**Aspects of Biochemical Engineering**
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**Lecture - 53**
**Medium sterilization – I**

Welcome back to my course Aspects of Biochemical Engineering. In the last 2 lectures I try to discuss the air sterilization process. Now, this particular lecture and coming lecture, I am going to discuss the medium sterilization. Because, the medium sterilization is appears to be the one very important aspects as per biochemical industry is constant, because the purpose I let me repeat it again that purpose of this sterilization is to allow your desired organism to grow and that is why you want to make the environment to totally free from other organism so, that your desire organism can grow.

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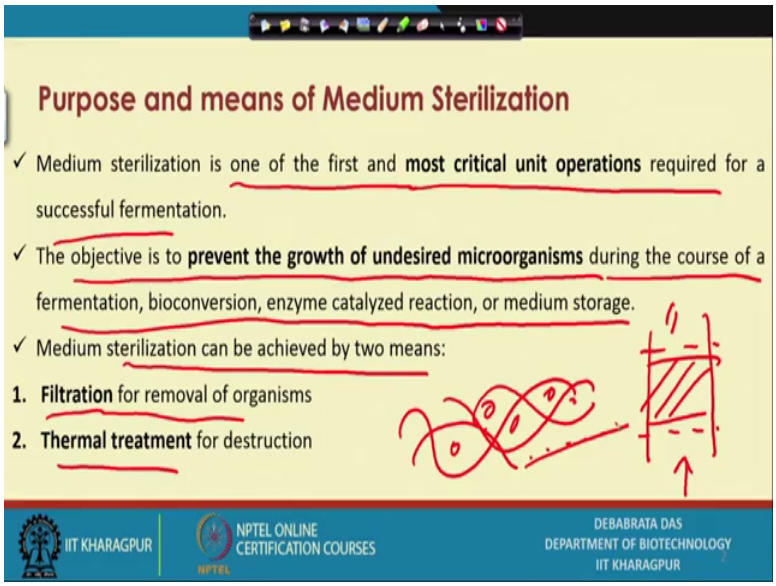


Now, here if you look at that what is the purpose of the medium sterilization; medium sterilization is the one of the most the one of the first and most critical unit operation required for successful fermentation. The objective is to prevent the growth of undesired microorganism during the course of a fermentation and bioconversion enzyme catalyzed reaction and medium storage.

So, you know that these are the main purpose as I told you the medium sterilization can be achieved by two means; one is filtration and there is the thermal treatment for

discussion. Now filtration I have we have shown previously in the filtration what we do we physically remove the microorganism? I what I told in that suppose do you have fiber like this. In between the fiber here when you when you suppose these have this is filter I told you this is filter and this is the supporting material. So, when you pass your air like this is the fiber and in between the fiber your organism will be entrapped am I right.

And in case of another type this is the fiber in case of membrane we have pore size on the basis of that we can, will not allow the bigger size particle to enter into the system this is how we can do that? And thermal treatment is the, we heat it so, that you know your organisms are killed. So, I told you that high temperature the protein undergo denaturation process and due to denaturation of the process the process denaturation of the protein, the biochemical activities of the organism will be stopped and your organism will be killed that ok.

Now, that now in case of media that since the media is a good conductor of heat, that we considered the heat is the best media for the sterilization of med the medium, but in not like air sterilization because air sterilization I mention the air is a non-conductor of heat.

So, there that is why we consider the filtration technique for air sterilization. Now, in case of medium sterilization since we are it is a aqueous base water base and water is good conductor of heat. So, we go for thermal sterilization process.

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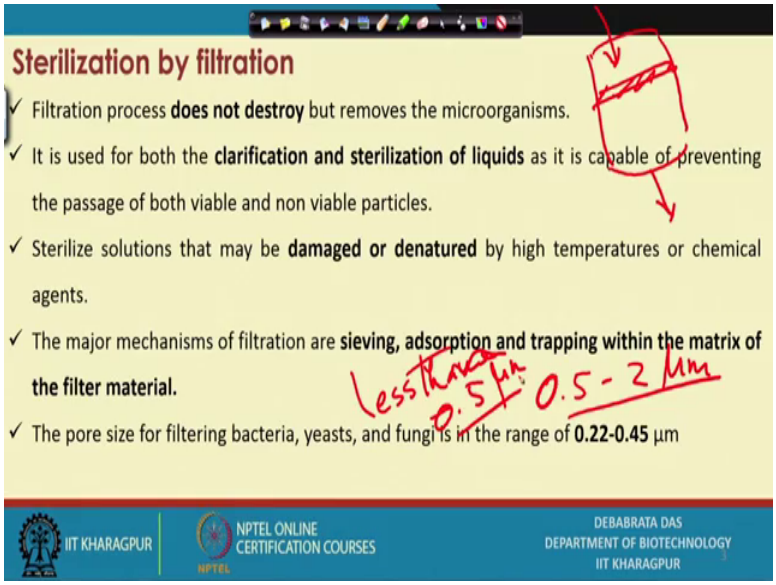

