THERMAL DESTRUCTION OF MICROORGANISMS

Thermal processing involves heating, holding and cooling process of foods in hermetically sealed containers for a specific time at a specific temperature to eliminate the microbial pathogens that endanger public health and microorganisms (spoilage m.o.) and enzymes that deteriorate food during storage.

Food processors look for more energy-efficient, cost-effective, and high-speed processing technologies.

High-Temperature-Short-Time (HTST) techniques have primarily evolved to minimize the severity of heat treatment and promote product quality.

Continuous aseptic processing and packaging further minimize the heat severity by quick heating and cooling of the food, prior to packaging, under aseptic conditions.

Microwave, radio frequency, and ohmic heating techniques have gained attention as alternate and nonconventional rapid heating techniques (novel heating techniques).

PRINCIPLES OF THERMAL PROCESSING

Most food processing techniques involve heat transfer operations of some kind. Some involve the application of heat (thermal processing), some involve the removal of heat (refrigeration), while some others involve simultaneous exchange of heat and moisture (evaporation, concentration, baking, drying, frying, etc.).

Thermal processing is not designed to destroy all microorganisms in a packaged product. Such a process would result in low product quality due to the long heating required.

Instead, all pathogenic and most spoilage microorganisms in a hermetically sealed container are destroyed, and an environment is created inside the package that does not support the growth of the more resistant thermophilic bacteria not fully destroyed by the heat treatment.

In order to determine the extent of the heat treatment, several factors must be known:
1) type and heat resistance of the target m.o., spore, or enzyme present in the food,
2) pH of the food,
3) heating conditions,
4) thermophysical properties of the food and container shape and size,
5) storage conditions following the process.

The growth and activity of microorganisms are also largely pH dependent. From a thermal processing standpoint, foods are divided into three major pH groups as shown in the table below.

Classification of Foods Based on pH		
Group	**pH**	**Examples**
High acid	**< 3.7**	**Fruit juices, apples, berries, cherries (red sour), plums, sour pickles, sauerkraut, vinegar**
Acid or medium acid	**3.7-4.5**	**Fruit jams, fruit cocktail, grapes, tomatoes, tomato juice, peaches, pimientos, pineapples, potato salad, prune juice, vegetable juice**
Low acid	**≥ 4.5**	**All meats, fish, vegetables, mixed entries, and most soups**

The temperature and pH requirements of some common spoilage microorganisms are summarized in the table below.

Spore-Forming Bacteria Important in Spoilage of Food		
	Acidity of Food	
Approximate Temperature (°C) Range for Vigorous Growth	**Acid (3.7<pH<4.5**	**Low Acid (pH≥4.5)**
Thermophilic (55–35°C)	***B.coagulans, S.Thermophilus, L. bulgaricus***	***C. thermosaccharolyticum, C. Nigrificans, B. Stearothermophilus,***
Mesophilic (40–10°C)	***C.butyricum, C.Pasteurianum, B.Mascerans, B.polymyxa***	***C. botulinum (A and B), C. Sporogenes, B. Licheniformis, B.subtilis***
Psychrophilic (35–<5°C)	***Pseudomonas, Micrococcus***	***C. botulinum E, S. aureus***

Most food industries dealing with thermal processing devote special attention to *C. botulinum*. It is the microorganism of public health concern in low-acid foods, and, due to its high heat resistance, temperatures of 115 to 125°C are commonly employed for processing of these foods. It has been generally accepted that *C. botulinum* doesn't grow and produce toxin below a pH of 4.5.

With reference to the acid and medium-acid foods, the process is usually based on the heat-resistant, spoilage-type vegetative bacteria or enzymes that are destroyed easily even at temperatures below 100°C. The thermal processes for such foods are, therefore, normally carried out in boiling water.

THERMAL RESISTANCE OF MICROORGANISMS

The first step prior to establishing thermal processes is the identification or designation of the most heat-resistant or target microorganism or enzyme on which the process should be based. This requires the microbiological history of the product and conditions under which it is subsequently stored.

Heat is lethal to microorganisms, but each species has its own particular heat tolerance. During a thermal destruction process, such as pasteurization, the rate of destruction is logarithmic, as is their rate of growth. Thus bacteria subjected to heat are killed at a rate that is proportional to the number of organisms present. The process is dependent both on the temperature of exposure and the time required at this temperature to accomplish to desired rate of destruction. Thermal calculations thus involve the need for knowledge of the concentration of microorganisms to be destroyed, the acceptable concentration of microorganisms that can remain behind (spoilage organisms, for example, but not pathogens), the thermal resistance of the target microorganisms (the most heat tolerant ones), and the temperature-time relationship required for destruction of the target organisms.

<u>How # of Survivors is Determined (Tube Method)</u>

- **Inoculated menstruum (water, buffer solution, culture medium or food material) is distributed in small diameter (7 to 10 mm) test tubes and sealed. Volume of tubes are 1 to 4 ml,**
- **Transfer heat sealed test tubes in oil bath,**
- **At predetermined intervals (periodically) replicate tubes are removed and plunged into cold water. After cooling, the tubes are opened and their contents are transferred to tubes of steril culture medium and the # of survivors is determined. From these data, the survivor curve can be plotted on either a normal millimetrically divided paper (Figure 1) or on a semi-log paper as logN vs time (Figure 2).**

A typical thermal death curve for a heating process is shown below (Table 1 and the Figure 1). It is a logarithmic process, meaning that in a given time interval and at a given temperature, the same percentage of the bacterial population will be destroyed regardless of the population present. For example, if the time required to destroy one log cycle or 90% is known, and the desired thermal reduction has been decided (for example, 12 log cycles), then the time required can be calculated. If the number of microorganisms in the food increases, the heating time required to process the product will also be increased to bring

the population down to an acceptable level. The heat process for pasteurization is usually based on a 12D concept, or a 12 log cycle reduction in the numbers of this organism.

Table 1. Microbial population over time at constant temperature

Time (min)	# of survivors (or microbial population)
0	$1{,}0x10^6$
4	$1{,}1x10^5$
8	$1{,}2x10^4$
12	$1{,}2x10^3$

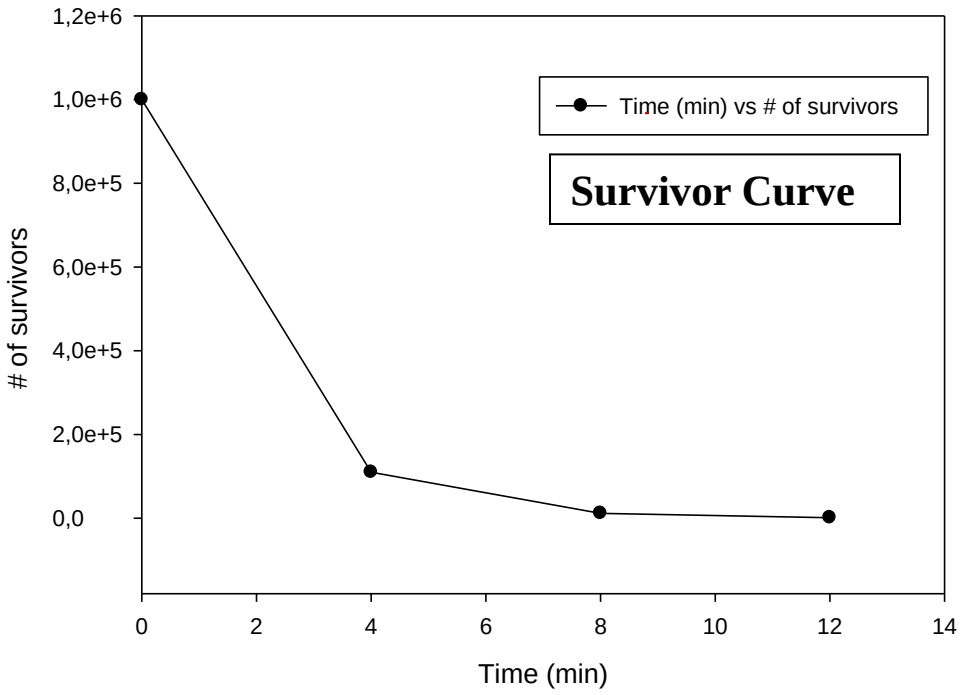


Figure 1. A typical plot of microbial population over time at constant temperature.

By ploting the same data on a semi-log ordinates, a straight line is obtained as shown below. The slope of the straight line is related to directly Decimal Reduction Time (D).

Semi-log paper; y-axis is the log scale, the x-axis is the regular scale.

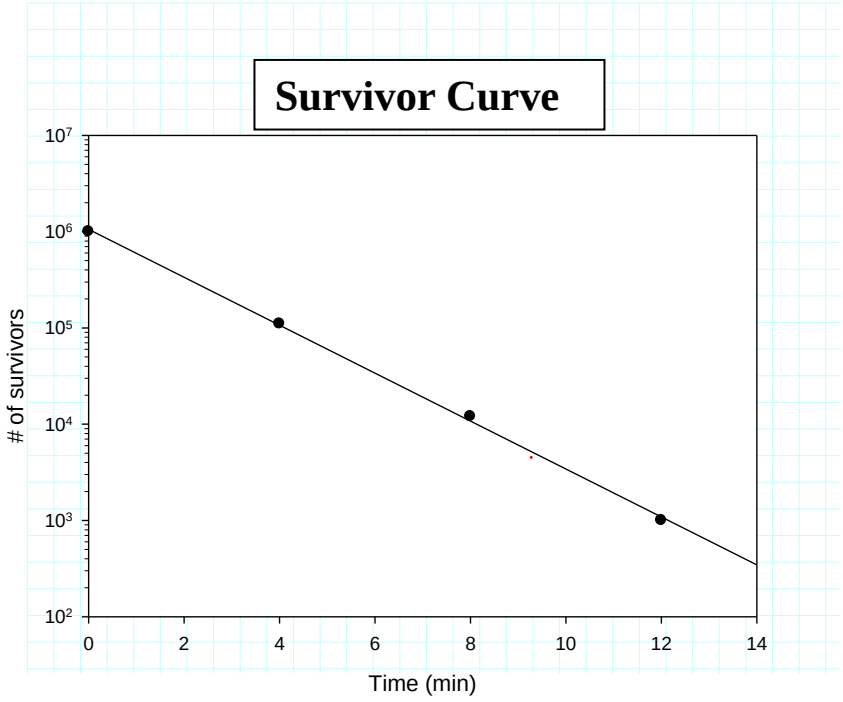


Figure. 2

DECIMAL REDUCTION TIME (D-VALUE)

The decimal reduction time is dependent on the temperature, the type of microorganism and the composition of the medium containing the microorganism.

