Journal of Theoretical Biology 364 (2015) 428–432

Contents lists available at ScienceDirect

Journal of Theoretical Biology

journal homepage: www.elsevier.com/locate/yjtbi

Maximizing yields of virulent phage: The T4/*Escherichia coli* system as a test case

Ira Aviram [a], Avinoam Rabinovitch [a,*], Arieh Zaritsky [b]

[a] Department of Physics, Ben-Gurion University of the Negev, POB 653, Be'er-Sheva 84105, Israel
[b] Faculty of Natural Sciences, Ben-Gurion University of the Negev, POB 653, Kiryat Bergman, Be'er-Sheva 84105, Israel

HIGHLIGHTS

- Maximum phage titers calculated.
- Calculations carried out by a novel hybrid model.
- T4 phage/*Escherichia coli* host system used as test case.

ARTICLE INFO

Article history:
Received 18 July 2014
Received in revised form
7 September 2014
Accepted 14 September 2014
Available online 26 September 2014

Keywords:
Maximal bacteriophage titer
Doubling time
Multiplicity of infection
Probability
Delayed-differential equations

ABSTRACT

A hybrid mathematical model was devised to obtain optimal values for bacterial doubling time and initial phage/bacteria multiplicity of infection for the purpose of reaching the highest possible phage titers in steady-state exponentially growing cultures. The computational model consists of an initial probabilistic stage, followed by a second one processed by a system of delayed differential equations. The model's approach can be used in any phage/bacteria system for which the relevant parameters have been measured. Results of a specific case, based on the detailed, known information about the interactions between virulent T4 phage and its host bacterium *Escherichia coli*, display a range of possible such values along a highlighted strip of parameter values in the relevant parameter plane. In addition, times to achieve these maxima and gains in phage concentrations are evaluated.

1. Introduction

Bacteriophages were discovered a century ago (Twort, 1915; Duckworth, 1976). The idea that they could be exploited as a means to get rid of damaging bacteria was introduced but was abandoned almost completely soon afterwards due to various reasons (reviewed in Adams, 1959). The appearance and fast spread of drug resistant pathogenic bacteria revived the so-called Phage Therapy arena (Thiel, 2004; Sulakvelidze and Pasternack, 2010; Barr et al., 2013), which has been maintained only in a small number of laboratories in Georgia and Poland during the decades of temporary reduced interest in the subject (Parfitt, 2005; Miedzybrodzki et al., 2012; Gorski et al., 2009). Nevertheless, phage biology was highly instrumental in developing Molecular Biology and Genetic Engineering in the 1940s–1960s (Cairns et al., 1966).

Various methods have been devised to obtain high titers of phage suspensions when large quantities/high concentrations of phage are needed (Su et al., 1998); extensively reviewed and described in Carlson and Miller (2004). To meet this end, a steady-state growing bacterial culture (Fishov et al., 1995) is infected and the crop of phage is harvested after a certain period (e.g., Hadas et al., 1997). The experiments are designed so that the final phage titer is as high as possible. To achieve this goal, the required doubling time of the culture τ, and the phage multiplication parameters have hitherto been estimated and considered usually just 'by eye'.

Levin and coworkers (see e.g., Levin et al., 1977) were the first to analytically examine a system of coexisting bacteria and bacteriophage. The latest reports describe phage multiplication under exceedingly slow host's growth rates (Golec et al., 2014) and stochasticity of adsorption rates (Galet et al., 2012) or lysis time (Dennehy and Wang, 2011) of certain mutants, but to the best of our knowledge none has been concerned with the question posed here: what growth conditions would bring about highest titers in wild-type strains.

Recent deciphering of the relationships between τ and the latent period L (time after infection to the consequent phage burst by cell lysis) and the burst size β (the number of new phage

* Corresponding author. Tel.: +972 8 6461172.
E-mail address: avinoam@bgu.ac.il (A. Rabinovitch).

http://dx.doi.org/10.1016/j.jtbi.2014.09.020
0022-5193/

appearing after lysis of a single bacterium) in the T4/*Escherichia coli* system (Rabinovitch et al., 1999, 2002) allows the derivation of a simple computational model as a reasonable first approximation allowing an experimental control of this yield.

