Industrial Biotechnology
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Module 05
Lecture No 23
Upstream Processing Medium Sterilizer (Contd)

Welcome to my course Industrial Biotechnology. Now I was discussing about the medium sterilization and in the in the last class I concentrate on this why the heat is considered as effective way for sterilizing the media and we have shown you that how temperature plays very important role for killing the microorganisms, and we have shown that as you increase the temperature your sterilization time drastically reduce and also I try to discuss that why in the industry, the batch sterilization is not preferable as compared to continuous sterilization process because batch sterilization process not only require the higher amount of steam, but also time of sterilization also about double as compared to the continuous sterilization process.

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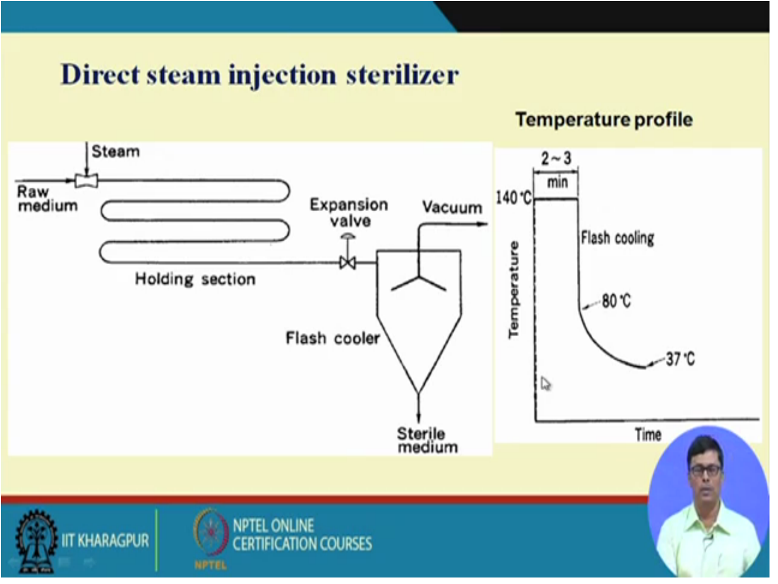

Now, this class I am going to share with you that continuous sterilization process how that is in practise in the industry. Now, if you look at the continuous sterilization process we have direct steam injection sterilization we have plate heat exchanger sterilization. Now let me explain this process because direct steam injection means you directly you mixing the steam with the media. Now you can see it here that raw media is coming one way and steam is coming in other way and this is this is actually we call it holding section.

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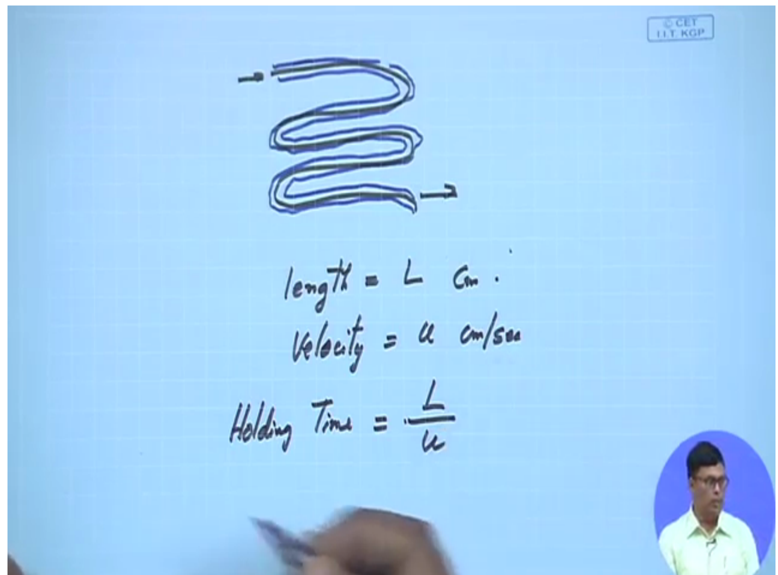

You hold the this is the pipeline and this pipeline would be insulated that you know I want to show draw it like this and this pipeline is basically they are insulated that means it is insulated like this so that heat loss this is the insulation that we have the outside the pipeline, so that heat loss should be minimum so that is in practice because we pass media here and now if you if you know the length of the pipe, suppose length of the pipe is about L and if you know the velocity of the liquid, what is velocity is u what is the unit of velocity is centimetre per second, am I right? And what is the unit of length it is the centimetre.

