Bioreactor Design and Analysis
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Lecture – 29
Heat Transfer Operations in Bioreactors – Part 2

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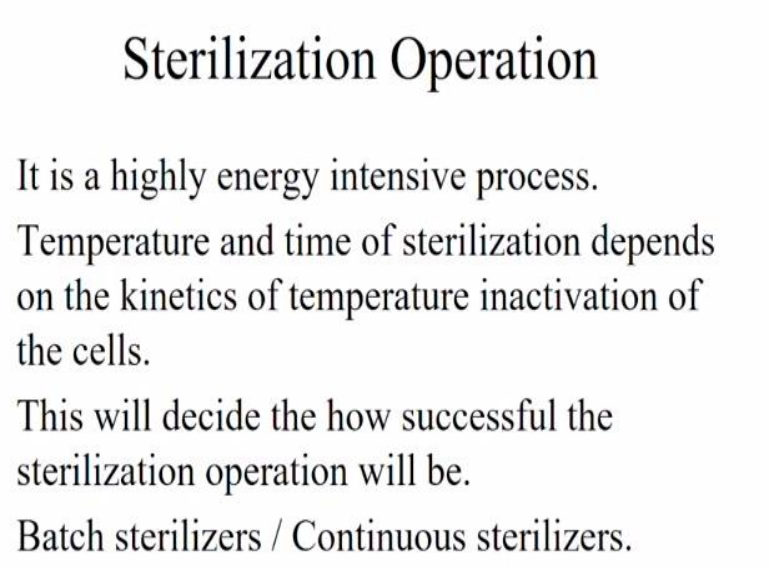

So we know that steriliaation process or operation this is highly energy intensive process. Temperature and time of sterilization these two depends on the kinetics of the temperatur inactivation of the cells. This will decide how successful the sterilisation operation will be. So there are two kinds of sterilizers in use, batch sterilizers and continuous sterilizers.

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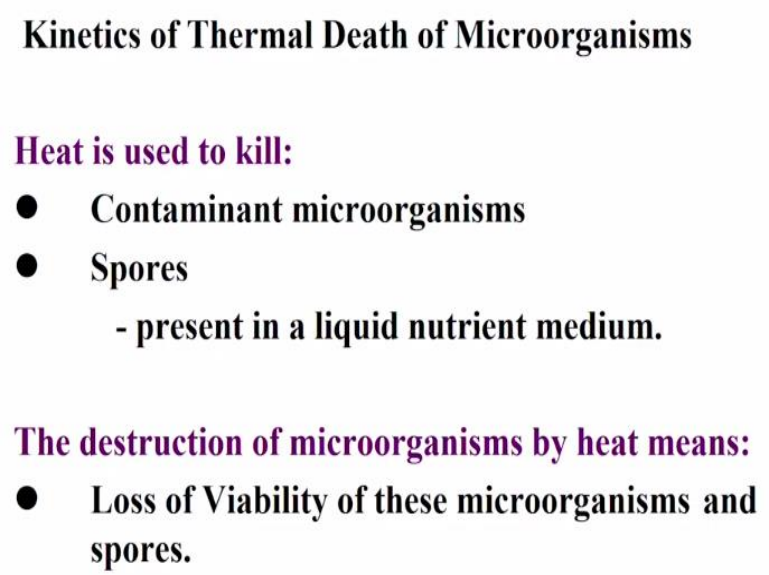

So in order to find the profile for sterilization the temperature time profile, we first need to understand the kinetics of death of the microorganisms to determine the time required for sterilization. Now heat is generally used to kill the contaminants or to sterile the medium. Destruction of microorganism by heat means loss of viability of these microorganisms and spores even.

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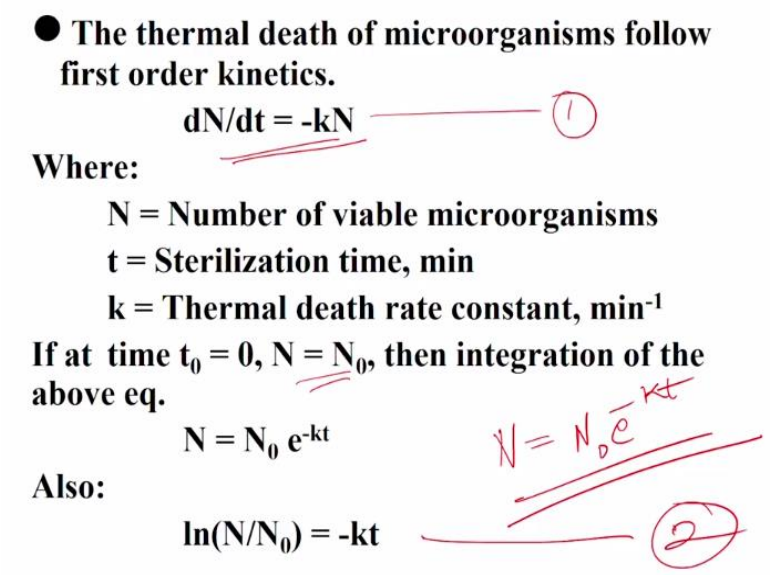

So the thermal death of microorganisms is assumed to follow first-order kinetics. If N is the number of viable microorganisms, t is the sterilization time and k is the thermal death rate constant, so then using equation 1 shown on the slide we can characterize the rate at which the viable cells will reduce with time due to heat thermal death.