D-value is the amount of time that it takes at a certain temperature to kill 90% (1-log cycle or 1D) of the target organisms being studied. Thus after an organism is reduced by 1D, only 10% of the original organisms remain. The population number has been reduced by one decimal place in the counting scheme. When referring to D values it is proper to give the temperature as a subscript to the D. For example, a hypothetical organism is reduced by 90% after exposure to temperatures of 300°F for 2 minutes, Thus the D-value would be written as D_{300F} = 2 minutes.

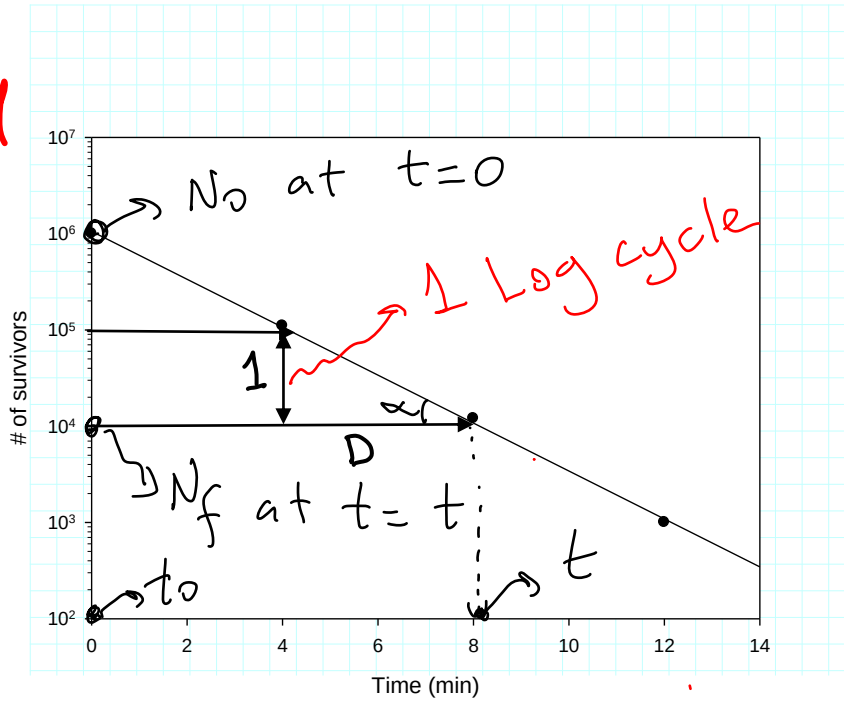
It is often more convenient to use the D-value as a measure of rate of microbial inactivation. For example, a D value at 72°C of 1 minute means that for each minute of processing at 72°C the bacteria population of the target microorganism will be reduced by 90%.

The D-value may be estimated graphically (see graph below) or mathematically from the equation derived below.

Initial

Reduction

Final



$$\text{Slope} = \text{tg}\alpha \quad \text{or} \quad \text{Slope} = \frac{\Delta y}{\Delta x} = \frac{y_2 - y_1}{x_2 - x_1}$$

$$-\frac{1}{D} = \frac{\log N_f - \log N_0}{t - t_0} \xrightarrow{t_0 = 0}$$

$$-\frac{1}{D} = \frac{\log N_f - \log N_0}{t} \Rightarrow$$

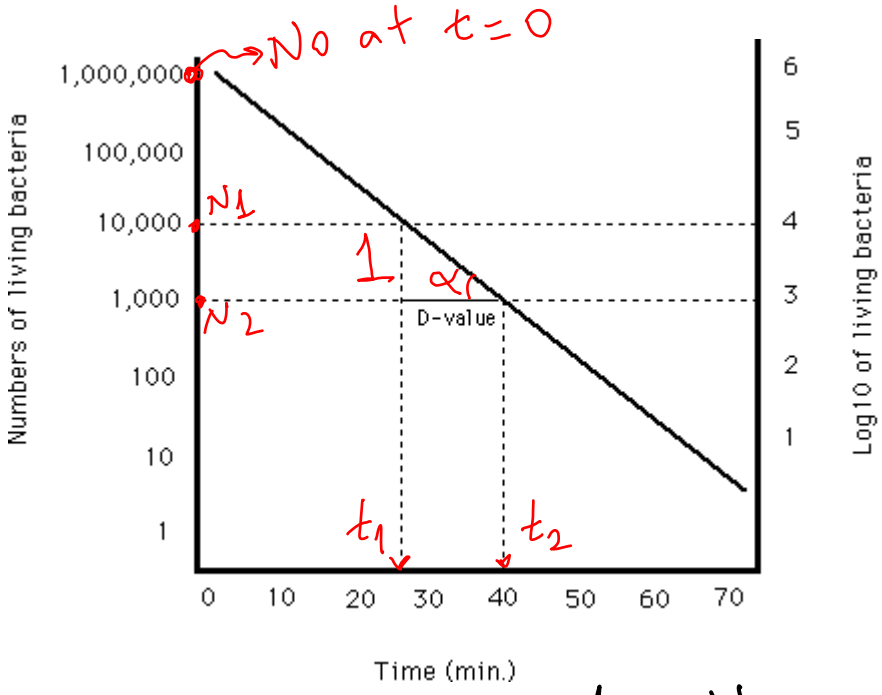
$$\frac{1}{D} = \frac{\log N_0 - \log N_f}{t} \Rightarrow D = \frac{t}{\log N_0 - \log N_f} \Rightarrow$$

$$\boxed{D = \frac{t}{\log \frac{N_0}{N_f}}} \Rightarrow \boxed{t = D \times \log \frac{N_0}{N_f}}$$

vc

$$\boxed{\log \frac{N_0}{N_f} = \frac{t}{D}} \Rightarrow \boxed{\frac{N_0}{N_f} = 10^{t/D}} \text{ or } \boxed{\frac{N_f}{N_0} = 10^{-\frac{t}{D}}}$$

In the illustration below, the D value is 14 minutes (40-26) and would be representative of a process at 72°C.



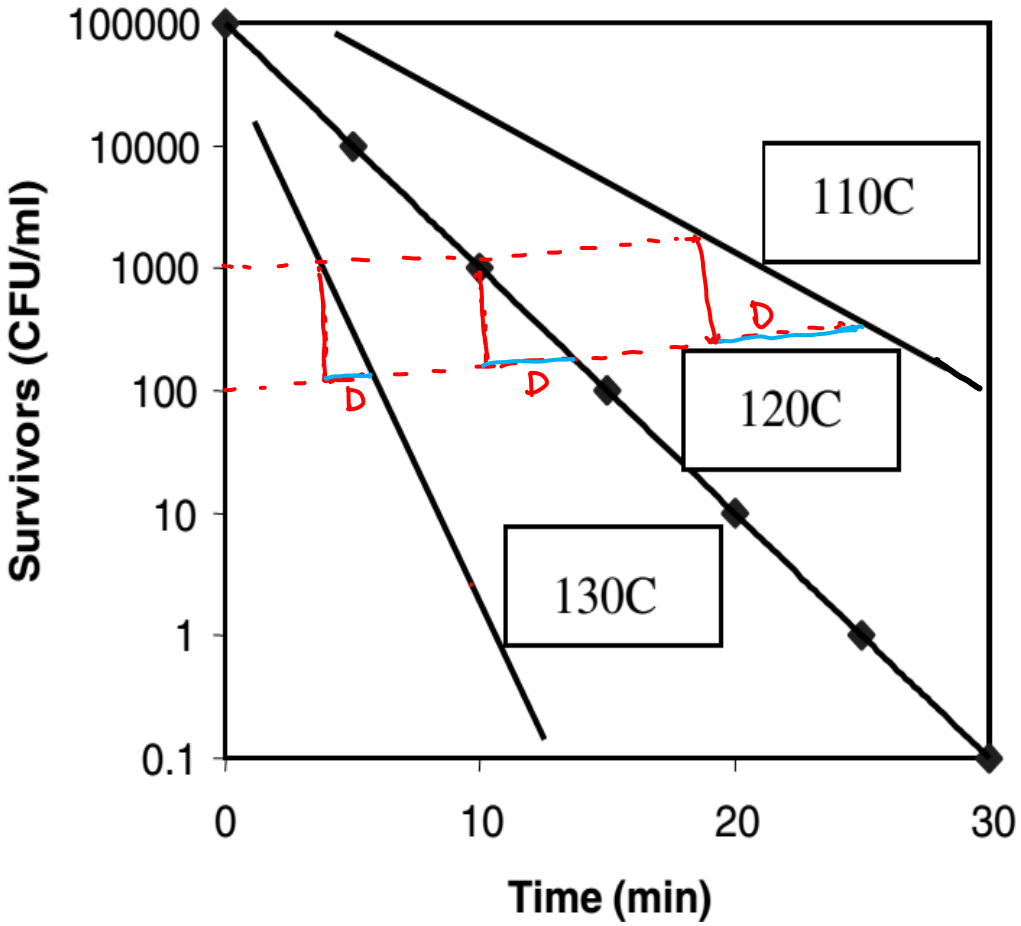
$$Slope = -\frac{1}{D}$$

$$Slope = tg\alpha = \frac{\Delta y}{\Delta x} = \frac{y_2 - y_1}{x_2 - x_1}$$

$$-\frac{1}{D} = \frac{\log N_2 - \log N_1}{t_2 - t_1}$$

$$-\frac{1}{D} = \frac{\log(1000) - \log(10000)}{40 - 26} \Rightarrow D = 14 \text{ min.}$$

Exposure of the microbial population to higher temperatures results in a decrease in the D value.



$D_{130} < D_{120} < D_{110}$ for 1 log (90%) reduction of m.o.

Figure of survivor curves as influenced by temperature.

