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# Inactivation kinetics and thermodynamics assessments of *Geobacillus stearothermophilus* during thermal sterilization for products safety

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ABSTRACT

Chemical kinetics and thermodynamics provide modes and mechanisms for the thermal death of microbial spores. In this study, the effect of thermal inactivation on *Geobacillus stearothermophilus*, a highly heat-resistant bacterial species, was studied over the temperature range of 95, 100, 105, and 110 °C and holding sterilization periods of 10, 15, 20, 25, 30, and 45 min by the application of mathematical analysis of kinetic and thermodynamic properties. Thermal death rate constant, k, for the best kinetic order of sterilization ranged between 0.0431 to 0.1581 $min^{-1}$ with the energy of activation, $E_a$, estimated to be 115.96 kJ/mol. Two primary thermal death kinetic models were applied (log-linear first order and the nonlinear Weibull). Weibull's model provided more reliable kinetic parameters to predict the effect of thermal treatments. Concave curves ($\alpha > 1$) were predicted with the Weibull's model for 100, 105, and 110 °C (1.57, 1.26, and 1.22 respectively), indicating the susceptibility of spores to lethal treatment. The rate parameter, $\phi$ (first reduction time) decreased with increasing thermal heating (28.80 min (95 °C), 21.08 min (100 °C), 14.61 min (105 °C), and 9.65 min (110 °C)) following the paths of the D-values (about 7 min to attain 88% spores' destruction after 110 °C heating) of the log-linear kinetic model. Thermal death time (TDT) for the complete destruction of spores was predicted to be after 40 min at 110 °C. The z-value was 23.31 °C, indicating the sterilization temperature that must be attained for one log destruction of spores. The heat of activation showed endothermic reactions for all temperatures ($\Delta H$ ranged 112.90 – 112.78 kJ/mol), Gibb's free energy of activation, $\Delta G$, ranged from 325 – 333.74 kJ/mol (indicating a non-spontaneous reaction), and the entropy of activation ($\Delta S$) showed reversibility of reaction ($\Delta S < 1$) for all the thermal temperatures.

## 1. Introduction

Microorganisms can contaminate food, cosmetics, poultry, and pharmaceutical products, thus, causing spoilage and health risks when such materials are used or consumed. The growth of these microorganisms must be prevented to ensure product safety (Stavropoulou, and Bezirtzoglou, 2019). One of the most widely used methods for preservation is dry heat or thermal (steam) sterilization. Dry or steam sterilization (which may involve boiling in water, using steam, or autoclaving) is capable of inactivating most microbes and enzymes, and subsequent death of such microorganisms (Cebrián et al. 2017). This provides a contaminant-free environment devoid of any life forms (Chiruta, 2000). Because of the usefulness of microorganisms in the conversion of substrates (especially biological) into new products that are metabolic in nature (Ayeni et al., 2016; Ayeni et al. 2019), a sterile (contaminant--free) environment is needed for efficient products preservation and formation. When applied, sterilization leads to loss of microorganisms, and a reduction in the number of viable cells (Chiruta, 2000). The temperature in which microorganisms are resident is a crucial factor to their growth or survival (Deegenaars and Watson, 1998). At a higher temperature, the likelihood of microorganisms surviving follows a particular regime and the time of exposure. In addition to temperature, for safety and bioproducts preservation, the pH of the medium plays another critical role, especially during spores' production (Blocher and Busta, 1983). Thermal death time, a kinetic parameter of the sterilization process determines how long it takes to kill a specific microorganism at a specific temperature. Therefore, a prior thermal death study is needed to prevent excessive heating which may denature or degrade

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the proteins or other nutrients in the medium. The kinetics of cell death is an important consideration in the design of sterilization processes. The design of a sterilizer can be likened to the continuous flow reactor where the thermal inactivation of contaminating microorganisms is regarded as the reaction medium (Chiruta, 2000). The three main protocols in a continuous sterilizer are the heating (temperature build-up to 121 °C), holding (normally where the destruction of cells take place, the medium is held at 121 °C and 0.1 MN/m$^2$), and cooling sections (from 121 °C to room temperature) (Chiruta, 2000). Direct injection and indirect heating are two ways heat can be applied to continuous sterilizers. For practical realization of rapid heat up, direct steam injection is better suited than indirect heating by having a greater heat transfer coefficient and elimination of heat exchange over the surfaces during the heating section (Chiruta, 2000). The applications and the principles of kinetic model development for biological systems offer valuable insight into products ' understanding, control, and prediction of changes in food quality occurring during thermal transformation (Haefner, 2005).

