Bacteriophage T4 Thermal Inactivation Kinetics

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***Abstract*—Antimicrobial resistance has rekindled interest in bacteriophages, viruses that specifically infect and lyse bacteria. Understanding phage viability under various conditions is vital for assessing their potential applications across industries and for developing effective phage control measures. This study investigates the thermal inactivation kinetics of the T4 bacteriophage at different temperatures. Samples of T4 phage were exposed to 55°C, 60°C, and 70°C, with constant agitation at 200 RPM. The inactivation process followed a first-order kinetic model, which was used to determine the rate constants (k) and activation energy (E_a). The rate constants were calculated as −0.004485 ± 0.001466, −0.01323 ± 0.001546, and −0.05356 ± 0.01161 min^{-1} at 55°C, 60°C, and 70°C, respectively. The activation energy (E_a) was found to be 151.92 kJ/mol, confirming the thermal stability of T4 within this temperature range. High positive free energy of inactivation (ΔG) and negative entropy (ΔS) values further indicated the structural and ordered thermo-stability of T4. The study highlights significant thermal inactivation at elevated temperatures, with rapid inactivation occurring at 70°C. These findings not only underscore the importance of understanding thermal inactivation kinetics for bacteriophage stability but also provide valuable insights for optimizing their use in industrial applications and informing effective phage control strategies across various environments.**

***Keywords*—Bacteriophage T4, thermal inactivation, kinetic model, inactivation thermodynamics**

I. Introduction

Antimicrobial Resistance (AMR) has been declared by the World Health Organization as a serious threat to global public health [1]. This growing crisis has renewed interest in bacteriophages—viruses capable of specifically infecting and lysing bacteria—as a promising alternative for bacterial control. Their precise targeting ability opens avenues for diverse applications; however, scaling up phage production to meet commercial demands poses significant challenges. Bioprocessing technologies offer solutions for large-scale production, but exposure to elevated temperatures can denature proteins and inactivate bacteriophages, undermining their viability [2].

This study investigates the thermal inactivation kinetics of the T4 bacteriophage under varying temperature conditions. It evaluates widely accepted kinetic models and determines key parameters, including the rate constant of inactivation (k), D-values, temperature coefficient (Q), and activation energy (E_a). Additionally, thermodynamic parameters such as entropy change (ΔS) and Gibbs free energy change (ΔG) are calculated to provide deeper insights into the thermal stability of T4 phage.

II. Literature Review

Bacteriophages are viruses that are made of proteins which is susceptible to denaturation by UV-irradiation [3], mixing [4], phytochemicals [5], various chemical disinfectants [6], and elevated temperature [2]. The thermal inactivation of bacteriophages was initially postulated to follow a fist-order decay, but it was found to be more complex. Thermal inactivation of bacteriophage T4 and λ at 65°C initially showed a very rapid decline, followed by a relatively slower logarithmic behavior [7]. They suggest 2 mechanisms of thermal inactivation: the degradation of genetic material and another due to DNA packing within the capsid. The empirical model for thermal inactivation of bacteriophage PDR1 is a first-order decay model with a time constant that involves two parts, a constant and a temperature-dependent term [8]. A study on the effect of microwaves and thermal heating in a microwave oven showed higher inactivation due to thermal heating. They suggest that capsid rupture is the likely mechanism of inactivation [9]. This underscores the multifaceted nature of bacteriophage inactivation mechanisms, which vary depending on environmental conditions and the specific factors influencing their structural integrity.

III. Materials and Methods

A. Phages and Host

Bacteriophage T4 was acquired from the National Institute of Molecular Biology and Biotechnology, University of the Philippines – Diliman. T4 phage was propagated in SM buffer containing 1.0 M Tris (pH 7.4), 2.9 g NaCl, and 1.0 g $MgSO_4 \cdot 7H_2O$. High-titer phage preparations were obtained via harvesting from Double-

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Layer Agar (DLA) assays. Plaques formed through DLA were purified by PEG-precipitation [10].

The host cell culture used was *E. coli* ATCC 11303. They were grown in Tryptone Soya Broth (TSB), prepared according to the manufacturer's specifications. A single colony was grown to mid-exponential phase by shaking at 200 cycles per minute for 7 hours at 37°C. *E. coli* cells were centrifuged at 4000×g for 5 minutes and suspended in SM buffer prior to a plaque assay. All work was performed inside a Biosafety cabinet.

B. Thermal Treatment

Phage stock (1×10^{-4} PFU/mL) was prepared and exposed to temperatures of 27.6°C (control), 55°C, 60°C, and 70°C, and agitated at 200 RPM. Samples were collected and immediately immersed in an ice bath for 1 minute to halt thermal effects. Bacteriophage concentration was quantified using Double-Layer Agar (DLA) method to quantify the concentration of phages following thermal treatment. All experiments were conducted in triplicate.