Now sterilization of filtered, now sterilization is filtered with sometime we used to do because let me tell let me tell you the, for why we do not use this filtration technique for medium sterilization, because again it depends on the on the on the on the type of fermentation you are looking for.

Because, why because suppose I use some kind of membrane filter am I right, now in case of membrane filter this is the membrane and you want to pass your material bonding then take out the material form here other end. Now, suppose size of the bacteria is 0.5 to 2 microns am I right.

So, your what is this what is the pore size of the membrane, it should be less then, it should be less then less than 0.5 micron less than 0.0 0.5 microns am I right. So, now if you pass air that that media through this through this membrane there will be tremendous pressure drop across the across the membrane. And due to the tremendous pressure drop your operational cost of the process increase very high. So, your operational and not only that you know due to high pressure difference, what will happen that the life of the filter that membrane also is a very important factor because life of the membrane will be reduced am I right.

Now, let me discuss about the how the sterilization process is they affected by filtration. Filtration process does not destroy, but remove the microorganism It is used both for the clarification and sterilization of the liquid as it is capable of preventing the passage of both viable and non-viable particles. Sterilized solution may be damaged or denatured by a high temperature or chemical agent. This is the, this is one of the reason, where we call we can consider that that you know that filtration by that sterilization by filtration in case of medium sterilization process. And we have we have we have come across 2 type of byproducts, we have in the market one is high value products; another is the low value products. High value products means per unit cost of the product is very high.

So, when we handle the high value products; that means, your volume of the liquid volume the, that your product that will be very less. There we can we can think for this sterilization through this filtration process. Now, major may the mechanism of filtration by sieving adsorption and trapping within the matrix of the filter mat material. The pore size of the filtering bacteria yeast and fungi is in the range of 0.22 to 0.45 microns.

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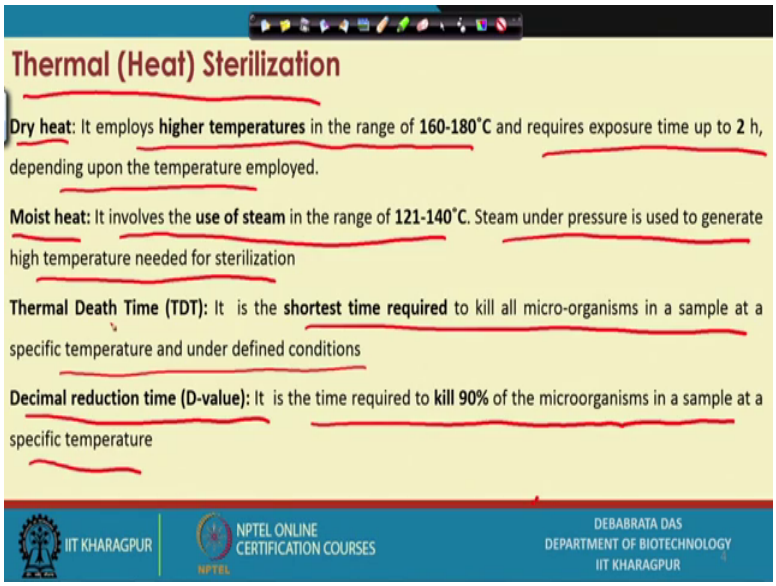


Now, let us see what are the advantage of using this that you know thermal sterilization process. Now thermal sterilization as I told you that in case of thermal sterilization process, because why we use because water is a good conductor of heat that is one of the reason and it when you heat it your bacteria or you know organism that protein present inside the organism that undergo denaturation.

So, that the activity of the enzyme will be lost and organism will lose their biochemical characteristics. Now, this can be done into 3 2 ways by using one is by using dry heat and moisture heat dry heat means it employs the higher temperature in the range of 160 to 180 degree centigrade. And required the exposure time 2 hours depending upon the that temperature employment, but in case of moist it is quite effective this is 100 and 21 to 140 degree centigrade and steep the steam under pressure is used to generate higher temperature needed for sterilization. Now because; obviously, the moisture is much much more effective as compared to dry heat that I told you that and dry heat means by using hot air we do that and moisture it by the help of steam we do that.