The mechanism for quality changes and complete kinetic models can be demonstrated at the molecular level in the areas of thermodynamics and chemical kinetics. Kinetic parameters can be estimated in traditional and emerging thermal inactivation kinetics (Dolan, 2003; Bermudez-Aguirre and Corradini, 2012). Thermodynamics help to determine and predict the feasible region for the performance of a system (be it physical, chemical, or microbial), which leads to having information in the calculation of the system reactions and processes (Tagade et al. 2021). The enthalpy (ΔH), entropy (ΔS), and Gibbs free energy (ΔG) information define the targets and limits, and also assist to optimize and improve the performance of such processes (Hildebrandt et al. 2009). In microbial systems, these thermodynamics parameters are useful for analyzing the growth and metabolisms of microorganisms (von Stockaret et al. 2006)

*Geobacillus stearothermophilus* (formerly *Bacillus stearothermophilus*) is a thermophile, that is heat-resistant when compared with other organisms (Thwaite and Atkins, 2012). *Geobacillus stearothermophilus* causes spoilage in food products. It causes flat sour spoilage of low-acid canned foods (Batt and Tortorello, 2014).

In this study, to characterize temperature effects on the kinetic parameters during sterilization, a modified logarithm order of death model was used to evaluate the order of reaction (Jang, 1991) and the Arrhenius relationship was used to estimate the activation energy for the thermal sterilization of *Geobacillus stearothermophilus.* In addition, other parameters for describing the sterilization process were also obtained by the application of thermal death kinetic primary models (having influence only on temperature) of first-order (log-linear) kinetics (to estimate the D- value which measures organism reduction by 90% or one log at a given temperature, the z- value which relates the resistance of an organism at different temperatures and is the increase in temperature required to reduce D value to 1/10 its value or reduce it by one log cycle when D is plotted against temperature, and the thermal death time (TDT), which is how long it takes the death of a particular organism at a specific temperature. Weibull's survival non-linear model was also used to determine the kinetic parameters. Finally, the thermodynamics properties of the sterilization process were determined.

## 2. Materials and methods

### 2.1. Experimental procedure

Strains of *Geobacillus stearothermophilus* were obtained from FOWM Biotechnology Limited, Lagos, Nigeria and were transported to the Department of Biological Science Laboratory, Covenant University, Ota, Nigeria. Further tests were carried out for proper identification and characterization of the isolated organisms. Nutrient broth was freshly prepared and dispensed into McCartney bottles. Each bottle contained 10 ml of the sterile nutrient broth and inoculated with the strains of *Bacillus stearothermophilus* which were later incubated at 37 °C for 24 h. Sets of inoculated nutrient broth were brought out of the incubator and placed in an autoclave for temperatures ranging from 95 to 115 °C (with 5 °C interval). These final temperatures were held for six different periods, 10, 15, 20, 25, 30, and 45 min. Colony counts (using a colony counter) were changed to colony-forming units (CFU) accordingly and used for data handling. All reported data are mean of replicate measurements at each sampling time.

### 2.2. Estimation of the best kinetic order and activation energy for thermal treatments

The reaction order most suitable for the prevailing heating conditions was determined. In developing a kinetic model to describe thermal death of *Geobacillus stearothermophilus*, a classical logarithmic kinetic model approach was used to determine the order of reaction (Wang et al., 2002), and then the evaluation of the activation energy was based on the dependence of reaction rate on temperature.

