

Original Article

Technical Errors Negatively Affect Investigation of Effect of Time-Temperature Relation in Growth of *Escherichia coli*

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Abstract

Thermal treatment is an important method for controlling microbial growth in food, clinical and biotechnological systems. This study evaluated the effect of heat exposure on the growth response of *Escherichia coli* using optical density (OD)-based measurements. Bacterial suspensions were exposed to temperatures ranging from 30°C to 75°C for 10 minutes, followed by incubation at 37°C for 24 hours. Growth was quantified spectrophotometrically and cell concentrations were estimated from OD values. Temperature dependent variations in bacterial growth were observed following thermal treatment. Post-incubation OD values ranged from 0.44 to 0.80, indicating differential responses of *E. coli* to heat stress. The highest growth response was observed at 55°C (OD = 0.80), whereas the lowest was recorded at 45°C (OD = 0.44). No complete growth inhibition was detected within the tested temperature range. These observations suggest that optical density analysis serves as a convenient and efficient approach for assessing the response of *E. coli* to thermal stress and offers initial evidence regarding the impact of temperature on its growth dynamics.

1. Introduction

Thermal processing remains one of the most widely used methods for ensuring food safety and extending shelf life through the inactivation of pathogenic and spoilage microorganisms. Conventional technologies such as pasteurization and sterilization continue to play a central role in the food industry by reducing microbial populations and maintaining product quality [1]. The

effectiveness of thermal treatments depends on microbial heat susceptibility and the accurate prediction of survival under processing conditions. Foodborne bacteria are frequently exposed to environmental stresses, including desiccation, osmotic stress, nutrient limitation and temperature fluctuations. Such stresses can induce physiological adaptations that alter microbial survival and thermal resistance. In particular, desiccation-stressed pathogens may exhibit

increased tolerance to subsequent heat treatments, resulting in inactivation kinetics that differ from those of unstressed cells. Understanding these responses is essential for quantitative microbial risk assessment and the maintenance of food safety standards [2].

Among foodborne microorganisms, *Escherichia coli* is one of the most extensively studied bacterial species because of its importance in microbiology, biotechnology and public health. This Gram-negative, facultative anaerobic bacterium has served as a model organism for molecular biology and microbial physiology for more than six decades [3].

Beyond its role as a model system, *E. coli* is also a significant pathogen associated with diverse infections. Its pathogenicity is enhanced by virulence factors such as toxins, adhesins, siderophores, capsules, antimicrobial resistance mechanisms and biofilm-forming ability, all of which contribute to persistence and disease severity [4].

Quantitative microbial risk assessment relies on predictive Microbiology, which employs mathematical models to describe microbial growth, survival and inactivation under varying environmental conditions encountered during food processing, storage, transportation and consumption [5]. Although these models improve risk estimation, their predictive capability depends on experimentally determined parameters and must account for variability among bacterial strains exposed to identical environmental conditions [5].

Thermal resistance is commonly characterized using thermal death parameters such as Decimal Reduction Time (D-value), Thermal Death Time (TDT) and Thermal Death Point (TDP). The D-value represents the time required at a specific temperature to achieve a 90% reduction in microbial population and is widely used to quantify heat resistance. These parameters provide essential information for evaluating thermal process efficacy and must be determined experimentally from microbial survival data [5]. Although extensive compilations of thermal resistance data have been reported microbial inactivation remains an active research area because of emerging food products, novel processing technologies and increasingly complex microbial responses, including non-linear inactivation behavior such as shoulder and tailing effects [5].

Traditionally, thermal resistance studies have relied on isothermal experiments, with results extrapolated to

non-isothermal industrial processes. However, bacterial cells exposed to gradually increasing temperatures may undergo stress acclimation, developing enhanced thermal tolerance during heating. Consequently, microbial survival may exceed predictions based solely on isothermal models, potentially creating food safety risks. To address these limitations, mathematical models incorporating stress adaptation have been developed. Notably, Garre et al. (2018) [5] proposed a model based on the Bigelow log-linear approach that distinguishes between static thermal resistance and dynamic resistance arising from stress acclimation, thereby improving the prediction of microbial behavior under changing thermal conditions.

