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Bacteria Ecology: A Model of Population Dynamics Among Bacterial Communities and Bacteria-Phage Interaction

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MCB 136L

Introduction

Unicellular microorganisms are the most versatile and adaptable forms of life on Earth, and they have existed here for some 3.5 billion years. Indeed bacteria alone ruled the planet for the first 2 billion years, colonizing every ecological niche from the polar caps to hydrothermal vents of the deep sea. As these early bacteria evolved, they developed major metabolic pathways as well as defensive mechanisms to survive in a hostile world. Today, our research into bacterial biochemistry and physiology reflects several billion years' worth of genetic responses to an ever changing-world, unveiling some of Nature's secrets. In brief, understanding the biology of bacteria (along with viruses) has changed our perception of and relation to life. However, these tiny, unicellular creatures are life-forms, and, like all life-forms, have a selfish motif to reproduce. On occasions, they cause disease. Fortunately, medical science has responded to the threat of bacteria through an intensive campaign—using an armada of drugs such as β-lactams, aminoglycosides, macrolides, sulphonamines—that have been notably successful. Truly, our understanding of bacteria is responsible for this century's revolution in human health. In spite of such progress, we are still threatened by some bacteria. For instance, thousands of infants are dying from a disease called salmonellosis. You won't see much about this on the evening news because it's not a problem of medically sophisticated nations. But in Third World countries, a lack of sanitary facilities and clean drinking water condemn millions of infants to death each year. The culprit of the disease is any of several species from the genus *Salmonella*, which are mobile, Gram-negative bacilli. (*Salmonella* species are also responsible for one of the most common types of food poisoning and for the highly lethal typhoid fever.) In addition, there are *Clostridium botulinum*, *Mycobacterium tuberculosis*, *Streptococcus pyogenes*, and *Escherichia coli* outbreaks in developed countries such as the United States causing botulinum, tuberculosis, scarlet fever, and diarrhea, respectively. So why are they deadly? How can we control them? Again, understanding the biology of bacteria is crucial. Let us examine how bacteria interact with one another, with the environment, and even with predators such as bacteriophages. A note of warning: little

data on bacteria in their natural habitat has been published, therefore the values appearing in this paper are derived from laboratory observations.

Growth on Laboratory Media

Many liquid media are available for growing bacteria. A common one is bouillon or broth prepared from meat while others are simply water consisting of sugars, amino-acids, certain salts, and, on occasion, specific growth factors. Either cases, the bacterial growth cycle consists of three recognizable phases: (1) the lag phase before cell division commences, (2) the exponential (or logarithmic) growth phase when cell division occurs at a constant rate—i.e. a steady state growth, and (3) the stationary phase, when cell division has ceased. Between the first two phases is an acceleration of growth and between the last two is a deceleration of growth (Fig. 1).

Stationary and lag phases. In the usual practice cells are inoculated into a given volume of medium and allowed to grow until an essential nutrient (e.g. sucrose) has been exhausted or until metabolic by-products (e.g. ethanol) accumulate and inhibit further multiplication. Moreover, other factors such as lack of space, lack of oxygen, and changes in ion concentration (especially pH) prevent further growth. The total number of cells then remain constant. The culture is now in the stationary phase. During this period, the activities of various enzymes decline. Also, essential metabolites of low molecular weight will be lost from the cell by diffusion (Stanier, 1970). Both changes effect biochemical reactions as well as concentration gradients; both changes abate cell division. We can represent the stationary phase by the differential equation

$$dN/dt = -bN^2 \qquad (1)$$

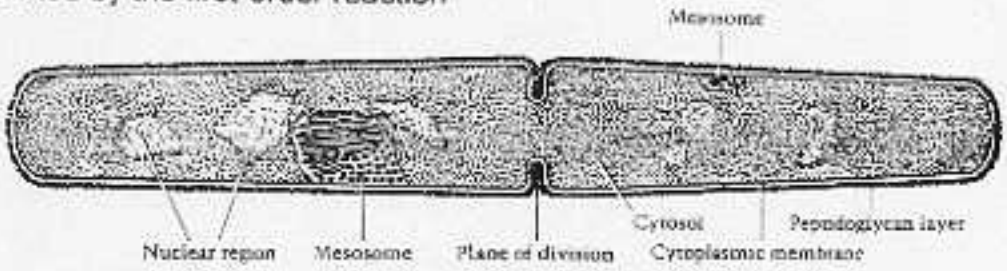
where N is the number of cells at time t, and b is the specific death rate constant. The formulation of equation (1) follows a second-order reaction. This is because a given cell N detects the toxic effect of itself and of all N cells. In other words, the toxic effect of all N cells is N times the effect on one cell and hence proportional to $N \times N$. Should we express equation (1) in terms of half-life (after integration), the result is

$$t_{1/2} = 1/b[N_o] \qquad (2)$$

where N_o is the original number of cells. Equation (2) is useful in determining the specific death rate constant *b*. For *Escherichia coli*, *b* is approximately 2.78×10^{-9} per CFU[1] per minute per mL (Dean, 1966).

If cells from stationary phase is transferred to fresh media, steady state growth can only be re-established after a time interval known as the lag phase. The lag phase is a period of metabolic adjustment, the duration of which depends on a number of factors. Here, various intermediates are accumulated once more in the requisite concentrations, and the activities of the enzymes are restored. The expected sequence of events is indeed observed when cells are inoculated into fresh medium (*see Fig. 1*). The lag phase will not be investigated hereafter.

Exponential phase. Bacteria multiply by elongation and binary fission, represented by the first-order reaction



$$N \longrightarrow 2N$$

Therefore the exponential phase, frequently regarded as a steady-state system, can be expressed by the growth law

$$dN/dt = kN \qquad (3)$$

where *k* is the specific growth rate constant. Equation (3) suggests, with regular division and a constant environment[2], each cell grows at the same rate as its parent (Koch, 1995). And for a large number of cells in random phases of their individual generation periods, the change in numbers is strictly proportional to the total number. The equation above can be re-written, after integration and rearrangement, as

$$\ln N = kt + \ln N_o \qquad (4)$$

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[1] CFU = colony forming unit, which is equivalent to one cell.

[2] As cells grow, they continuously modify the composition of their medium, consuming nutrients and excreting waste products. Thus, the growth law does not represent the true growth curve but is generally sufficient.

where N_o is the original number. This law is in fact rather closely followed (with understandable deviations) over quite wide ranges of growth. The steady state during which the above law is followed is also known as the logarithmic phase of growth. A plot of *ln N* vs. time *t* gives the slope constant *k* (Fig. 2). However, a more convenient quantity by which to characterize their growth is the mean generation time; that is, the time required for the number of cells to double ($N = 2N_o$) during the exponential phase:

$$\ln (2N_o/N_o) = \ln 2 = k(t_1-t_2) = kT$$

$$T = \ln 2/k = 0.693/k \qquad (5)$$

Here *T* is the mean generation time. At optimal conditions, the mean generation time for *E. coli* is *T* = 30 minutes (Poindexter, 1989). Thus, its specific growth rate constant is *k* = 0.0231 CFU per minute. Note either equations (4) or (5) can be used to derive the value of *k*.

But what about sub-optimal conditions when the concentration of nutrients is low? How is the specific growth rate constant affected? Interestingly, the nutrient concentration and the growth rate constant are related by Michaelis-Menten's equation (Thingstad, 1997):

$$k = (k' [Nutrient]) / (n + [Nutrient]) \qquad (6)$$

where *k*, *k'*, *n*, and [*Nutrient*] are analogous to *V*, V_{max}, K_m, and [*S*], respectively. Indeed, nutrient uptake and its conversion to a product has the reaction

$$\text{bacterium + nutrient} \rightleftharpoons \text{bacterium/nutrient} \longrightarrow \text{bacterium + product}$$

which is similar to Michaelis-Menten's enzymatic reaction

$$E + S \rightleftharpoons ES \longrightarrow E + P$$

Here, the bacterium is comparable to an enzyme. Note, the formation of the product is essential for bacterial growth, thus represents the rate limiting step. As an illustration, the glucose concentration at which *k* is ½ *k'* for *E. coli* is 4 μg/mL (Fig. 3).

Integrating the equations. The survival of a bacterium depends on its ability to grow at a rate sufficient to balance death caused by starvation and other natural causes. On the whole, combining equations (1) and (3) give us the growth cycle of bacteria in the laboratory:

$$dN/dt = kN - bN^2 \qquad (7)$$

where the first term on the right side of the equation represents growth and the second term represents death. Notice when N is small, the growth rate is greater than death; when N is large, the death rate is greater. The former describes cells transferred into fresh media whereas the latter occurs when nutrients are scarce. As an example, consider the growth curve of a bacterium in favorable nutrient concentration (Fig. 4). The population density equilibrates at 8,000,000 CFU/mL after 20 hours, a value that agrees with empirical data. After 20 hours, the growth rate balances the death rate; the culture is in stationary phase. Interestingly, the initial inoculum does not influence the equilibrium value. An inoculum of 1 or 1,000 CFU/mL will equilibrate at 8×10^6 CFU/mL even with sufficient nutrient (Fig. 5). The limiting factors are lack of oxygen and space.