To evaluate the optimal conditions that can achieve this phage maximum, a *hybrid* mathematical model of the phage population growth was developed here, consisting of two time stages. The first is based on probabilistic arguments, in preparation of the second stage involving *delayed* differential equations (DDE), in which the lysing latent period L for bacteria implies delay. The reason for combining these two methods is that the entire process is normally of short duration, never exceeding two or three latent periods, and without achieving a steady type of dynamics. The first L period is treated probabilistically in order to provide a "history" for the DDE system of equations in the second stage, if needed. The detailed description is given below.

2. Method: The hybrid model

As implied in the Introduction, a lytic phage growth procedure is usually realized in a laboratory by bringing bacteria and bacteriophage into interactive contact during a relatively short period of time, while the bacteria need not compete with each other for the provided nutrient. The process is not expected to attain persistent dynamics, its termination being externally controlled by the phage population reaching a maximum.

In order to investigate this process we chose here a *hybrid* mathematical model, constituted of two time periods, describing the process during which phage infect bacteria, and new phage are produced by bacterial delayed lysis occurring at a finite latent period of time L after infection. The model is set up as follows: the occurrences during the delay between the *first* infection and the *first* lysis are treated probabilistically since this "history" is not known ab initio. This is denoted as the 'first phase'. If, however, the process does continue beyond this time, which, as shown subsequently, will be the case when the bacteria doubling time is significantly longer than L, the 'second phase' of the process is treated by a relatively simple system of two *delayed differential equations* (DDE) of the prey/predator type in which the historical values are provided by the probabilistic initiating phase. For simplicity, it is also assumed that L is constant, that all bacteria are equally susceptible to be infected, and all phage are equally able to infect.

The DDEs are presented here first, followed by the description of the probabilistic phase.

2.1. DDE

The model includes two non-linear delay differential equations (DDE), as follows:

$$\frac{dN(t)}{dt} = \mu N(t) - \gamma N(t)V(t)$$
$$\frac{dV(t)}{dt} = -\gamma V(t)(N(t) + \beta\gamma N(t-L)V(t-L) \quad (1)$$

where N and V [ml^{-1}] are the concentrations of free (susceptible) bacteria, and infecting phage, respectively; t is the time [min]; τ[min] $= \ln(2/\mu)$ is the doubling time of the phage free bacteria where μ [min^{-1}] is the exponential growth rate; γ [ml $cell^{-1}min^{-1}$] is the adsorption rate of phage onto susceptible bacteria; L [min] is the latent period of time between infection and lysing; β is the number of phage (burst size) released into the system at time t by the lysis of a bacterium infected at time $t-L$. It was shown (Rabinovitch et al., 2002) that in a T4/*E. coli* system, the parameters L, β and γ, depend on the doubling time τ (the respective empirical relations are given in App. 1).

The first equation describes the rate of change of the bacterial concentration N: it increases by division and decreases by phage infection, which is proportional to the product of the phage and bacterial concentrations. The second equation refers to the rate of change of the phage population V: it decreases by the immediate infection but is more than replenished by β times the number of infected bacteria L minutes earlier. For simplicity, the model does not address infected bacteria explicitly: by analyzing their inclusion into the model, it was found out not to make much difference. Hence, we opted for the simple differential model, with only two state variables: susceptible bacteria and phage. The presence of infected bacteria is accounted for only by the lysis process.

Here, phage growth is explored for a test case of a system of T4/*E. coli* in terms of two externally controllable parameters: the doubling time τ of the bacterial culture in the range 20 – 100 [min], realized by varying the nutrients, and the initial (subscript 0) multiplicity of infection (MOI) denoted $m_0 = V_0/N_0$ in the range $10^{-7} - 1$ (phage $cell^{-1}$). The main purpose of the work is to find the maximal yield of the phage, and the conditions to achieve this maximum. In addition, results provide the time T_f when this maximum occurs and the virus gain G, the ratio V_{max}/V_0 between the final and initial virus concentrations.