Using the ratio of change of microbes' survivals ($N_t$) to initial number ($N_o$) during thermal treatments, we have the fundamental kinetic model (Wang et al., 2002):

$$\frac{d\left(\frac{N_t}{N_o}\right)}{dt} = -k\left(\frac{N_t}{N_o}\right)^n \tag{1}$$

where n is the kinetic order of reactions. Integrating Eq. (1), for different reaction orders:

$$\ln\left(\frac{N_t}{N_o}\right) = -kt + c(n=1) \tag{2}$$

$$\left(\frac{N_t}{N_o}\right)^{1-n} = -kt + c(n \neq 1) \tag{3}$$

k is the thermal death (inactivation) rate constant, t is the sampling time after sterilization.

The activation energy, $E_a$, for the thermal death was determined by using the Arrhenius relationship between k and the temperature, T. The Arrhenius equation relates the initiation energy to the rate at which the reaction occurs. The rate constant, k, is then indicated by:

$$k = Ae^{-\frac{E_a}{RT}} \tag{4}$$

A is a constant, T denotes the absolute temperature (K), $E_a$ is the activation energy in J/mol, R stands for the universal gas constant (8.314 J/mol.K). Taking the natural logarithm of Eq. (4) above gives a linear relationship of ln k and 1/T

$$\ln k = \ln A - \frac{E_a}{RT} \tag{5}$$

### 2.3. Kinetic parameters for effective thermal treatment

Thermal sterilization kinetics for disease-causing microorganisms can be described by primary models (which is influenced by temperature, e.g., log-linear and Weibull), secondary models, to global approach (omnibus models) by considering the process operating factors such as heating rate, temperature, pH, time, salinity, water activity (Rui et al., 2018; Villa-Rojas et al., 2013). The decimal reduction time (D) value, which is the time required for 90% destruction of spore population in a particular environment, that is, a reduction from 100% to 10% of the initial value, thermal death time (TDT) value, which is the time required for complete reduction of spores in a particular environment, and the thermal resistant point (z-value) are important parameters for describing the effectiveness of heating. A simple form of the primary first-order kinetic model can be given as follows (Rui et al., 2018; Peleg, 2006):

$$\frac{dN}{N} = -kt \tag{6}$$

Where N is the population of the microbes, t is the heating time (min) at constant temperature conditions, k is the rate constant in $min^{-1}$.

Integrating Eq. (6) gives the form:

$$t = (\log N_0 - \log N)x\frac{2.303}{k} \tag{7}$$

$$t = (\log N_0 - \log N)x\,D \tag{8}$$

$$D = \frac{t}{\log N_o - \log N} \tag{9}$$

where $N_0$ represents the initial microbial population (survival at time 0) and N is the final microbial population (survival at time t = t) after heat treatment. The liability of pathogens to thermal treatment at a particular temperature is governed by the D-value (which is the time in minutes for one log reduction at isothermal conditions). D-value compares heat resistances. A plot of log D-values against temperature often shows a linear relationship, called the thermal death time (TDT) curve. TDT is related to the D-value as given in Eq. (10)

$$TDT = \text{Thermal death time} = D\,(\log No - - \log N) \tag{10}$$

Where, No = survival at time 0, N survival at time t = t

A z-value (thermal resistant point) is obtained as the increase in temperature (°C) that makes one-log reduction of D-value:

$$z = \frac{T_{2\ -\ T_1}}{logD_1 - logD_2} \tag{11}$$

where $D_1$ and $D_2$ are measured D-values at $T_1$ and $T_2$ respectively. The z-value can be estimated from the –1/slope when the log D-value is plotted against temperature (Rui et al., 2018). The resistances of pathogens can be related to different temperatures through the z values.