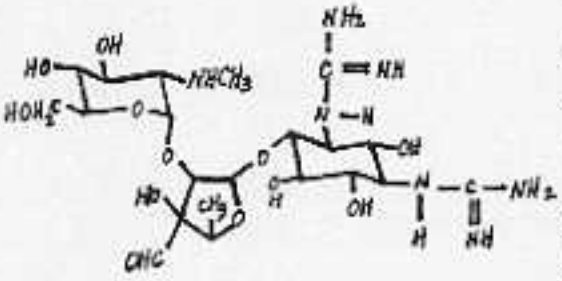
In the proceeding model (Fig. 6), N describes wild-type (W) and mutant (M) bacteria populations. Both populations will have the same specific growth rate (k) and death rate (b) constants. The only difference is their ability to survive on inhibitory drugs.

Adaptation to Drugs

The term drug means essentially a substance, other than a normal intermediate metabolite, which influences the growth and function of a cell. In this sense, both stimulant and inhibitory compounds are included, however only the latter will be investigated. From the kinetic point of view, one of the most interesting aspects of drug action is the way in which cells become resistant, a phenomenon known as adaptation. This occurs, according to Levy (1998), when the cell inherits an ability to deactivate the drug by such mechanisms as modification (e.g. phosphorylation of aminoglycosides), cleavage (e.g. hydrolysis of penicillin), and extrusion (e.g. active efflux of tetracycline). Interestingly, in some cases, growth of resistant strains actually depend on the presence of these inhibitory compounds to fight off competing sensitive strains (Fig. 7). So how do drugs affect the growth rate of bacteria? In the simplest case, the effect of drug is given by a linear relationship

$$k = k' - d[Drug] \qquad (8)$$

where d is a constant having dimensions of mL/minute/μg. Note the second term on the right side of the equation is negative, as expected for inhibitory compounds. Equation (8) only applies to drugs without a threshold of tolerance. It is important to realize that various compounds influence growth rate differently. For example, *E. coli* still grows, albeit slowly, when the concentration of sodium-dodecyl-sulfate (SDS) is over 1200 μg/mL. In contrast, a minute 2μg/mL of streptomycin[3] causes cell death immediately. Hence, there is no threshold of tolerance for streptomycin (Fig. 8). For streptomycin in *E. coli*, d is 0.0157 mL/minute/μg (Jack, 1996). In this model, only the wild-type population W is sensitive to streptomycin.



Streptomycin

As mention, one of the most remarkable phenomenon in bacteriology is the rapidity and persistence with which cells become resistant to the further action of drugs. The adaptive process, i.e. mutation rate, can be described by a linear equation under optimal conditions as

$$dM/dt = uW \qquad (9)$$

Here u is the fractional mutation rate per unit time at which wild-type cells (W) convert to mutant cells (M). (Back reversions, M to W, are not considered in the model.) So how does the mutation rate determine the value of M? Consider two bacterial populations, W and M, which are related genetically so that M cells arise from W cells by mutation. Assuming growth conditions are favorable for both types, the population density of W is immensely greater than M (Fig. 9). W simply out competes M, as shown by combining equations (1), (3), and (9)

$$dM/dt = kM - bM^2 + uW - dW/dt \qquad (10)$$

where $kM\text{-}bM^2$ is the net growth rate of M, uW the mutation rate, and dW/dt the competing growth rate of W. Only when the net growth rate dW/dt of wild-type population reaches zero, as in the case of streptomycin inhibition, the mutant population becomes the dominate phenotype (*see Fig. 7*). Aberrant ribosomes allow M to escape

[3] Streptomycin is an aminoglycoside. It targets the 30S ribosomal subunit of prokaryotes. Spontaneous mutants have altered ribosome structure, allowing them to escape inhibitory effect of aminoglycosides.

streptomycin inhibition but, at the same time, lower the growth rate such that *M* cannot compete against *W* in absence of streptomycin. The mutation rate constant *u* for *E. coli* on streptomycin is 9.80×10^{-4} (Nakajima, 1995).

Interacting Virus-Bacteria Populations

Let us consider a simple example of two interacting populations for which the level of one population depends on the level of another. Parasitic viruses are born from an infected host bacterium that is killed in the process. How do host and parasite populations vary in time? Let *W* and *V* represent the number of bacteria and viruses, respectively, at time *t*. The infection of host by invading parasite depends on the probability of them encountering, and is therefore proportional to *VW*. There is also a constant of proportionality *f*, so that the rate at which viruses adsorb to the bacteria is *fVW*. In addition, the viral efficiency of entry after adsorption is given by the constant *e*. The product *efVW* thus represents the parasite-killing rate, assuming that entry always lead to death of host. Therefore, the host population is governed by the equation

Entry of T4 phage into bacterium. C. K. Mathews. 1977. Comprehensive Virology, ed. H. Plenum Press, New York.

$$dW/dt = kW - bW^2 - efVW \qquad (11)$$

and the parasite population is governed by

$$dV/dt = refVW - xV \qquad (12)$$

where *r* is the specific replication rate constant, and x is the specific decay rate constant (Goyal, 1987). Note the term *xV* describes a first-order decay rate according to the second law of thermodynamics. Viruses decompose outside its host because they lack energy input to sustain an ordered structure. For T-even phages, *e* is 0.15 per PFU, *f* is 1×10^{-8} PFU per CFU, *r* is 0.0768 per minute, and *x* is 9.83×10^{-4} per minute (Krukonis,

1995). Consider the consequences when a viral population of 100 PFU/mL is introduced to a wild-type bacterial population (initially at 1 CFU/mL) undergoing exponential growth (Fig. 10). Immediately after inoculation, the viral population increases until most of the host population is exhausted. At 500 minutes, after 3.3 hours of viral growth, viral decline occurs even before the bacterial population reaches zero. This early decline illustrates a low occurrence of adsorption—i.e. a low *fVW*. A different way to graph the virus-bacteria interaction is shown in Figure 11. It is a diagram of the population density of bacteria as a function of population density of virus. Notice the start of bacterial decline occurs when the virus population reaches $V \cong 200$ PFU. Also, viral decline can be seen before the bacteria population reaches zero, at $W \cong 110$ CFU. In a perfect predator-prey system that permits coexistence, Fig. 11 would be a closed loop. Here, the broken loop indicates a high virulence that ultimately leads to death of both populations. This can be advantageous in medicine, should bacteriophages be used as antibacterial agents.

Conclusion

The discussion above is a short attempt in modeling the ecology of bacteria. Needless to say, many parameters—such pH, temperature, and osmotic pressure—were not included. Furthermore, the values used are derived from laboratory studies rather than natural observations. Henceforth, the model may not depict the true activity of bacterial populations. Nevertheless, some valuable insights can be construed. For instance, application of a drug can lead to resistant mutants (*see Fig. 7*), therefore preventative measures—such as use of multiple drugs—should be taken to inhibit the occurrence of mutants. Second, drugs should be applied immediately after infection. A delay allows bacterial population to reach astronomical numbers upon which a high drug dosage cannot abate infection (Fig. 12). Finally, virulent bacteriophage can be used as antibacterial agents, even when the host population is high. As Fig. 10 shows, a virulent bacteriophage ultimately kills all of its host. Therefore, use of bacteriophage is an attractive means in controlling bacteria. On the whole, continuing research will uncover

the true ecology of bacteria and allow us to attenuate, hopefully, the diseases caused by these ancient life-forms.