So, if at time t 0 which is 0, N = N 0 then if we integrate this equation given the initial conditions the amount of cells remaining after time t can be given as an function exponentially decreasing function. So, this can be further written in the form of equation 2.

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- **The term decimal reduction time, D, is used to characterize the death rate constant.**

- **D is defined as the sterilization time required to reduce the original number of viable cells by one tenth.**

$$N/N_0 = 1/10 = e^{-kD}$$

$$\ln(0.10) = -K_d$$

$$D = 2.303/k$$

Term decimal reduction time which is shown as capital D, this is used to characterize the death rate constant. Now it is defined as the sterilization time required to reduce the original number of viable cells by one tenth. So, in the previous equation 2, the condition is that N should become N 0 by 10. So, if we substitute this, we can find the decimal reduction time in terms of the death rate constant.

Which is given here as k and here it has been changed to K d. So, then your decimal reduction time is nothing but 2.303 by the death rate constant.

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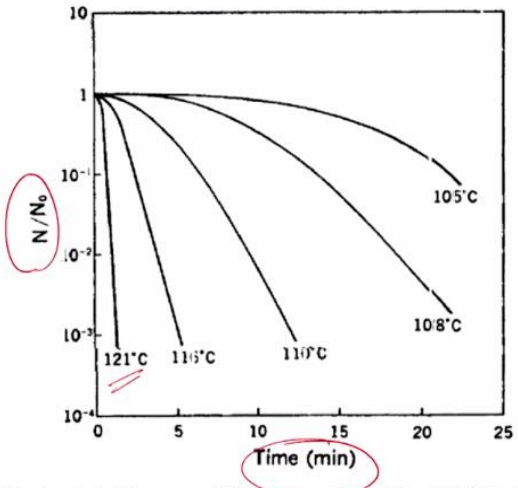

Typical thermal death rate data for spores of *Bacillus stearothermophilus* Fs 7954 in distilled water where N = number of viable spores at any time, N_0 = original number of viable spores.

If a plot is made between N by N 0 ratio versus time at different temperatures one can find that as the temperature increases the rate at which this ratio decreases keeps on increasing and at 121 degrees c as shown here the reduction is very rapid. So, this plot is for the bacterial

spores of Bacillus Stearothermophilus in distilled water where N was the number of viable spores at any time and N 0 was the original number of viable spores.

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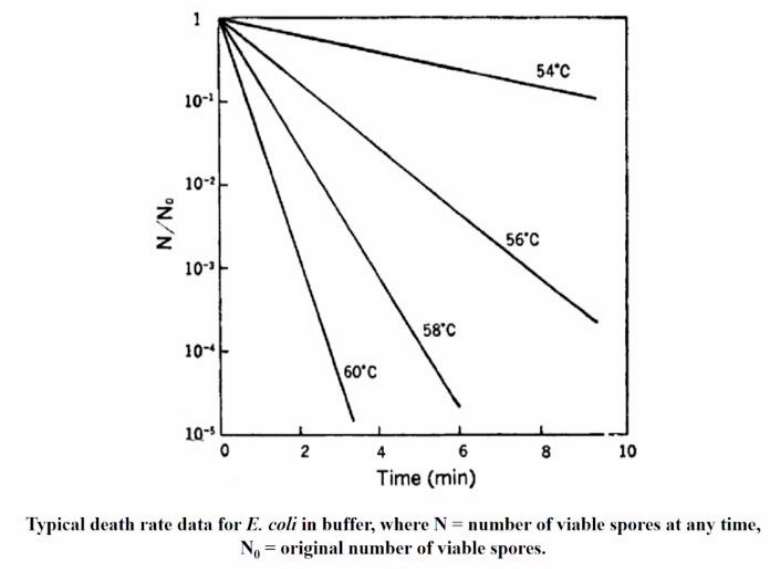

Typical death rate data for *E. coli* in buffer, where N = number of viable spores at any time, N_0 = original number of viable spores.