Sometimes, microbial inactivation for novel and conventional heat technologies do not obey the first-order kinetics. It occasionally shows either upward or downward concavities. Weibull's distribution model can be used to describe the survival trends during microbial inactivation. Thermal inactivation kinetic models, such as Weibull's survival model (Eq. (12)) is often used to characterize the non-linearity of survival curves (Rui et al., 2018).

$$\ln\frac{\mathbf{P}}{\mathbf{P_o}} = -\left(\frac{t}{\phi}\right)^{\alpha} \tag{12}$$

$P_o$ is the initial microbial count, P is the momentary microbial count, $\phi$ is a rate parameter known as the first reduction time which is similar to the D-value of the linear thermal inactivation, and $\alpha$ is a measure of the semi-logarithmic survival curve's concavity (shape parameter, linked to the geometrical shape of the curve) (Bevilacqua et al., 2015). When $\alpha < 1$, the curve is convex, meaning that some microorganisms can be more resistant than others (governed by different factors), making them survive under the prevailing environmental conditions or the remaining population becomes more robust to thermal condition. When $\alpha > 1$, the curve presents concave form, pointing to accumulated damage which makes the surviving cells more exposed to destructive treatment. If $\alpha = 1$, a linear semi-logarithmic survival curve is established.

Non-linear regression analysis was used to estimate the kinetic parameter values of the nonlinear survival curves (Weibull's model) by finding the minimum objective function, the sum of the square error.

### 2.4. Determination of thermodynamics properties of thermal inactivation

The thermodynamic properties were calculated following the theory of absolute reaction rates after obtaining the death rate constant, k (Amaha and Sakaguchi, 1957). The governing equations for estimating the heat of activation ($\Delta H$), the entropy of activation ($\Delta S$), and the Gibbs free energy of activation ($\Delta G$) are given below:

$$\Delta H = E_a - RT \tag{13}$$

$$\Delta G = E_a + RT\ln\left(\frac{K_b\,T}{hA}\right) \tag{14}$$

$$\Delta S = \frac{\Delta H - \Delta G}{T} \tag{15}$$

Where $E_a$ is the activation energy, $K_b$ is Boltzmann constant ($1.381 \times 10^{-23}$ J/K); *h* is Plank constant ($6.63 \times 10^{-34}$ J s); T is the temperature in Kelvin, *A* is the pre-exponential factor.

## 3. Results and discussion

### 3.1. Thermal death inactivation kinetics parameters estimation

Most microorganisms can withstand heat much greater than many other living matters (Deindoerferi, 1957). For bacterial spores, heat tolerance varies amongst species and strains. In addition, varied numbers of environmental conditions (during spores' formation and thermal exposures) affect their heat resistance (Deindoerferi, 1957). Thermal death kinetics parameters were estimated by the log-linear (determination of the order of reaction (n), inactivation rate constants (k), the energy of activation ($E_a$), D-values, z-value, thermal death time, TDT), and Weibull (determination of first reduction time ($\phi$), and the measure of microbial resistance to heat ($\alpha$), the measure of concavity) models.

### 3.2. Modified logarithm order of thermal death and activation energy

By applying Eq. (3), the coefficient of determination ($R^2$) for the different reaction orders for the different heat treatments were estimated (Table 1). The 0.5th order was the best amongst others to describe the most suitable heat sterilization temperature of 100 °C ($R^2 = 0.9737$). However, considering the overall heat sterilization range (95 °C and 110 °C), first order reaction (average $R^2 = 0.9190$) was the most applicable of the reaction orders for *Geobacillus stearothermophilus* inactivation or sterilization process. The closest order to this is 0.5th with average $R^2$ value of 0.9101. Furthermore, the rate constants at the different heat temperatures for the first order reaction are higher than the 0.5th order indicating that the reaction occurs speedily with the first order model. A small rate constant indicates a slower reaction while a larger rate constant indicates a faster reaction.

The thermal death curve (survival rate) for the sterilization process based on the reaction order, n = 1, is given in Fig. 1. The inactivation rate constants (in parentheses), k in $min^{-1}$, at different temperatures are presented in Table 1. The reaction rate constants (k) generally increased with increasing temperature.