Heat treatment is also widely applied for the disinfection of food products, water, wastewater, sewage and biosolids. Microbial inactivation depends on the combined effects of temperature and exposure time, with higher temperatures generally requiring shorter treatment durations. Understanding these time–temperature relationships is critical for optimizing process design, energy efficiency and pathogen control [6]. Although time–temperature pathogen reduction curves are frequently used to define safe treatment conditions, uncertainties associated with detection limits and incomplete quantification of microbial reductions can complicate their application in quantitative risk assessment [6].

Recent studies have investigated thermal inactivation kinetics of foodborne pathogens under a variety of processing conditions. However, microbial heat resistance is influenced by multiple factors, including strain variability, physiological state, food composition, pH, water activity, salt concentration, fat content and processing environment. Consequently, advanced primary, secondary and omnibus models have been developed to improve predictions of microbial survival under diverse conditions [7].

Spectrophotometry has long been used as a rapid method for estimating microbial population density through optical density (OD) measurements, typically at 600 nm. Although OD provides a convenient and non-destructive estimate of cell concentration, bacterial cells scatter rather than absorb light, limiting the direct applicability of the Beer–Lambert law. Accurate determination of cell density, therefore, requires calibration for each microbial species and instrument configuration [7]. The introduction of microtiter plate

readers has facilitated high-throughput monitoring of microbial growth; however, calibration remains necessary because optical path length and plate geometry influence OD measurements [8].

Despite the widespread use of plate-count methods for determining thermal death parameters, these approaches are labor-intensive and time-consuming. Optical density measurements offer a rapid alternative for monitoring microbial population changes during thermal exposure. However, limited information is available regarding the application of OD-derived data for estimating TDT, TDP, D-values, and related kinetic parameters, and the reliability of OD-based approaches for thermal inactivation studies remains insufficiently explored [7,8].

Therefore, the present study investigated the thermal inactivation of *Escherichia coli* over a temperature range of 30–75°C using optical density-based measurements. The study aimed to determine Thermal Death Point (TDP), Thermal Death Time (TDT), Decimal Reduction Time (D-value) while evaluating the suitability of OD measurements as a rapid method for assessing microbial thermal resistance. The findings are expected to contribute to a better understanding of microbial thermal tolerance and the applicability of OD-based approaches for thermal death parameter estimation in food safety, biotechnology and environmental Microbiology.

2. Materials and Methods

2.1 Microorganism and Culture Conditions

Escherichia coli was used as the model organism in this study. A pure culture of the organism was maintained on nutrient agar slants and subcultured prior to experimentation to ensure culture viability and purity. An actively growing 24-hour-old culture was prepared in sterile nutrient broth and used as the inoculum. All experiments were conducted in the Microbiology Laboratory of Jaywantrao Sawant Commerce and Science College.

2.2 Media and Reagents

Nutrient broth and nutrient agar were prepared according to standard microbiological procedures. The media were sterilized by autoclaving at 121°C for 15 minutes prior to use [9].

2.3 Preparation of Bacterial Suspension

To obtain a standardized bacterial suspension, 1 mL of a 24 hours old *E. coli* culture was aseptically inoculated into sterile 100 mL nutrient broth. The

resulting suspension was mixed thoroughly to ensure a homogeneous distribution of bacterial cells, thereby minimizing variations during subsequent thermal exposure treatments [9].

2.4 Thermal Exposure Protocol

10 ml Aliquots of the prepared bacterial suspension were dispensed into each sterile test tubes. The tubes were exposed to a controlled temperature gradient ranging from 30°C to 75°C using a calibrated water bath. All samples were exposed to their respective temperatures for 10 minutes under controlled conditions. A non-heated control was maintained to serve as a baseline for comparison. Following thermal exposure, all samples were immediately cooled to room temperature to prevent further heat-induced cellular damage [9].