THE 12D PROCESS

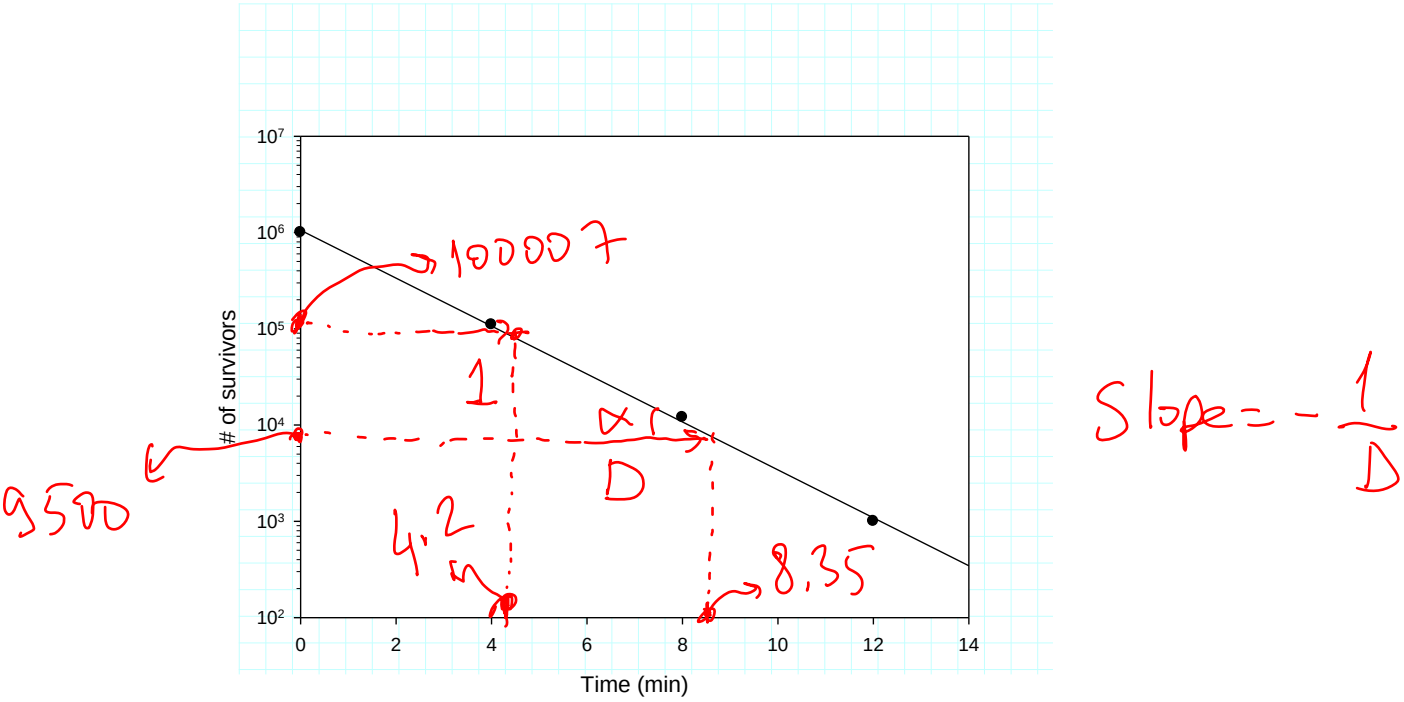
Canned foods are susceptible to the spores of the organism *Clostridium botulinum*. This is the organism that causes botulism. These bacterial spores can survive many heat treatment processes. However, in modern food production, canned foods are subjected to a time-temperature process that will reduce the probability of the survival by the most heat-resistant *C. botulinum* spores by 12 logs or 12D at the reference temperature of 250°F (the temperature used in the calculation of most commercial 12D processes is 250°F, and the D-value for this organism at 250°F is 0.21 minutes). This process is based on the assumption of the number of surviving spores in one can. If we assume that there are 10 surviving spores in one can initially, then we can calculate the time for a 12D process to occur by using the following formula:

- **$F_0 = D_{250°F}(\log No - \log N_f)$, where No = initial population and N_f = final population.**
- **So $F_0 = 0.21\text{min}.(\log 10^1 - \log 10^{-11})$, we move down 12 log values (1 - (-11)) = 12**
- **So, $F_0 = 0.21\text{min}.(1 - (-11))$, or 0.21 x 12 = 2.52 minutes.**

OR simply, Fo=Do.12 = 0.21 x 12 results in a 12-D process.

Example: **The data given in Table 1 (previously given above) were obtained from thermal resistance experiment conducted on a spore suspension at 112°C. Determine the D value.**

Solution:



First way:

$$D = \frac{t}{\log \frac{N_0}{N_f}} = \frac{12}{\log \frac{1 \times 10^6}{1.2 \times 10^3}} = 4.1 \text{ min}$$

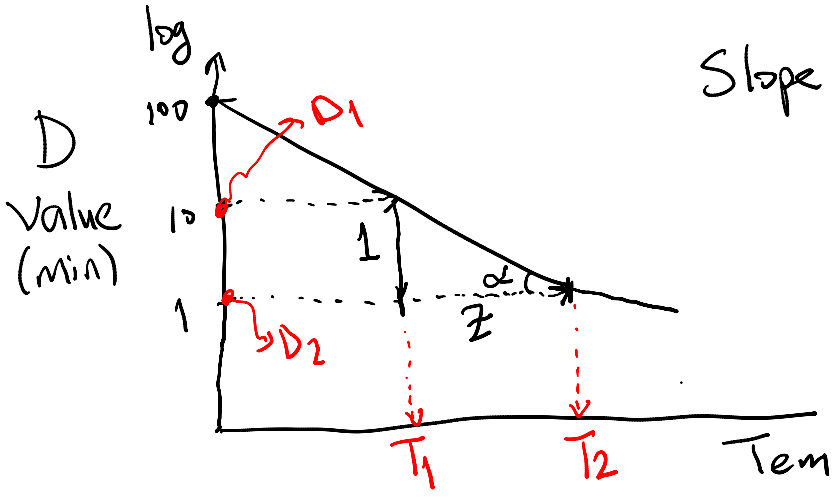
Second way: Calculate from slope: 1/D = [log(100007)-log(9500)]/(8.35-4.2)= 4.06 min

THE Z-VALUE (THERMAL RESISTANCE CONSTANT)

The Z-value is a unique factor describing thermal resistance of the bacterial spores. It is defined as the increase in temperature necessary to cause 90 % reduction in the Decimal Reduction Time D. It is a measure of the change in death rate with a change in temperature.

It is the number of degrees Fahrenheit or Centigrade required for a thermal death time curve to traverse 1-log cycle. This is the temperature increase required to reduce the thermal death time by a factor of 10. The z-value gives an indication of the relative impact of different temperatures on a microorganism, with smaller Z values indicating greater

sensitivity (high temperature dependence) to increasing heat. The large Z values require large changes in temperature to reduce the time. The D values for different temperatures are plotted on a semi-log coordinates and the temperature increase for a 1-log cycle in D values represents the Z value. Z value is determined graphically (see graph below) or mathematically from the equation derived below.



$$\text{Slope} = -\frac{1}{z} = \frac{\Delta y}{\Delta x} = \frac{y_2 - y_1}{x_2 - x_1}$$

$$-\frac{1}{z} = \frac{\log D_2 - \log D_1}{T_2 - T_1} = -\frac{(\log D_1 - \log D_2)}{T_2 - T_1} \Rightarrow$$

$$z = \frac{T_2 - T_1}{\log D_1 - \log D_2} \Rightarrow \log D_1 - \log D_2 = \frac{T_2 - T_1}{z} \Rightarrow$$

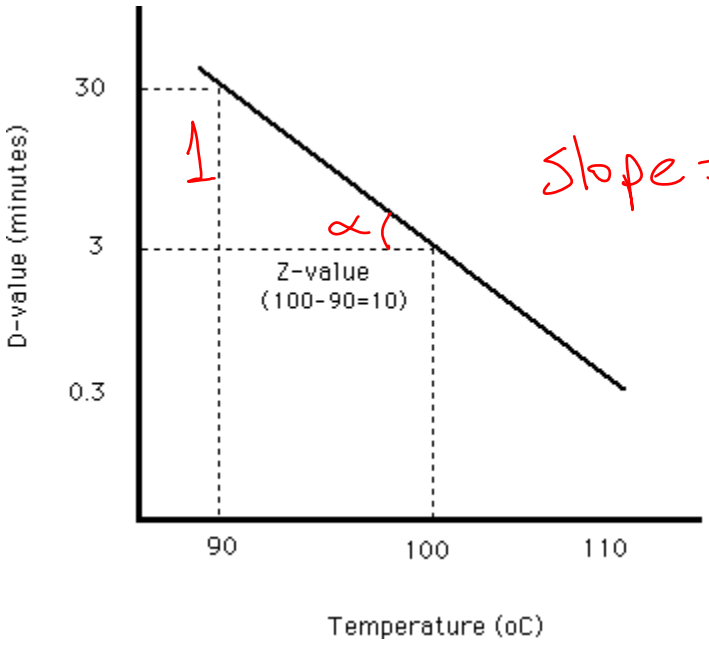
$$\log \frac{D_1}{D_2} = \frac{T_2 - T_1}{z} \Rightarrow \frac{D_1}{D_2} = 10^{\frac{T_2 - T_1}{z}}$$

D_2 = D value corresponding to T_2 and the time required to destroy 90 % of the cell population when exposed to T_2.

D_1 = D value corresponding to T_1 and the time required to destroy 90 % of the cell population when exposed to T_1.

While the D-value gives us the time needed at a certain temperature to kill an organism, the z-value relates the resistance of an organism to differing temperatures. So, the z-value allows us to calculate a thermal process of equivalency, if we have one D-value and the z-value. So, if it takes an increase of 10°F to move the curve one log, then our z-value is 10.

So then, if we have a D-value of 4.5 minutes at 150°F, we can calculate D-values for 160°F by reducing the time by 1 log. So, our new D-value for 160°F is 0.45 minutes. This means that each 10°F increase in temperature will reduce our D-value by 1 log. Conversely, a 10°F decrease in temperature will increase our D-value by 1 log. So, the D-value for a temperature of 140°F would be 45 minutes.