Similarly, this is a plot shown for E. coli in buffer. So, you can see that the rate of fall is much faster at lower temperatures in E. coli whereas Bacillus Stearothermophilus is more temperature tolerant.

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The thermal death rate constant k follows the typical Arrhenius equation.

$$K = A\, e^{-E/RT} \quad (1)$$

Where:

A = empirical constant
E = Activation energy for thermal death of microorganism
T = Absolute temperature, °K
R = Gas constant = 1.98 cal/g mole °K

For spores of *B. stearothermophilus*, the following kinetic parameters apply:

$A = 7.94 \times 10^{38}$ min^{-1}
$E = 68.7 \times 10^{3}$ cal/g mole

The higher the value of E, the more difficult it is to kill by thermal denaturation a microorganism or spore.

So thermal death rate constant K is further known to be a function of temperature and is governed by the Arrhenius equation given here on the slide where A will be your empirical constant, E is the activation energy for thermal death of the microorganism, T is the absolute temperature and R is the universal gas constant. Now for example the spores of Bacillus Thermophilus the kinetic parameters in this model A and E are shown here.

So higher is the value of the activation energy one can make out more difficult it will become to kill by thermal denaturation.

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- The value of activation energy, E, due to thermal denaturation (death) for vegetative microbial cells and spores is in the range of 50 to 100 kcal/g mole.
- For the thermal denaturation of enzymes, vitamins, and other fragile nutrients, the activation energy, E, is in the range of 2 to 20 kcal/ g mole.
- For a given liquid medium containing both, it is easier (faster) to denature thermally, enzymes and vitamins and other nutrients, and more difficult (slower) to denature (kill) vegetative cells.

So, for vegetative microbial cells it varies in the range of 50 to 100 kilocalorie per gram mole. While for enzymes, vitamins and other thermolabile components, the activation energy varies in a lower range which is shown here between 2 to 20 kilo calorie per gram mole. Now for a given liquid medium which contains both it is easier to denature thermally the enzymes and vitamins and other nutrients and more difficult to denature the vegetative cells.

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- In order to find the value of k for any system (spores and vegetative cells, nutrients) it is important to know both A and E in the Arrhenius Eq.
- Sterilization at relatively high temperatures with short sterilization times is highly desirable because it favors the fast killing of vegetative cells and spores with minimal denaturation of nutrients present in the liquid medium.

So, in order to find the thermal death rate constant for any system whether vegetative cells and spores with nutrients it is important to know both the parameters in the equation or in the

model the A and the E. So, sterilization at relatively high temperatures with short sterilization times are highly desirable because this can favour the fast killing of the vegetative cells and spores with minimum denaturation of the nutrients or thermolabile components in the medium.

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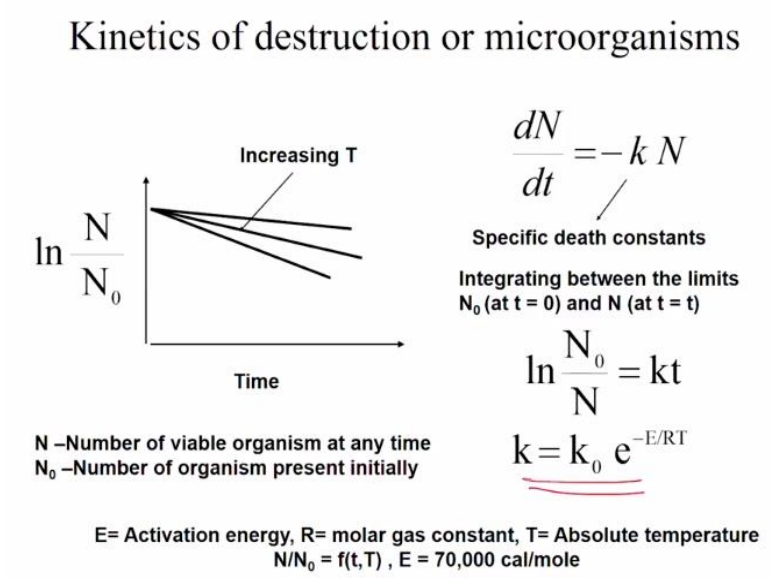

So, as we have already seen earlier this is the kinetics of destruction of microorganisms where it has been assumed to be a first-order kinetics and k which is the rate constant it is following the Arrhenius equation.