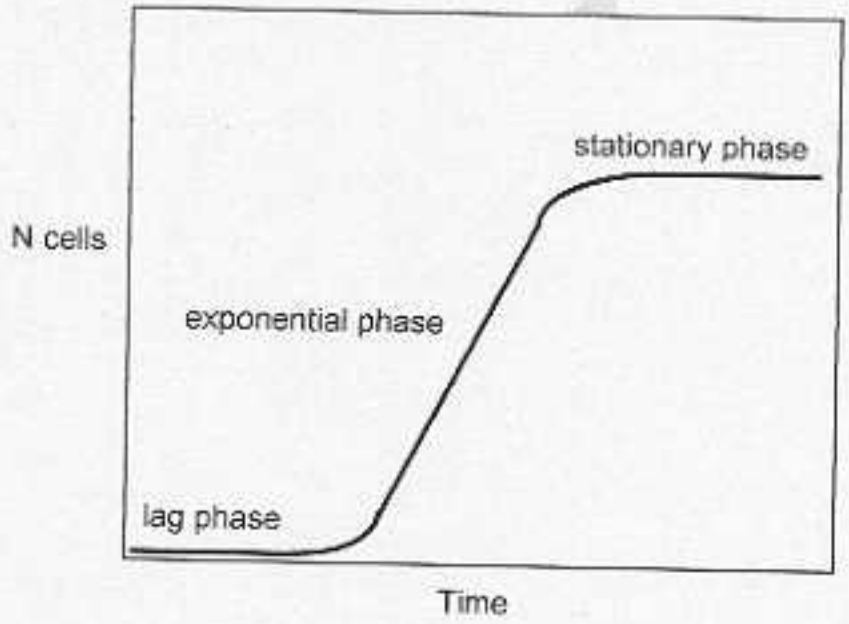


Figure 1 *Growth curve of bacteria in nutritive media.*

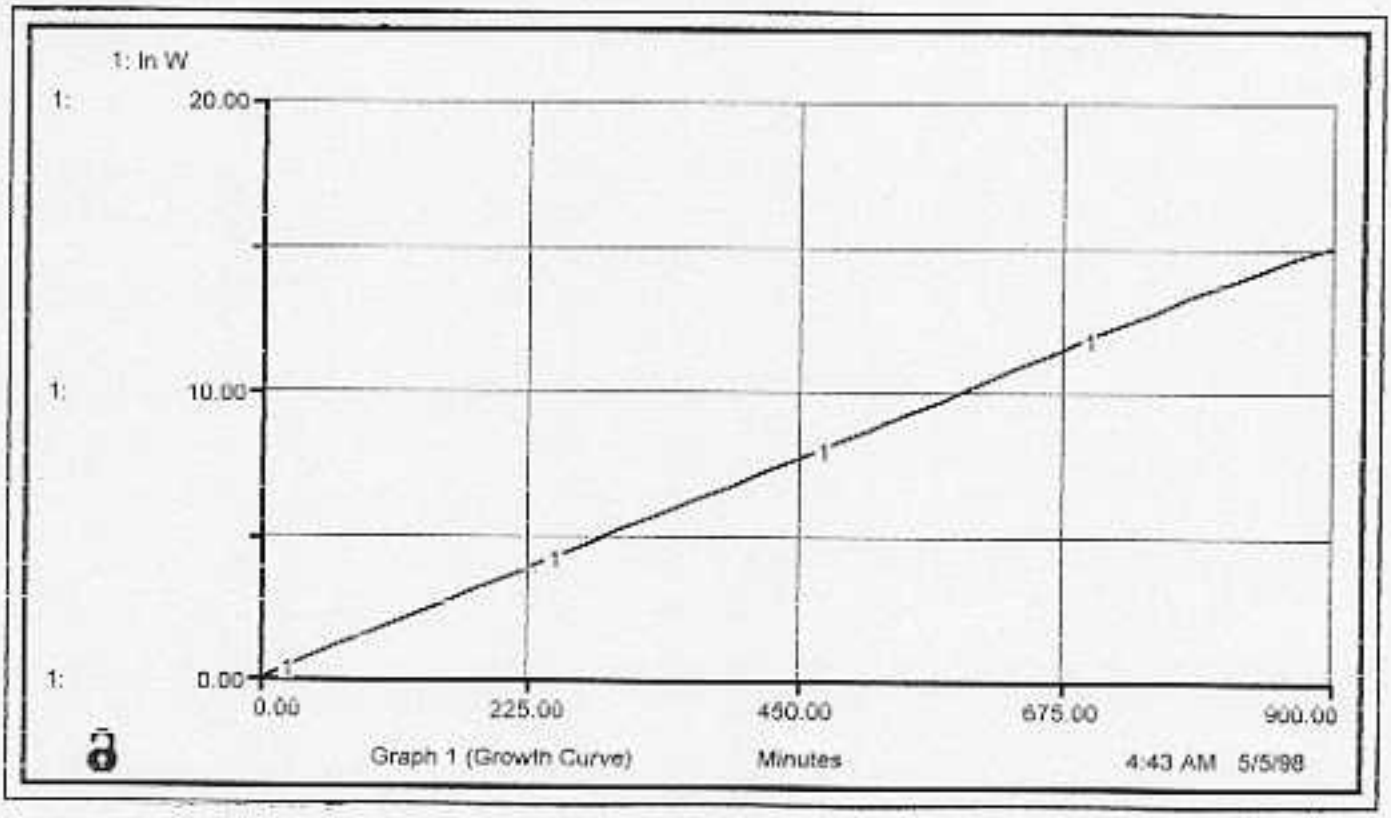


Figure 2. Graph of ln W [CFU/mL] as a function of time t [minutes]. The slope of the line is the specific growth rate constant k, given by the equation ln w = kt + ln W_o where W_o is 1 CFU/mL.

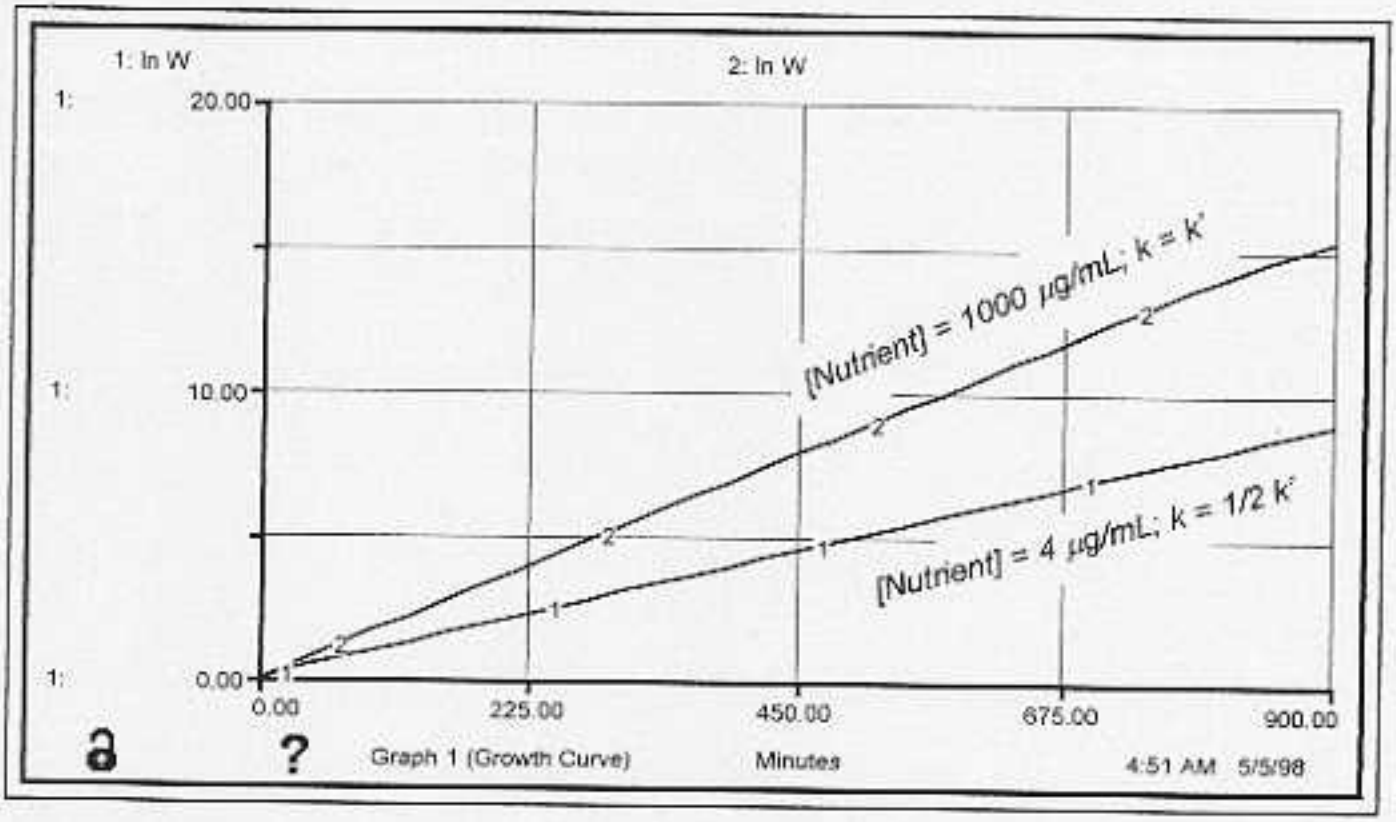


Figure 3. Graph of ln W [CFU/mL] as a function of time t [minutes]. The slope of the line is the specific growth rate constant k, given by the equation ln w = kt + ln W_o where W_o is 1 CFU/mL. Here, line 1 represents k in presence of nutrient concentration of 4 µg/mL, the concentration at which k is half that of k'. Line 2 represents k in medium saturated with nutrient such that k is approximately that of k'.