Now the time the holding time the time and the liquid resides in the in the holding section will be what this is L by u so divide by so you can easily find out. Suppose you want to have 2 to 3 minutes holding time so you can adjust accordingly, this is like this

And then there is the expansion valve and this through this expansion they expand in the particular when a chamber what you call is flash cooler then what happens that you know from the top vapour will be released and concentrative liquid will be falling like this. Now here there is a problem that if you do this that there will be loss of moisture that will take place, another most important thing is that when you mix the steam with the raw medium the steam undergo condensation am I right, in the in the particular pipeline.

And since the condensation of the steam occurs then the viscosity and density of the liquid will be changed. Then as we know the flow characteristics of the fluid depends on the that largely depends on the viscosity and the density, I can give the examples of the Reynolds number is equal to what Rho by Mu so the density and Mu that plays very important role. As

your density and the and the viscosity changes it is very difficult to maintain a particular temperature inside this holding section and if you unable to maintain a particular temperature then your sterility is very difficult to maintain. So that is the major problem that we have this direct steam injection process and that is why the industry this is not in practise.

Now here is a temperature time profile a temperature profile of this system you can see that as soon as the media injected the instantaneously temperature rises because that is the major there is no heat loss basically that you know your whole steam is directly the heat is directly transferred to the media. So you know that instantaneously temperature will rise to 140 degree centigrade and you can hold it then you can do the flash cooling like this 80 degree centigrade then slowly you can cool it down to 37 degree centigrade, this is what we have but this is not in practise in the industry.

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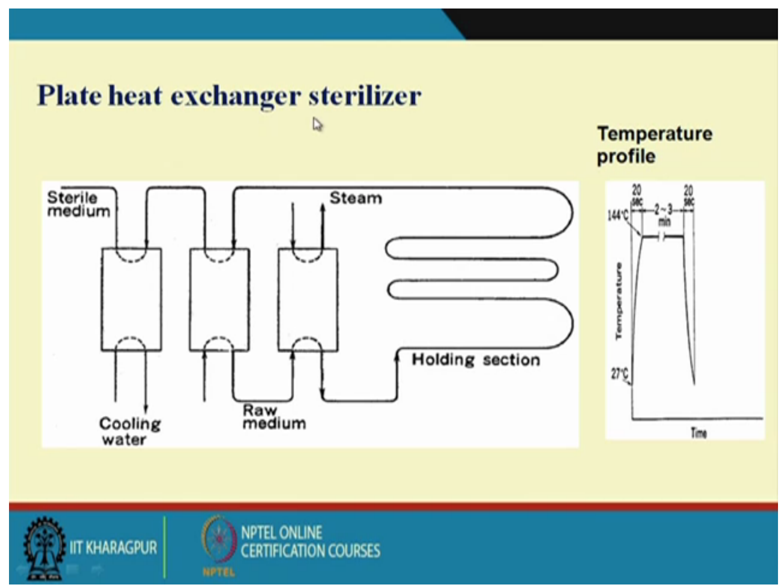

And industry sterilization technique that is in operation that is we call plate heat exchanger sterilization. Now here we have three heat exchanger 1, 2, 3 you can see the three different heat exchanger. Now this particular heat exchanger we call it economizer and that this is this we this we consider as the economizer, the reason is that you know I can explain that the raw media is going like this and it is coming out like this again going through the this is the steam injection and after going through the holding section it is, so what I wanted to tell that here you raise the temperature to 140 degree centigrade and then you are keeping in the holding section you cool it down

And this system you are passing through the heat exchanger and to you are utilising your raw media here just to use this heat for creating the media so you save some energy here that is we call it economizer. This is called the you say some kind of heat we had and then your temperature will come back come around say 140 to 80 degree centigrade, then we pass through the chiller to have this is you pass the cooling water, now then we get the sterilizing media. Now let me explain what is called how the how the pate heat exchanger looks