**Table 1**
Estimation of the best kinetic order (n) for the thermal inactivation of *Geobacillus stearothermophilus* at different temperatures comparing their coefficients of determination, $R^2$.

| Temperature (°C) | $R^2$ values n = 0 | n = 0.5 | n = 1.0 | n = 1.5 | n = 2 |
|---|---|---|---|---|---|
| 95 | 0.8909 (0.0172)[a] | 0.9267 (0.0117) | 0.9489 (0.0431) | 0.8443 (0.0194) | 0.6284 (0.0147) |
| 100 | 0.9225 (0.0209) | 0.9737 (0.0179) | 0.9238 (0.0724) | 0.8222 (0.0207) | 0.5225 (0.0139) |
| 105 | 0.8232 (0.0207) | 0.9162 (0.0195) | 0.8928 (0.0930) | 0.6913 (0.0197) | 0.3586 (0.0119) |
| 110 | 0.7592 (0.0245) | 0.8240 (0.0247) | 0.9103 (0.1581) | 0.7193 (0.0230) | 0.5446 (0.0160) |

[a] Values in parentheses are the thermal inactivation rate constants (k, $min^{-1}$) for the order of reaction at the different temperatures

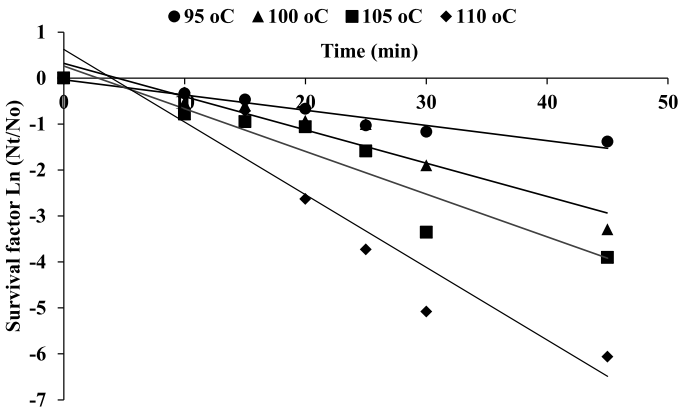


**Fig. 1.** Heat survival of *Geobacillus stearothermophilus* with time at different sterilization temperatures for reaction order, n = 1.

From the inactivation (death) rate constants, the energy of activation, $E_a$, was calculated from Eq. (5) using the linear graph of lnk against 1/T (Fig. 2). The energy of activation was estimated to be 115.96 kJ/mol and the Arrhenius constant was $9.50 \times 10^{-16}$ $min^{-1}$. These results are in consonance agree with literature values. Lund (1977) reported that for thermal treatments (between 100 °C–130 °C) of spores of different microorganisms, activation energy can vary from 222–502 kJ/mol. The lower range of thermal treatments (95 °C–110 °C) chosen in this study as well as environmental operating conditions during treatments may have caused the differences. A larger value of $E_a$ means that more energy is required to inactivate the microorganism. Therefore, with the lower value of activation energy (115.96 kJ/mol) obtained in this study, minimal energy is needed for the microorganisms' inactivation.

### 3.3. Influence of D-value and z-value on thermal sterilization temperature

D-values, decimal reduction time (Eq. (9)) was obtained from the general form of the primary first-order kinetics (Eq. (8)). Different thermal temperatures and environments with the history of microorganisms affect the D-values (Cebrián et al., 2017) and the z-values (obtained from the linear plot of log D value against temperature). The higher the temperature the lower the D-values (Table 2). The first 5 °C increase in thermal temperature (95 °C and 100 °C) resulted in over 54% spores' destruction (measured by the D-values). At a further higher temperature of 110 °C, the microbial population attained about 80% destruction. Similar trends were discovered for thermal inactivation rates of enterohemorrhagic *Escherichia coli* in wheat flour by Forghani et al. 2018. In their study, spores' destruction of the different strains of *Escherichia coli* varied between 80% to 88% from the lowest to the highest thermal treatment (Forghani et al. 2018). Therefore, at increasing temperatures, the microorganisms become less resistant to