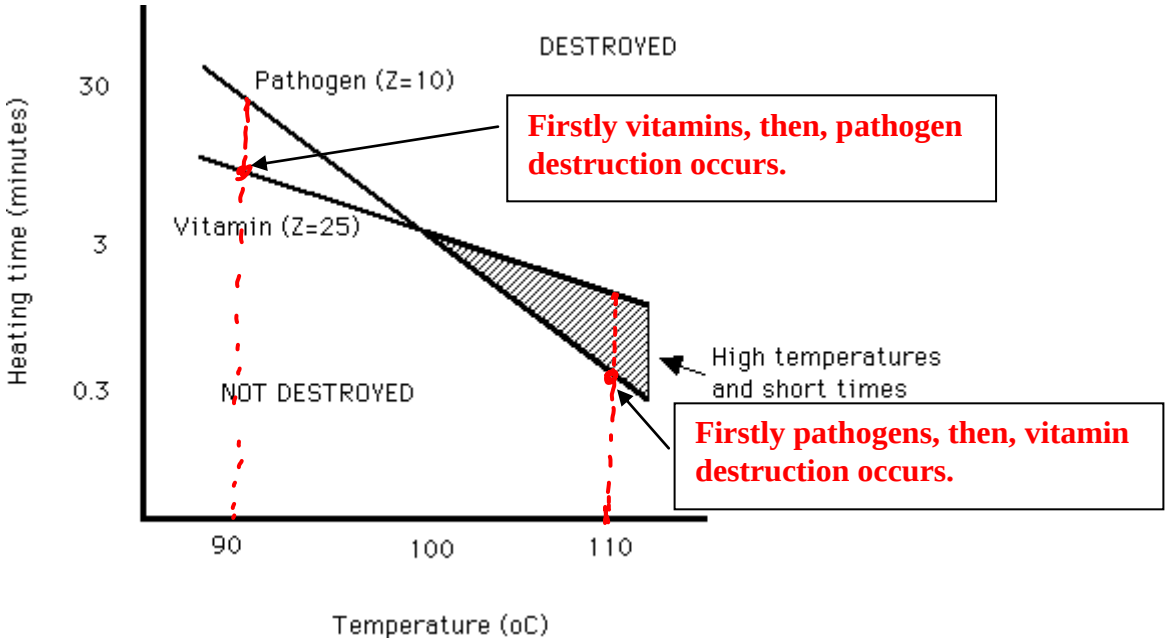
Slope $= -\frac{1}{Z}$

In this figure the Z value is 10°C.

A Z value of 10°C is typical for a spore forming bacterium. Heat induced chemical changes have much larger Z values that microorganisms, as shown below.

	Z (°C)	D_{121} (min)
bacteria	**5-10**	**1-5**
enzymes	**30-40**	**1-5**
vitamins	**20-25**	**150-200**
pigments	**40-70**	**15-50**

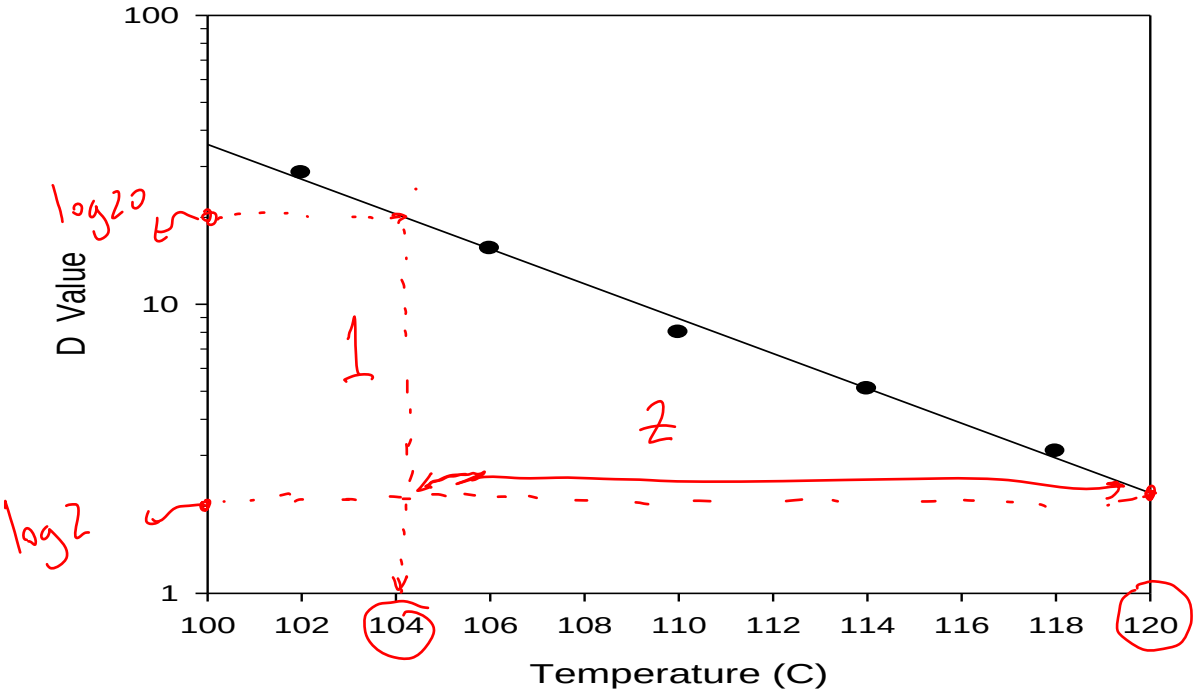
The figure below illustrates the relative changes in time temperature profiles for the destruction of microorganisms. Above and to the right of each line the microorganisms or quality factors would be destroyed, whereas below and to the left of each line, the microorganisms or quality factors would not be destroyed. Due to the differences in Z values, it is apparent that at higher temperatures for shorter times, a region exists (shaded area) where pathogens can be destroyed while vitamins can be maintained. The same holds true for other quality factors such as colour and flavour components. Thus in milk processing the higher temperature, shorter time (HTST) process (72°C/15 sec) is favored compared to a lower temperature longer time (batch or vat) process since it results in a slightly lower loss of vitamins and better sensory quality.



Alkaline phosphatase is a naturally-occurring enzyme in raw milk which has a similar Z value to heat-resistant pathogens. Since the direct estimation of pathogen numbers by microbial methods is expensive and time consuming, a simple test for phosphatase activity is routinely used. If activity is found, it is assumed that either the heat treatment was inadequate or that unpasteurized milk has contaminated the pasteurized product.

<u>Example:</u> Determine Z value for a spore suspension if the following D values were obtained for different temperatures.

T (°C)	102	106	110	114	118
D value	28.5	15.6	8	5.1	3.1

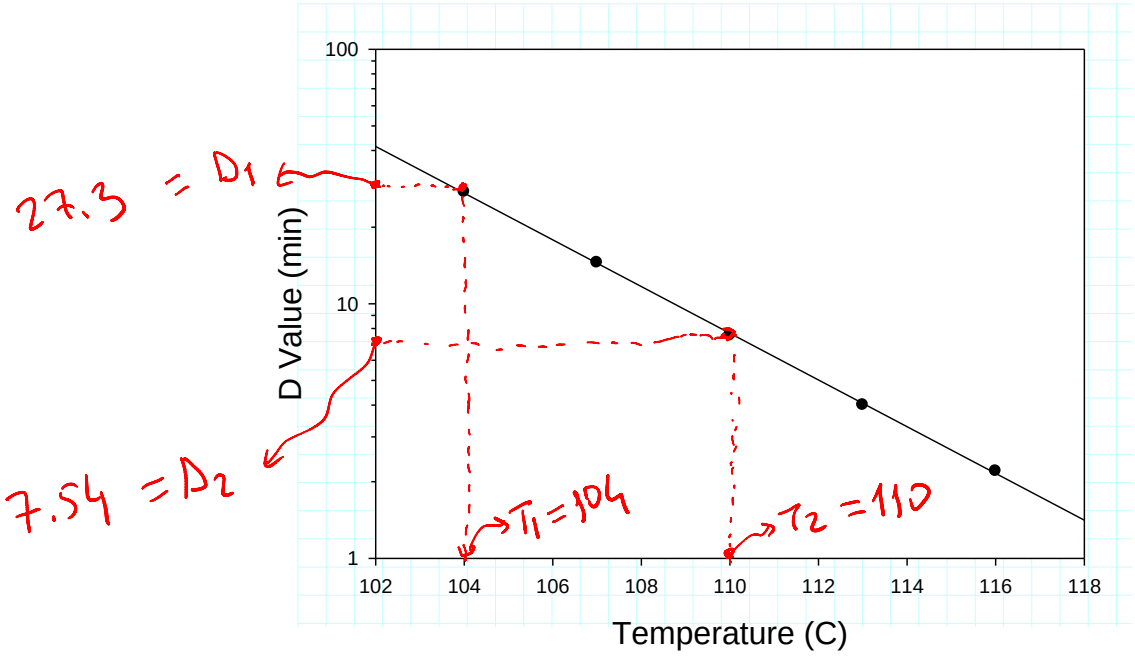


Slope = - 0.0603 = - 1/Z, then, Z = 16.58°C (slope – 0.0603 is obtained from linear regression of the line)

-1/Z = [log20-log2]/(104-120) = -1/16 ⟹ Z = 16°C.

Homework: The decimal reduction times (D) for a spore suspension were measured at several temperatures as given below. Determine thermal resistance constant Z value.

T (C)	104	107	110	113	116
D value (min)	27.5	14.5	7.5	4	2.2



$$z = \frac{T_2 - T_1}{\log D_1 - \log D_2} = \frac{110 - 104}{\log(27.3) - \log(7.54)} \cong 11$$

METHODS FOR HEATING PROCESSING

1. **Processing at temperatures below 100°C (Pasteurization)**
   It is usually applied to high acid foods
2. **Processing at temperatures above 100°C (Sterilization, UHT, etc.)**
   It is usually applied to low acid foods (most food materials are low acid foods)

PASTEURIZATION AND STERILIZATION DEFINITIONS

Pasteurization

Pasteurization is one type of preservation by heat that most people are familiar with. Pasteurization is a mild heat treatment given to foods, with the purpose of destroying selected vegetative microbial species (especially the pathogens) or inactivating the enzymes. Because the process does not eliminate all vegetative bacteria-and almost none of the spore formers-pasteurized foods need to be contained and stored under conditions of refrigeration, with or without chemical additives or modified atmosphere packaging, to minimize microbial growth.