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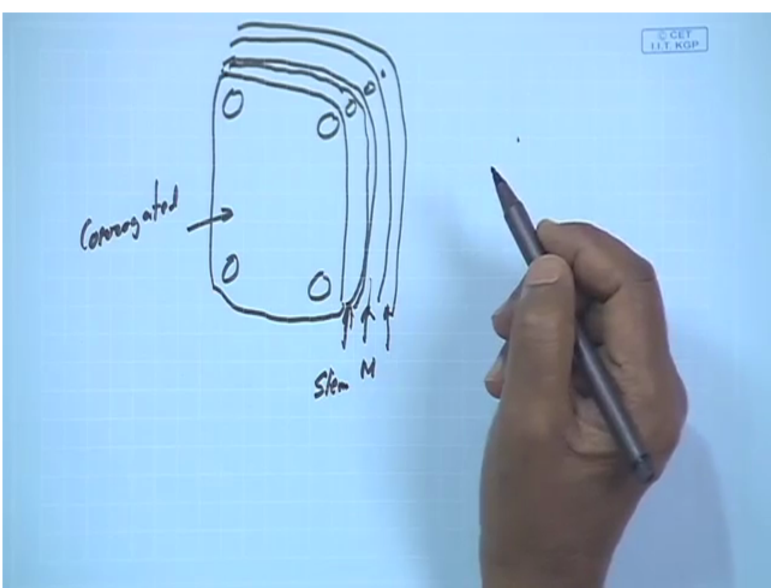

Plate heat exchanger looks like this, so basically it is a plate like this. So several plates they are bunching together like this, so we have this different plates they are bunching together. Now this surface is corrugated, the surface is corrugated, why it is corrugated, so if you make the surface corrugated then what will happen the surface area will increase. We know the rate of mass transfer depends on the surface area, more surface area more will be mass transfer.

Now what we do, suppose you are passing your media here suppose your media here and you passing your hot the heat may be steam you are passing through these or hot media you passing though this, so when you pass this plate will come in contact with each other and then it will heat your desired media and take it out from this particular sterilizer. So all these plate heat exchanger three plate heat exchanger this is like this, similar this is parallel the plates are they are bunch together, several plates may be 50, 100 plate they are bunched together

Now problem is that this particular sterilizer has severe problem what is the problem that this plates one plates when binds with each other you put a gasket here in between this we put a gasket then we can tie it together and since suppose we are using the steam as very high

pressure. There is a every possibility when you pass the steam at a high pressure then leakage of the your gasket may bust. That you know if the gasket burst then what is there will some steam leaking is there suppose you in between the when you pass your liquid

If some leakage in the gasket is there some kind of cracking in the gasket is there, then also your media can goes out. Those kind of problem that we have, another problem is that suppose this is the steam that means it is the high temperature this is the liquid so there is the possibility that the surface will be there is skill formation on the surface of the plate, now if there is the skill formation on the surface of the plate then what will happen.

The conductivity of the material will be lost; if the conductivity of the material lost then what will happen more steam will be required more heat will be required for heating the media. So that is not desirable, so what you have to do, you have to open this plate and clean the plate time to time so that you can have the effective heating and cooling in the system. So this is the major drawback of this particular process but this is largely in operation with the industries.

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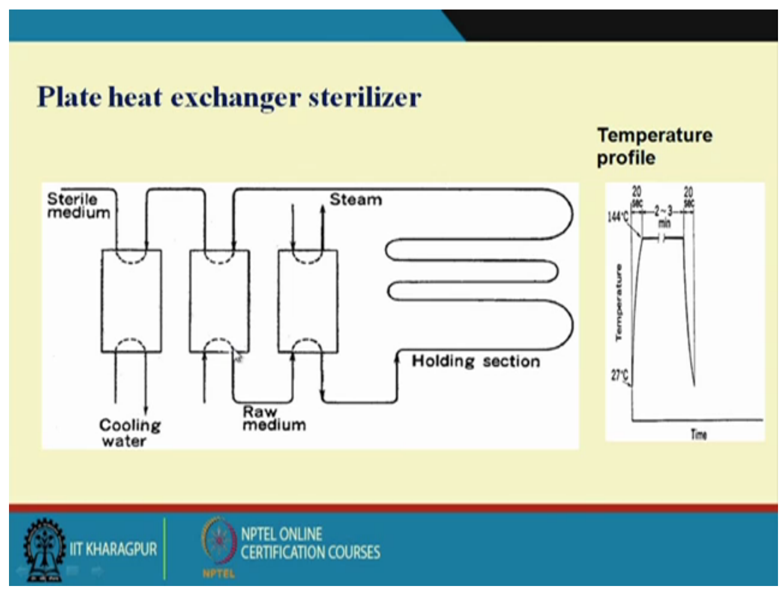