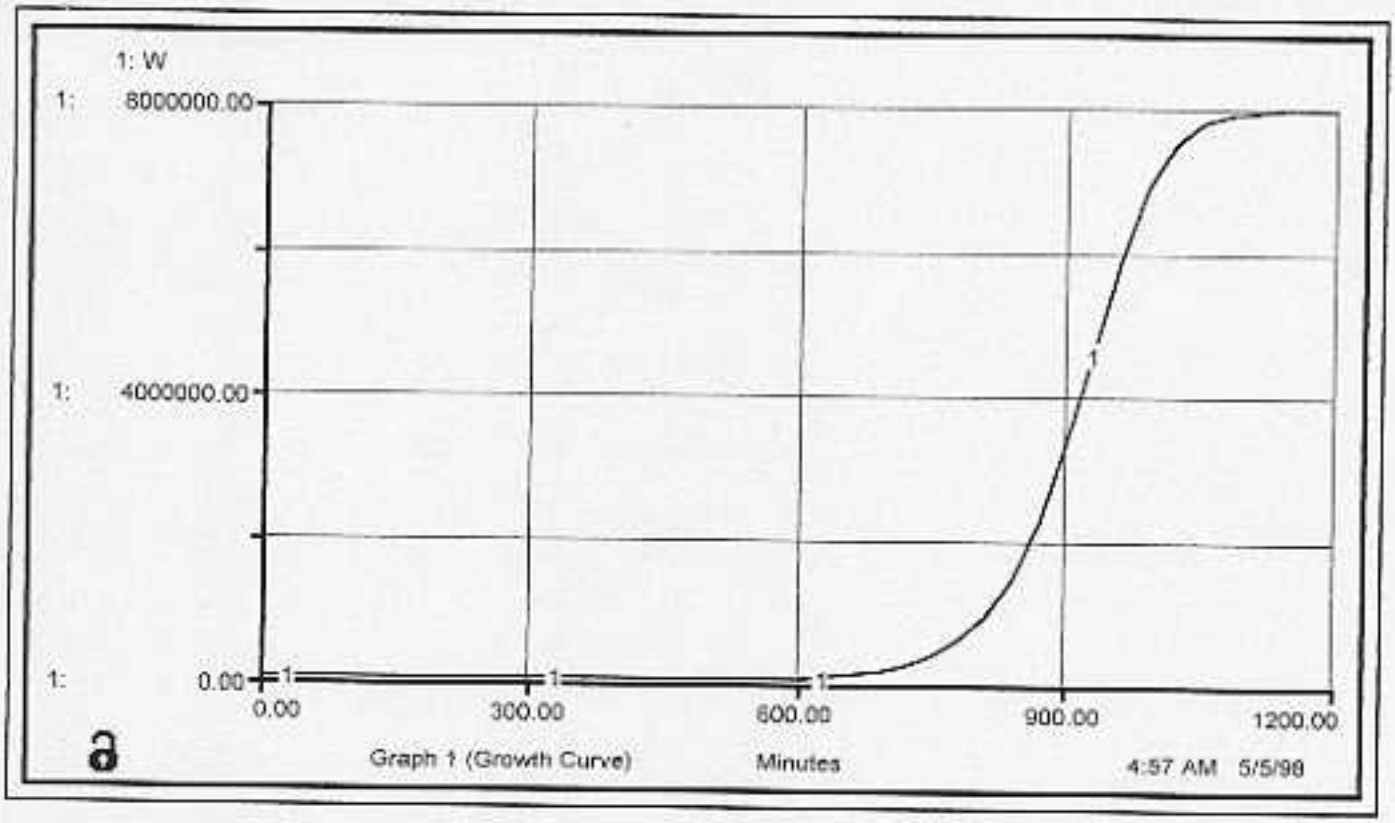


Figure 4. Growth curve of E. coli in laboratory conditions, a plot of W [CFU/mL] versus time t [minutes. The lag phase is not represented even though the curve seems to remain constant in the first 600 minutes. Reproduction is occurring during this period, but undetectable. Note stationary phase starts as 1200 minutes, or 20 hours. This agrees with empirical data.

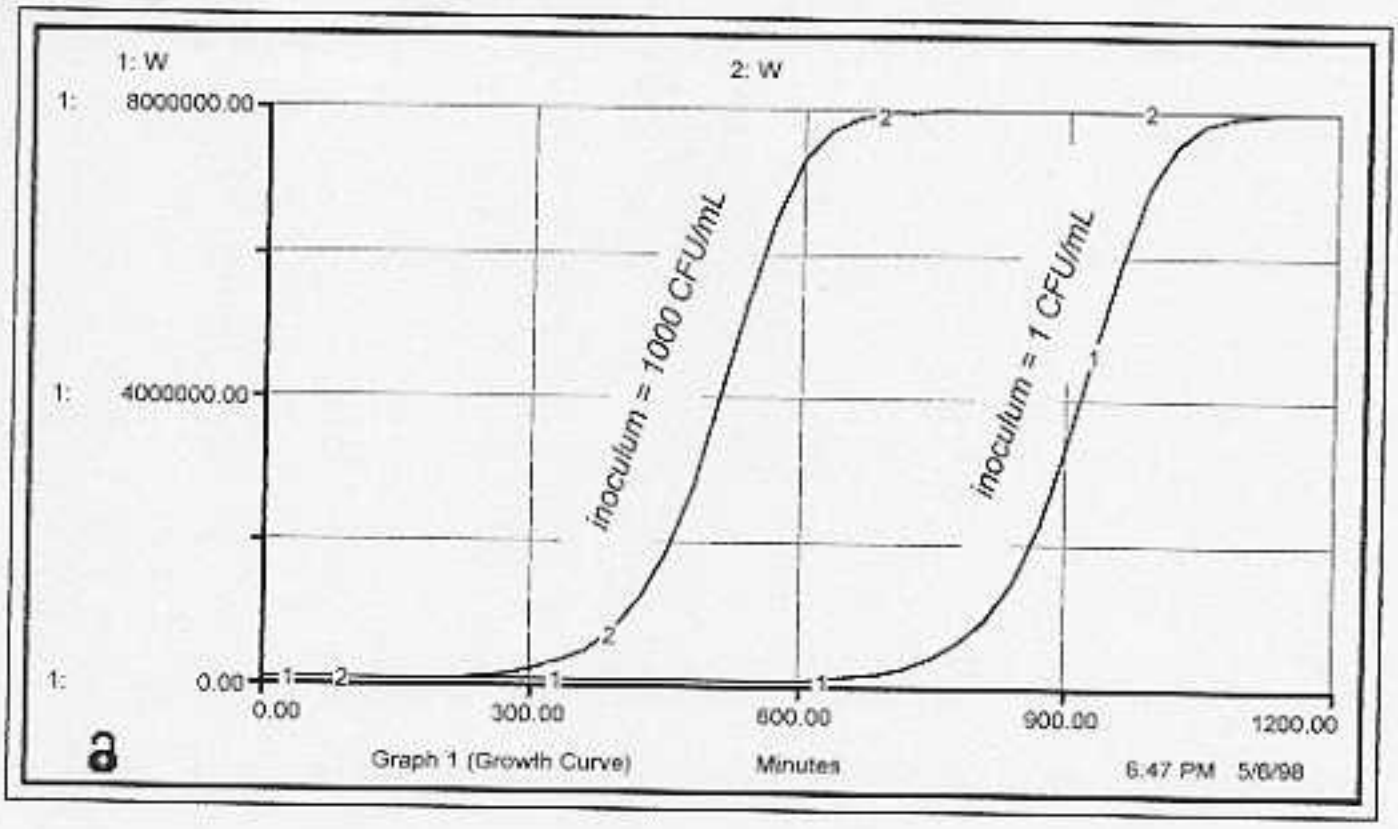


Figure 5. Growth curve of wild-type bacteria [CFU/mL] with initial inoculum of 1 CFU/mL (line 1) and 1000 CFU/mL (line 2). Notice both have a saturation density of 8,000,000 CFU/mL; the only difference is the speed in which equilibrium is reached.