The system in Eq. (1) is infinite dimensional in the sense that a continuum of values of the variables N and V in the (delay) time interval $[t–L, t]$ preceding t are required in order to specify their condition at t. For the numerical solution of the 2D system Eq. (1) the continuum is approximated by embedding into this range an ordered finite number of values of the variables, effectively transforming the differential system into a multi-dimensional iterated map: divide the time range L into a sufficiently large number j of small intervals Δt such that $L = j\Delta t$ (see e.g. Sprott, 2003). A standard Runge–Kutta 2 (RK2) (Sprott, 2003) algorithm written for MATLAB, was used here for the numerical solution of the $2+j$ variables of the ensuing map.

The initial (at $t=0$) bacteria concentration here is fixed at $N_0 = 10^9$ [cell ml^{-1}], while V_0 will be determined by one of the initial MOIs in consideration: $m_0 = 10^k$, where $k = -7, -6, -5, \ldots, 0$. Now, in order to complete the information needed for running the time series RK2 algorithm, one has to set up the historical values of N and V at the j time steps after $t=0$ prior to the first lysis at $t = L$. This is an important step in the context, for the entire time evolution of the system is expected to be short lived, as explained above, the interesting dynamics never attaining here a long term, persistent, finite, non-trivial attractor which has managed to "forget" the transient initial history. However, these vital initial quantities cannot be calculated by Eq. (1). We therefore use here a well-defined probabilistic approach for a well posed, self-contained formulation of this period as described below.

Note that the probabilistic formulation can also be employed as a first approximation to the whole process instead of Eq. (1), but is applied here only between $t=0$ and $t=L$.

2.2. History and probabilities

The entire time axis is divided into a sequence of time intervals of finite length L ("boxes"), indexed by the letter n. Each box is viewed as a whole, indivisible object (time discretization). A probability distribution function is addressed which defines the probability $p_{s,n}$ that precisely s phage infect a single bacterium in the time box n. The first time box, and the only one important in the hybrid model, $n=0$, contains at the onset a concentration of N_0 bacteria and V_0 phage, with $m_0 = V_0/N_0$. The concentration of *uninfected* bacteria in this box is thus $N_0 p_{0,0}$, which then multiplies exponentially in the box reaching the level N_1 at $t=L$:

$$N_1 = N_0 p_{0,0} a, \quad (2)$$

where $a=\exp(L\ln 2/\tau)$ is the exponential bacteria growth factor. The number of bacteria infected by one or more phage is, obviously, $N_0(1-p_{0,0})$. Thus, the phage concentration in this box (at time L) is given by

$$V_1 = N_0(1-p_{0,0})(\beta-\bar{s}) \tag{3}$$

where $\bar{s}$ is the mean number of the infecting phage per bacterium in the box. The probabilistic approximate process may then be advanced along the box chain by iterating these steps in box $n=1$, with N_1, V_1 and m_1, and so on:

$$N_{n+1} = N_n p_{0,n} a$$
$$V_{n+1} = N_n(1-p_{0,n})(\beta-\bar{s})$$
$$m_n = \frac{V_n}{N_n} \tag{4}$$

As mentioned previously, this procedure presents a direct first approximation for the complete process, namely for the final numbers of N, and V. However, for the more accurate hybrid model treated here, only the first box $n=0$ needs to be addressed for setting up the initial history before $t=L$. In view of the essentially exponential nature of the variation of N and V, it is most convenient to set the $j-1$ embedded values by *interpolating linearly* between log N_0 and log N_1, as well as between log V_0 and log V_1.

It is assumed that the bacteria are indistinguishable among themselves, the same being true for the phage. Then if $V, N\to\infty$ so that their average ratio tends to $m=V/N=const.$, the probability that any given bacterium was infected by precisely s phage is the geometric (Feller, 1968; Pitman, 1993) distribution:

$$p_s = \frac{m^s}{(1+m)^{s+1}} \tag{5}$$

yielding $p_0=1/(1+m)$.