Now I here I have given you the temperature profile as the here you see that when your raw media is coming it requires some time to heat the partially heating and then it is heating to 140 degree centigrade, this is the so you require some time about 20 seconds is required for heating purpose, then 2 to 3 minutes for holding maintaining the temperature 140 degree centigrade here it is 144 degree centigrade. They have mentioned then this is 20 second for

cooling purpose this goes here and then pass through the chiller, the whole thing that requires the time required for that is 20 seconds

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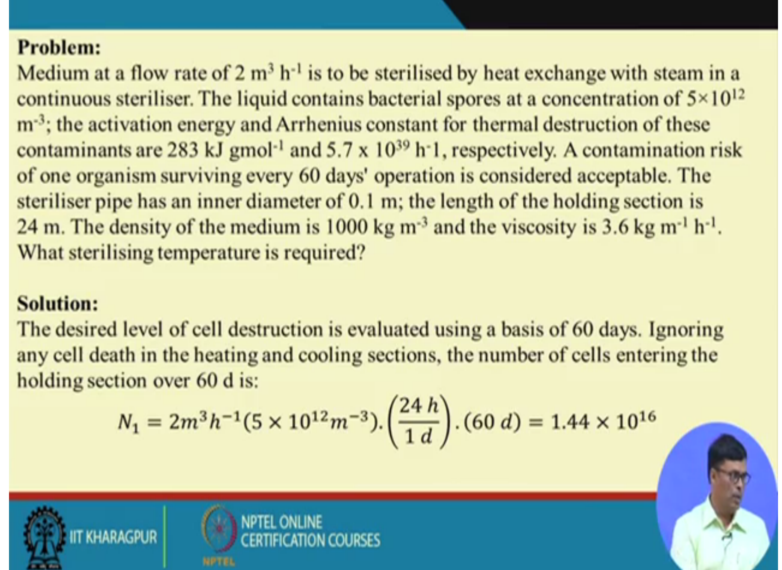

So this is how the sterilization is done medium sterilization done in the industry. Let me discuss some problem related with the medium sterilization process so that so that we can have better understanding on the process. Now the problem is like this, media has a flow rate 2 metre cube per hour is to be sterilized by heat exchanger with steam in a continuous sterilizer. The heat contains bacterial spores as a concentration of 5 into 10 to the power 12 per cubic metre

The activation energy and Arrhenius constant for thermal destruction of these contaminants are 283 kilo Joules per gram moles and 5.7 into 10 to the power 36 hour inverse respectively. A contamination risk of one organism surviving in 60 days operation is considered to be acceptable, now the steriliser pipe has an inner diameter 0.1 metre. The length of the holding section is 24 metre, the density of the media is 1000 kg per cubic metre, the viscosity is 3 point 6 kg per metre per hour, what is the sterilization temperature required? So this is I hope you understand this problem because what I suggest that we should have an understanding on this process that how it how it actually in practise.

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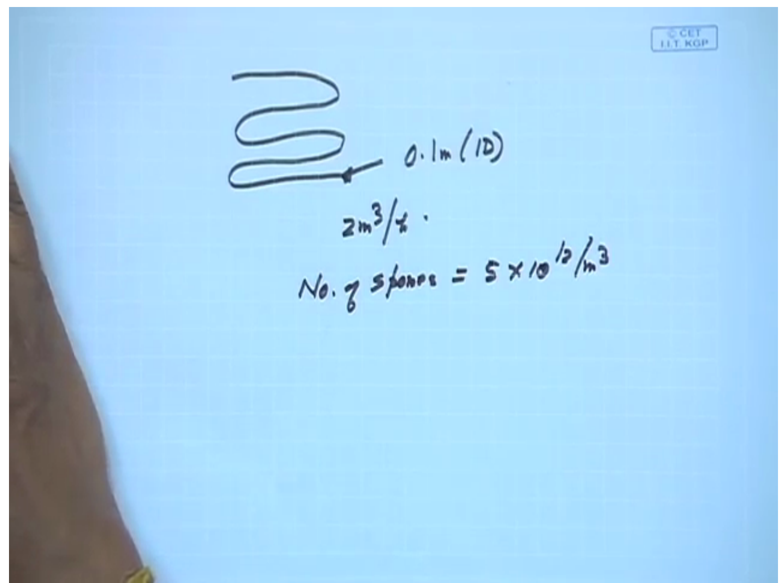

