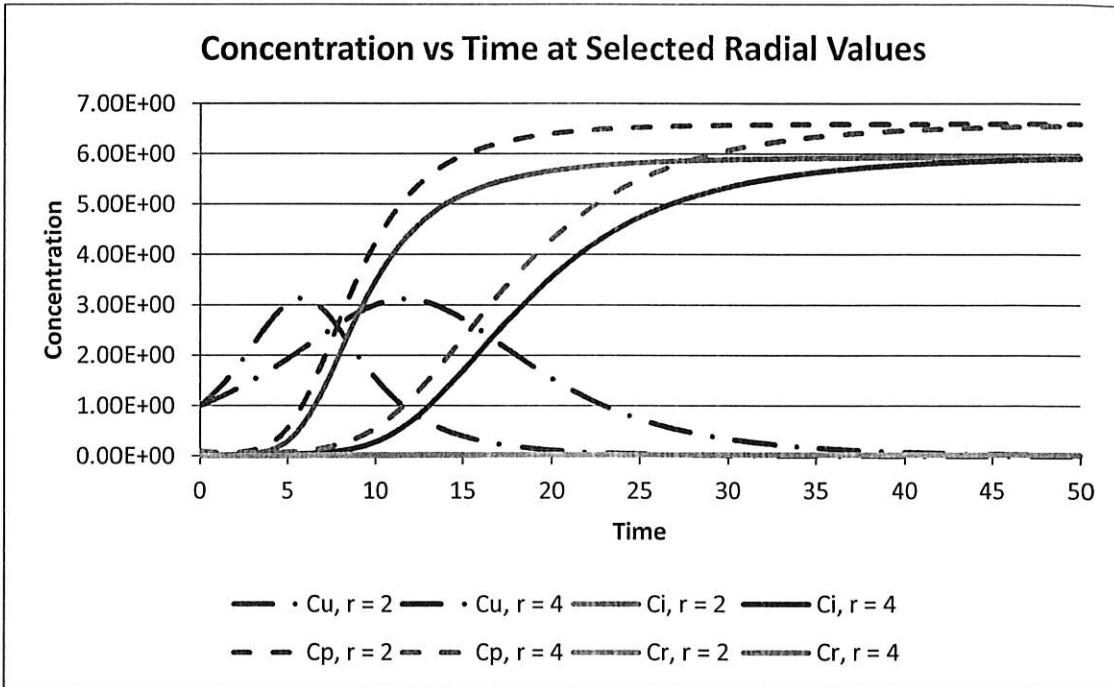


Figure 6. Fitted parameters yield expected system behavior.

Without actual kinetic parameters, the values for binding and unbinding of phage to the pili of the cell can range wildly depending on the physical nature of the system. Lower bounds on the forward binding rate, assuming the process is diffusion limited are $O(10^{-12})$ ml molecules $^{-1}$ sec $^{-1}$. The upper limit on the reverse binding rate should be around $O(10^{10})$ hour $^{-1}$. Given the somewhat arbitrary nature of specifying the initial conditions, and the insufficient numerical capabilities of excel to handle excessively large (or small values), the range of these values was narrowed for implementation to yield realistic system behavior (rather than realistic values). In figure 6, the value used for the forward binding constant, kf, is 1.5 and the value used for the reverse binding constant, kr, is 1e-5. Clearly, the phage particles are much more likely to outnumber the bacteria in the population, so the number of phage per unit volume should be much higher than the number of bacteria per unit volume. Introducing a linear scaling into the output may be sufficient to obtain this expected result while retaining the essential system behavior. The current output is thus insufficient in this way.

Two of the most sensitive parameters in the model are the phage infection rate, kPh, and the carrying capacity of the system, Kmax. Because Kmax depends on a number of factors including the self-limiting nature of bacterial growth and death in a closed volume system and the availability of nutrients within the system, perturbing this value even nominally leads to substantial numerical instability in the Excel implementation of this program. Currently, a

nominal value of 4 is used for the output in figure 6. Though this is only a factor of 6 times the initial concentration of bacteria in the system, if we change the initial concentration such that there are 1000-fold fewer initial bacteria, the model behavior remains nearly the same. In this scenario, the number of uninfected bacteria rapidly diminishes as they are overtaken by phage, and the infected bacterial population rises in response (Figure 7).

