# Aspects of Biochemical Engineering

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## Lecture -54
## Medium sterilization – II

Welcome back to my course Aspects of Biochemical Engineering. Now, I was discussing one very important aspects of biochemical engineering that is the medium sterilization. Previously, I discussed the here sterilization and I told you that sterilization, plays very important role as their bio chemical industries concern, because we shall have to uh, make the environment totally free from the other micro organism.

So, that our design, micro organism can grow. In the last lecture, I try to discuss the principles behind the medium sterilization process and I told you that two type of techniques may be used for medium sterilization process one is filtration and another is the heat.

You know when you use the filtration process, filtration appears to be the best method, the reason is that that there is no degeneration of nutrient and they present in the media; so, but we know heat; obviously, has some kind of demeasuration of the order or some kind of that loss of nutrient might be, might taken place.

So, but the problem is that, when we use any kind of filtration process particularly, membrane filtration, we have, we have seen that across the membrane, there is a tremendous pressure drop and as you know that any kind of operation cost, any kind that you know depends on the, the pressure drop and if the pressure drop is more, operation cost will be more.

So, not only that membrane since, the pressure increases, because if the pore size of the membrane is less than 0.5 microns, because if we target to remove bacterial cells, we know the size of the bacteria we had is from 0.5 to 2 microns.

So, naturally that, that you know the pressure drop across the membrane will be and since, it is very high and your membrane material mostly based on the polymeric material. Am I right? And polymeric material, they cannot withstand very high pressure, though we put some kind of supporting material perforated discs above and below this

membrane, but we find that you know, made the life of the membrane depends on the pressure difference as well as the fouling of the membrane that is another important factor that we have.

So, for that you know that I told you that when you think about the biochemical industry, we have come across two different type of products. One is high value products and these low value products, high value products means per unit cost of the product is, is very high, I can give you the example of that, you know that Hepatitis B vaccine, then we have insulin, then we have the Kanamycin.

These are very Kanamycin antibiotics, they are very costly medicine, but if you, if you, if you go for the liars at the low value products as far as the alcohol cyclic acid, acetic acid, all the low value products.

So, in case of low value products, we have to handle huge volume of the liquid and when we use the huge volume of the liquid, is very difficult to use the membrane type of filter, but when you use small amount of liquid, small amount of media will handle then we think for some kind of filtration techniques.

So, in the industry, in the biochemical industry mostly, we use the heat here, as a media (Refer Time: 04:12) for removing all the micro organism. I told you, when you heat then what is happening? The protein inside the micro microbial cells, then undergo denaturation and due to denaturation of the protein, the biochemical activity of the neuroorganism will be lost and here well be killed.

Now, now when, when you go for heating, because why in case of media sterilization, why you use the heating? One of the important reason is that water is good conductor of heat, because in case of we have seen, in case of beer sterilization process we, we do not use this heat, because the air is non conductor of heat it. Now, when we consider the heat, we find that the removal of the organism that depends on this, is this follow the first order kinetics.

We have come across the thermal death rate constant and we also come back to us one parameter called decimal reduction time and if you look at the decimal reduction time and the thermal death rate constant, they are inversely proportional to each other and we

have seen as you increase the temperature at the k d value or thermal death rate constant that will increase very high. So, your decimal reduction type decrease drastically.

Now, in our lab we use 121 degree centigrade, temperature for 15 minutes for sterilization purpose, but the industry we use, usually we use 140 degree centigrade that is hardly 1 to 2 minutes, for the still. A borehole is that is, that detention time of the liquid in the holding section. So, this is, because we actually, we have seen that you know that high temperature, short time technique that is usually followed for medium sterilization process; one important reason is that bitumen and amino acid.

They, they denatured they deactivated at a very low temperature, that is why the activation energy of this bitumen amino acid within the range of 80 to 90 kilojoules per mole and whereas, in case of micro as high as 300 through 400 micro use form also, it is quite high, because. So, at a high temperature, it is more detrimental on the death of the microorganisms rather than the bitumen or amino acid, because bitumen amino acid that we deactivated even at the low temperature.

So, that is why H T H T technique is use, because we are holding the medium for a very short time. So, that loss of the media quality will be very less. Now, when you go for the sterilization process, we find two type of sterilization process is available with us. One is called bad sterilization and these continuous sterilization process, the best sterilization process is suitable, when you work with a very small volume of the liquid, but when you go for the high volume of the liquid, the best realization is no good, because the reason is that time required for the sterilization process is very high.