C. Double-Layer Plaque Assay

Bacteriophage concentration was determined by the Double-Layer plaque Assay (DLA). *E. coli* in mid-exponential phase suspended in SM buffer (200 µL) was infected with 20 µL of phage-containing samples. After an incubation period of 15 minutes, this was mixed with 3 mL of soft agar and was poured onto prepared TSA plates. The soft agar was prepared from TSB with 0.70% agar. All solutions used were autoclaved at 121°C for 20 minutes and cooled prior to the addition of bioactive agents. All assays were conducted in triplicate.

D. Kinetic Model

Inactivation of phage by heat was based on first-order kinetics [11, 12] as shown in Eq. (1).

$$C(t) = C_0 e^{-kt} \tag{1}$$

where *C(t)* is the concentration of active phages in PFU/mL at time t in minutes, C_0 is the initial concentration of phages in PFU/mL before thermal treatment, k is the first-order rate constant.

Temperature coefficients were also evaluated to ascertain the extent of rapid thermal inactivation resulting from the temperature increases of 5°C and 10°C [11] as depicted in Eq. (2).

$$\frac{k_{60}}{k_{55}} = Q_5 \qquad \frac{k_{70}}{k_{60}} = Q_{10} \tag{2}$$

Additionally, decimal reduction time, or D-value, was derived from the rate constant as outlined in Eq. (3). This metric quantifies the duration required to decrease the phage population by one log10, providing insights into phage stability under specific conditions [13].

$$D = \frac{k}{2.3} \tag{3}$$

From the derived rate constants, activation energy was approximated using Arrhenius law as shown in Eq. (4). The activation energy of a reaction provides the temperature dependence of the rate constant [14].

$$\ln k = -\left(\frac{E_a}{R}\right)\left(\frac{1}{T}\right)\ln A \tag{4}$$

where E_a is the activation energy in kJ-mol^{-1}, $R = 0.008314$ kJ (mol K)$^{-1}$ the universal gas constant, T is the absolute temperature in K, A is the pre-exponential factor.

IV. Result and Discussion

A. T4 Phage Concentration

Fig. 1 shows that an overall decrease in phage concentration is evident as time progresses. The drop in concentration is higher at increased temperature. This is consistent with the expected thermal inactivation effect noted in previous studies [7, 11, 15]. This trend confirms the susceptibility of T4 bacteriophage to higher temperatures, leading to a reduction in viable bacteriophages over time.

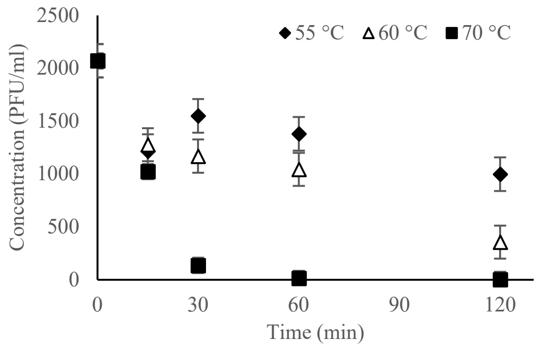

Fig. 1. Concentration profile of T4 phage for 0–120 min at temperatures 55°C, 60°C, and 70°C.

The concentration at 70°C is generally lower relative to lower temperatures of 55°C and 60°C. This indicates a difference in the rate of thermal inactivation of T4 bacteriophage. Statistical analysis using a two-factor ANOVA with replication reveals significant effects of both time and temperature on the phage concentration ($p < 0.0001$ for both factors). Additionally, a significant interaction effect between time and temperature ($p < 0.0001$) suggests that the impact of one factor on phage concentration is dependent on the level of the other, indicating the necessity of considering these factors in conjunction when studying thermal inactivation dynamics of bacteriophages.

B. Kinetic Model

Table I summarizes the first order rate constant and the r^2 value. Higher k-values indicate faster inactivation, with the process notably accelerating at 70°C.

TABLE I. Summary of Rate Constants at 55°C, 60°C, and 70°C.

Temperature (°C)	k (min^{-1})	r^2
55	−0.00448 ± 0.001466	0.61 ± 0.29
60	−0.01323 ± 0.001546	0.94 ± 0.07
70	−0.05356 ± 0.011610	0.89 ± 0.12

The experimental data closely matches the model predictions, illustrating the model's accuracy. As shown in Fig. 2, at 55°C, the decrease in phages concentration is linear, while at 60°C and 70°C, it exhibits an exponential decay, more pronounced at 70°C. This indicates that higher temperatures accelerate the thermal inactivation of phages, validating the model's effectiveness across varied thermal conditions. The thermal dependence of the inactivation constants is fully explained by the Arrhenius equation with an r^2 value of 0.9989 and an activation energy of 151.92 kJ/mol.