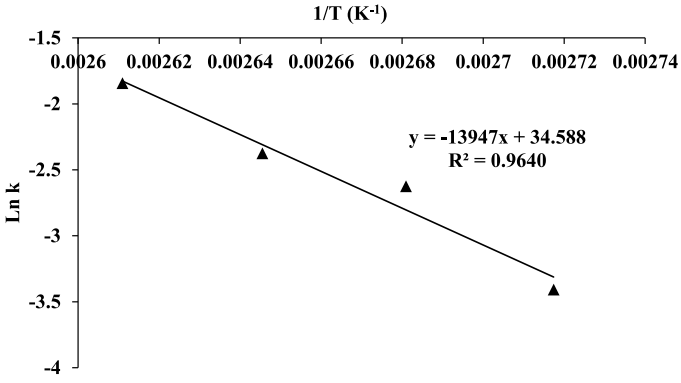


**Fig. 2.** Arrhenius plot for the determination of activation energy.

**Table 2**
Thermal inactivation kinetic parameters for the log-linear and Weibull models.

| Temperature (°C) | log-linear k ($min^{-1}$) | D (min) | $R^2$ | Weibull ϕ (min) | α | $R^2$ |
|---|---|---|---|---|---|---|
| 95 | 0.0331 | 30.21 | 0.9489 | 28.80 | 0.87 | 0.9564 |
| 100 | 0.0724 | 13.81 | 0.9238 | 21.08 | 1.57 | 0.9772 |
| 105 | 0.0930 | 10.75 | 0.8928 | 14.61 | 1.26 | 0.9038 |
| 110 | 0.1581 | 6.33 | 0.9103 | 9.65 | 1.22 | 0.9107 |

heat, leading to their death. Microbial growth increases at reduced stages of heating before thermal death sets in. This value is the susceptibility of pathogens to heat at a specific temperature (one log reduction at a constant temperature). Fig. 3 is a linear relationship that allows the estimation of the z-value or the TDT curve (for different lethal times) (Table 3), the thermal death time curve. It is the increase in temperature required to reduce the D value to 1/10 of its value or to reduce it by one log cycle when D is plotted against temperature. The D-values (Table 2) and the z-value (calculated to be 23.31 °C as given in Fig. 3) obtained in this study for *Geobacillus stearothermophilis* and some other microorganisms follow similar trends as reported (Cebrián et al., 2017; Forghani et al., 2018; Smelt and Brul, 2014). Reducing the D-value, the treatment temperature must be increased by 23.31 °C (the z-value). In other words, to achieve a reasonable time for 90% destruction of spores' population, the thermal temperature must be increased by 23.31 °C, for one log destruction. z is related to the thermal resistance of an organism at different temperatures (Eq. (11)).

### 3.4. Thermal death time (TDT) estimation

The developed model for the first-order kinetic (Eq. (2)) was used to estimate and predict the lethal time (thermal death time) from 95%, 99%, and 99.67% destruction (Table 3) for each treatment temperature by imputing 0.05, 0.01, and 0.0033 for Nt/No (microbial survival ratio) in the first-order kinetic model (Eq. (2)). 99.67% closely corresponded to the exposure that produces a complete death of all the microorganisms (Gazit et al., 2004).

Table 3 indicates, for example, that at 95 °C thermal treatment, complete destruction (thermal death) of spores (at 99.67%) will occur after about 172 min compared to 40 min if thermal treatment was carried out at 110 °C. At 110 °C thermal heating, 95% of spores would have been destroyed after about 23 min. Complete mortality of spores was achieved after holding at temperatures 95, 100, 105, and 110 °C for 172, 83, 64, and 40 min respectively. As expected, the percentage of

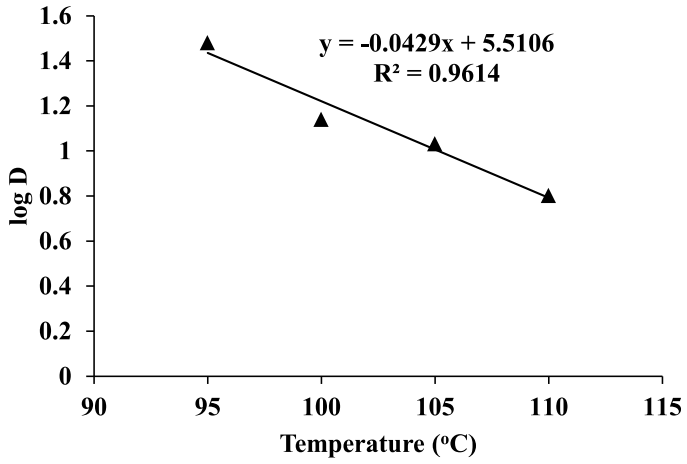


**Fig. 3.** Relationship between the logarithmic value of the decimal reduction time (thermal death time required to completely destroy microbial population) and heat treatment temperatures. The z-value was estimated to be 23.31 °C from this curve (using Eq. (11)).