So, as you know in the industry, not only time requirement, but also the amount of steam that is consumed in, in case of continuous reliable sterilization process is drastically reduced.

So, both the factors plays very important role as for industries concerned. Now, when you go for the continuous sterilization process, we come across two type of continuous sterilization process. One is direct heat steam injection and there is a parallel plate heat exchanger, the direct heat steam injection process appears to be the best one, because the instantaneously temperature rises to 140 degree centigrade. There is no lack of heating or heating that take place and then you can keep the media, the, in the holding section part,

but a desired amount of time and, and then do the flash cooling, to reduce the temperature.

But, the problem is that since, you are directly injecting the steam with the media there is a possibility of condensation of the steam with the media and sin the condensation of the steam take place in the media. The, the viscosity as well as the density of the media will be changed and we know the flow characteristics of the fluid, depends on the, on the density and the viscosity of the media. So, it will be very difficult to maintain the temperature of the sterilizer that is that, that is in other way that is the operational problem well be facing.

And that is why, we industry, we usually follow the plate parallel, plate heat exchanger. It is very simple, we have three different exhale heat exchanger; one heat exchange, we considered at the economizer, because we preheat the media with the and the heating media.

Here, the outgoing and those temperature of the, of the sterilization liquid and then we increase the temperature with the help of steam and 140, then we put it in the holding section for the desired period of time. Then finally, after, after economize that it passes through the chiller to reduce the temperature of the media to the dessert temperature.

So, this is the, this has the things, I discussed in the last lecture that. Today, I want to discuss some kind of numerical problems involved with this particular process, because particularly we are interested to find out that how you can find out the temperature of the stabilizer that you know we, we shall find out or any other related parameters with this particular process. Let us let us try for that.

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## Problem

A 15 $m^3$ chemostat is operated with dilution rate 0.1 $h^{-1}$. A continuous sterilizer with steam injection with steam injection and flash cooling delivers sterilized medium to the fermenter. Medium in the holding section of the sterilizer is maintained at 130° C. The concentration of contaminants in the raw medium is $10^5$ $mL^{-1}$; an acceptable contamination risk is one organism every 3 months. The Arrhenius constant and activation energy for thermal death are estimated to be 7.5 X $10^{39}$ $h^{-1}$ and 288.5 kJ $gmol^{-1}$, respectively. The sterilizer pipe inner diameter is 12 cm. Assuming perfect plug flow, determine the length of the holding section.

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Now, first problem that I want to know, tell you, this is 115 cubic meter of chemistry, is operated with dilution rate of 0.1 hour, inverse the, a continuous sterilizer with steam injection, with steam, with steam injection and flash cooling delivered the sterilized media to the fermenters, the media is, in the holding section of the sterilizes, that it maintaining 130 degree centigrade that is the holding section. I told you previously, it is 140 degree centigrade, but in this problem, they mentioned 130 degree centigrade.

The concentration of the contaminants of the raw material is 10 to the power 5 per milliliter, an acceptable contamination risk of one organism. Every 3 months, I told you that whenever we, we, we, we do for, we walk for any kind of sterilization process, we always, we have a basis, basis is there how much contaminant you are allowing with respect to how much of organism. Now, here in this problem, they are saying the raw material contents 10 to the power 5, the number of cells per milliliter and acceptable contamination readings is one organism, every 3 months operation.

So, we can easily find out, we know the flow rate, we hope that we can find out how much organism? How, how much organism, one organism will be allowing in this particular sterilization process? The Arrhenius constant and activation energy for the thermal death, that the death rate constant are estimated to be this, the, the two value is given respectably and sterilization say like that pipe inner diameter is 12 centimeter and

assume the perfect path flow determine the length of the holding section. So, this is the problem that we, we have given and let us see how we can solve this problem.

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**Solution:**

Given: $V = 15\ m^3$, $D = 0.1\ h^{-1}$, $A = 7.5 \times 10^{39}\ h^{-1}$ and $E_d = 288.5$ kJ $gmol^{-1}$, $T = 130°$ C, $d = 12$ cm $= 0.12$ m

For a chemostat, we know that:

$D = F/V$

Therefore, $F = DV = (0.1)(15) = 1.5\ m^3\ h^{-1}$

The linear velocity ($u$) in the holding section of the sterilizer can be determined by dividing F by the pipe cross sectional area (SA)

Thus, $u = \frac{F}{SA} = \frac{F}{\pi r^2} = \frac{1.5}{\pi (0.06)^2} = 132.62\ m\ h^{-1}$