So you can you can write a sterilizer like this and where they have mentioned that inner diameter of the pipe this 1 metre this is the inner diameter of the pipe so ID what you call inner diameter is considered as ID. Now, the flow rate of the media is about say about 2 metre cube per hour that is given and media contains about number of spores is how much, number of spores bacterial spores is about 5 into 10 to the power 12 per cubic metre am I write. Now activation energy that is given the contamination this is very important a contamination risk of one organism surviving every 60 days operation is considered, now in a continuous flow reactor then first you have to calculate how many organisms is present if you operate this for 60 days

So what we can have this is 2 metre cube per hour is the flow rate, am I right? And then how much is the organism present 5 into 10 to the power 12 per metre cube, so this is that means how much organism per hour is going, this is you multiplied by this factor. Now you can convert it to per day am I right if you multiply by 24, 1 day is equivalent to 24 hours, so if we can multiply by 20 days you can per day how much organisms are entering into the system, so he is saying that 60 days operation so you multiply by 60 days so how much organism will be there, 1.44 into 10 to the power 16 this is the number of organism.

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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:

$$u = \frac{2m^3h^{-1}}{\pi(\frac{0.1\ m}{2})^2} = 254.6mh^{-1}$$

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

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

Now he is saying that one organism out of that 60 days operation is acceptable that means I can write N2 by N1 value 1 by this and this is equal to 6.9 into 10 to the power – 17 that we can write. Now from the flow rate because we know the inner diameter this of the pipeline is 0.1 metre and this the what is the cross section of this is Pie r square Pie r the cross sectional area.

Now if you divide by flow rate divide by cross sectional area, suppose flow rate is the volume by cross sectional area what you will get you will get volume per unit time and cross sectional area you will get that you will get here you will get metre per hour that means length per hour you will get some length per hour that is exactly whether that is metre cube and that is metre square so this will be metre per hour, so velocity you can easily find out

Why we want to find out velocity, because we want to have the Reynolds number the Reynolds number is a Dv Rho by Mu so if you know I know the diameter of the tube is 0.1 metre and velocity is 250.6 that metre per hour and Rho is 1000 kg per Rho of the water is like this per metre cube and viscosity is 3.6 kg per metre per hour then this Reynolds number is coming about 707 into 10 to the power 3.

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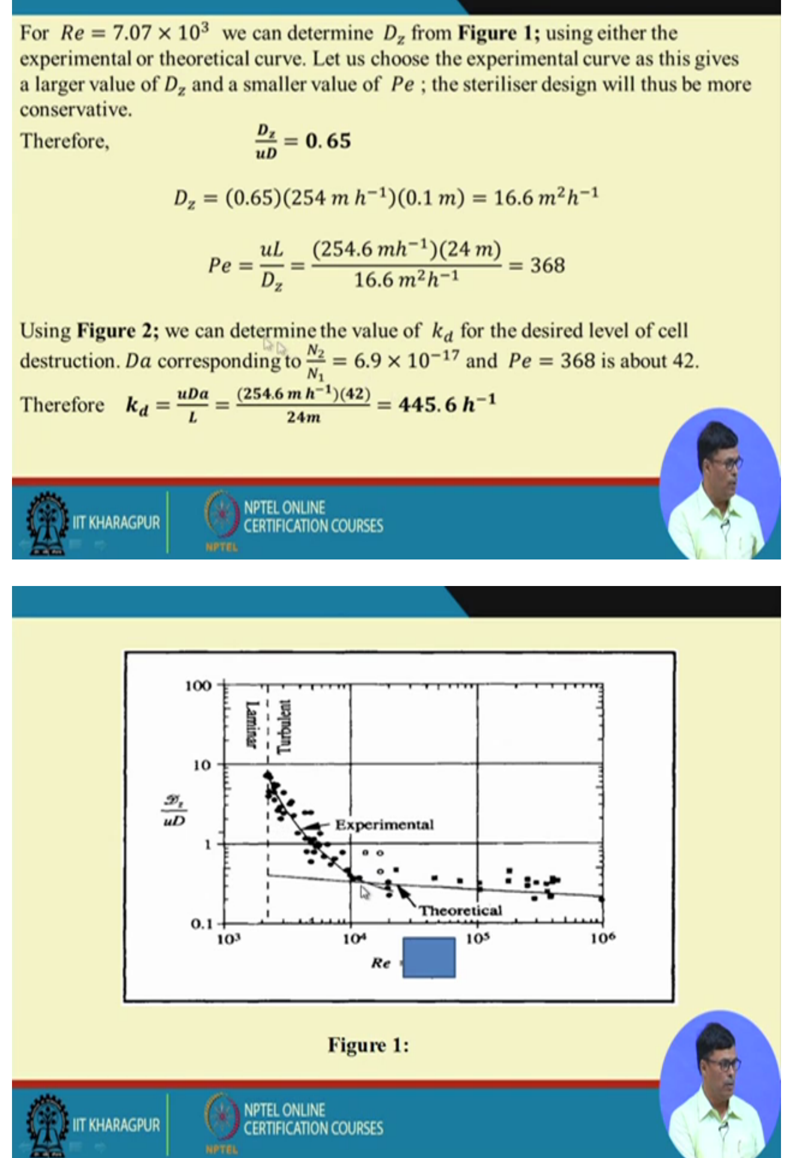