Note that if, in addition, m is sufficiently small, p_s may be approximated by the Poisson distribution:

$$p_s = \frac{m^s}{s!}e^{-m} \tag{6}$$

where $p_0=\exp(-m)$. The mean is $\bar{s}=m$ for both distributions. Note also that the value of m changes from box to box.

3. Results

Fig. 1 shows a typical time series for $N(t)$, $V(t)$, $m(t)$ produced by the hybrid model combining the probabilistic history with the DDE Eq. (1), with $\tau=30$, and the initial state values $N_0=10^9$, $V_0=3.2\times10^5$, $m_0=3.2\times10^{-4}$. According to Rabinovitch et al. (1999, 2002), the burst size and lysis period are: $\beta=128$, $L=28$ [min] and $\gamma=2\times10^{-8}$ [ml cell^{-1} min^{-1}]. The V_{max} output in this case is $V_{max}=1.3\times10^{12}$ [cell ml^{-1}], attained at time $T_f=60$ [min]. The final gain here is: $G=V_{max}/V_0\approx4\times10^6$. Some features are noticeable: After the probabilistic history has been built up till $t=L$, the phage concentration increases at the expense of bacteria. When $m(t)$ becomes of the order of 1–10, the susceptible bacteria concentration "formally" drops sharply by ~15 orders of magnitude, while, for another time interval of about L minutes, phage production continues, originating from the bacteria infected during their last latent period. The system now contains only infected bacteria which are not addressed explicitly in this model, rendering the "lysing from without" practically irrelevant. According to our relatively simplistic model, susceptible free bacteria have formally disappeared at this point of time. Bearing in mind that infected bacteria are not directly accounted for in the model equations, and that the model does not provide a mechanism of self-destruction of phage, the derivative $\dot{V}(t)$ in Eq. (1) also "formally" vanishes after attaining T_f (Fig. 1). It should be understood that the sharp drop of N accompanied by the surge of m, by some 15 orders of magnitude respectively, are both computational artifacts, lacking any physical significance.

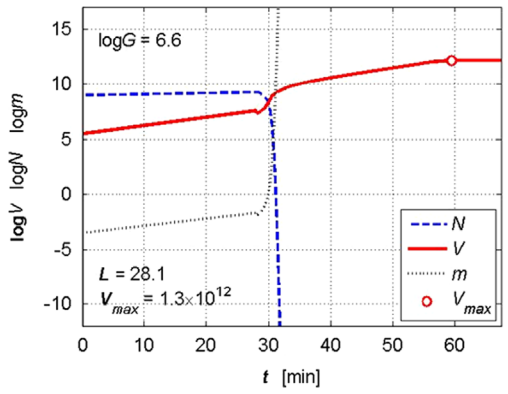

Fig. 1. A typical time series, produced with $\tau=30$ min., and $m_0=3.2\times10^{-4}$.

Table 1 contains the relevant results of this study, including, in each table case, the values of the following four quantities, in this order:

Maximum phage yield: V_{max} in units of 10^{11} ml^{-1},
Growth duration to V_{max}: T_f [min],
Lysis time delay: L [min],
Decimal logarithm of the gain: log$G$=log(V_{max}/V_0).

Data contained in grey shaded cases were obtained with only the probabilistic formulation, Eqs. (2)–(4). On the other hand, cases with white background, including the heavy lined contour and gold shading were computed with the complete hybrid model. The reason for this is that the large initial m_0 in the grey shaded cases causes $V(t)$ to catch up rapidly with $N(t)$, increasing rapidly past the value of 1 before the completion of the first lysing, and making the use of the DDE's unnecessary in these cases.

The most relevant feature of this study is reflected in the six cases of the table, highlighted by the heavy lined contour. They contain several highest maxima of V_{max}, as Fig. 2a clearly shows. It represents in 3 dimensions the surface V_{max} as a function of τ and

Table 1
Results for the T4/*E. coli* system (Explanations in text).