The specific death rate constant $k_d$ can be calculated as:

$$k_d = Ae^{-\frac{E_d}{RT}} = (7.5 \times 10^{39})e^{-\frac{2.885 \times 10^5}{(8.314)(130+273.15)}} = 313.1\ h^{-1}$$

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So, as I pointed out, before that when we try to solve the problem, first we should drop down, what are the parameters is given, this is the volume. This is a 15 cubic meter dilution rate of 1.1 or hour inverse activation that Arrhenius constant 7.5 into 10 to the power 39 hour inverse E d is the deactivation energy required is 288.5 kilo joules per gram mole and temperature is 130 degree centigrade and diameter of the tube is 12 centimeters. So, we shall have to write down all this thing.

Now, if you look at the dilution rate, how you define the dilution rate? Dilution rate equal to F by V now here. So, I can write F by V is there. So, we or we can write F equal to D into V, am I right. There, this is what is written here, the D we know the dilution rate, we know the volume. So, we can find out the volumetric flow rate of the liquid, the linear velocity how you can calculate? Suppose, in the pipeline this liquid is flowing, this is 1.5 cubic meter per hour, it is flowing like this.

So, we if you, if you know the cross sectional area, this a, if you divide by a then cross sectional area, then it will be recorded with a unit meter per hour that I, I can do that. So, this is exactly what we have done. This is, this is, this the flow rate and this is the cross sectional area pi r squared and, and this the diameter is 12 centimeter and 12 centimeter

is equal to what? 0.12 meter, am I right. This is in cubic meter. So, radius will be 0.06 meters. So, this is square and the, your velocity is coming about 132.62 meter per hour.

Now, we know the Arrhenius equation, what is the Arrhenius equation? Arrhenius equation is k d equal to e to the power A into e to the power E d by R T that is per day what is given here, am I right. A is the Arrhenius constant Ed is the deactivation energy requirement, h r is the gas content, constant and t is the temperature of sterilization.

So, every, all parameters are given here. So, we can easily calculate, what is the death rate constant? Now, once we know that the death rate constant.

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Within a period of 3 months i.e. 90 d, the number of cells $N_1$ entering the sterilizer can be given as:

$N_1 = 1.5\ m^3 h^{-1} \times (10^5 \times 10^6\ m^{-3}) \times (90 \times 24)\ h = 3.24 \times 10^{14}$

Now, within the same 3 month period, the acceptable number of cells remaining at the end of the sterilization ($N_2$) is 1 (given).

For a perfect plug flow, with no axial dispersion, the holding time can be given as:

$$t_{hd} = \frac{\ln \frac{N_1}{N_2}}{k_d} = \frac{\ln \frac{3.24 \times 1014}{1}}{313.1} = 0.107\ h$$

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Now, we know the equation that, what is the equation, that we have I told you this is d N by d t equal to k d into N, am I right. So, this is equal to d N by N equal to k d into d t. So, this is in 0 to N and this is 0 to t d or you know what are you holding, holding time t.

So, we can write this is equal to k d into t and what if we can write here l n N by N 0 and this is minus. So, I can write l n N 0 by N equal to k d into t. The time will be, what time will be equal to l n N 0 by N divided by k d. So, we can wait, this is what we have written here.

So, so first we shall have to find out, what is the value of N 0, am I right. The, how you can find out N 0, the 3 month operation, 3 month operation means how many days? It is

the 90, 90 days, 90 days. How much hours will be there 90 into 24 in the hours and this is the flow rate 1.3 cubic meter.

So, if you multiply by this, you will get that that you know that, how much, how much volume of the liquid, we shall have to sterilize and from that do you know how much cell that you know from the concentration of the organisms that is there, and if you multiply that we will find that how much total microorganism present in the, in the media and in 10 month or 3 months period.

So, this we can consider as N 0. Am I right or N 1 anyone or N 0, whatever you have and then we can put this value and this out of 1 N. N value will be 1 N 2 or N 2 value is 1 N 1 value is this and this kd value will you already will determine. So, we can easily find out what is the time required for the sterilization, that holding time, that is required in the sterilization, that we can easily calculate.

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To allow the medium to remain for this period of time in the holding section of the sterilize pipe, the length of the pipe required can be estimated as

$$L = u\, t_{hd}$$
$$= 132.62 \text{m h}^{-1} (0.107 \text{ h})$$
$$= 14.2\ m$$

**Thus, the length of the holding section is 14.2 m**

Now; however, our problem is something different. Our problem is that, that you know that we are, that we are injecting the steam. This is media and here is the steam. Am I right, then it is going to the holding section, this is the holding section.