Now, and now there is a there is a term called thermal death time what is thermal death time means the shortest time required to kill the microorganism in a sample at a specific temperature, under never defined conditions. This is the this is the this is the how to get thermal death time and decimal death reduction time is what it is the time required to kill, that 90 percent of the organisms in a sample at a specific temperature. So, 2 type of

time we consider one is thermal death time that to kill the microorganism how much time is required and that is the decimal reduction time to reduce the organism to one tenth that is that is why we call it decimal reduction time.

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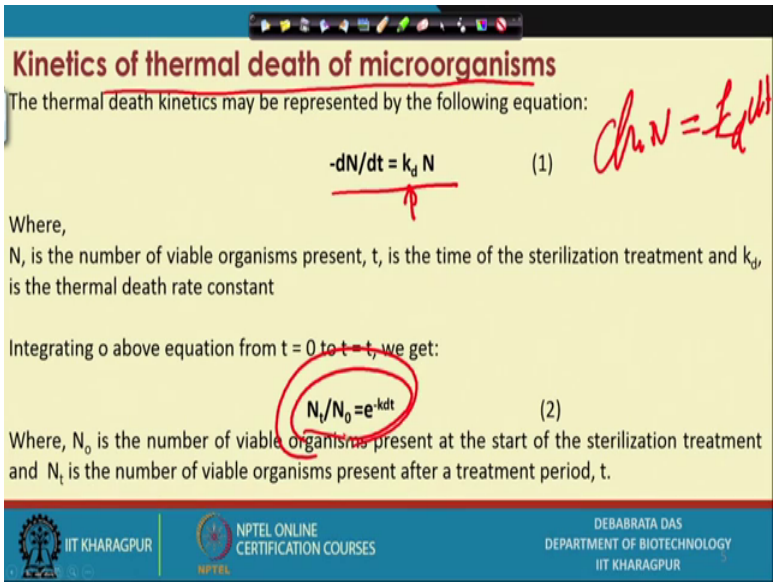


Now, the let us see the equation the kinetics of thermal death rate of microorganism. Now, d N by d t this is the first order kinetics k d is the thermal death rate constant and N is the number of viable organisms this is this is the how we can write it. Now, this we can we can express easily in this form that you know this is d l N d l N N equal to k d into d t am I right. So, this we can write in this form if you write this is minus so, we can write in this form.

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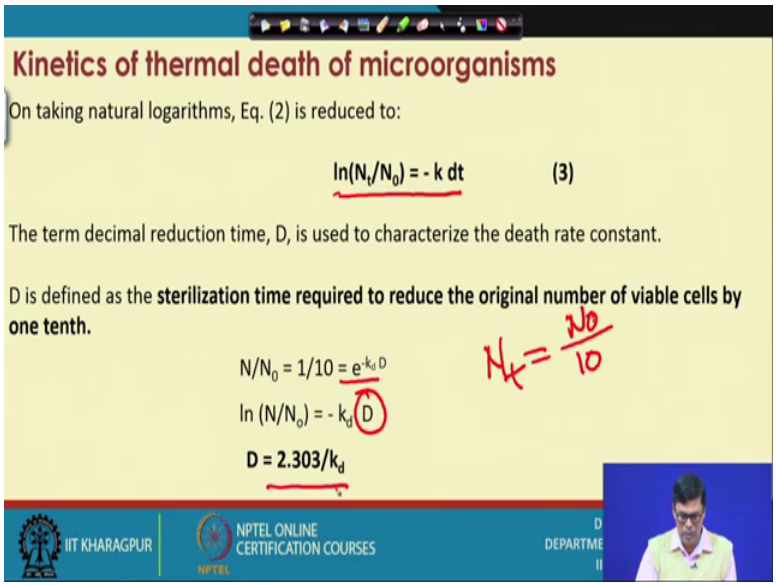


Then from this equation we can we can we can we can come this equation the l N I told you this is the l N l N t l N t by a N 0 k into d t and decimal reduction time is what then I will told you that that N t whatever is there it should be this will be what N 0 by 10 decimal reduction time a 1 10th of that. So, you know that. So, this is exactly that you know we can we can N by N 0 that will be one by 10th, that will be equal to that e to the power k one e to the power k D into D that D is the decimal reduction time. So, this will be equal to 2.3 0 3 by k D.