$m_0=$	10^{-7}	10^{-6}	10^{-5}	10^{-4}	$3.2\cdot10^{-4}$	10^{-3}	10^{-2}	10^{-1}	1
$\tau=20$	V_{max}= 16.0	12.0	13.0	40.0	110.0	320.0	10.0	6.1	2.1
	T_f= 63	55	48	43	41	40	20	40	40
	L= 20	20	20	20	20	20	20	20	20
	log$G$=10.2	9.1	8.1	7.6	7.5	5.6	4.8	3.9	2.3
30	6.0	4.2	3.5	5.2	13.0	35.0	*330.0*	1.8	0.7
	102	87	73	60	60	58	*58*	56	28
	28	28	28	28	28	28	*28*	28	28
	9.8	8.6	7.5	6.7	6.6	6.5	*6.5*	3.3	1.8
40	3.7	2.5	1.9	2.5	4.6	15.0	100.0	9.1	0.4
	104	119	98	82	76	73	70	69	35
	35	35	35	35	35	35	35	35	35
	9.6	8.4	7.3	6.4	6.2	6.1	6.0	2.9	1.5
50	2.6	1.8	1.3	1.5	2.4	5.4	45.0	390.0	0.3
	173	146	119	98	90	85	81	80	40
	40	40	40	40	40	40	40	40	40
	9.4	8.3	7.1	6.2	5.9	5.7	5.7	5.6	1.4
60	2.0	1.4	1.1	1.0	1.4	3.0	23.0	200.0	0.2
	201	169	139	113	102	96	90	88	44
	44	44	44	44	44	44	44	44	44
	9.3	8.2	7.0	6.0	5.7	5.5	5.4	5.3	1.3
80	1.3	0.9	0.7	5.8	0.7	1.1	6.8	57.0	240.0
	242	207	171	136	122	111	99	96	96
	48	48	48	48	48	48	48	48	48
	9.1	8.0	6.8	5.8	5.3	5.0	4.8	4.8	4.4
100	0.7	0.6	0.4	0.4	0.4	2.0	1.0	15.0	62.0
	266	228	190	153	130	100	56	95	85
	46	46	46	46	46	46	46	46	46
	8.9	7.8	6.6	5.5	5.1	4.3	2.7	4.2	3.8

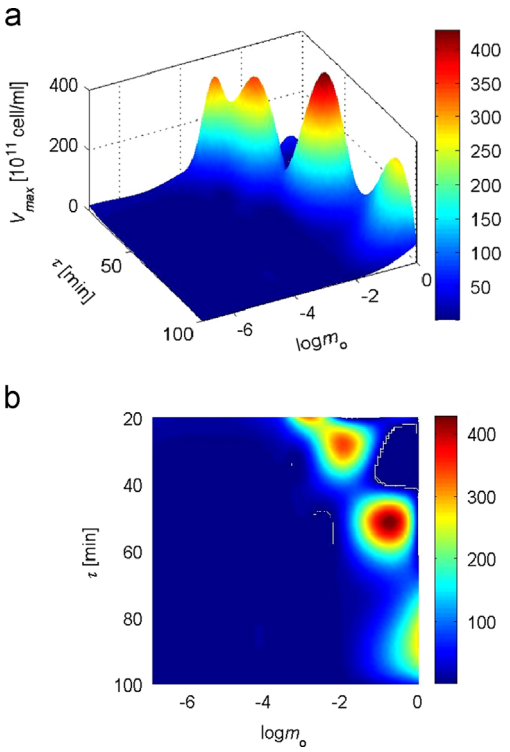

Fig. 2. (a) V_{max} surface. (Color bar for V_{max}) (b) projection of the V_{max} surface on the (logm_0, τ) plane. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

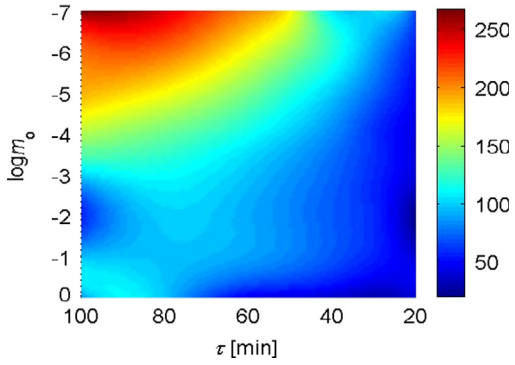

Fig. 3. Projection of the surface $T_f(\tau, \log m_0)$ [min]. (Color bar for T_f.). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

logm_0, showing a chain of "mountain peaks". Beyond this ridge lie the grey cases.