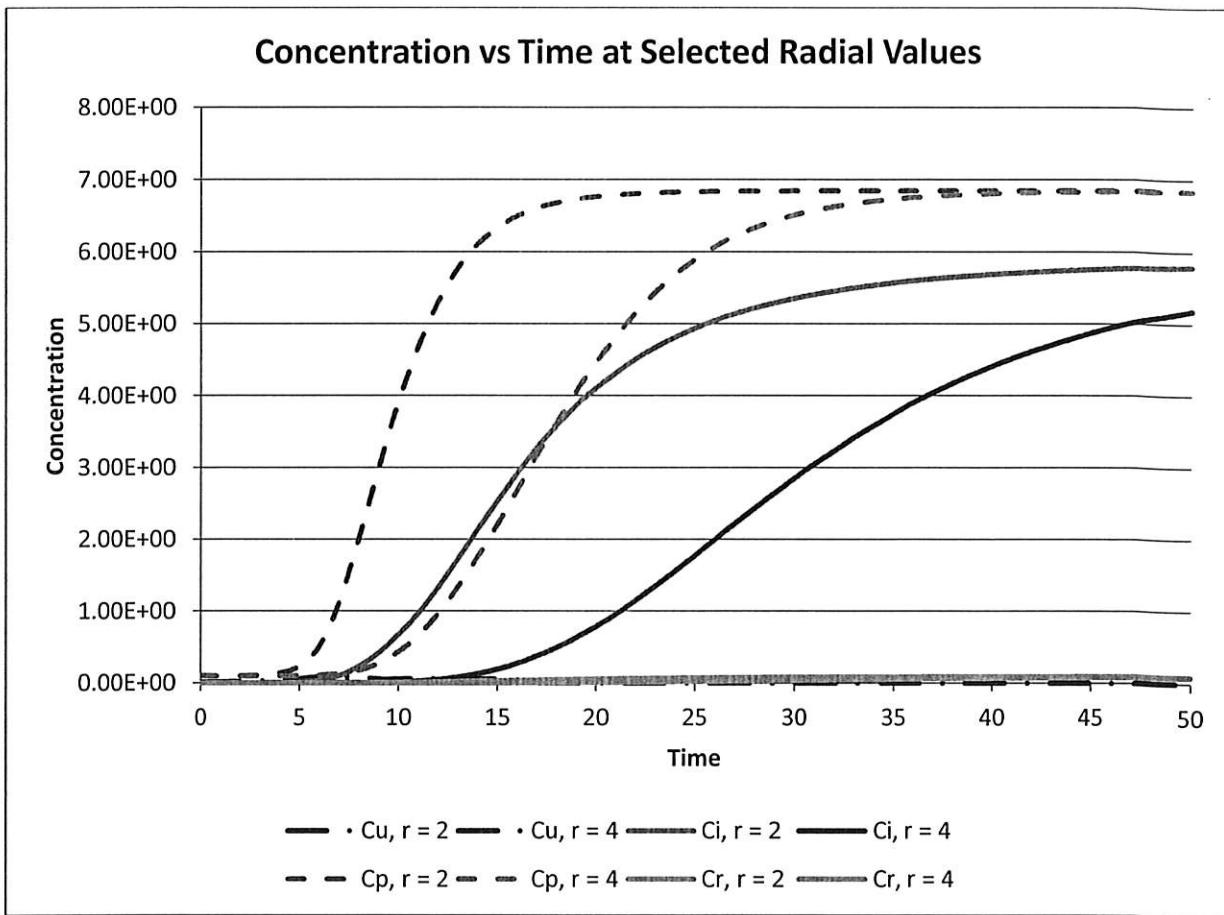


Figure 7. System dynamics with initial uninfected bacterial concentration 1000-fold lower than K_{max} .

The kPh value induces system instability based on the physical nature of the constant. As it represents the entire process outlined in the introduction (phage infection and genome insertion), it has substantial importance in the context of the model. Increasing the value corresponds to an increase in the capacity of the phage to infect the host once bound. Because binding events are relatively common at normal concentrations of phage and bacteria, increasing or decreasing the infectivity drives system instability, resulting in failure of the model to produce reasonable output. By decreasing this parameter, phage concentration in the long

term actually increases. Once the viruses have infected all the cells in the system, infecting more cells would simply deplete the population of phage particles. Consequently, the maximum number of phage in the system is lower than the maximal if the infection rate were lower.

Changes in the other parameters of the system only nominally change model behavior, with the exception of diffusivity, which can cause massive system instability. This problem results from the numerical limitations of Excel. Having employed the finite difference method, the time-step and radial step values must be confined within a suitable range.

Though there are a number of limitations to the use of appropriate values in this model due to instability and presentation of the output, the model quite reasonably predicts the behavior expected of bacteria and non-lytic phage in a closed volume environment. The variety of model outputs further confirms the robustness of the model in this context. Using non-dimensional constants in the model, it may be possible to improve its stability and increase its accuracy. Further steps for the improvement of the model would include introducing these dimensionless constants and finding lumping parameters in such a way to produce numerically manageable values. This procedure would enhance the feasibility of the model while preventing context-specific exceptions.