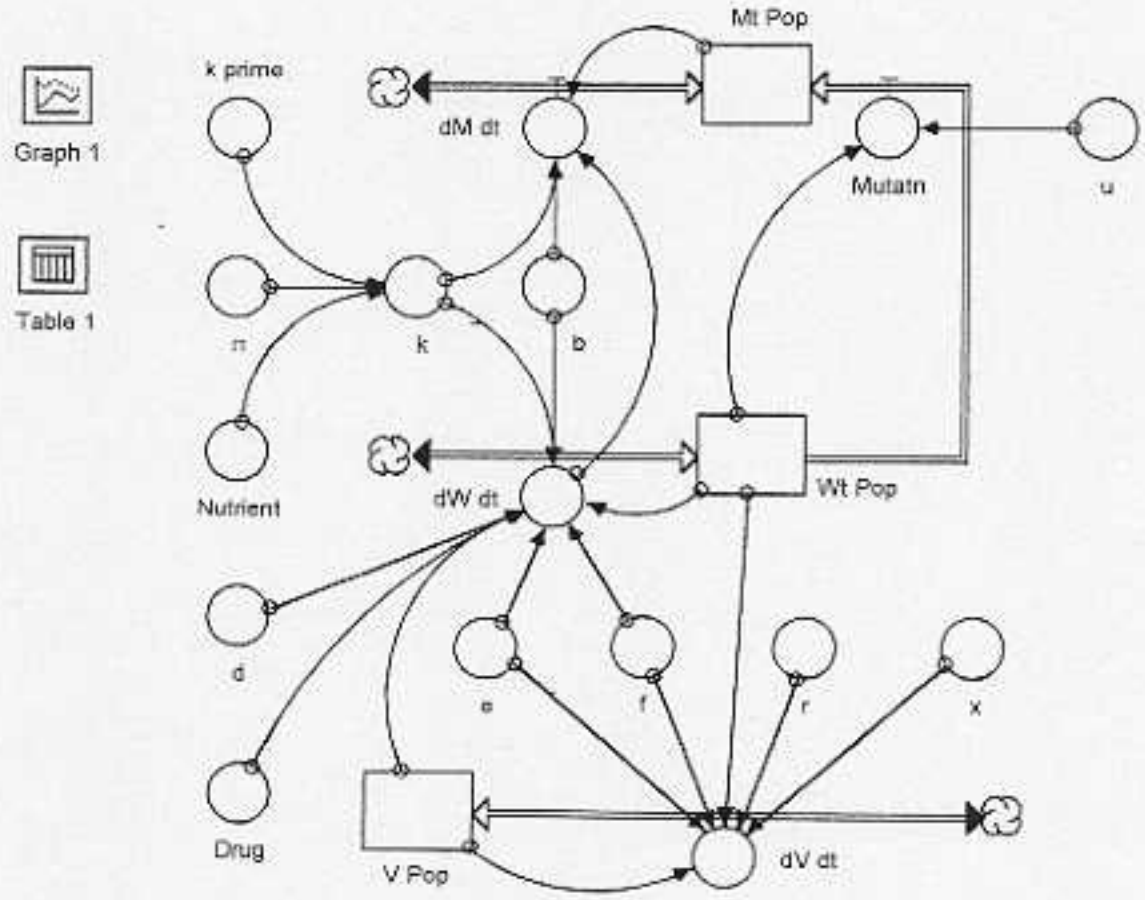


Figure 6. Model of bacteria ecology, showing interaction among wild-type (Wt Pop) and mutant (Mt Pop) bacterial populations, as well as wild-type and phage (V Pop) populations. Note drug and phage infection only affect the wild-type bacteria.

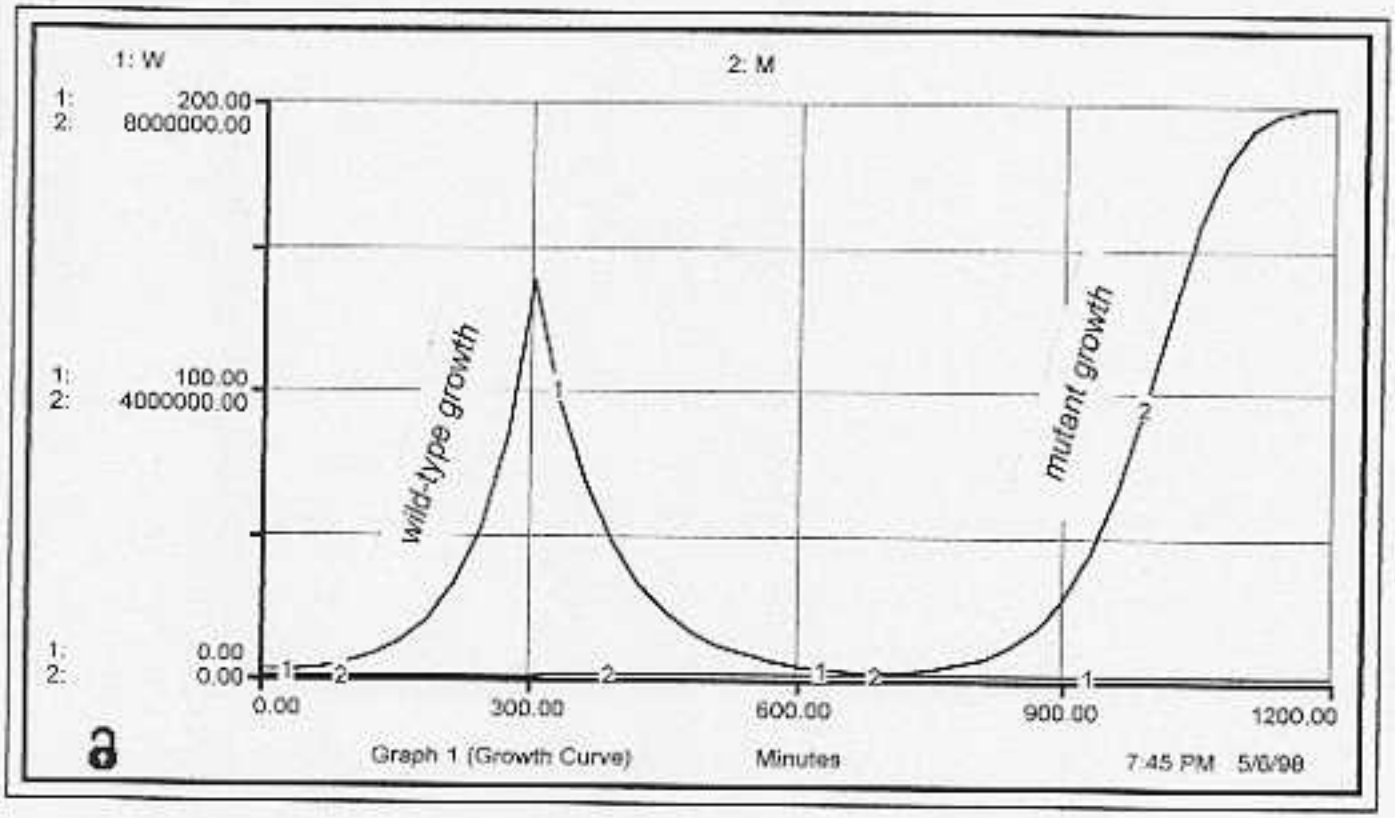
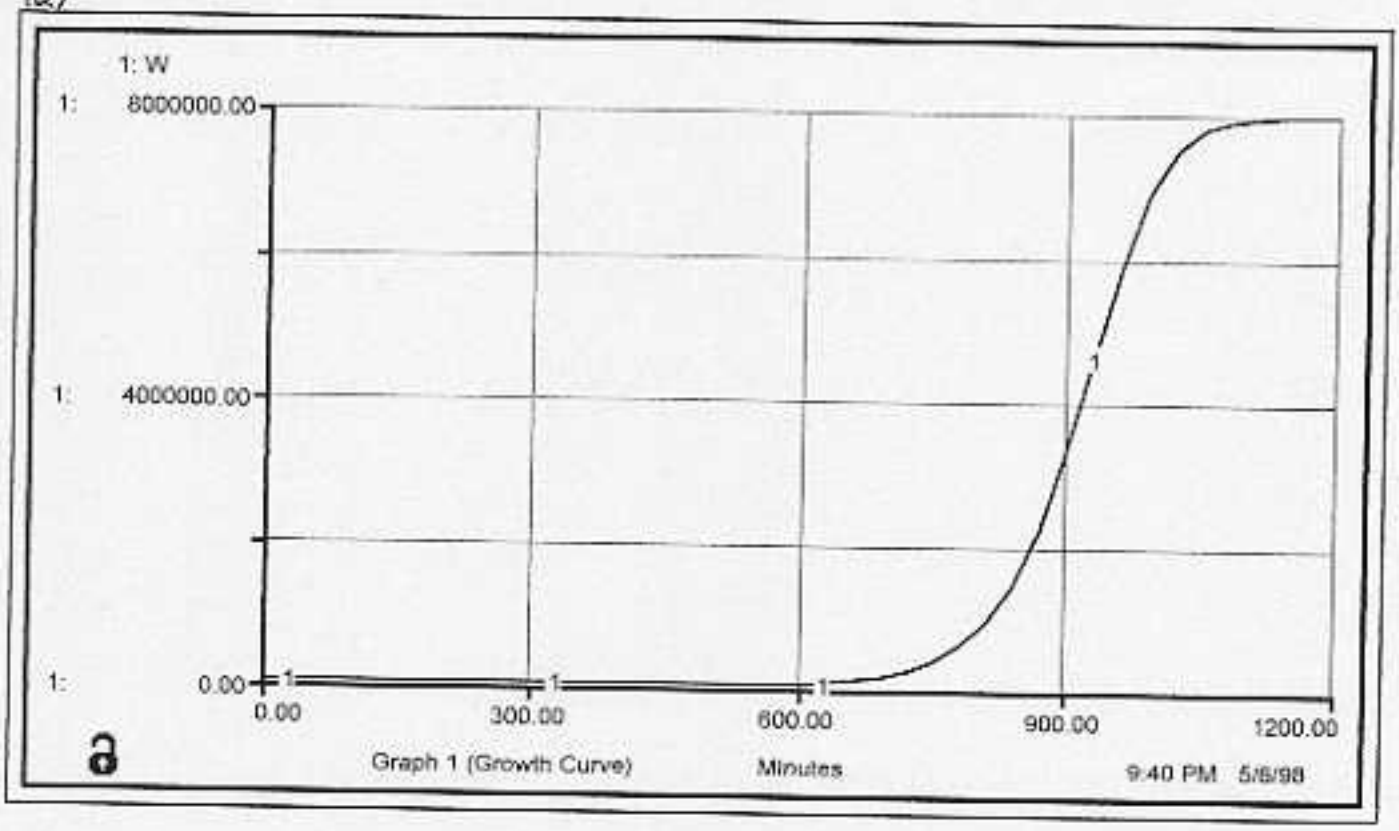