Now the Peclet number we can from this we can calculate the Peclet number, Peclet number how we can calculate we can calculate the Peclet number equation is that u in L by Dz, Dz is the that that Dz value the we can calculate from this equation, dispersion coefficient that we can calculate with this we have a plot between the Reynolds number and this dispersion coefficient here that this is diffusivity this is the velocity

This is kind of (())(17:54) so if you know the Reynolds number your corresponding value you can find it out and if you know that that from this Reynolds number is 7.07 into 10 to the power 3 we can determine the Dz value that either experimental let us choose the so this is this is from this figure we can have this from this we can have this value there somewhere here and then we find out the z value which is coming about 16.6 square metre per hour. Then

your Peclet number is equal to uL by Dz , u is the velocity that is like this 254.6 metre per hour into 24 metre is the length of the tube then divide by Dz value you will get 368.

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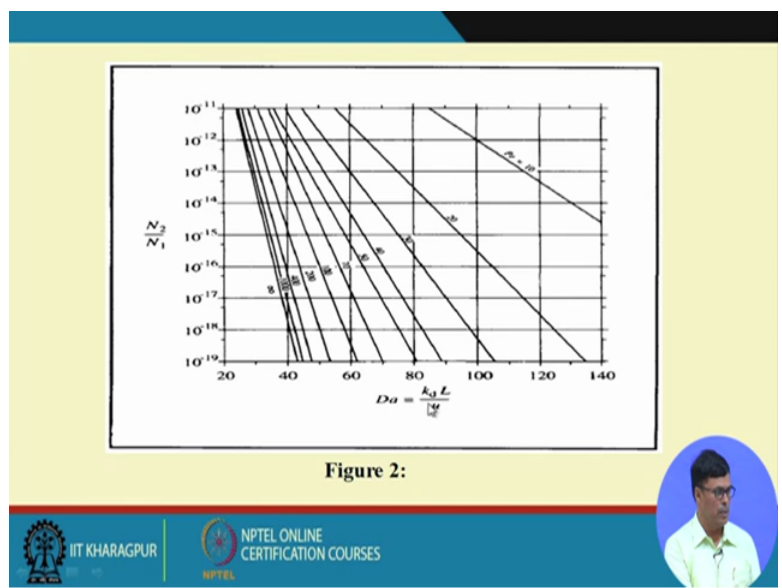

Now figure if you look at the figure 2, it is the plot between the N2 by N1 against the this is the what you call damn polar number KdL by u, so you have different values here so you can you can you can find out the if you know these values corresponding value you can find it out. So N by this value N2 by N1 value is 6.9 into 10 to the power – 17 and Peclet number is this, then Da Da value is coming about 42. If it is 42 and actually that if you look at this is different Peclet number, Peclet number 10, 20, 30, 40, 50, 70 like this. As your Peclet number changes, this will be becoming steeper and steeper, so we can find out this value 42 then Kd equal to what is this equation equal to u if you look in this equation, Da equal to Kd Kd is the thermal death rate constant L by MU so if you if you know the Da value and you know L value you know u value, so you can easily find out the value of Kd Kd is coming about for 445 into 0.6 into hour inverse.