2.5 Incubation and Growth Assessment

Following thermal treatment, the samples were incubated at 37°C for 24 hours to allow recovery and growth of surviving bacterial cells. Bacterial growth was assessed by measuring optical density (OD), which served as an indirect indicator of biomass accumulation and cellular viability [9].

2.6 Optical Density Measurement and Cell Quantification

The optical density of the samples was measured spectrophotometrically before and after incubation. Optical density measurements at 600 nm were used for bacterial cell quantification and D-value determination. The obtained OD values were converted into cell concentrations using the established relationship of $1 \text{ OD} = 2.4 \times 10^9 \text{ cells/mL}$ by formula, $\text{CFU/mL} = \text{OD}(600) \times 2.4 \times 10^9$. This conversion enabled quantitative estimation of bacterial populations under each temperature treatment. Optical density measurements at 540 nm were used for the determination of Thermal Death Time (TDT) and Thermal Death Point (TDP) [9].

2.7 Determination of Thermal Death Parameters

Thermal death characteristics were evaluated by analyzing changes in bacterial cell density in response to temperature variation. Thermal Death Time (TDT) was determined based on the reduction in viable cell numbers following exposure to specific temperatures, as assessed using OD540 measurements. Thermal Death Point (TDP) was estimated as the lowest temperature at which no detectable bacterial growth occurred after 10 minutes of exposure and subsequent incubation. The D-value was determined from OD600 measurements and

represented the time required at a given temperature to reduce the bacterial population by one logarithmic cycle. These parameters were calculated using observations obtained from the experimental dataset (Table 1).

2.8 Experimental Controls and Data Reliability

All experimental procedures were performed under aseptic conditions to prevent microbial contamination. A non-heated control was included to establish baseline microbial growth and validate the reliability of the experimental outcomes. Experimental observations were recorded systematically and analyzed to ensure consistency, accuracy and reproducibility throughout the study

3. Results

3.1 Baseline Cell Density and Experimental Consistency

The pre-incubation optical density measurements demonstrated a consistent baseline across all experimental conditions, as calculated using the established OD conversion factor. The uniformity of these values confirmed the reliability of the inoculum preparation procedure and ensured that subsequent variations in cell density could be attributed primarily to thermal exposure rather than experimental variability. The observed consistency in the initial bacterial population provided a standardized basis for evaluating the effects of temperature on the growth and survival of *E. coli* in TDP and TDT.

3.2 Temperature-Dependent Growth and Survival Response

Post-incubation optical density measurements demonstrated a temperature-dependent response in *E. coli* growth and survival. Following thermal exposure and subsequent incubation, bacterial growth was observed across all temperature treatments, although the magnitude of growth varied with temperature. The highest optical density value was recorded at 55°C (OD = 0.80), corresponding to a cell concentration of 1920×10^6 cells/mL. Relatively high optical density values were also observed at 65°C (OD = 0.77), 75°C (OD = 0.73), 60°C (OD = 0.72), and 50°C (OD = 0.71), indicating substantial recovery and proliferation of bacterial cells following thermal exposure.

In contrast, lower optical density values were observed at 40°C (OD = 0.45) and 45°C (OD = 0.44), suggesting comparatively reduced bacterial growth under these treatment conditions. The optical density profiles indicated that *E. coli* retained the capacity for

growth and recovery over a broad temperature range. However, variations in optical density among treatments suggest that thermal exposure influenced bacterial viability and subsequent proliferation. The observed differences in growth response may reflect the physiological effects of temperature on cellular metabolism, membrane integrity and enzyme activity. Overall, the results demonstrate that thermal treatment significantly affected bacterial growth dynamics, as evidenced by changes in optical density and cell concentration following incubation (figure 1).