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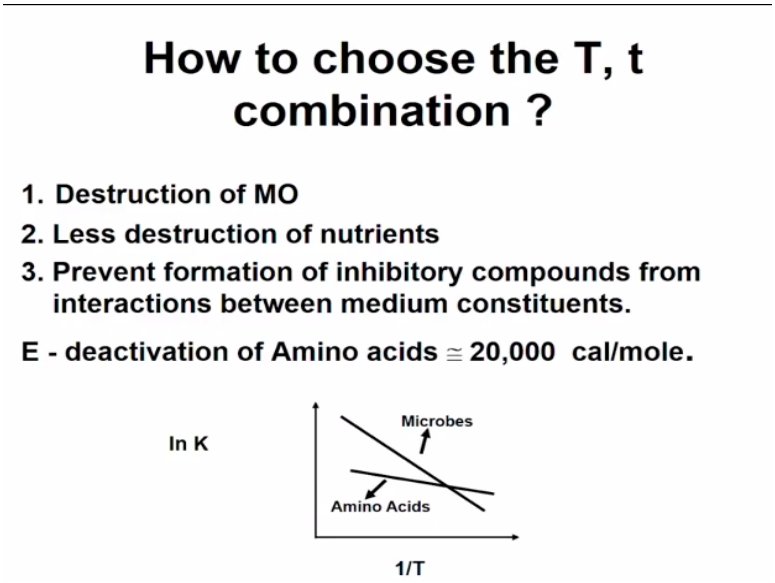

Now how do we choose the temperature and the time for sterilization? What combination should we use? So, what has to be kept in mind is that we need to destroy the microorganisms to achieve sterility. We want minimum destruction of the nutrients and prevent the formation

of any inhibitory compounds due to the interactions between the medium constituents during sterilization.

So, if the medium components contain very heat sensitive components which have very low deactivation energies like in case of amino acids it is nearly 20,000 calorie per mole.

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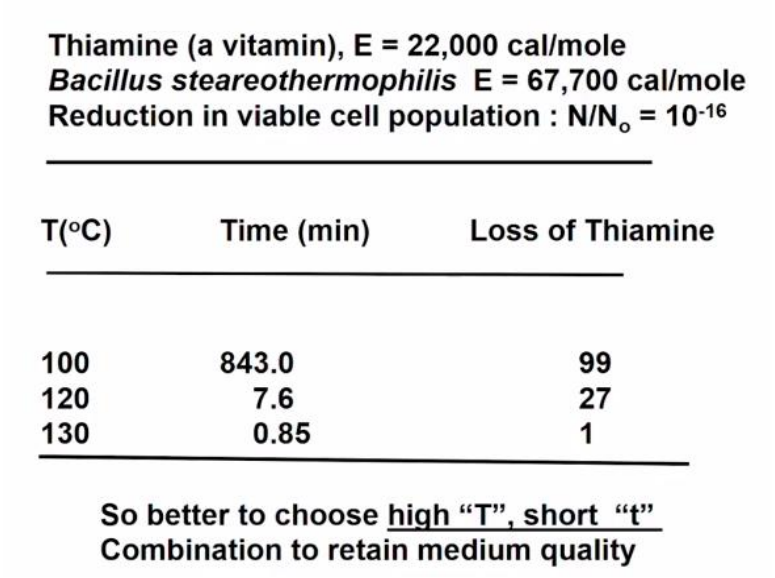

So, there is an example shown here on the slide where different combinations of temperature and time can be used to achieve the desired sterility. Here, the reduction or the sterility desired the fraction is equal to 10 to the power of –16 in Bacillus Stearothermophilus and the media is said to have a heat labile component which is thiamine where the deactivation energy is 22,000 calorie per mole while for the bacterial spore it is 67,700 calorie per mole.

So, if you see the chart given here for different combinations as the temperature increases from 100 to 130 the time which is required to reach to the desired sterility is reduced from 843 minutes to 0.85 minutes. And if you see the loss of thiamine, the loss of thiamine is less when the time of exposure is less of the medium to the high temperature. So, therefore it is best to choose high temperature with short time as the combination to retain the medium quality.

So, in this case the combination of 130 at 0.85 minutes seems to be working best to retain the medium quality

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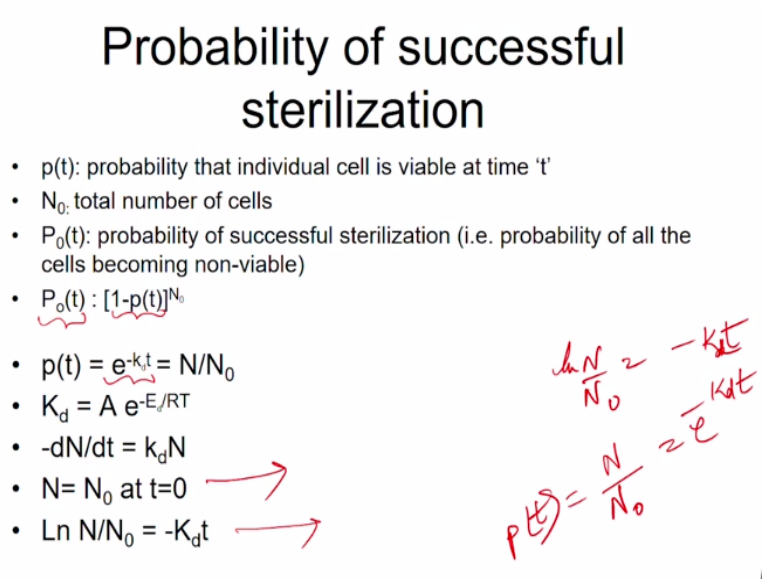