This process involves heating a particular food to a certain temperature and keeping that temperature over a specific amount of time (holding time) to kill the organisms *Mycobacterium tuberculosis* and *Coxiella burnetii*. These two organisms are the most heat resistant of pathogens that are not spore forming. Milk is a product that most people know

is pasteurized. There are many different time/temperature combinations that can be used in the pasteurization of milk. The LTLT (low-temperature-long-time) process involves bringing the milk to a temperature of 145°F (63°C) for 30 minutes. Conversely, the HTST (high-temperature-short-time) method brings the milk to a temperature of 161°F (72°C) for 15 seconds. Both of these processes accomplish the same thing: the destruction of *Mycobacterium tuberculosis* and *Coxiella burnetii*. So, you can see that not only is temperature important, but the time at that temperature is also important.

Organisms that can survive pasteurization temperature belong to the groups of organisms referred to as thermodurics and thermophiles. Thermoduric organisms are those that can survive at high temperature, but dot necessarily grow and reproduce at those temperatures. Thermophiles are organisms that can grow and reproduce at high temperatures.

Sterilization

Sterile: Free from viable micro-organisms

Sterilization is any physical or chemical process which destroys all life forms, with special regard to microorganisms (including bacteria and sporogenous forms), and inactivates viruses.

Some products are referred to as commercially sterile (Shelf stable food). This means that no viable organisms can be grown from traditional culture methods. In other words, the product should have been subjected to a heat treatment having a sufficiently high lethal effect so that - after incubation at 30°C or 35°C for 5 days - no spoilage occurs and the changes in flavor, odor, color and nutritional value are minimized. In addition to ensuring the destruction of microorganisms, the heat treatment of milk also results in a number of other reactions and changes occurring.

The main changes are:

- **Inactivation of enzymes**
- **Denaturation and complex formation**
- **Maillard browning reactions**
- **Losses of vitamins**
- **Losses of amino acids**

Sterilization is a more severe heat treatment intended to destroy microorganisms of both spoilage and public health concern, after packaging the food in a hermetically sealed container. In essence, it represents a thermal process in which foods are exposed to a high enough temperature for a sufficiently long time to render them commercially sterile. Sterilization, implying destruction of all viable microorganisms, is not the appropriate word to be used for thermal processing of foods, because these foods are not sterile. The success of thermal processing does not lie in destroying all viable microorganisms. The heat process given is designed to kill microorganisms of spoilage and public health concern

and is combined with vacuum sealing, hermetic packaging, and moderate storage temperatures to limit the growth of other microorganisms (especially the thermophiles). The sterilization process takes into account the heat resistance of the spore formers in addition to their growth sensitivity to oxygen, pH, and temperature.

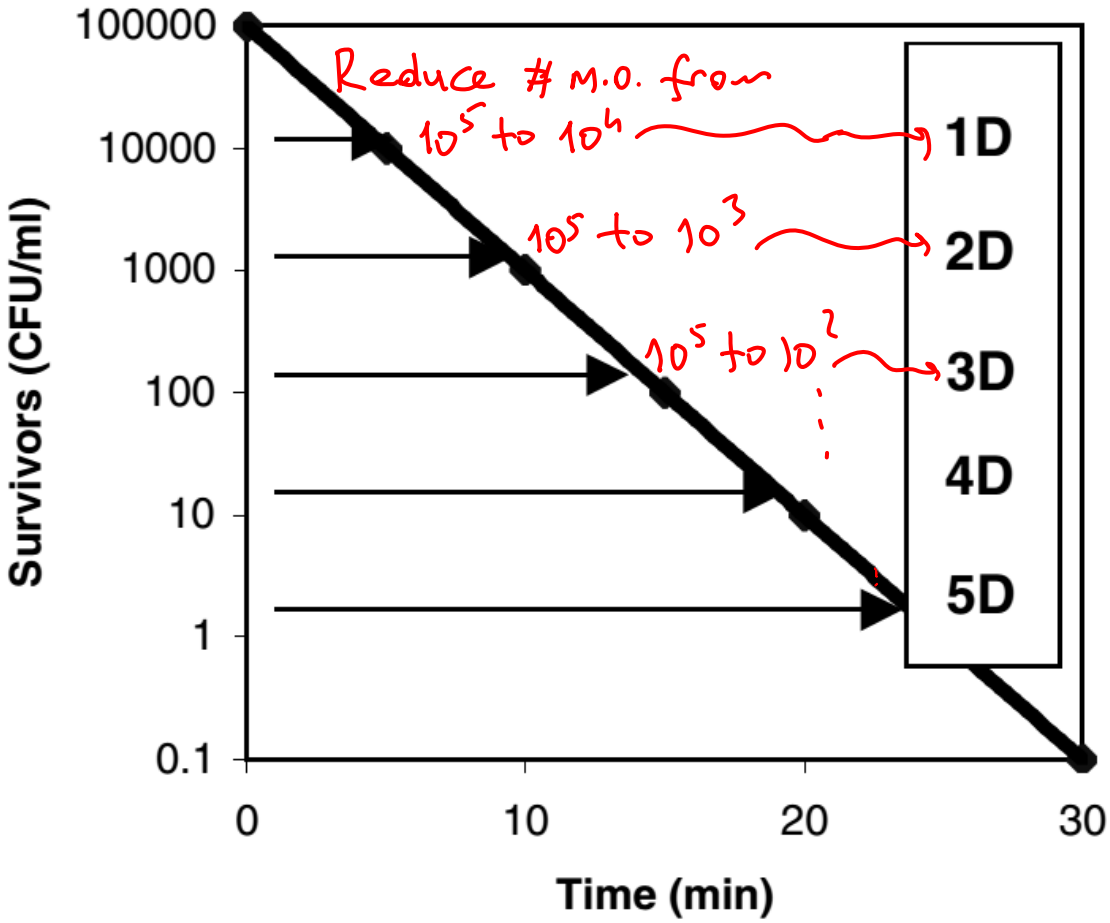
THERMAL DEATH TIME (TDT)

TDT is the heating time required to cause complete destruction of a microbial population at any temperature. Such data are obtained by subjecting a microbial population to a series of heat treatments at a given temperature and testing for survivors. TDT then represents a time between the shortest destruction and the longest survival times. The difference between the two is sequentially reduced or geometrically averaged to get an estimate of TDT. The "death" in this instance generally indicates the failure of a given microbial population, after the heat treatment, to show a positive growth in the subculture media. Comparing the TDT approach with the decimal reduction approach, one can easily recognize that TDT value depends on the initial microbial load (whereas D value does not). Further, if TDT is always measured with reference to a standard initial load or load reduction, it would simply represent a certain multiple of D value. For example, if TDT represented the time to reduce the population from 10^5 to 10^1, then TDT is a measure of 4-D values. On the other hand, if it is based on 10^6 to 10^{-6}, it would represent a 12-D value. In other words:

TDT = n D, where n is the number of decimal reductions (# of log cycles).

TDT is expressed as a multiple of D values. For example, a 99.99% reduction in microbial population is equivalent to 4 log cycle (4D) reductions.

Typically, in thermal processing of shelf-stable foods the value used for TDT is F = 12D with the D value for *C. botulinum*.



Thermal Death Time Graph

STERILIZING EFFECT OR LETHALITY CONCEPT (F VALUE)

The sterilizing effect, which is also called lethality or death rate, indicates the effect of a heat treatment, expressed as the number of decimal reductions in the number of microorganisms.

We need a procedure to compare effect of different heating treatments at different time periods to reduce microbial population. For this purpose, we determine Process Lethality also called F value or thermal death time.

The criterion for the adequacy of a process must be based on two microbiological considerations:
1) destruction of the microbial population of public health significance
2) reduction in the number of spoilage-causing bacteria.

For low-acid foods, the microorganism of public health significance is *C. botulinum* and, hence, the destruction of the spores of this organism is used as the minimal criterion for

processing. Once again, it has been arbitrarily established that the minimum process should be at least as severe to reduce the population of *C. botulinum* through 12 decimal reductions (12D).

F-VALUE

F value is the time required to destroy a given # of microbial population at a specific temperature. Lethality (F value) is a measure of the heat treatment or sterilization processes. In order to compare the relative sterilizing capacities of heat processes, a unit of lethality needs to be established. For convenience, this is defined as an equivalent heating of 1 min at a reference temperature, which is usually taken as 250°F (121.1°C) for the sterilization processes. Thus, the F value would represent a certain multiple or fraction of the D value, depending on the type of microorganism; therefore, a relationship like Equation (**) below holds good also with reference to F value.

Remember; $z = \dfrac{T_2 - T_1}{\log D_1 - \log D_2} \Rightarrow D_1 = D_2 \times 10^{[(T_2 - T_1)/z]}$

If $T_2 = T_{ref} = T_0 \Rightarrow D_2 = D_{ref} = D_0$, then

$D = D_0 \times 10^{[(T_0 - T)/z]} \Rightarrow \dfrac{D_T}{D_0} = 10^{[(T_0 - T)/z]}$

Similarly, $F = F_0 \times 10^{[(T_0 - T)/z]} \Rightarrow \dfrac{F_T}{F_0} = 10^{[(T_0 - T)/z]}$

$\boxed{\dfrac{D_T}{D_0} = \dfrac{F_T}{F_0} = 10^{[(T_0 - T)/z]}}$ → eqn (**)

OR $F_0 = F \times 10^{[(T - T_0)/z]} \Rightarrow F_0 = t \times 10^{[(T - T_0)/z]}$ (where $F \rightarrow t$)

The Fo in this case will be the F value at the reference temperature (To). It is the number of minutes required for a process to kill a known population (specified) of microorganisms at the reference temperature of 121.2°C (250°F) when Z is 10°C (18°F).

It is possible to express the lethal effects of any time-temperature combination in terms of equivalent minutes at 250°F or lethality. It is assumed that the heating to the appropriate temperatures and the subsequent cooling are instantaneous. For real processes where the food passes through a time-temperature profile, it should be possible to use this concept to integrate the lethal effects through the various time-temperature combinations. The combined lethality so obtained for a process is called the process lethality and is also represented by the symbol Fo.