So, question comes here, this holding section whether we want to maintain the temperature higher than 130 degree centigrade. Now, question comes that how, what

should be the length of this holding section, the how you can find out? We know the velocity, velocity that you and time required in this holding section in this.

So, velocity what is the unit or the meter per unit time and if you, if you, if you multiply it by time then mean time, time will cancel, then we will get the length, of the length, of the holding section. So, well be getting about 14.2 meters. So, it is very-very easily. We can find out the length of the holding section of a particular strain like that.

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**Problem**

Medium at a flow rate of 2 $m^3 h^{-1}$ is to be sterilised by heat exchange with steam in a continuous steriliser. The liquid contains bacterial spores at a concentration of $5\times10^{12}$ $m^{-3}$; the activation energy and Arrhenius constant for thermal destruction of these contaminants are 283 kJ $gmol^{-1}$ and $5.7 \times 10^{39}$ $h^{-1}$, respectively. A contamination risk of one organism surviving every 60 days' operation is considered acceptable. The steriliser pipe has an inner diameter of 0.1 m; the length of the holding section is 24 m. The density of the medium is 1000 kg $m^{-3}$ and the viscosity is 3.6 kg $m^{-1} h^{-1}$. What sterilising temperature is required?

**Solution:**

The desired level of cell destruction is evaluated using a basis of 60 days. Ignoring any cell death in the heating and cooling sections, the number of cells entering the holding section over 60 d is:

$$N_1 = 2\, m^3h^{-1}(5\times10^{12}m^{-3}).\left(\frac{24\,h}{1\,d}\right).(60\,d) = 1.44\times10^{16}$$

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Now, let us go to the another problem, I think if you, if you, if you look at this problem, I think your conception will be little bit better, that the media at a flow rate to this is the continuous dilation, 2 cubic meter per hour is to be sterilized by heat exchanger, with a steam in a continuous sterilize.

The liquid contains the bacterial spores at a concentration 5 into 10 to the power 12 per cubic meter. The activation energy and the Arrhenius constant or thermal destruction of this contaminants are 283 reduce gram moles per gram mole and 5.7 into 10 to the power 39 per hour respectively.

A contamination risk, one survival every 60 days operation is considered acceptable, because in the last problem we, we have, we have three days and here it is, it is, it is the last problem that we try to find out, how much population is there with 3 months operation. We did it, I can remember and this is also similar, to that 60 days. So, a

contamination brings one organism survival surviving every 60 days operation. The every 60 days operation, we are allowing one organism to parka late through this.

Here, sterilizer and the sterilizer pipe has an inner diameter of 0.1 meter and length of the holding section is 24 centimeter, when the last problem you can remember, we try to find out what is the length of the holding section. Now, here the length of the holding section is given. This is 24 centimeter and density of the media is 100 k g per cubic meter is same as the water and viscosity is 3.6 k g per meter, per hour. What we shall have to find out the sterilize strain sterilizing temperature, that is required for this sterilizing the media. So, this problem is little bit different as compared to the previous problem.

Now, let us see how we can do that the desired level of cell destruction is six degrees. So, like you know previously, we try to solve it, 60 days operation that we have and what is the flow rate 2 cubic meter per hour then our 2 cubic meter means. You have to find out part A. What is the flow rate, the one day is 24, if you multiply by 24 hours, you will get meter cube per day and then you multiplied by 60 days. You will get that how much cubic meter of liquid you are handling and this liquid, what is the microorganism sport present 5 into 10 to the power 12. So, if you multiply that. So, you get this N 1 value is 1.44 into 10 to the power 12.

Now, if you want to calculate N 1 by N 2, what will be this? Well, be 1.44 into 10 to the power 16 divided by 1. There is the ratio is like this. So, we can easily find out the ratio between N 1 and N 2.