Now if we want to characterize the probability of successful sterilization how do we do that? Now let us do some probability here. Let us assume small p t stands for probability that an individual cell will remain viable at time t. Total number of cells given are N 0 and capital P 0 which is a function of t is the probability of the successful sterilization. So, probability of successful sterilization which means probability that all the cells will become non-viable.

So, your capital P 0 can be given as 1 minus the probability of individual cell being viable. So, this will become whatever given inside the bracket is the probability of the individual being non-viable and there are N 0 number of cells. So, if all these cells will become non-viable, then this will be equal to the probability of successful sterilization. So, therefore capital P 0 t can be given as 1 – small p to the power of N 0.

Now this probability of individual cells remaining viable at any time t can be given as e to the power of –k d t, how? Because we know that ln N by N 0 = –k d t during sterilization, is not it is the thermal death rate? So, the probability of survival is N by N 0 which is e to the power of –k d t, which is equal to your probability of survival which means the cell remaining viable. Now the initial number of cells given here are N 0 so ln N by N 0 is–k d t, this we know from the first-order reaction.

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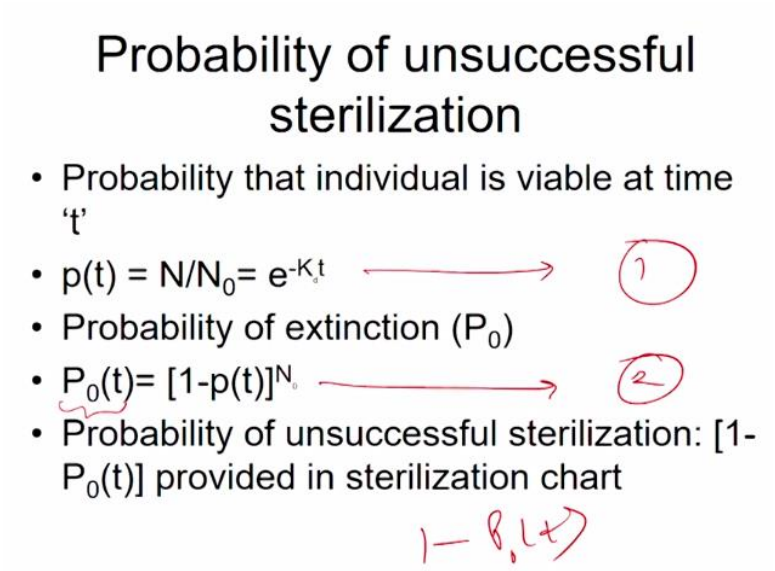

So, probability that the individual is viable at time t is we have now given it as e to the power of –k d t. So, probability of extinction which means to get extinct is given as P 0 t as shown last time in the last slide which we know is now can be written as minus small p 0 to the power of N 0. So, this is the probability of extinction for all the cells. Now the probability because even if one cell survives, it is an unsuccessful sterilization. So, the probability of unsuccessful sterilization can then be given as 1 – capital P 0 t.

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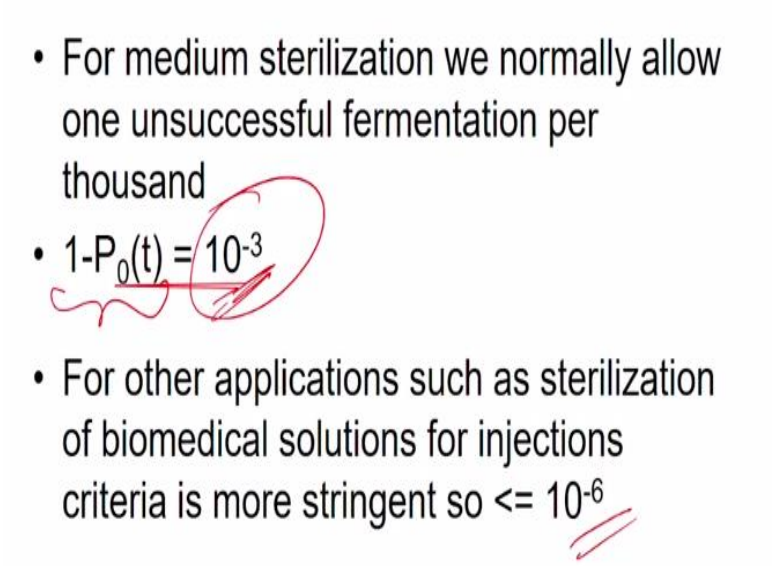

Now this for acceptable limits let's assume is 10 to the power of –3 which is the probability of unsuccessful sterilization, so which means the probability of unsuccessful sterilization let us assume out of the thousand runs one run gets contaminated. So the probability of unsuccessful sterilization would be 1 by 1000 which is equivalent to 1 – P 0 t.