The range of highest V_{max} for $N_0=10^9$ is between 3 and 4×10^{13} occurring for τ and m_0 pairs of: $(20,\ 10^{-3})$; $(30,\ 10^{-2})$ and $(50,\ 10^{-1})$, while different values of τ or m_0 lead to lower maxima. The precisely computed highest peak, $V_{max}=4.25\times10^{13}$, is located at $m_0=0.8$, and $\tau=48$, see also Fig. 2b.

Fig. 3 represents a projection of the final time to maximum on the $(\tau,\ \log m_0)$ plane, and Fig. 4 shows the gain, log G, as a function of τ and log m_0 indicating that for sufficiently low values of m_0 the gain is almost independent of the doubling time τ.

4. Discussion

A hybrid mathematical model was used to obtain optimal combinations of bacterial doubling time τ and multiplicity of phage infection m_0 to achieve highest yields of viruses. Results

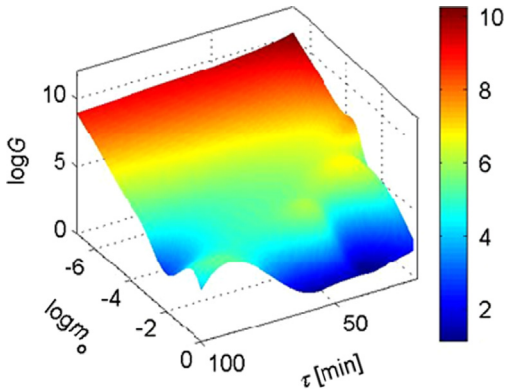

Fig. 4. The surface log $G(\tau,\ \log\ m_0)$. (Color bar of logG). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

show rather sharp peaks around three pairs of these values [(20, 10^{-3}); (30, 10^{-2}) and (50, 10^{-1})]. The pair (48, 0.8) that yields the maximum phage concentration, $V_{max}=4.25\times10^{13}$, is surprising because faster growth conditions are generally exploited for this purpose and it accentuates the importance of the present calculations.

Under faster bacterial growth conditions (shorter τ's), cells are larger than at poorer media (e.g., Zaritsky et al., 1979) and reach the stationary phase at cell concentrations significantly lower than the 10^9 ml^{-1} taken here as N_0 for all media. And since slower growing cells, such as during the stationary phase, produce less phage progeny (according to the equations displayed in the Appendix, derived in (Rabinovitch et al., 2002), the real V_{max} will be smaller than the values derived here which assume constant, optimal conditions all through growth up to the high concentration of 10^9 ml^{-1}. Refining our model would thus be possible only when the exact changes in instantaneous τ during the growth curve are known. On the other hand, even when this knowledge is gained, cell lysis L minutes after infection complicates matters further because it results in pouring into the growth medium of the remaining live cells breakdown materials thus enhancing their growth rate.

The phenomenon of lysis inhibition (LIN), which can significantly raise phage yield (Bode, 1967), was also not considered here due to lack of solid, parametric relationships: lysis of phage-infected bacterial generally exploited for this purpose. Doubling time of about 48 min are achieved in *E. coli* at defined salt solution medium with glucose as the sole carbon source, which is more tedious to prepare than rich, undefined media such as LB. No previous articles are known to us that have addressed this problem in other phage/host systems. To apply a similar computational model, the values of these parameters as functions of τ should first be measured. Experiments are evidently required to test these results and to enhance them to treat different phage-bacteria couples. It is assumed here that, once τ is determined, all these parameters remain constant. Otherwise, all the parameters' experimental values need to be separately obtained.

As mentioned in Section 2, the model does not address infected bacteria explicitly. Also of lesser importance was estimated to be the effect of immunity towards infection of a small set of the bacterial population. All these, and other secondary effects may be taken into consideration in a more elaborate study.