Figure 7. Growth curve of wild-type and mutant bacterial populations [CFU/mL] in presence of inhibitory drug streptomycin. The drug (60 μg/mL) is introduced at time t=300 minutes. Note abatement of wild-type growth (line 1) along with a small jump in mutant population (line 2) at 300 minutes. Also, the mutant population dominates the ecology only when wild-type population reaches zero.

(a)



(b)

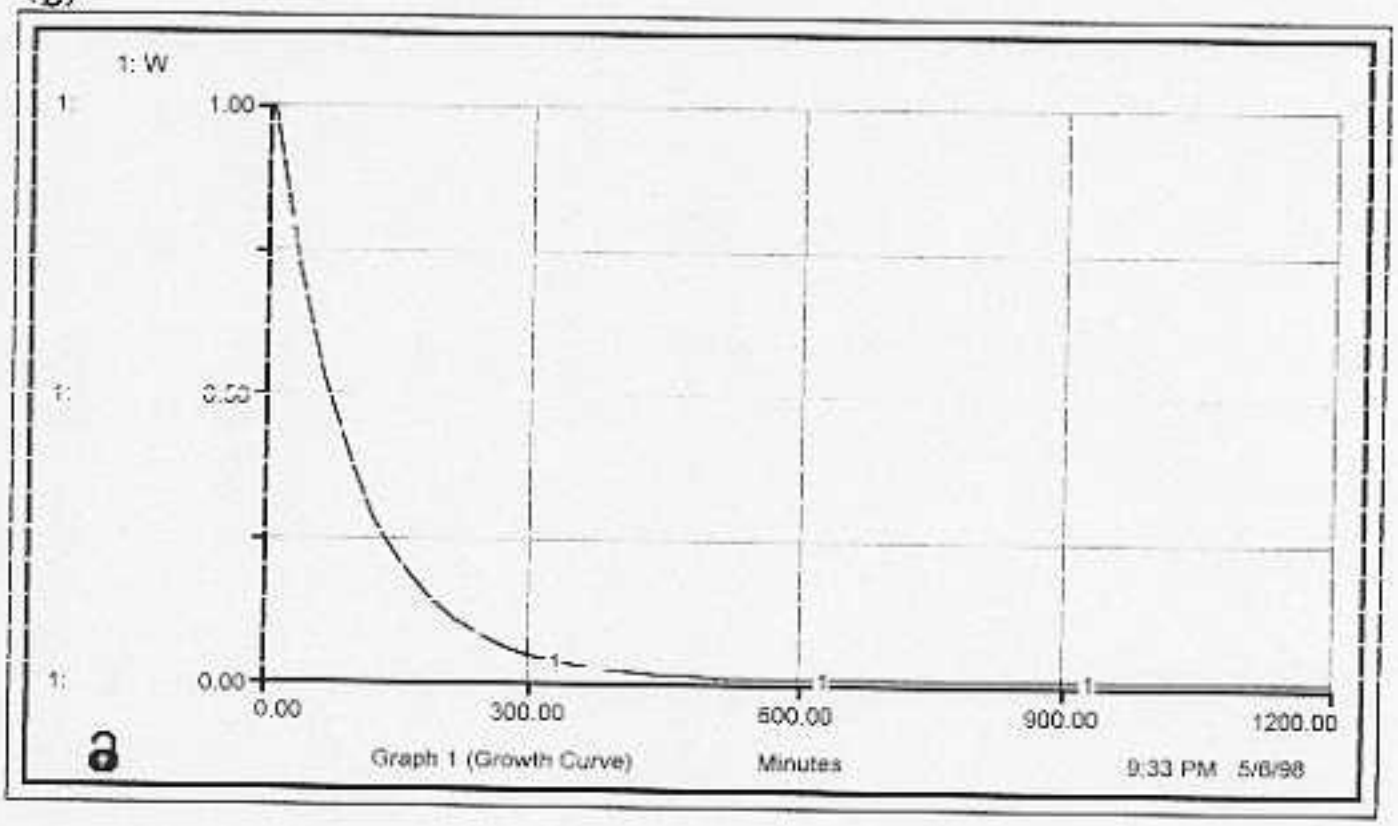


Figure 8. Comparison of wild-type bacterial growth in absence (a) or presence (b) of streptomycin, 2 μg/mL. The graphs suggest there is no threshold of tolerance for streptomycin in E. coli.

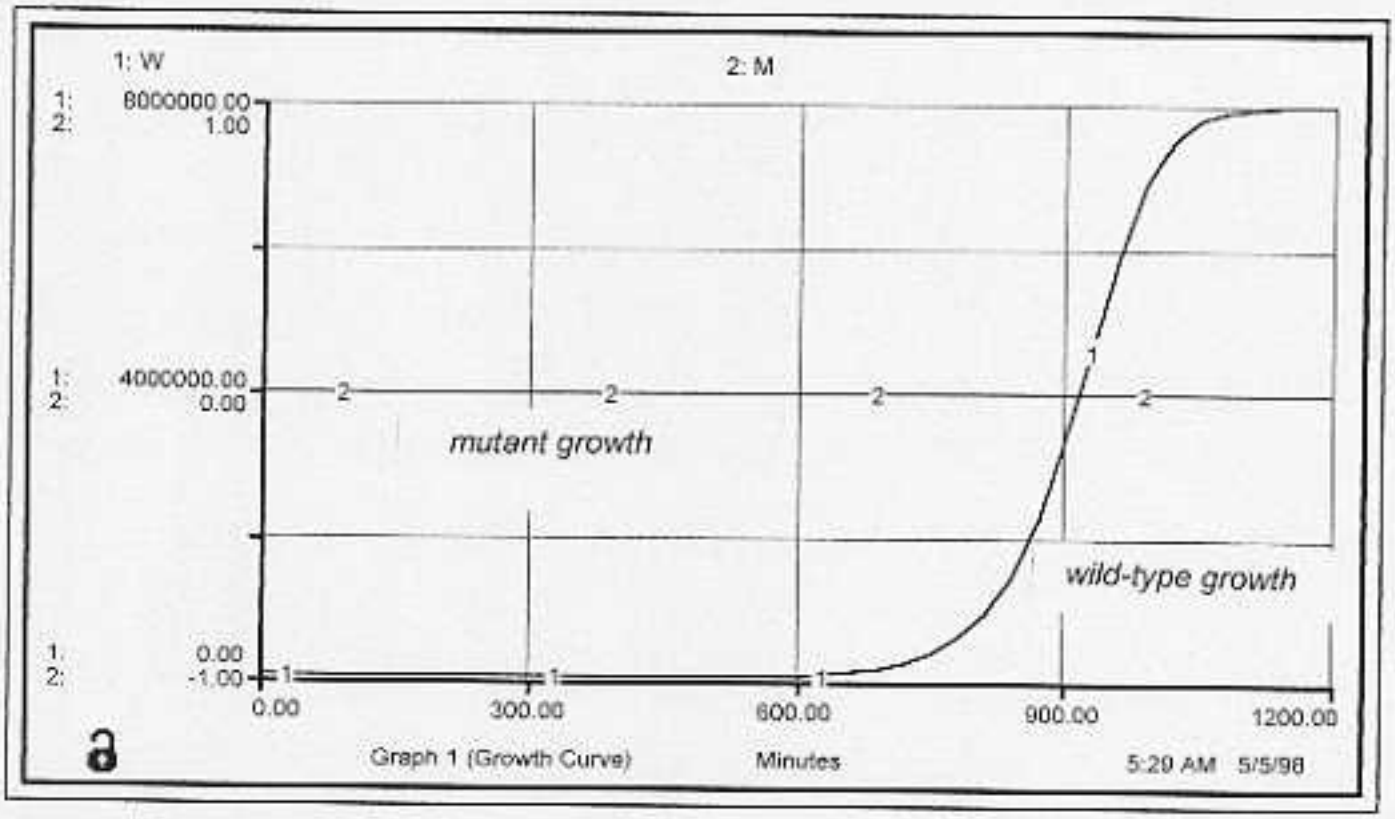


Figure 9. Competition between wild-type and mutant bacteria [CFU/mL]. Note growth of mutant is hindered by the presence of wild-type population. Compare with figure 6. Line 1 is wild-type and line 2 is mutant.