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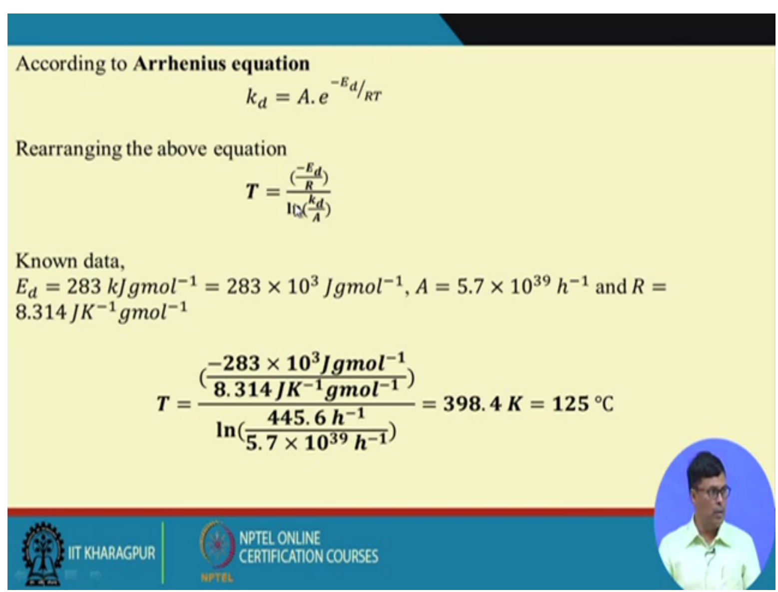

Now once you get that now your situation is little bit simpler because we know as per Arrhenius equation this equation is there the Kd equal to A into e to the power – Ea by RT, Ea this will be Ea by RT is a activation energy. Now energy required for deactivation of the cell Ed also sometimes we write so we can do this and find out the what is the temperature required for the sterilization. So all these values is given here, your that the activation energy required for killing the organism death of the cells is given, Arrhenius constant is given gas constant we know then you put this value we will find this temperature, so the temperature of the sterilization is coming about 125 degree centigrade

So this is how we can calculate the temperature of the medium sterilization, we can easily calculate. Now let me show you how we can transfer the because now we have the information with us how media can be sterilized, how air can be sterilised, now things that remaining with us that the culture we prepare in the lab and that is to be aseptically transferred in the inoculum vessel because I told you the volume of the inoculum vessel usually 5 to 10 per cent of the production fermenter.

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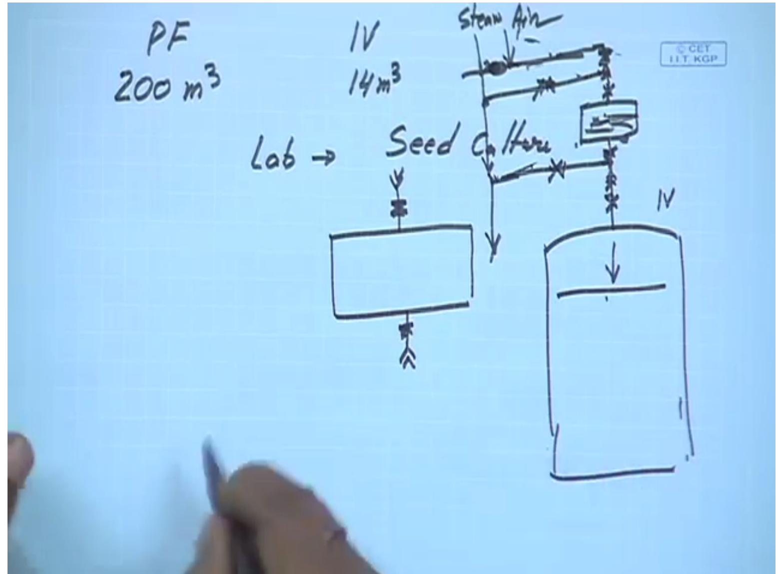

And I work with industry where the capacity of the production fermenter was 200 cubic metre and inoculum vessel that is where we prepared the inoculum is about 5 to 10 per cent in our case it was 14 cubic metre, so 14 cubic metre is 14000 litres, the 14000 litres culture we cannot prepare in the lab. So that is why you have to prepare this inoculum in the fermentation plant itself. So what we do in the lab what we prepared the seed culture.