Several issues related to the results obtained here are in order:

The absolute values of the results in real life would obviously depend on various parameters that were not considered here. For example, under faster bacterial growth conditions (shorter τ's) LIN will likely operate due to the spread of L in the infected population provided the MOI is larger than 1 but smaller than the value causing "Virion-Mediated" lysis-from-without (Abedon, 2011).

Long term effects were not addressed here since the whole process discussed is short and these effects are unlikely to appear during this period.

The analysis performed here does not consider temperate bacteriophage, which can lysogenize their bacterial hosts. The question of how are these anticipated to behave, and what method should be used in order to maximize their yield upon induction, remains moot. Still, defining the time during culture growth at which artificial induction is initiated to reach high titers, will no doubt benefit from the outcome of our analysis. In one sense, imposing induction by a physical or chemical agent is even better regulated because it affects the whole population simultaneously, whereas infection (particularly at low MOI) is much less synchronized (and see stochasticity in lysis time by Dennehy and Wang (2011), Galet et al. (2012).

Other methods to maximize phage yield that have been attempted are cumbersome and may not be reproducible. For example, the use of soft agar layer on agar plate (Swanstrom and Adams, 1951), which results in up to 10^{12} ml^{-1} T4r phage, can be exploited with our rigorous analysis for raising the yield further. Similarly, the final yields may be concentrated by sedimentation in the presence of polyethylene glycol (Yamamoto and Alberts, 1970). The rigorous analysis described here can be exploited to simplify these other methods.

5. Conclusions

Maximum titers can appear at several parameter values which are not obvious under a first glance. Usually labs carrying out such experiments apply a trial and error approach to find the best values. A simple calculation along the lines of the present model can reduce the searching time. Thus, if the bacteria doubling time, the adsorption rate, the latent period and the burst size of a system are known, a procedure based on Eqs. (1)–(4) can be carried out and the values of the highest titers calculated. Of course, as in the case of *E. coly*/T4 system, if some other parameter values depend on the doubling time, final evaluation would become easier, being dependent only on two parameters, τ and the MOI. Note that a first approximation to the maximum titer can be derived from the probabilistic approach (Eqs. (2)–(4)) continued for the additional time boxes, as discussed following Eq. (4).

Appendix

The formulae for L, β and γ as functions of τ used in the present work, were established (Rabinovitch et al., 1999, 2002) for the bacteriophage T4 development in *E. coli*, as follows:

$$L \cong 1.14\tau - 0.0068\tau^2 \text{ [min]; (used here in the range } 20-46)$$

$$\beta \cong (0.25\tau - 0.0017\tau^2)\exp(92.1/\tau); \text{ (range } 430-20)$$

$$\gamma \cong 5.26\times 10^{-8}\exp(-0.0315\tau) \left[\text{ml cell}^{-1}\text{ min}^{-1}\right];$$

(range $2.8\times 10^{-8} - 2.3\times 10^{-7}$).

The ranges correspond to the values of τ in increasing order.

References

Abedon, S.T., 2011. Lysis from without. Bacteriophage 1, 46–49.

Adams, M.H., 1959. Bacteriophages. Interscience Publishers, Ltd, London p. 592.

Barr, J.J., Auro, R., Furlan, M., Whiteson, K.L., Erb, M.L., Pogliano, J., Stotland, A., Wolkowicz, R., Cutting, A.S., Doran, K.S., Salamonm, P., Youle, M., Rohwer, F., 2013. Bacteriophage adhering to mucus provide a non–host-derived immunity. Proc. Natl. Acad. Sci. U.S.A. 110, 10771–10776.

Bode, W., 1967. Lysis inhibition in *Escherichia coli* infected with bacteriophage T4. J. Virol. 1, 948–955.

Cairns, J., Stent, G.S., Watson, J.D., 1966. Phage and the Origins of Molecular Biology. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Carlson, K., Miller, E.S., 2004. Working with T4. In: Karam, J.D. (Ed.), . Molecular Biology of Bacteriophage, T4; 2004.