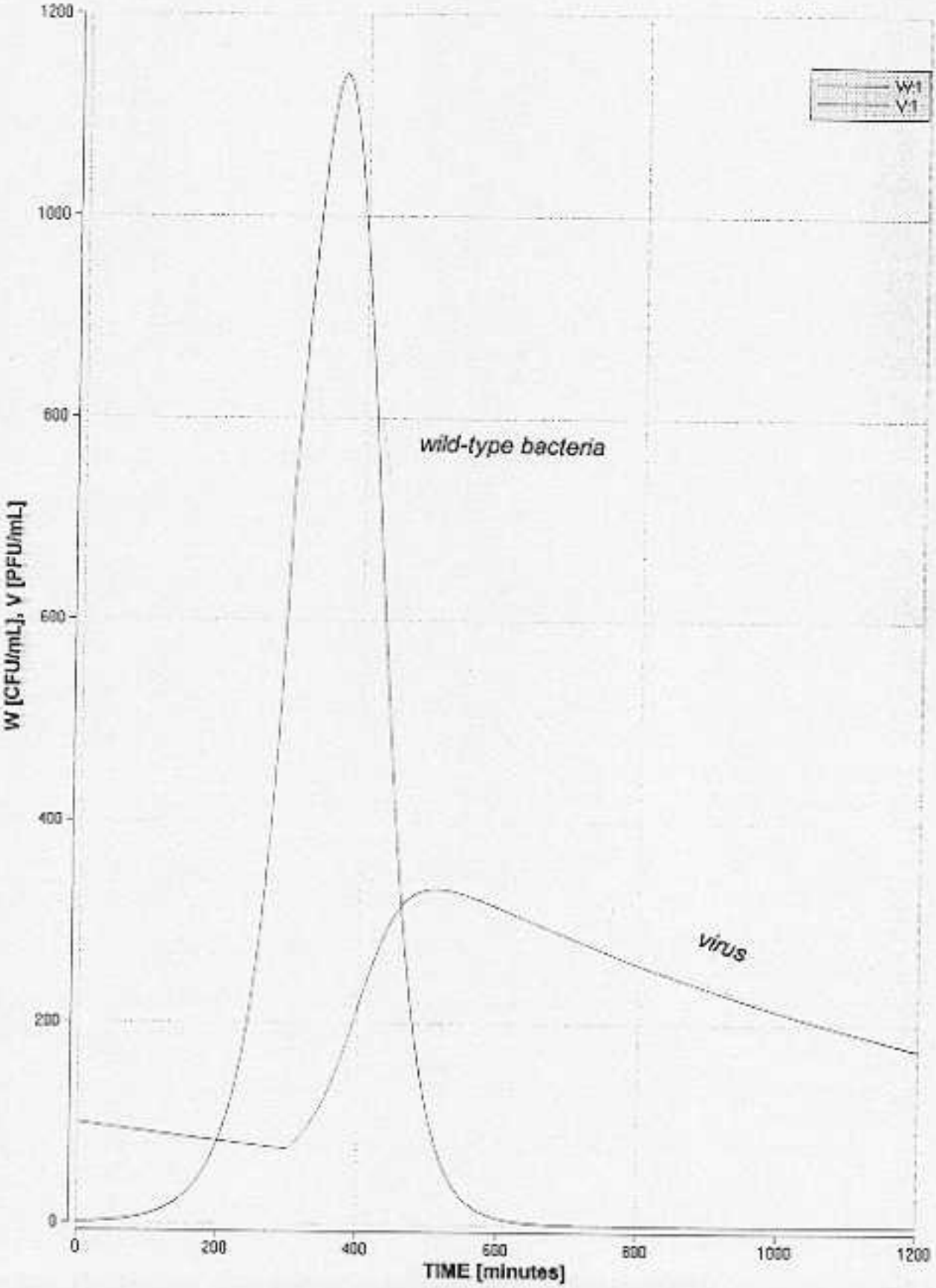


Figure 10. Interaction between phage [PFU/mL] and wild-type bacteria [CFU/mL]. Note introduction of phage at time t = 300 minutes stops bacterial growth as phage population reaches approx. 200 PFU/mL (at 380 minutes). Also, the phage population declines when there is no viable bacteria in medium.

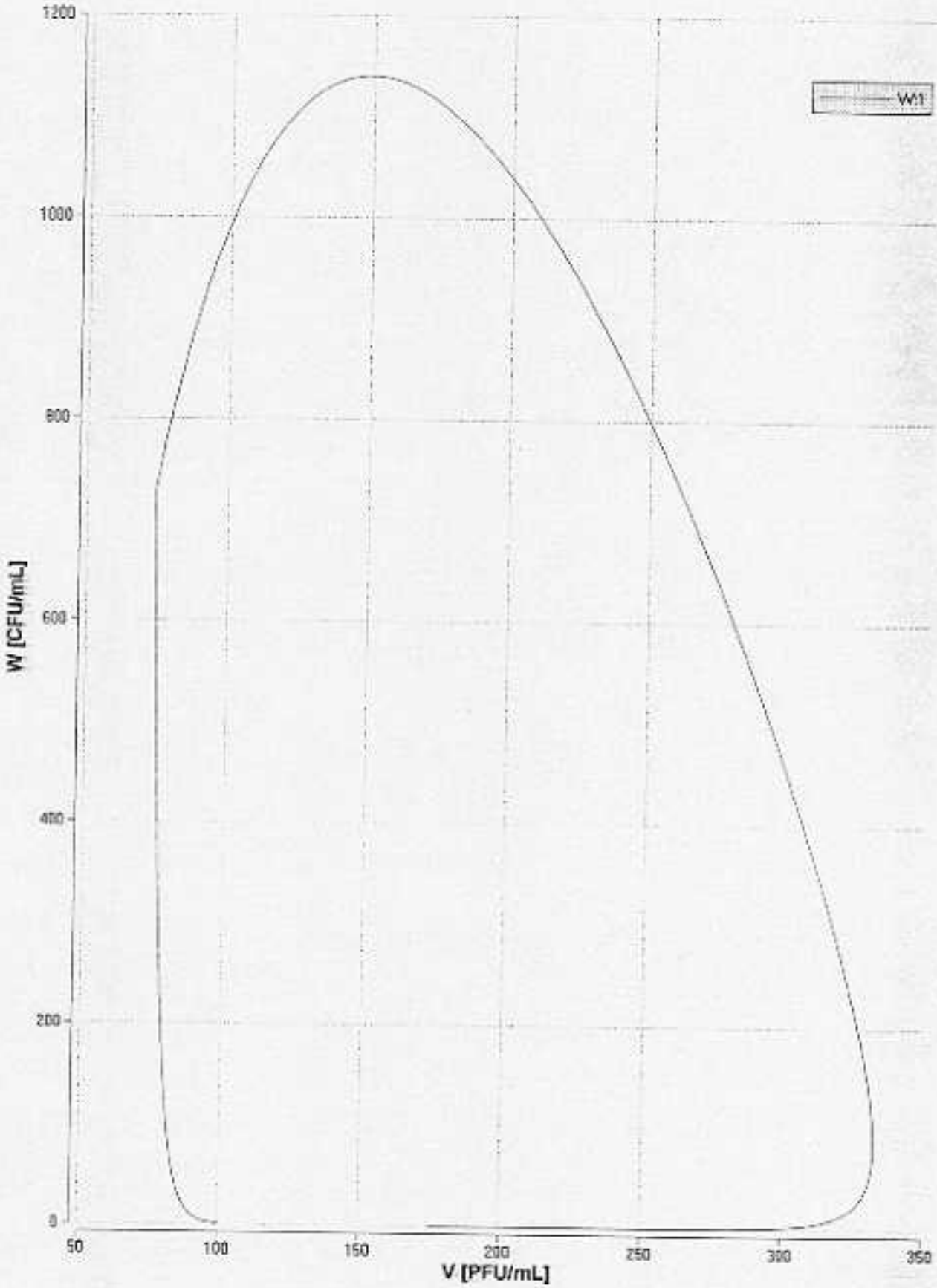
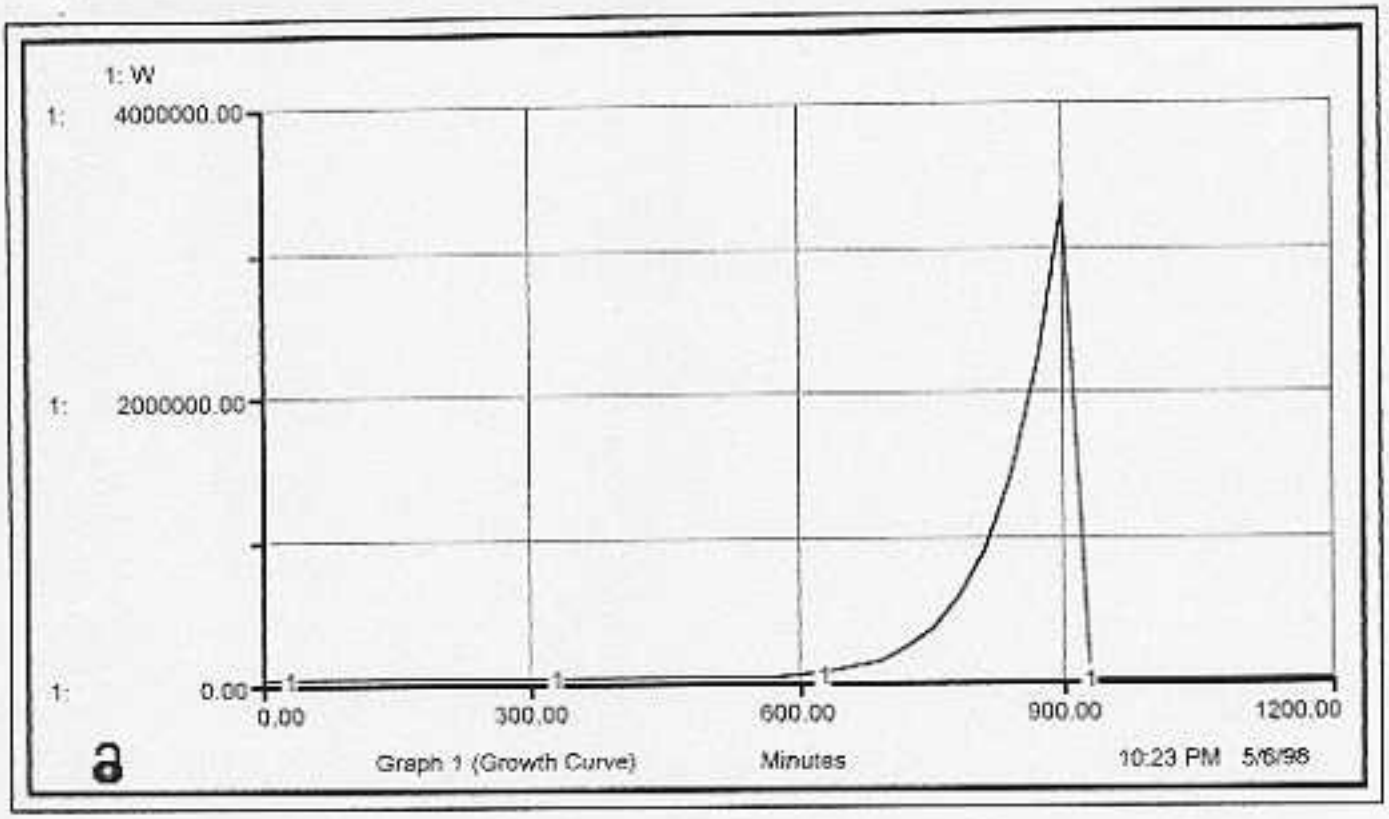