4. Discussion

The present investigation elucidated the thermal response of *Escherichia coli* under a controlled temperature gradient. The results demonstrated a temperature-dependent variation in bacterial growth and survival, highlighting the complex interaction between cellular adaptation and thermal stress. Although *E. coli* is generally recognized as a mesophilic bacterium with optimum growth near physiological temperatures (approximately 37°C), the post-incubation optical density measurements obtained in the present study revealed substantial bacterial recovery and growth across a broader temperature range. Previous studies in predictive Microbiology have reported that the optimum metabolic activity of *E. coli* occurs at temperatures close to 37°C owing to favorable enzyme kinetics and membrane fluidity [10]. These observations support the classification of *E. coli* as a mesophilic organism exhibiting temperature-dependent growth characteristics.

The variations in bacterial growth observed at intermediate temperatures may be associated with the onset of sub-lethal thermal stress. Previous investigations have demonstrated that exposure to elevated temperatures induces a cellular heat-shock response characterized by the up-regulation of molecular chaperones such as DnaK and GroEL, which contribute to protein stabilization and protection against thermal damage [11]. These adaptive mechanisms enable bacterial cells to tolerate transient thermal stress; however, their protective capacity becomes progressively limited under prolonged or more severe heat exposure, leading to cumulative cellular injury.

The alterations in bacterial growth observed at temperatures above 60°C are consistent with established mechanisms of thermal inactivation [12].

Previous studies have reported that elevated temperatures induce irreversible damage to multiple cellular targets, including protein coagulation, ribosomal disruption and destabilization of membrane lipid bilayers [13]. Furthermore, microscopic and molecular investigations have identified increased membrane permeability and intracellular leakage as key indicators of irreversible cellular injury during thermal stress [14,15]. These mechanisms collectively contribute to the reduction in microbial viability under high-temperature conditions. Consequently, optical density should be interpreted as an indirect indicator of bacterial biomass rather than a direct measure of cell viability.

Thermal exposure produced temperature-dependent variations in the growth response of *E. coli*, as indicated by changes in post-incubation optical density. The highest OD value was observed at 55°C (0.80), while the lowest was recorded at 45°C (0.44), suggesting differential physiological responses to heat stress. Reduced growth at certain temperatures may reflect the inhibitory effects of thermal stress on cellular metabolism.

Unexpectedly high OD values were observed at temperatures between 50°C and 75°C. These results differ from the expected thermal sensitivity of *E. coli* and may be attributed to experimental limitations, including variations in temperature control, exposure duration, sample handling or OD measurement. Additionally, optical density does not distinguish between viable and non-viable cells and may be influenced by cell debris and turbidity.

Overall, the findings demonstrate that OD-based analysis is a rapid method for evaluating bacterial responses to thermal exposure. However, future studies should incorporate viable cell count methods, such as colony-forming unit (CFU) analysis, to validate thermal response patterns and reduce experimental uncertainty.

5. Conclusion:

Thermal exposure influenced the growth response of *Escherichia coli*, as evidenced by variations in optical density across the tested temperature range. However, unexpected results at higher temperatures suggest that technical factors, including temperature control, exposure time and sample handling, may have affected the observations.

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Appendices

Abbreviations:

OD-Optical Density, **TDP**-Thermal Death Point, **TDT**-Thermal Death Time, **CFU**-Colony Forming Unit, **/ml**- per milliliters



Figure 1:Tubes after incubation in Thermal death point experiment.



Figure 2: Tubes after incubation in Thermal death time experiment

Table 1: Pre-incubation and post-incubation optical density measurements against different temperatures for cell concentrations of Thermal Death Point (TDP)

Temperature (°C)	Pre-incubation OD	Cell Concentration	post-incubation OD	Cell concentration
Control	0.14	336×10^6	0.60	1440×10^6
30	0.14	336×10^6	0.61	1464×10^6
35	0.14	336×10^6	0.54	1296×10^6
40	0.14	336×10^6	0.45	1080×10^6
45	0.10	240×10^6	0.44	1056×10^6
50	0.12	288×10^6	0.71	1704×10^6
55	0.15	360×10^6	0.80	1920×10^6
60	0.14	336×10^6	0.72	1728×10^6
65	0.16	384×10^6	0.77	1848×10^6
70	0.16	384×10^6	0.62	1488×10^6
75	0.14	336×10^6	0.73	1752×10^6