So, for other applications such as sterilization of biomedical solutions which are required for pharma industry this probability is much more stringent, it can go as low as 10 to the power of –6 for unsuccessful sterilization.

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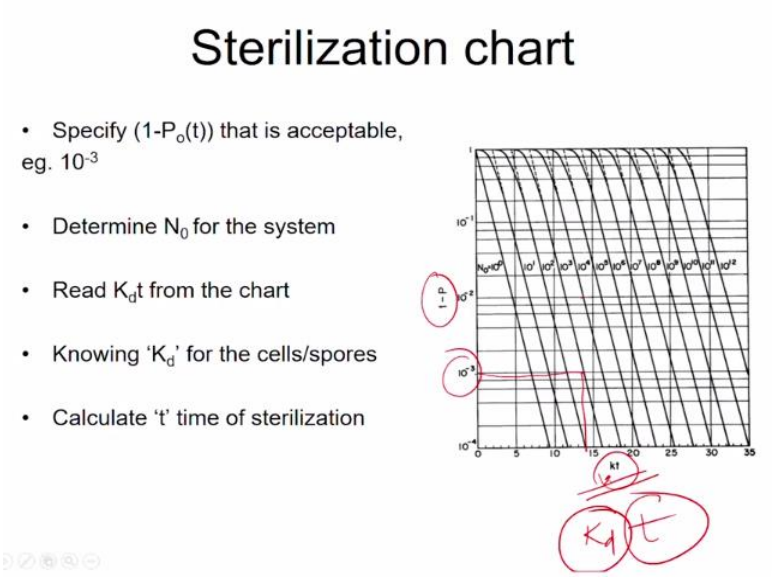

So, when the probability of unsuccessful sterilization is plotted against k times t for different initial number of cells if you see this plot let us see we know the initial number as 10 to the power of 4 and the acceptable the probability of unsuccessful sterilization is 10 to the power of –3. So, depending on the initial number of cells contaminants present in the medium, we can find the product of k t. Now if we know the thermal death rate constant which is given as k here the rate constant, then we can find the time of sterilization.

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So, batch sterilization of liquid medium it can be carried out in batch mode by direct steam sparging or by electrical heaters. The sterilization cycles they are composed of heating, then holding and then cooling.

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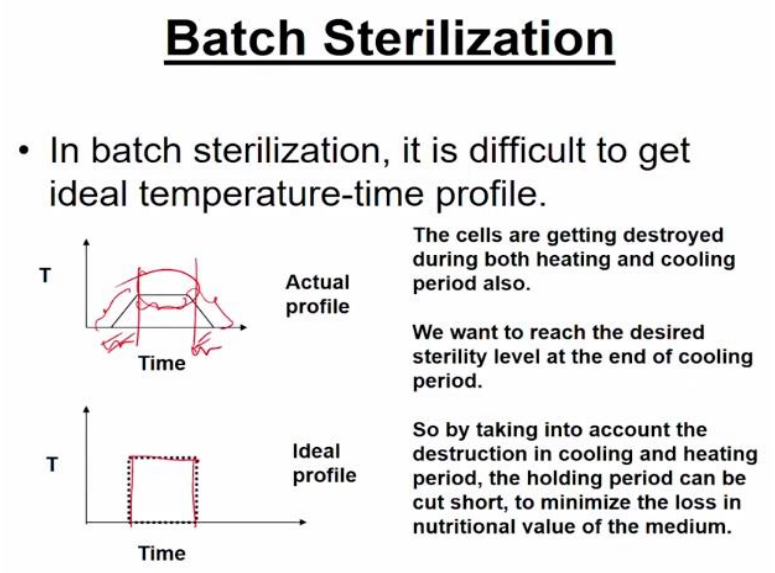

So, in batch sterilization process it is difficult to get the ideal temperature time profile. So, the ideal profile would be as soon as you give the steam it reaches directly the sterilization temperature, then you hold at that temperature and then you cool down. So, there is no time spent in heating and cooling, but actually it takes time for the temperature to reach to the sterilization temperature and then further to cool down from the sterilization temperature to become normal.