**Table 3**
Comparing the lethal times (LT in min) of the first-order kinetic models at different heat treatments.

| Temperature °C | $LT_{95}$ | $LT_{99}$ | $LT_{99.67}$ |
|---|---|---|---|
| 95 | 89.43 | 138.05 | 171.54 |
| 100 | 45.8 | 68.03 | 83.34 |
| 105 | 35.04 | 52.34 | 64.26 |
| 110 | 22.91 | 33.09 | 40.10 |

microbial death increased with increasing thermal treatments. This follows the same trend as reported in the literature, that as the thermal temperature increases, the faster it takes to achieve 100% mortality. In their study (Gazit et al., 2004), the thermal death time of fruit fly infestation of citrus fruit at sterilization temperature of 46–52 °C (46, 48, 50, and 52 °C) for 100% mortality occurred at 60, 15, 4, and 1 min respectively.

Wang et al., 2002 (for fifth-instar *Cydia pomonella* thermal heating) and Chiruta 2000 (for *Pseudomonas Fluorescens and Escherichia coli* thermal heating), also reported the same trend of sterilization temperature increase with decreasing the minimum time required to achieve 100% mortality.

### 3.5. Weibull's survival non-linear death kinetic model

Since most thermal processes do not follow the first order inactivation kinetics, Weibull's non-linear model (Eq. (12)) was applied to evaluate the inactivation kinetics parameters.

Fig. 4 gives the non-linear survival curves for *Geobacillus stearothermophilis* at the different inactivation temperatures. Weibulls model presumes that spores in the microbial population can resist thermal treatment differently (which is governed by the value of $\alpha$) and that a progressive form of death can be presented (Forghan et al, 2018). On the other hand, the log-linear first order kinetic model assumes that each spore in the population has the same chance of dying (Ma et al., 2009; Forghan et al, 2018). Looking critically from Table 2, $\phi$ (a rate parameter called first reduction time with similarity to the D-value of the log-linear kinetic model when the shape factor, $\alpha$, becomes 1) decreased with increasing temperature. The coefficients of determination, ($R^2$), for the Weibull's model (Table 2) were closer to unity (one) than the log-linear model in describing the kinetics of the thermal treatment. In terms of precision, the average value (using Weibulls non-linear model) of $R^2$ was 0.9370 compared to 0.9190 for the log-linear first order model kinetics. The observations gathered in this study compare favourably with other reported findings (Forghan et al, 2018; Santillana, et al., 2014; Rachon et al., 2016) that the Weibull's model is a better basis for the development of anti-microbial sterilization involvements than the log-linear

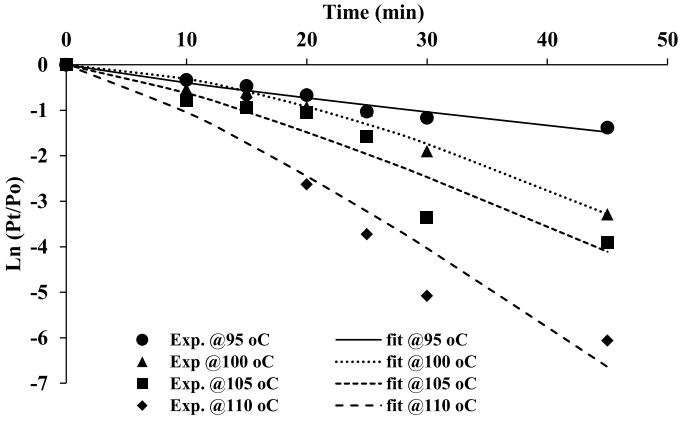


**Fig. 4.** Thermal inactivation kinetic curves for *Geobacillus stearothermophilis* at different temperatures using the Weibull non-linear model (Eq. (12)). Markers represent the experimental values.