Figure 11. Graph of wild-type bacterial population [CFU/mL] in relation to phage population [PFU/mL]. Note bacterial population increases until it reaches 1140 CFU/mL. At this point, phage growth occurs exponentially and thereby lyse bacteria. Also, notice phage growth declines at 470 PFU/mL, the point when low abundance of bacterial host in conjunction with low adsorption frequency allows phage decay to be greater than reproduction.



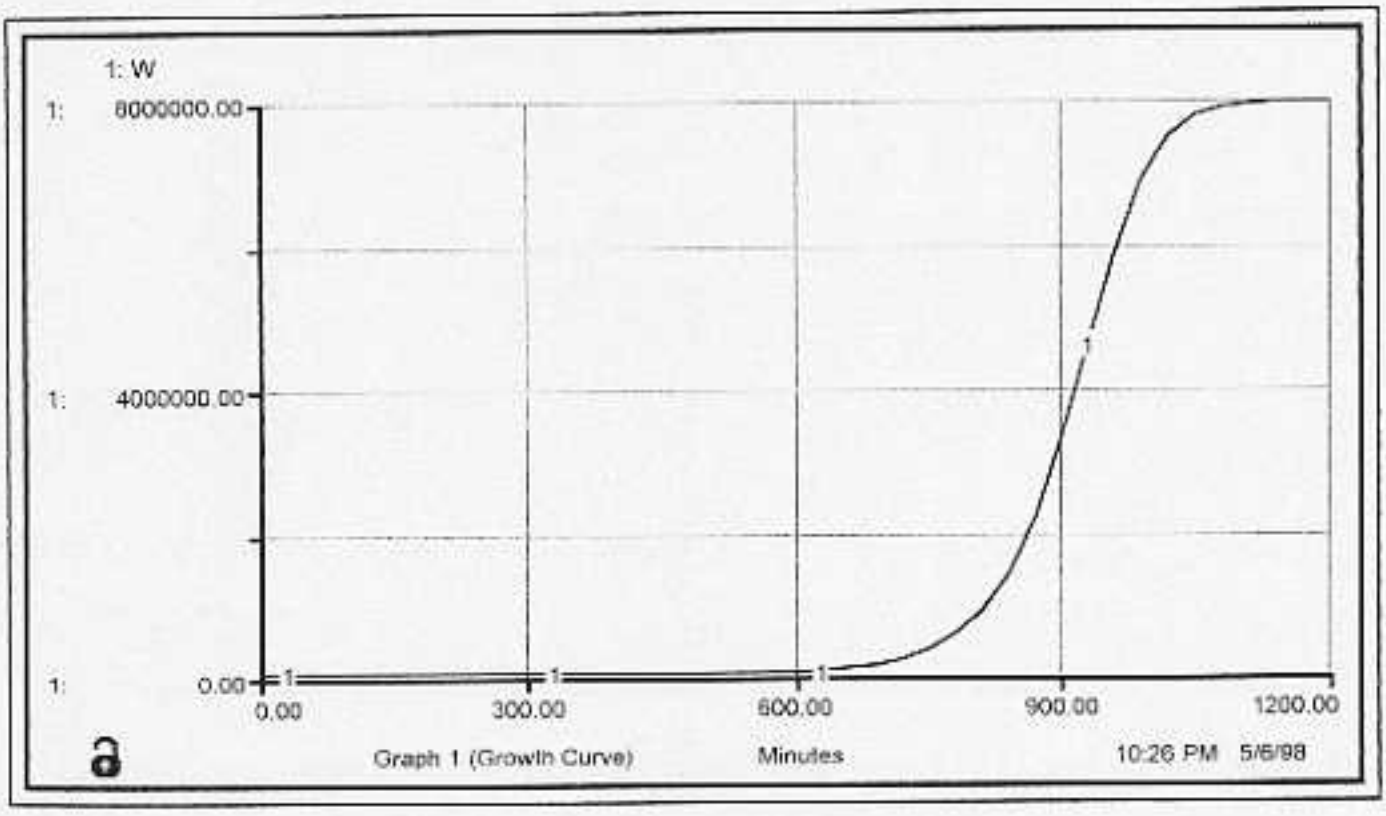


Figure 12. Application of streptomycin at 900 minutes (a) versus 1200 minutes (b). Only 1000 μg/mL is required to stop growth at 900 minutes, whereas 10,000 μg/mL is not enough to stop growth at 1200 minutes.

{ INITIALIZATION EQUATIONS }

u = 9.80E-4
INIT W = 1
Mutation = u*W
INIT M = 0
k_prime = 0.0231
Nutrient = 100
n = 4
k = (k_prime*Nutrient)/(Nutrient+n)
d = 0.0157
Drug = 0
b = 2.78E-9
e = 0.15
f = 1E-3
INIT V = 100
dW_dt = ((k-(d*Drug))*W)-(b*(W^2))-(e*f*V*W)
dM_dt = (k*M)-(b*(M^2))-(dW_dt)
r = 0.0768
x = 9.83E-4
dV_dt = (r*V*W*f*e)-(x*V)

{ RUNTIME EQUATIONS }

W(t) = W(t - dt) + (dW_dt - Mutation) * dt
M(t) = M(t - dt) + (dM_dt + Mutation) * dt
V(t) = V(t - dt) + (dV_dt) * dt
Mutation = u*W
k = (k_prime*Nutrient)/(Nutrient+n)
dW_dt = ((k-(d*Drug))*W)-(b*(W^2))-(e*f*V*W)
dM_dt = (k*M)-(b*(M^2))-(dW_dt)
dV_dt = (r*V*W*f*e)-(x*V)

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