Now the cells are getting destroyed not only during this holding period, but also during this heating and cooling down period. So, if we want to reach the desired sterility level at the end of the cooling period, so we must take into account the destruction in the cooling as well as the heating period. This can minimize the time which is required to hold it the medium at the sterilization temperature to reach to the desired sterility level thereby we can also maintain the nutritional quality of the medium.

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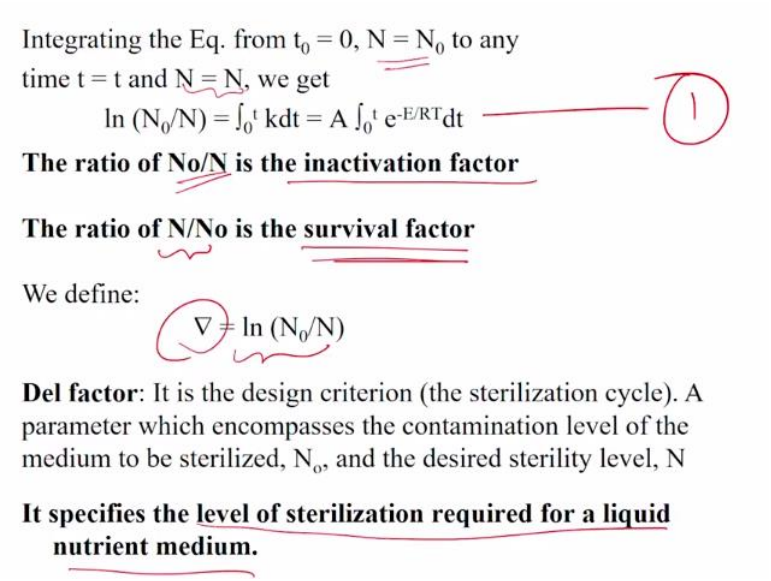

So, if suppose the initial number of cells present in the medium was N 0 and at time t the number of cells remaining were N during the sterilization, then we know by the first rate of thermal death rate equation we can have ln N 0 by N as k dt and k can further be substituted by the Arrhenius equation as shown in this equation 1. So rather than writing ln N by N 0, the denominator has been brought up and the negative sign has been removed.

So, the ratio of N 0 by N here is called as the inactivation factor and this ratio N by N 0 is nothing but the survival factor. So, for sterilization we take into account the inactivation factor which is N 0 by N del factor which is ln N 0 by N. Now this del factor it is design criteria which is used in the sterilization cycles. It is a parameter which encompasses the contamination level of the medium to be sterilized.

Which is the initial number of cells and the desired sterility level N, which means how many minimum numbers of cells are required after sterilization or can or acceptable after sterilization. So, it specifies the level of sterilization required for a liquid nutrient medium.

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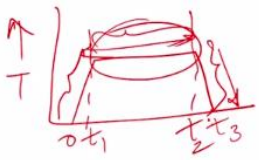
During batch sterilization, there are three periods of sterilization:

- Period for Heating of the liquid medium
- Period for Holding at constant temperature
- Cooling period

During each period, a separate value of ∇ is calculated:

$\nabla_{Total} = \ln(N_0/N) = \nabla_{heating} + \nabla_{holding} + \nabla_{cooling}$

- $\nabla_{heating} = \ln(N_0/N_1) = \int_0^{t1} k dt$
- $\nabla_{holding} = \ln(N_1/N_2) = \int_{t1}^{t2} k dt$
- $\nabla_{cooling} = \ln(N_2/N) = \int_{t2}^{t3} k dt$



Where: N = No. of contaminants after sterilization
N_0 = No. of contaminants before sterilization
N_1 = No. of contaminants after heating period t_1
N_2 = No. of contaminants after holding period t_2
t_1, t_2, t_3 = Sterilization times during, heating, holding and cooling.

Total batch sterilization time, t, is given as $t_1 + t_2 + t_3$

During bath sterilization, there are three period of sterilization. As we have seen in the plot there is a period of heating of the liquid medium, so there is a period of heating, period of holding which is at constant temperature and then period of cooling. So, during each of these periods, a separate del can be calculated which is the deactivation factor. So, ln N 0 by N which is the del will be the del during the heating.

The del factor during the holding and the del factor obtained during the cooling. So, during the heating let us assume that the number of cells which survive become equal to N 1. After the holding process the number of cells remaining become N 2 and after cooling down the number of cells remain N finally. So, the time during the heating is the t 1 time, the time for holding is between t 1 and t 2.

This is t 1, t 2. So from 0 to t 1 is your death rate due to heating, from t 1 to t 2, it is death during the holding period and from t 2 to t 3 it is the death during the cooling period. And the del factor can be obtained as shown in the equations here during all these three periods.