So, here I want to point out that when decimal reduction time is inversely proportional to k d.

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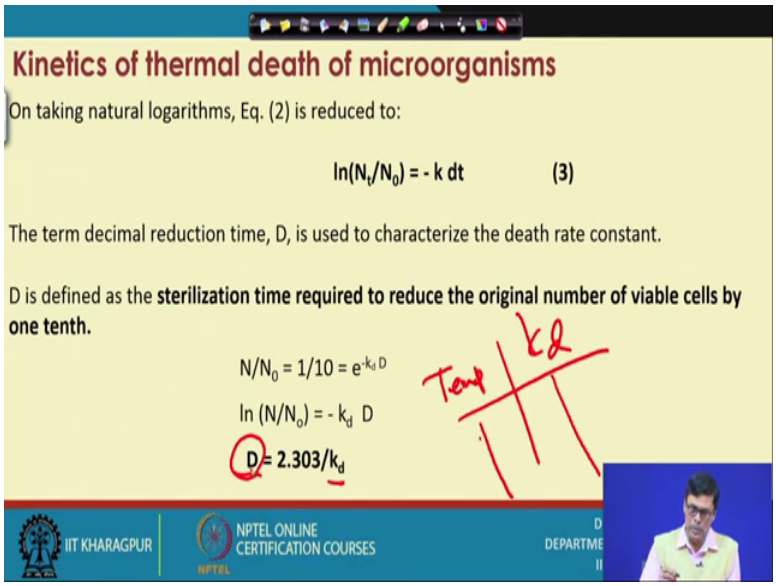


That means as your temperature increases suppose you have this temperature and this is k d value as you increase the temperature you will find the k d value also increases. Now as your k d value increases then thermal decimal reduction time will reduce significantly. And, this is the advantage of this is the advantage that we use in case of continuous realization process I shall explain you later.

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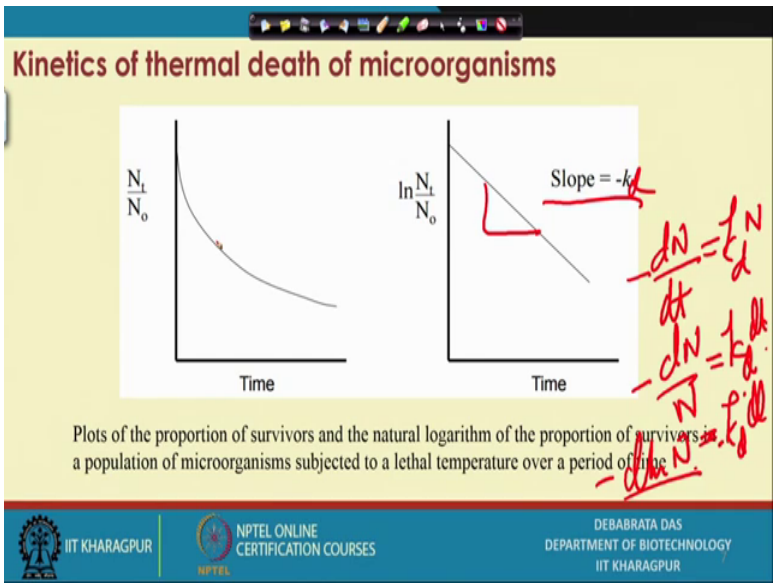


Now, this is the plot that we have this is exactly that I I discussing about that minus d N by d t equal to k d into N am I right. So, this is the straight line plot if you if you if you if

you write like this d N by N is equal to k d into d t I want to know. Now, if you plot and this is what this is equal to d l N n minus this is equal to k d into d t. So, this is negative this will be negative plot here if you take it out it will negative.

So, it is negative plot there it will be straight line basically this is y equal to m x. So, basically this will be a straight line and slope slope will give you the value of k d this is k d is the decimal reduction time. Now, if you if you if you if you have normal plot N t by N 0, then you will be having this kind of plot.