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$N_2$, the acceptable number of cells leaving during this period is 1. Therefore:

$$\frac{N_2}{N_1} = \frac{1}{(1.44 \times 10^{16})} = 6.9 \times 10^{-17}$$

The linear velocity u in the steriliser is equal to the volumetric flow rate divided by the cross-sectional area of the pipe:

$$u = \frac{2 m^3 h^{-1}}{\pi (\frac{0.1\, m}{2})^2} = 254 \text{ m h}^{-1}$$

To calculate $Pe$, we must first determine $D_z$ using **Figure 1**;

$$Re = \frac{Dv\rho}{\mu} = \frac{(0.1\, m)(254\, mh^{-1})(1000\, kg\, m^{-3})}{3.6\, kg\, m^{-1} h^{-1}} = 7.07 \times 10^3$$

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This is exactly this, we, we here it is done in other way N 2 by N 1. So, you divide by this and we get this, this ratio as 6 0.9 into 10 to the power 17. Now, then question comes, what is the, the flow rate is given 2 cubic meter per hour and called that diameter of the tube also given 0.1 meter. So, here what is the u u equal to flow rate divided by area. Am I right? What is the flow rate is, 2 cubic meter per hour and area is 2 pi R square.

The that you know that, if 0.1 meter is the diameter of the tube. So, radius will be 0.1 divided by 2. So, if you do that you will get 254 meter per hour. This is the velocity of the fluid. Now, if you know the velocity of the fluid, we can calculate the Reynolds number. What is the Reynolds number? Do you know by mu? So, you know that, this is the diameter of the tube, this is the velocity of the fluid, this is the density, this is the viscosity. So, easily we can easily calculate the Reynolds number.

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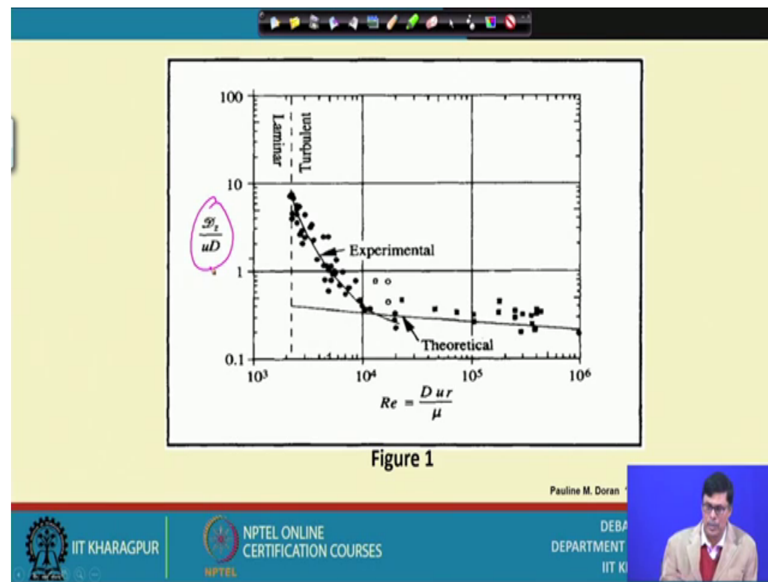


Now, one thing is that, that if you know the Reynolds number then we can find out the respective value of D z, this is, this is what D z u by D z u by D.

Now, what is D z is the axial dispersion coefficient, am I right, use the velocity of the fluid and these are the diameter of the tube. So, you know that this you can easily find out the respecting value you can find out this is what we have done here.

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For $Re = 7.07 \times 10^3$ we can determine $D_z$ from **Figure 1**; using either the experimental or theoretical curve. Let us choose the experimental curve as this gives a larger value of $D_z$ and a smaller value of $Pe$; the steriliser design will thus be more conservative.

Therefore, $\frac{D_z}{uD} = 0.65$

$$D_z = (0.65)(254\ m\ h^{-1})(0.1\ m) = 16.6\ m^2 h^{-1}$$

$$Pe = \frac{uL}{D_z} = \frac{(254.6\ mh^{-1})(24\ m)}{16.6\ m^2 h^{-1}} = 368$$

Using **Figure 2**; we can determine the value of $k_d$ for the desired level of cell destruction. $Da$ corresponding to $\frac{N_2}{N_1} = 6.9 \times 10^{-17}$ and $Pe = 368$ is about 42.

Therefore $k_d = \frac{uDa}{L} = \frac{(254.6\ m\ h^{-1})(42)}{24m} = 445.6\ h^{-1}$

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That D z by this is at this particular Reynolds number that 7 points. So, something it is close to this that, we shall have to find out where it is. We can find out this ratio and once

we do that, this is the, this is this, then D z value the axial dispersion coefficient. We can easily calculate and this will come about 16.6 square meter per hour and.

Once you know that, then this is the peclet number. What is peclet number u ? L by D z, where u is the velocity of the fluid, L is the length of the holding section, and D z is the axial dispersion coefficient. So, if you put all these values well get the peclet number. Now, once we know the peclet number then we can find out.