And through our research and developmental work we try to determine that what is the optimum concentration of cell required for the optimum amount required for the preparation of the media that we shall have to determine and once we have this value, I can give a very typical example that in case of bacterial fermentation process particularly for the unicellular cells we do not have any problem because unicellular cells you can count the number under the microscope and you have haemocytometer so you can count the number what is the concentration of cells. And even you know if you if you know the mass total mass of the cells and if you know what is the weight of individual cells from that also you can convert it that how many cells are present there. It is not very difficult either you can take the mass or you know the density of the cells you can easily find out how many cells are there, so now question that is we face in case of the fungi

Fungi is the multicellular organism it has filamentous growth so there it is not a unicellular cell so how we can count the numbers so in case of fungi we use the spores because under stress condition it produces spores and spores can be counted because we can count the spores. We can under the haemocytometer we can count so you have to quantify that how

many spores is required, so in case of unicellular cell we do not have any problem we can use directly unicellular cell or the or the yeast cells we do not have any problem we can count the number and we can find out that what exact number of cells is required q what number of cells are exactly required for the preparation of the inoculums.

So that number you have to maintain then that so that we put it in the seed can like this you know seed can looks like this. What is the seed can it is here we have a valve and here we have a valve, now here we have some nut and bolt arrangement. Now in the industry what you do suppose this is the inoculum vessel, so if you want to transfer this here also we have valve so here when you bring this seed culture here.

So we can we can here we have valve here we have nut and bolt arrangement, here also we can we can plug together, here also we have valve and here we have nut and bolt and here we have another pipeline maybe it is connected with steriliser, so it is like this and also there is another line what you have steam line, steam line that is for sterilisation of the line that pipeline, so you have to you have to sterilise this pipeline before you transfer

We have to pass the line so that and take it out to insure that line is 100 per cent sterilised and then you have to sterilise this line also and then you take it out. This steam you can take it out and when we insured that your pipeline is 100 per cent sterilised then you close this valve, you close this valve then you open this valve here there is valve is there, there is nothing is there so if you open this valve the air will go here and here is closed and you open this valve open this valve open this valve.

This is closed then with this air pressure whatever seed culture is there that will come the inoculum vessel, this is IV you can you can transfer it and when it is completed you close it you close it you close it you close it again you pass the steam here so that in a pipeline whole pipeline will be sterilised and then you run this operation but when you get the culture now how you do that how you transferred this culture from IV that inoculum vessel to production fermenter.

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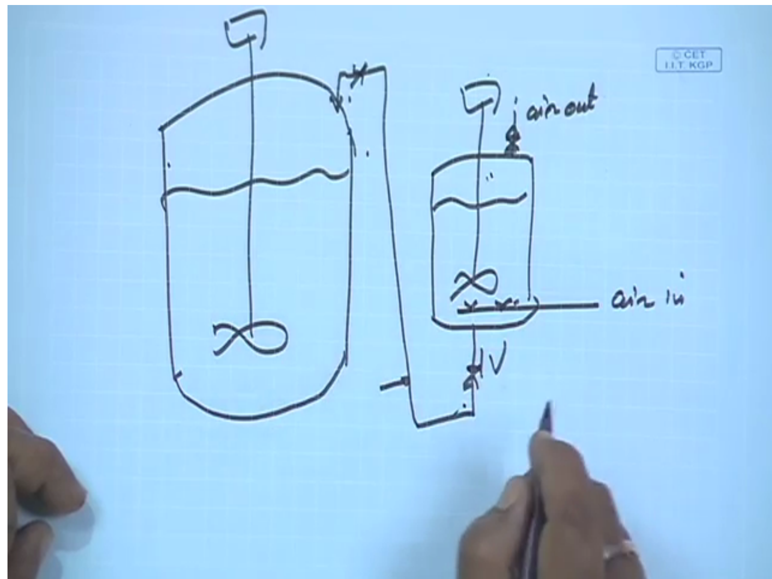

Suppose this is the small vessel small inoculum vessel this is inoculum vessel that we have and this is big production fermenter, this is IV and this is production fermenter. So here also you have like this, so what you do that here we do sparging this is air am I right, air in and this is air out. Now if you close this air then what will there will be tremendous pressure here air pressure, with that air pressure liquid will come here and you can fall it here so you can transfer the culture usually aseptically then you can have the valve here, you open this valve and drag the culture from this vessel to this vessel.

So this is how we can and then when it is transfer is taken place then we can close this valve close this valve and again we do the sterilisation of the valve. This is how we can aseptically transfer the media transfer the culture from the inoculum vessel to the production fermenter. So this is in practise in the industry and this is find very effective. Thank you very much