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During each period, a separate value of ∇ is calculated:

$\nabla_{Total} = \ln(N_0/N) = \nabla_{heating} + \nabla_{holding} + \nabla_{cooling}$

- $\nabla_{heating} = \ln(N_0/N_1) = \int_0^{t1} k dt$ (1)
- $\nabla_{holding} = \ln(N_1/N_2) = \int_{t1}^{t2} k dt = k(t_2-t_1)$ (2)
- $\nabla_{cooling} = \ln(N_2/N) = \int_{t2}^{t3} k dt$ (3)

$k = f(T)$ by Arrhenius equation.
$T = f(t)$

Where:

N = No. of contaminants after sterilization
N_0 = No. of contaminants before sterilization
N_1 = No. of contaminants after heating period t_1
N_2 = No. of contaminants after holding period t_2
t_1, t_2, t_3 = Sterilization time during, heating, holding and cooling.

So, during each period a separate value of del is calculated which is shown here for all the three periods as equation 1, 2 and 3 and k in turn is a function of temperature by Arrhenius equation.

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Temperature-Time Profile in Batch Sterilization.
The sterilization temperature is a function of time for batch sterilization using types of heat transfer and cooling.

Steam sparging	$T = T_0\left(1+\frac{\alpha t}{1+\gamma t}\right)$ (Hyperbolic) (9.30)	$\alpha = \frac{hs}{Mc_pT_0}$ $\gamma = \frac{s}{M}$
Electrical heating	$T = T_0(1+\alpha t)$ (Linear) (9.31)	$\alpha = \frac{q}{Mc_pT_0}$
Heating with steam	$T = T_H(1+\beta e^{-\alpha t})$ (Exponential) (9.32)	$\alpha = \frac{UA}{Mc_p}$ $\beta = \frac{T_0-T_H}{T_H}$
Cooling with coolant	$T = T_{co}(1+\beta e^{-\alpha t})$ (Exponential) (9.33)	$\alpha = \left(\frac{wc_p'}{Mc_p}\right)\left\{1-\exp\left(-\frac{UA}{wc_p'}\right)\right\}$ $\beta = \frac{T_0-T_{co}}{T_{co}}$

So, this slide gives you how the temperature profile changes with time with different types of heating processes or different type of batch sterilization processes where if you use steam sparging then the profile is more hyperbolic shown here. And if it is electrical heating, then the profile the temperature changes as a linear function of time.

So, in order to solve this if k which is a function of temperature in turn if we know how temperature profile is changing with time, we can substitute this function in k which is by

Arrhenius equation e to the power of –E by RT and this T is in turn a function of time and then these can be integrated.

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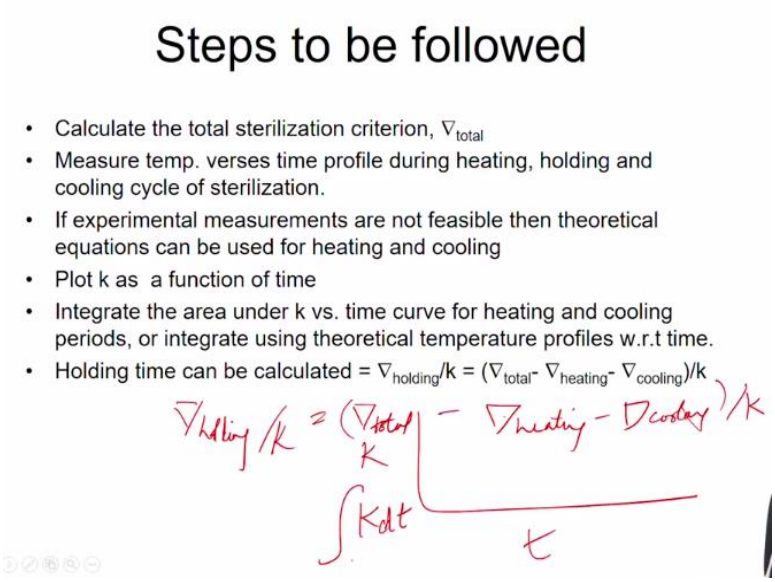

So, steps which have to be followed include first calculating the total sterilization criteria depending on the initial number of cells or contaminants and the final number of cells which are allowed to survive or acceptable. Measure temperature versus time profile during the heating, holding and the cooling cycle of temperature. So, you see how the temperature profile is changing with time.

If experimental measurements are not feasible, then use empirical relationships or theoretical equations which can be used to determine the temperature profiles with time during the heating and the cooling period. Then you make a plot of k as a function of time. Integrate the area under the k versus time curve for heating and cooling period which is k dt for the given time of heating or cooling period.

And then the holding time can be calculated as shown here. So, del holding by k will be equal to del total – del heating – del cooling divided by k.