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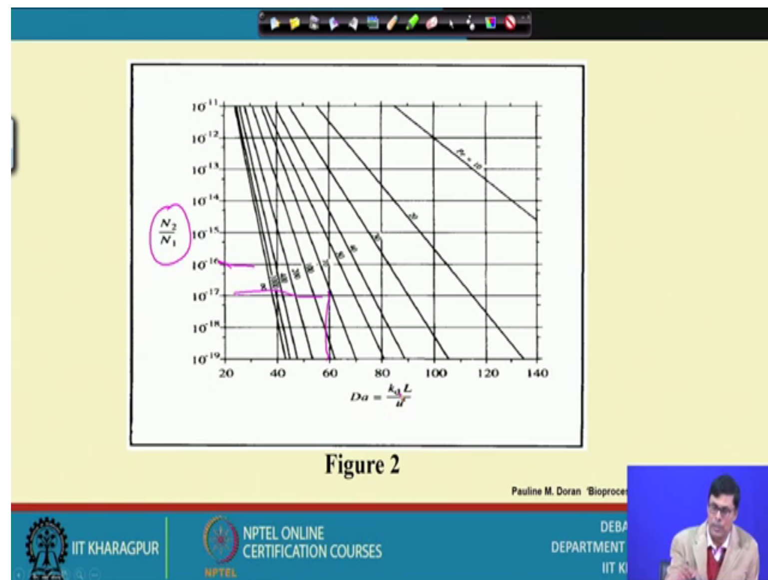


Then what we can find out? Now, here we can see that there were that here, if we have only the value of N 2 by N 2 by N 1, am I right about 10 to the power minus 17 and if you know the peclet number, we have different slope with their different peclet number. So, we can, we can find out. Suppose, here peclet number, if it is 70. So, it is coming like this. So, you can easily find out the dam coolant number.

So,. So, here what we can, we can do? We can, we can find out this dam coolent number value and from that we can find out the k d value. This k d value we can find out the u Da, D a by L L. So, you have all the values here the. So, this is, this value that from the plot, what do you get plot N 2 by N 1. So, versus, this is, this is what? This is the, what we have? Dam coolant number, dam coolant number equal to what k d L by u. Am I right, k d is the death rate constant, now you have this value.

So, we can easily find out what is the result. These value is coming about 42, this is 42 and then, if we put this value, we can find out the value of k d, k d is the thermal death rate constant is the 445.6 hour inverse.

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According to **Arrhenius equation**

$$k_d = A.e^{-E_d/RT}$$

Rearranging the above equation

$$T = \frac{(\frac{-E_d}{R})}{\ln(\frac{k_d}{A})}$$

Known data,

$E_d = 283\ kJgmol^{-1} = 283 \times 10^3\ Jgmol^{-1}$, $A = 5.7 \times 10^{39}\ h^{-1}$ and $R = 8.314\ JK^{-1}gmol^{-1}$

$$T = \frac{(\frac{-283 \times 10^3 Jgmol^{-1}}{8.314\ JK^{-1}gmol^{-1}})}{\ln(\frac{445.6\ h^{-1}}{5.7 \times 10^{39}\ h^{-1}})} = 398.4\ K = 125\ ^\circ C$$

Then now let us use the Arrhenius equation what is Arrhenius equation k d equal to A into e to the power E d by R T. Now, T equal to this divided by this all values are given, you just put the value, you can find out like this. Now, another way we can is suppose, we do not have that we are able to estimate the k d value.

Now, we, we estimate the k d value there, but we uh, we do not have the other values that the E d value is not there then, then if we have the correlation between k d and T values that as the, how the k d value. This oppose the k d value with respect to temperature T, temperature, how it is changes with respect to temperature. So, if you know the k d value, then from the plot, plot from the graph. Also, we can find out the temperature of sterilization.

So, this is the two way, we can find out the temperature of sterilization, is not very difficult. So, in this particular lecture, I try to solve two numerical problems on that medium sterilization, one problem deals with that. We shall have to find out the length of the holding section, but; that means, how long you want to maintain the temperature of this realization.

And then here, the temperature of sterilization was a 100, 130 degree centigrade and next case, next problem deals with that how we can find out the temperature of sterilization and also we can, if you know the temperature of sterilization and we can, we can also find out how much heat required for the sterilization process, because there that also we can, estimate it.

Thank you very much.