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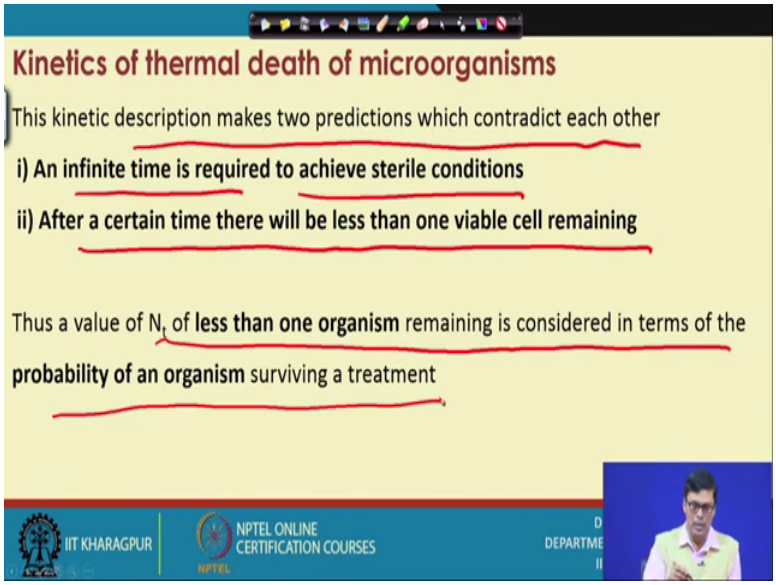


So, if you look at the kinetics description makes 2 prediction, which contradicts each other at a finite time at infinite time as the infinite time increase is required to achieve the steriliz sterile condition, and after certain time there will be less than one viable organism remaining, that is exactly I was try to point out that you know to get 100 percent sterility is very difficult to achieve.

So, what we do one how much organism will allow how with on the on the basis of how much organism input, that that we shall have to do to infinite that an infinite time we require to achieve the sterile conditions the value of N t less than one organism is considered in terms of probability of the organism of surviving of a treatment.

So, how much organism will be survived out of will how much organism input that we considered, that that determines the degree of sterility now thermal this is very interesting.

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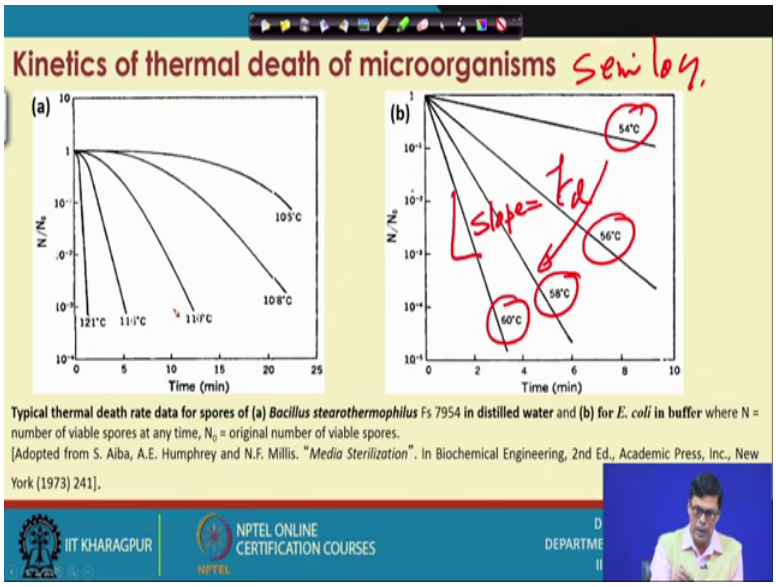


If, you look at that thermal this we have already shown this is log log graph paper you can see that (Refer Time: 14:17) 10 to the power 10 to the power minus this is this is log log log this is log semi log graph paper not this is semi log. So, this is this is the time. So, we find that as the temperature increases like this. So, this is 54 this is 56 58 50 60. Now, as you time this slope will be recovering steeper and steeper and this slope will be (Refer Time: 14:45) this slope I we have already find out this is k d. Then, k d value increases as the temperature increases am I right. And, if you normal flows normal plot will be this is kind of pattern we will get.

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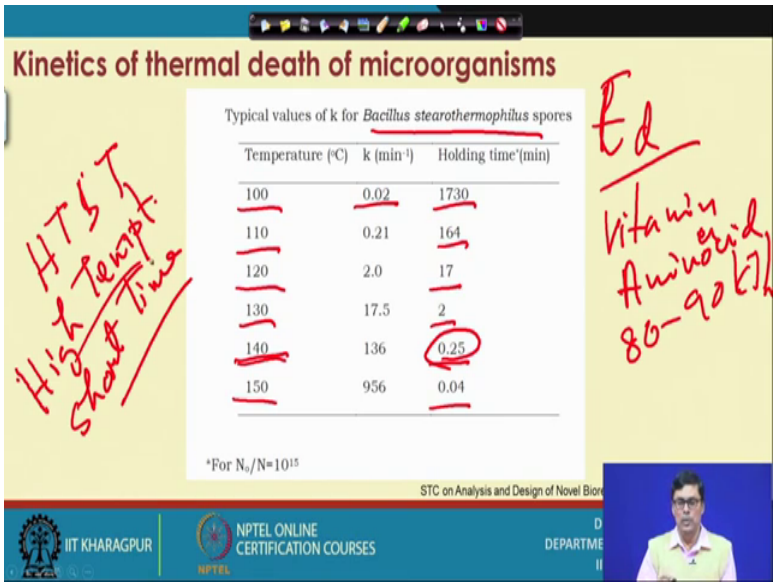