The value is used as a basis for comparing heat sterilization procedures. This F value is usually set at 12 D values to give a theoretical 12 log cycle reduction of the most heat-resistant species of mesophilic spores in a can of food. For example, if there were 10,000 spores of a species of spore in a can of food and a 12 D process was given, the initial 10,000 spores (10^4 spores) would be reduced to a theoretical 10^{-8} living spores per can, or again in theory, one living spore per 10^8 cans of product (one spore per one hundred million cans). To refer back to the original example where the D_{240} was 1 min., the F value for the process would be 12 min. or F_{240} = 12 min.

Remember, for *C. botulinum*, $D_{121.1}$ = 0.21 min, then, for 12D inactivation Fo = 0.12*12 = 2.52 min is required. The minimum process lethality (Fo) is therefore 2.52 min. Several low acid foods are processed beyond this minimum value.

It is customary to express F with a subscript denoting temperature and a subscript of the Z value for the microorganism involved.

$$F^{Z}_{T} \Longrightarrow F^{10}_{121} \text{ OR } F^{18}_{250}$$

SI → F^{10}_{121} ; British → F^{18}_{250}

When F is used without a subscript indicating temperature, 250ºF (or 121.1°C) is assumed.

For the references purposes, F^{10}_{121} is commonly used. It is simply written as F_0, represents the time for a given reduction in population of a microbial spore with a Z value of 10°C (18ºF) at 121°C (or 250ºF).

Fo value at a particular temperature other than 121°C is the time in minutes required to provide the lethality equivalent to that at 121°C for a stated time.

Fo value of a saturated steam sterilization process is the lethality expressed in terms of equivalent time in minutes at a temperature of 121°C delivered by the product in its final container with reference to microorganisms processing a Z value of 10.

The lethality of a thermal process is expressed as its Fo value. The Fo value of any thermal sterilization process is the number of minutes of heating at 121°C required to achieve the same thermal destruction ratio of a specified target microorganism, having a Z value of 10°C.

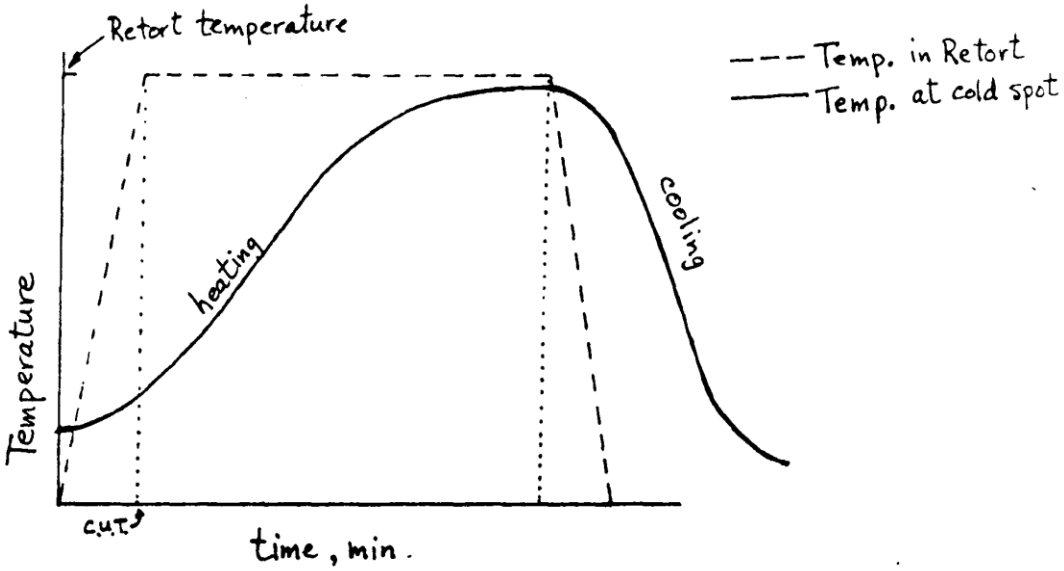
DETERMINATION OF THERMAL PROCESS TIME

The purpose of the thermal process calculations is to arrive at an appropriate process time under a given set of heating conditions, which will result in a target process lethality, or alternately, to estimate the process lethality of a given process. The method used must accurately integrate the lethal effects of the transient temperature response of the food undergoing the thermal processes, with respect to test microorganism of both public health and spoilage concern. The desired degree of lethality (Fo) in terms of an equivalent time at a reference temperature is generally preestablished for a given product, and processes are designed to deliver a minimum of this preset value at the thermal center. The process calculation methods are broadly divided into two classes:

1) General methods
2) Formula methods.

The General methods integrate the lethal effects by a numerical or graphical integration procedure based on the time-temperature data obtained from test containers processed under actual commercial processing conditions. Formula methods, on the other hand, make use of parameters obtained from the heat penetration data together with several mathematical procedures to integrate the lethal effects.

In order to determine the thermal process time, we must measure the temperature of the can at the slowest heating point periodically. For every temperature there is a sterilizing effect which we can calculate. A heat penetration curve for the slowest heating spot in a container processed in a batch steam retort may be similar to the following figure:



a) Numerical Integration Method

Remember; $t = D \times \log \frac{N_0}{N_f}$ at constant T.

Substituting T = 250°F or 121.1°C as the standard (reference) temperature into this equation and substituting F for t, then,

$F_0 = D_0 \cdot \log \frac{N_0}{N_f}$; $D_0 = D_T$: Decimal reduction time at Tref.

$F_0 = t \times 10^{(T-121.1)/z}$ ---→ SI , T in °C.

$F_0 = t \times 10^{(T-250)/z}$ ---→ English, T in °F.

This is the Fo value in min for the given thermal process at a given constant temperature T and a given heating time t in min. The effect of different but successive sterilization processes in a given material are additive. Hence, for several different temperature stages T_1, T_2, and so on, each having different times t_1, t_2, …, the Fo values for each stage are added to give theoretiacal Fo value.

Note that: The general equations used for pasteurization are similar to sterilization.

$$F_o = t_1 \times 10^{(T_1 - T_o)/z} + t_2 \times 10^{(T_2 - T_o)/z} + t_3 \times 10^{(T_3 - T_o)/z} + \ldots$$

Example: Cans of a given food were heated in a retort for sterilization. The Fo for Cl. Botulinum in this type of food is 2.50 min and Z = 18°F. The temperatures in the center of a can (the slowest heating region) were measured and were approximately as follows, where the average temperature during each time period is listed:

t_1 = 20 min, T_1 = 160 °F
t_2 = 20 min, T_2 = 210 °F
t_3 = 33 min, T_3 = 230 °F
Determine if this sterilization process is adequate. Use English units.

Solution:

$$F_o = t_1 \times 10^{(T_1 - T_o)/z} + t_2 \times 10^{(T_2 - T_o)/z} + t_3 \times 10^{(T_3 - T_o)/z}$$

$$= 20 \times 10^{(160-250)/18} + 20 \times 10^{(210-250)/18} + 33 \times 10^{(230-250)/18}$$

$$= 2.68 \text{ min.}$$

Hence, this thermal processing is adequate since only 2.50 min is needed for complete sterilization (i.e; F_o calculated > F_o standard (ref.))

2.68 min > 2.50 min

Example: If the Fo value given in the problem (Fo reference or Fo standard) were 2.80 min, then, what would be your decision ? Is the sterilization process adequate or not ? If not, what to do ? We need additional heating as short as possible in order not to loss nutritional value of the food.

Solution:

Since F_o calculated (2.68 min) < F_o ref. (2.80 min) $\Rightarrow$ the sterilization process is not adequate.

$\Rightarrow$ Heat at the highest $T = 230^\circ F$.

$2.80 - 2.68 = 0.12$ min of F_o (equivalent heating) is needed.

$$0.12 = t \times 10^{(230-250)/18} \Rightarrow t = 1.54 \text{ min.}$$

We need additional 1.54 min heating at 230°F.

Total heating time at 230°F = 33 + 1.54 = 34.54 min.

b) Graphical Integration Method

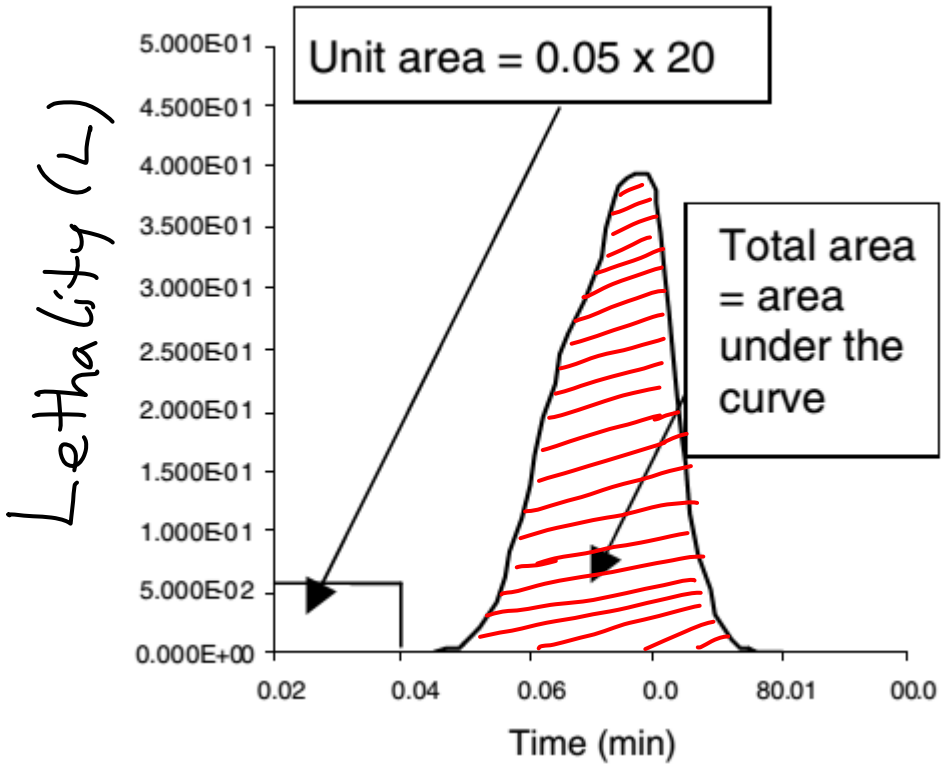
In general case when cans are being sterilized in a retort, the temperature is not constant for a given time period but varies continuously with time. For a continuously varying temperature T we take small time increments of *dt* min for each value of T and sum up. Then, the final equation is

$$F_o = \int_{t=0}^{t} 10^{(T-250)/z} \times dt = \int_{0}^{t} L \times dt \rightsquigarrow \text{English, eqn (1)}$$

$$F_0 = \int_{t=0}^{t} 10^{(T-121.1)/z} \times dt = \int_{0}^{t} L \cdot dt \rightsquigarrow SI,\ eqn(2)$$

where, L is the lethality, $L = 10^{(T-T_{ref})/z}$.