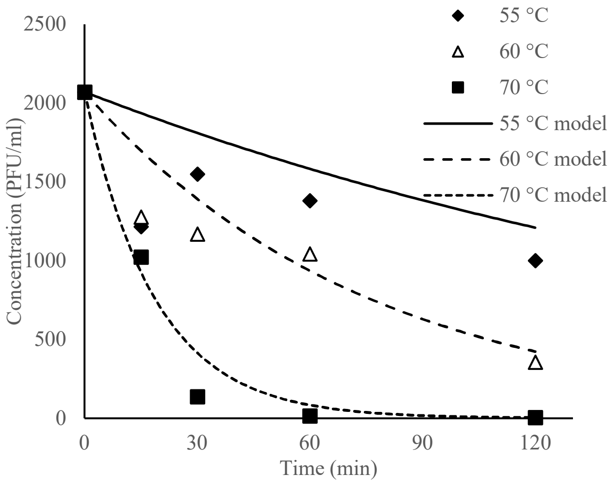

Fig. 2. Modeled and experimental concentration of T4 phage for 0–120 min at 55°C, 60°C, and 70°C.

Table II summarizes the D-values, and temperature coefficients for T4 phage at different temperatures. The D-values, representing the time required to reduce the phage population by one logarithmic unit, show a marked decrease with increasing temperature. This trend indicates that T4 phage's thermal resistance decreases significantly as the temperature increases, with the phage being much rapidly inactivated at 70°C compared to lower temperatures. In addition, the notable increase in the inactivation rate at higher temperature can be attributed to DNA melting which facilitates the release of phage DNA from its capsid, thereby exposing it to harsh external conditions [16].

Temperature coefficients, Q_5 and Q_{10}, calculated from the rate constants, demonstrate increased sensitivity to temperature increments. This suggests a greater acceleration in the rate of inactivation at higher temperature intervals, consistent with the substantial decline in D-values and the higher rate constants observed at 70°C. Similar kinetic parameters were also observed to thermal inactivation of different phages [7, 11, 17].

TABLE II. SUMMARY OF KINETIC PARAMETERS FOR THERMAL INACTIVATION OF T4 PHAGE.

Temperature (°C)	D-value (min)	Temperature Coefficients (Q)
55	512.81	
60	173.79	2.95
70	42.93	4.05

C. Thermodynamic Parameters

Table III summarizes the entropy change of activation (ΔS) and the Gibbs free energy change (ΔG), for the thermal inactivation of T4 phage across various temperatures.

The negative ΔS values at 55°C, 60°C, and 70°C indicate that the transitional form of the T4 phage exhibits an ordered activation state, even as the temperature increases. This may suggest coagulation and a loss of structural integrity, which could impair the phage's infectivity. Furthermore, the observed negative entropy change (ΔS) reflects the ordered nature of the transitional state, consistent with the structural constraints of the phage capsid [18]. In addition, negative entropy in microorganisms correlates with their self-reproducing rate, manifesting as a higher concentration of bacteriophages at lower temperatures [19].

Conversely, the consistently high positive ΔG values, ranging from 5305.60 kJ/mol at 70°C to 5453.61 kJ/mol at 55°C, signify that the thermal inactivation of the phage is non-spontaneous but thermodynamically feasible at the specified temperature [17].

TABLE III. SUMMARY OF THERMODYNAMIC PARAMETERS FOR THERMAL INACTIVATION OF T4 PHAGE.

Temperature (°C)	ΔS (kJ/K)	ΔG (kJ/mol)
55	−16.16	5453.61
60	−15.66	5367.72
70	−15.02	5305.60

V. CONCLUSION

The thermal inactivation of bacteriophage T4 is accurately predicted by a first-order decay model. The inactivation rate increases with temperature and aligns well with the Arrhenius equation, exhibiting an r^2-value of 0.9989 and an activation energy of 151.92 kJ/mol. D-values and temperature coefficients are consistent with expected trends at elevated temperatures. Thermodynamic analysis revealed a negative entropy change, indicating an ordered activation state, and a positive Gibbs free energy change, confirming the non-spontaneous nature of the process. These findings provide critical insights for optimizing bioprocessing strategies, enabling the preservation of bacteriophages through precise control of thermal conditions, whether by maintaining temperatures below 55°C or applying higher temperatures for shorter durations during processing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

SKCC, BKEG, and JBCP equally contributed to the methodology, validation, formal analysis, data curation, writing, visualization, and analysis of data; CMM contributed to the conceptualization, methodology, formal analysis, resources, supervision, project administration, funding, review, and editing of the manuscript; all authors had approved the final version of this manuscript.

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