Dennehy, J.L., Wang, I.-L., 2011. Factors influencing lysis time stochasticity in bacteriophage λ. BMC Microbiol 11174.

Duckworth, D.H., 1976. Who discovered bacteriophage? Bacteriol. Rev 40, 793–802.

Feller, W., 1968. An Introduction to Probability Theory and its Applications. vol. 1. Wiley, New York.

Fishov, I., Zaritsky, A., Grover, N.B., 1995. On microbial states of growth. Molec. Microbiol 15, 789–794.

Gallet, R., Lenormand, T., Wang, I.-N., 2012. Phenotypic stochasticity protects lytic bacteriophage populations from extinction during the bacterial stationary phase. Evolution 66, 3485–3494.

Golec, P., Karczewska-Golec, J., Tos, M., Wegrzyn, G., 2014. Bacteriophage T4 can produce progeny virions in extremely slowly growing *Escherichia coli* host: comparison of a mathematical model with the experimental data. FEMS Microbiol. Lett. 351, 156–161.

Gorski, A., Miedzybrodzki, R., Borisowski, J., Weber-Dabrowska, B., Lobocka, M., Fortuna, W., Letkiewicz, S., Zimecki, M., Filby, G., 2009. Bacteriophage therapy for the treatments of infections. Curr. Opin. Invest. Drugs 10, 766–774.

Hadas, H., Einav, M., Fishov, I., Zaritsky, A., 1997. Bacteriophage T4 development depends on the physiology of its host *Escherichia coli*. Microbiology 143, 179–185.

Levin, B.R., Stewart, F.M., Chao, L., 1977. Resource-limited growth, competition and predation—a model and experimental studies with bacteria and bacteriophage. Am. Nat. 111, 3–24.

Miedzybrodzki, R., Borisowski, J., Weber-Dabrowska, B., Fortuna, W., Letkiewicz, S., Rogoz, P., Ktak, M., Wojtasik, E., Gorski, A., 2012. Clinical aspects of phage therapy. Adv. Virus Res. 83, 73–121.

Pitman, J., 1993. Probability. Springer-Verlag, N.Y.

Parfitt, T., 2005. Georgia: an unlikely stronghold for bacteriophage therapy. Lancet 365, 2166–2167.

Rabinovitch, A., Hadas, H., Einav, M., Melamed, Z., Zaritsky, A., 1999. A model for bacteriophage T4 development in *Escherichia coli*. J. Bacteriol 181, 1677–1683.

Rabinovitch, A., Fishov, I., Hadas, H., Einav, M., Zaritsky, A., 2002. Bacteriophage T4 development in *Escherichia coli* is growth rate-dependent. J. Theor. Biol 216, 1–4.

Sprott, J.C., 2003. Chaos and Time Series Analysis; sec. 15.6. Oxford University Press, Oxford, UK.

Su, M.-T., Venkatesh, T.V., Bodmer, R., 1998. Large- and Small-scale preparation of bacteriophage lambda lysate and DNA. BioTechniques 25, 44–46.

Sulakvelidze, A., Pasternack, G., 2010. Industrial and regulatory issues in bacteriophage applications in food production and processing. In: Sabour, P.M., Griffiths, M.W. (Eds.), Bacteriophages in the Control of Food- and Waterborne Pathogens. ASM Press, Washington, DC.

Swanstrom, M., Adams, M.H., 1951. Agar layer method for production of high titer phage stocks. Exp. Biol. Med. 78, 372–375.

Thiel, K., 2004. Old dogma, new tricks—21st century phage therapy. Nat. Biotechnol 22, 31–36.

Twort, F.W., 1915. An investigation on the nature of ultramicroscopic viruses. Lancet 2, 1241–1243.

Yamamoto, K.R., Alberts, B.M., 1970. Rapid bacteriophage sedimentation in the presence of polyethylene glycol and its application to large-scale virus purification. Virology 40, 734–744.

Zaritsky, A., Woldringh, C.L., Mirelman, D., 1979. Constant peptidoglycan density in the sacculus of *Escherichia coli* B/r growing at different rates. FEBS Lett. 98, 29–32.