This equation can be used as follows. Suppose that the temperature of a process is varying continuously and a graph (heat penetration graph) or a table of values of T versus time is known. The equations 1 or 2 (given above) can be integrated graphically by plotting values of lethality (L) versus time (t) as shown below and taking the area under the curve. The graph paper; both y-axis and x-axes are the regular scale.



<u>Example</u>: In the sterilization of a canned puree, the temperature in the slowest heating region (center)of the can was measured giving the following time-temperature data for heating and holding time. The cooling time data will be neglected as a safety factor.

t (min)	0	15	25	30	40	50	64
T (°F)	80	165	201	212.5	225	230.5	235

The Fo value of *Cl. botulinum* is 2.45 min and Z value is 18°F. Calculate the Fo value of the process graphically and determine if the sterilization is adequate.

Solution:

Remember $L = 10^{(T-T_{ref})/z}$ $\Rightarrow T_{ref} = 250°F$

Let's calculate L values for each time t.

$t_1 = 0$ min, $T_1 = 80°F \Rightarrow L_1 = 10^{(80-250)/18} = 3.6 \times 10^{-10}$

$t_2 = 15$ min, $T_2 = 165°F \Rightarrow L_2 = 10^{(165-250)/18} = 0,0000189$

$t_3 = 25$ min, $T_3 = 201°F \Rightarrow L_3 = 10^{(201-250)/18} = 0,00189$

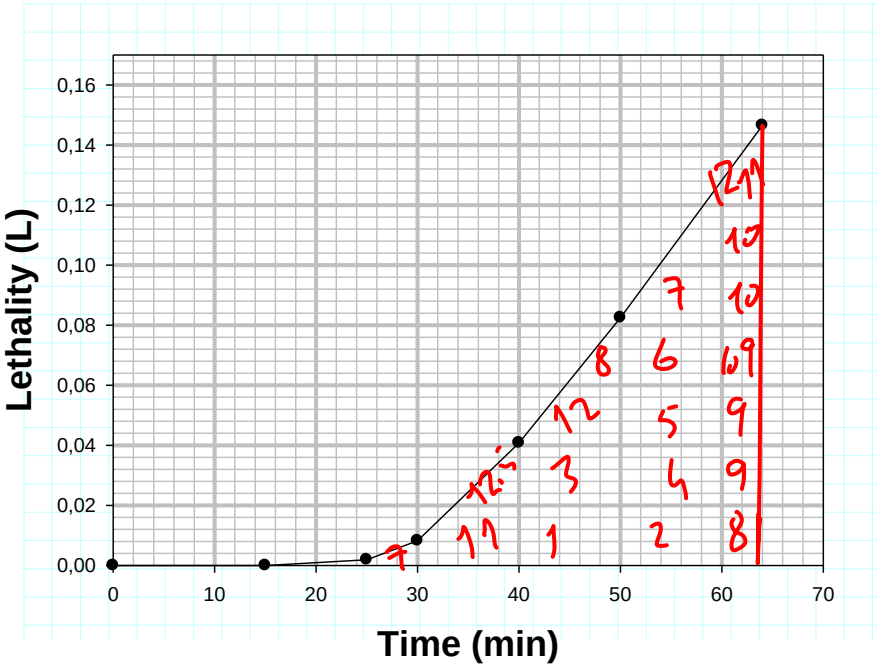
$t_4 = 30$ min, $T_4 = 212.5°F \Rightarrow L_4 = 10^{(212.5-250)/18} = 0,00825$

$t_5 = 40$ min, $T_5 = 225°F \Rightarrow L_5 = 10^{(225-250)/18} = 0,0408$

$t_6 = 50$ min, $T_6 = 230.5°F \Rightarrow L_6 = 10^{(230.5-250)/18} = 0,0825$

$t_7 = 64$ min, $T_7 = 235°F \Rightarrow L_7 = 10^{(235-250)/18} = 0.1465$

These Lethality (L) values are plotted versus time (t) as shown in the figure below. The sum of the areas of the various rectangles (under the curve) gives process Fo value in min.



Area under the curve is equal to process F_o value.

0,02 L ↕ 1 square ⇒ $A = 0,02 \times 10 = 0,2$

10 s, time

Total area = 12.5 squares $\times \frac{0,2}{\text{square}} \cong 2.5$ min.

Since the process time (2.5 min) is greater than the reference F_o value (2.45 min), then, the sterilization is adequate.

i.e., F_o calculated > F_o reference (required), the sterilization process is adequate.

<u>Example:</u> Fo value given for the thermal processing of milk in a tubular heat exchanger is F^{9}_{150} = 9 min and D_{150} = 0.6 min. Calculate the reduction in the number of viable cells for these conditions.

<u>Solution:</u>

$$F_o = D_o \times \log \frac{N_o}{N_f} \Rightarrow 9 = 0,6 \times \log \frac{N_o}{N_f} \Rightarrow 15 = \log \frac{N_o}{N_f}$$

$\Rightarrow \frac{N_0}{N_f} = 1 \times 10^{15} = \frac{10^{15}}{1} \Rightarrow$ the reduction is from 10^{15} cells to 1 cell at the final.

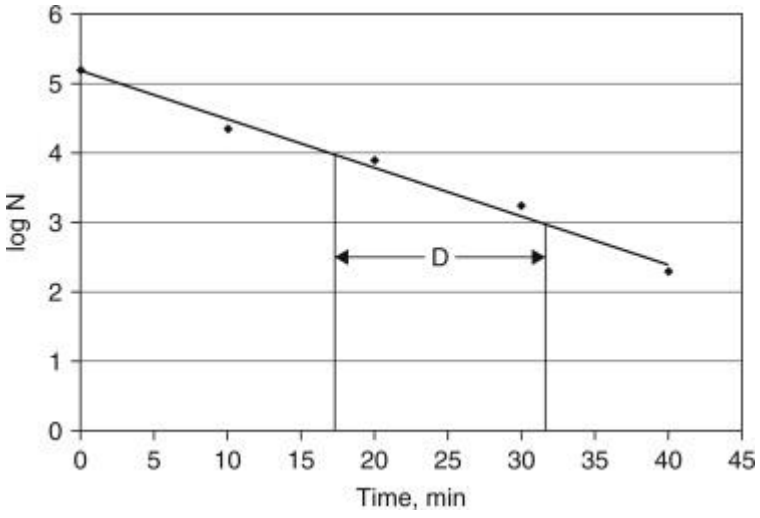
<u>Example</u>: A suspension of bacterial spores containing 160000 spores per ml is heated at 110°C. The number of survivors is determined in samples withdrawn every 10 minutes. The results are:

Heating time	N, survivors /ml
0	160 000
10	25 000
20	8000
30	1600
40	200

Assuming 'first order' kinetics, calculate the decimal reduction time.

<u>Solution</u>:

Log N is plotted against the heating time t. Good agreement with the first <u>order theory</u> is observed, within the range of the data. The decimal reduction time is determined from the regression line given in the figure. The result is: D=13.4 min



<u>Homework</u>: Solve the same example numerically.

SPOILAGE PROBABILITY

Remembert; t/D = log(N_0/N) = Sterilizing Value (SV)

The spoilage probability is used to estimate the # of spoiled containers within a total batch of processed product for total exposure of TDT, F.

log(N_0/N) = F/D

If r is the # of containers processed, N_0 = initial # of spores per container, then, the total microbial load at the beginning of the process = r.N_0

log(r.N_0/r.N) = F/D

If the goal of a thermal process is to achieve a probability of one microorganism in the entire batch, then,

$$\log(r.N_0) - \log(r.N) = \frac{F}{D} \Rightarrow \log(r.N_0) = \frac{F}{D}$$

$$r.N_0 = 10^{F/D} \Rightarrow \boxed{\frac{1}{r} = \frac{N_0}{10^{F/D}}} \rightsquigarrow \text{Probability of spoilage}$$

<u>Example</u>: Estimate the spoilage probability of a 50 min process at 113°C when D_{113} = 4 min and initial microbial population is 10^4 per container.

<u>Solution</u>:

$$\frac{1}{r} = \frac{N_0}{10^{F/D}} = \frac{10^4}{10^{50/4}} = 3.16 \times 10^{-9} \Rightarrow$$

$$\frac{1}{r} = 3.16 \times 10^{-9} \Rightarrow r = 3.16 \times 10^{9} \text{ cans}$$

$$\text{Probability of spoilage} = \frac{1}{r} = \frac{1 \text{ can}}{3.16 \times 10^9 \text{ cans}}$$

i.e., spoilage of 1 can in 3.16×10^9 cans can be expected.

OR

$$\log \frac{N_0}{N_f} = \frac{F}{D} \Rightarrow \log \frac{10^4}{N_f} = \frac{50}{4} = 12.5 \Rightarrow$$

$$\log 10^4 - \log N_f = 12.5 \Rightarrow 4 - 12.5 = \log N_f$$

$$-8.5 = \log N_f \Rightarrow N_f = 3.16 \times 10^{-9}$$

$\therefore$ Spoilage of 1 can in $3.16 \times 10^{+9}$ cans is expected.