model. Forghan et al. (2018) reported that the Weibull model kinetic parameters (with $R^2$-adjusted = 0.98) described the thermal sterilization of enterohemorrhagic *Escherichia coli* better than the log-linear model (with $R^2$-adjusted = 0.89), and that the $R^2$ values showed decreasing trends as the sterilization temperature increased. The concavities from 100 °C to 110 °C thermal treatments (Table 2) indicate $\alpha > 1$ (curve is concave). This means that more cells can be destroyed at this temperature range than at 95 °C, where $\alpha < 1$, indicating that the microorganisms at this temperature will be more resistant and stronger to thermal treatments.

### 3.6. Thermodynamic properties determination for thermal inactivation

The thermodynamic properties such as the heat of activation ($\Delta H$), the Gibbs free energy of activation $\Delta G$, and the entropy of activation ($\Delta S$) were estimated by applying Eqs. (11), (12), and (13) respectively. The directions of these properties for the thermal inactivation of *Geobacillus stearothermophilis* at the different heating temperatures show similarities in values (Table 4). However marginal reductions in ΔH were noticed as the heating temperature increased, which corresponded to destruction in spores.

ΔG and ΔS also increased marginally with increasing thermal temperatures accompanied by the reduction in spores (Table 4). Eze et al., (2010) reported marginal differences (using different sterilization temperatures (50–80 °C) in ΔH or ΔS for the thermal inactivation of the peroxidase enzyme in white yam. Thermodynamic properties of the same microorganism do not substantially differ in values due to the similarities in their molecular structure (Popovic, 2019). This follows that cell components (mostly composed of carbohydrates, proteins, water, lipids, and nucleic acids) of the same strain of microbe will always be exactly alike as long as the subculture medium is similar (Amaha and Sakaguchi, 1957). The negative value of ΔS shows that an additional driving force will be needed for the performance of the reaction (Singh et al., 2021). The values of the thermodynamic properties obtained in this study showed that the thermal sterilization process is endothermic, indicating a non-spontaneous process (Tagade et al. 2021).

## 4. Conclusion

Thermal sterilization (inactivation) of biological systems is an effective process that provides insights to understand, control, and predict products quality and safety. This study established that the kinetic order of reaction for the thermal inactivation process for a high heat resistant microorganism, *Geobacillus stearothermophilus*, was equal to one, under the prevailing experimental conditions. Through the Arrhenius relationship, the energy of activation was estimated to be 115.96 kJ/mol indicating mild energy utilization during the process. The primary nonlinear kinetic survival model of Weibull indicated the first reduction time decreased with increasing temperature with destruction of spores at increased temperatures. The thermal death time for complete destruction of spores was predicted to be 40 min for the highest sterilization temperature of 110 °C. The thermodynamics study of the process established endothermic, non-spontaneous, and reversible

**Table 4**
Heat of activation ($\Delta H$), the entropy of activation ($\Delta S$), and the Gibbs free energy of activation ($\Delta G$) thermodynamic properties for *Geobacillus stearothermophilis* thermal sterilization.

| Temp. (°C) | Nt (CFU/ml) | Ea (kJ/mol) | ΔH (kJ/mol) | ΔG (kJ/mol) | ΔS (kJ/mol) |
|---|---|---|---|---|---|
| 95 | $1.62 \times 10^5$ | 115.96 | 112.90 | 325.09 | -0.5766 |
| 100 | $2.40 \times 10^4$ | 115.96 | 112.86 | 327.97 | -0.5767 |
| 105 | $1.3 \times 10^4$ | 115.96 | 112.82 | 330.86 | -0.5768 |
| 110 | $1.5 \times 10^3$ | 115.96 | 112.78 | 333.74 | -0.5769 |

Nt denotes the number of life cells at any time t.

reactions for the thermal treatment process.

## Declaration of Competing Interest

The authors declare that they have no competing interests.

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