RELATIONSHIPS BETWEEN CHEMICAL KINETICS AND THERMAL PROCESS CALCULATIONS

Since the decrease in microbial population generally follows an exponential path, the change should follow first order kinetics.

$$\frac{dN}{dt} = \mp k \cdot N^{1} \quad (\text{order} = 1)$$

, k: rate of decrease OR k: " " increase

(+ means growth, − means decrease of m.o.)

$$\frac{dN}{dt} = -k \cdot N \Rightarrow \frac{dN}{N} = -k \cdot dt \Rightarrow \int_{N_0}^{N} \frac{dN}{N} = -k \cdot \int_{t=0}^{t} dt$$

$$\ln \frac{N}{N_0} = -k \cdot t \Rightarrow \frac{2.303 \log \frac{N}{N_0}}{2.303} = \frac{-k \cdot t}{2.303} \Rightarrow$$

$$\log N - \log N_0 = -\frac{k}{2.303} \cdot t$$

Remember $\log N_0 - \log N = \frac{t}{D} \Rightarrow \log N - \log N_0 = -\frac{t}{D}$

$$-\frac{t}{D} = -\frac{k}{2.303} \times t \Rightarrow \boxed{k = \frac{2.303}{D}}$$

Q_{10} AND Z VALUE

Q_{10} is often used to describe the effect of temperature on the reaction rate (k). i.e., it tells us about how the reaction rate or microbial growth rate or microbial inactivation rate changes when we increase or decrease the process temperature by 10°C.

$$Q_{10} = \frac{k_{T_2}}{k_{T_1}} = \frac{k_2}{k_1}$$

k_1: rate constant at T_1 (°C)

k_2: " " " T_2 (°C)

$$T_2 = T_1 + 10^\circ C$$

Remember $k = \frac{2.303}{D} \Rightarrow$

$$Q_{10} = \frac{k_2}{k_1} = \frac{2.303/D_2}{2.303/D_1} \Rightarrow \boxed{Q_{10} = \frac{D_1}{D_2}}$$

Also, $z = \frac{T_2 - T_1}{\log D_1 - \log D_2} \Rightarrow \frac{D_1}{D_2} = 10^{\left(\frac{T_2 - T_1}{z}\right)}$

For a temperature change $(T_2 - T_1)$ of $10^\circ C$ (from Q_{10}) $\Rightarrow$

$$Q_{10} = \frac{D_1}{D_2} = 10^{\frac{(T_2 - T_1)}{z}} = \boxed{10^{10/z} = Q_{10}}$$

Take log of both sides $\Rightarrow$

$\log Q_{10} = \log 10^{10/z} \Rightarrow \log Q_{10} = \frac{10}{z} \Rightarrow$

$$z = \frac{10}{\log Q_{10}}$$

REACTION RATE DEPENDANCE ON TEMPERATURE

The Arrhenius equation is used to describe the effect of temperature on the rate constant.

$k = B.\exp\left(\frac{-Ea}{R.T}\right) \rightsquigarrow$ Arrhenius eqn.

B: frequency factor,

R: gas constant = 8.314 J/mol.K

Ea: Activation energy in J/mol

k: reaction rate constant ($\frac{1}{time}, \frac{1}{s}, \frac{1}{min} \ldots$)

Take Ln of both sides $\Rightarrow$

$$\ln k = \ln B - \frac{Ea}{R.T}$$

For a temperature change from T_1 to $T_2 \Rightarrow$

$$\mp \ln k_1 = \mp \ln B \pm \frac{Ea}{R \cdot T_1} \quad \text{(multiply both sides by } -1) \Rightarrow$$

$$+ \quad \ln k_2 = \ln B - \frac{Ea}{R \cdot T_2}$$

$$\ln k_2 - \ln k_1 = -\frac{Ea}{R T_2} + \frac{Ea}{R T_1} \Rightarrow$$

$$\ln \frac{k_2}{k_1} = \frac{Ea}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right) \quad \text{OR}$$

$$\log \frac{k_2}{k_1} = \frac{Ea}{2.303 \cdot R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right)$$

Since $k = \frac{2.303}{D} \Rightarrow$

$$\log \frac{k_2}{k_1} = \log \frac{2.303/D_2}{2.303/D_1} = \log \frac{D_1}{D_2}$$

Remember $\frac{D_1}{D_2} = 10^{\frac{T_2 - T_1}{Z}} \Rightarrow$

$$\log \frac{k_2}{k_1} = \log 10^{\frac{T_2 - T_1}{Z}} \Rightarrow \log \frac{k_2}{k_1} = \frac{T_2 - T_1}{Z}$$

Substitute in $\log \frac{k_2}{k_1} = \frac{Ea}{2.303 \cdot R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right) \Rightarrow$

$$\frac{T_2 - T_1}{Z} = \frac{E_a}{2.303 * R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right) \Rightarrow$$

$$E_a = \frac{2.303 * R}{Z}\left[\frac{T_2 - T_1}{\frac{1}{T_1} - \frac{1}{T_2}}\right] \Rightarrow$$

$$E_a = \frac{2.303 * R}{Z} * (T_1 * T_2)$$

For small difference between T_1 and T_2 $\Rightarrow$

$T_1 * T_2 = T_1^2$ and $R = 8.314\ J/mol.K$ $\Rightarrow$

$$E_a = \frac{19.15}{Z} * T^2$$

IMPORTANCE AND DETERMINATION OF Fo VALUE

Generally sterilization is done at 121°C and it is a standard (reference) temperature for sterilization. If we want to sterilize the material at the temperature other than 121°C, then, we must change the time of sterilization.

Example: If for a certain food F^{10}_{120} = 10 min is needed for commercial sterility. Two alternative procedures:

1. **$Fo = F.10^{(T-\theta)/z} = 10x10^{(120-120)/10} = 10min.$**
   Heat food instantaneously to 120^0C, hold at this temperature for 10 min and cool instantaneously.
2. **$Fo= 0.1x10^{(140-120)/10} = 10min.$**
   Heat food inst. to 140^0C, hold at this T for 0.1min and cool inst.

This means; 0.1 min at 140^0C is equivalent to 10 min at 120^0C. Both give the same lethality (F0 = 10 min)

Suppose this food contains a valuable enzyme with a z-value of 50°C which requires 4 min at 120^0C for inactivation. At 140^0C time required for inactivation will be:

$$t = 4 \times 10^{(120-140)/50} = 1.6 \text{ min.}$$

	Processing time needed	Time needed for enzyme inactivation
Procedure 1:	**10 min**	**4 min**
Procedure 2:	**0.1 min**	**1.6 min**

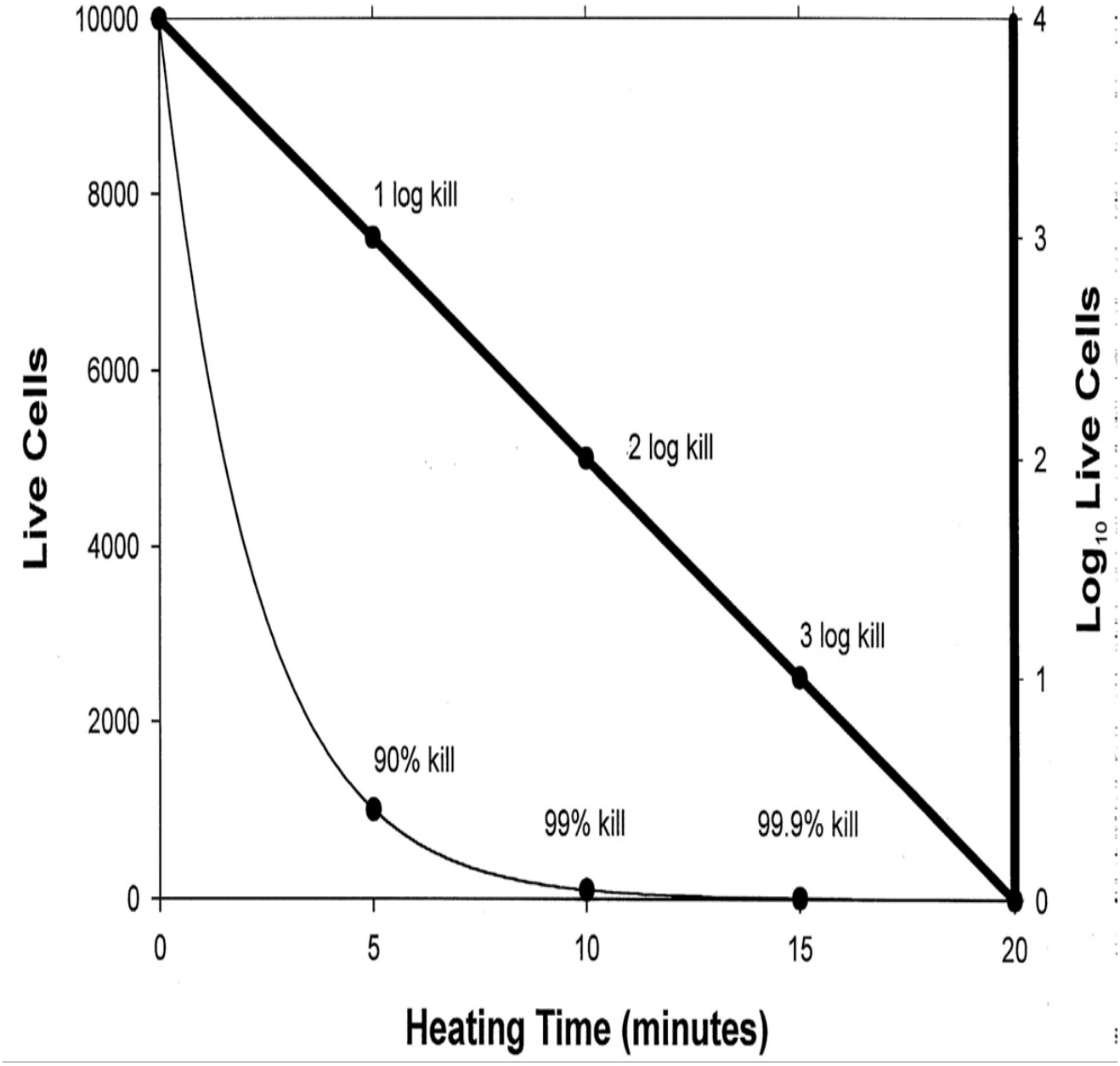


Figure. Thermal Death (Survivor) Curves (Y axis: Left is millimetrically divided scale, Right is semi-log scale; X axis is the process time).