Now, this is the this is very interesting thing that if you look at here and a 100 degrees, if you are 100 degrees sterilization temperature k d value or the thermal death rate constant value k is the thermal death rate constant, the holding time is a 1 1 1730 minutes; that means, this is the sterilization time that is required. Now, a 100 10th degree centigrade it is required 164 degrees centigrade this is by using the organism bacillus stearothermophilus.

Now, why we considered bacillus stearothermophilus because they use the heat stable organism that is why we have taken in to consider. The 120 degree centigrade it is 17 minutes 130 degree centigrade it is 2 minutes, 140 degree centigrade in 0.222 5 and 150 degree 0.0 0 4. Now, what does this signifies is signifies that as you temperature increases your time drastically reduce and that is very important. Why it is important, because if you look at that you know deactivation temperature of the vitamins or amino acids, it is the usually lie in between what 80 to 90 kilojoules per mole am I right. Now, if you look at the microorganism it is very high it is about 300 or something because close to 300 kilo joules.

So, maybe so, what I want to mean that even this a 100 degree centigrade means you heat it for very as a very small temperature. So, then you have to keep it for longer period of time. So, if you keep it for longer period of time that your k d the deactivation energy, that is required for the deactivation of the vitamin and amino acid that already

you supply. So, you know that that degradation of the medium quality will decrease to a great extent. Now and, but if you increase high temperature then you are reducing you are using very short time. And naturally that you are you are holding the media for the shorter period of time and the loss of the nutrient will be very less, that is why in the industry we use the technique what we call H H S T. H T S T means high temperature short time, this is largely used by in the media.

Because, if you expose because now I can give the example suppose in a media you have some nutrients and concentration is there let us assume the volume of the media is V, and the quantity of the ingredients that here were talking about the let us assume the amino acid concentration maybe V C.

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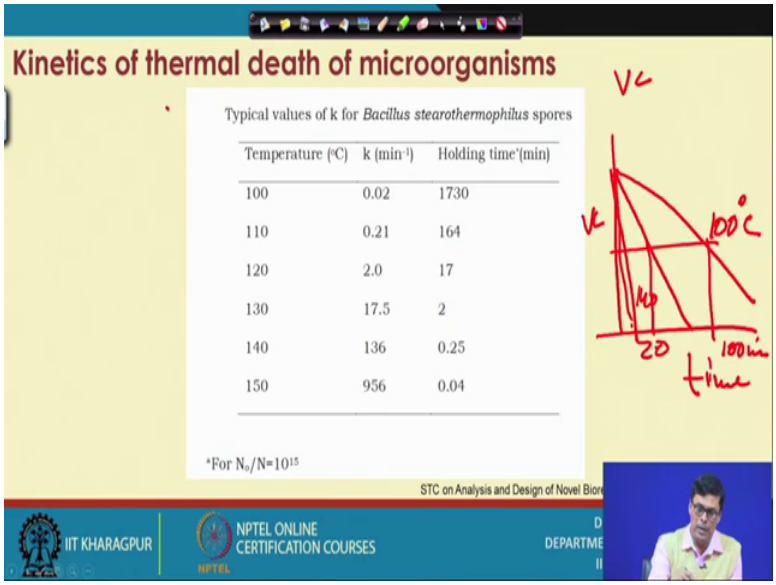


So, you know that. So, so this will like this, the obviously, it made and this is this may depends on temperature this is how the V c quality that you know decrease with respect to temperature. So, you can easily find out now suppose this is this is 100 degree centigrade. And this is maybe 100 and 40 degree centigrade, but it might be may maybe this is the maybe for 100 minutes this is might be for 20 minutes.

But, you know that if you go like this that you know that even at low lower value you will find that what I want to say I want to tell you that, if you go for higher temperature, then your exposure time will be very less that you drastically reduce. So, the quality of the vitamin will be will be remain like this.

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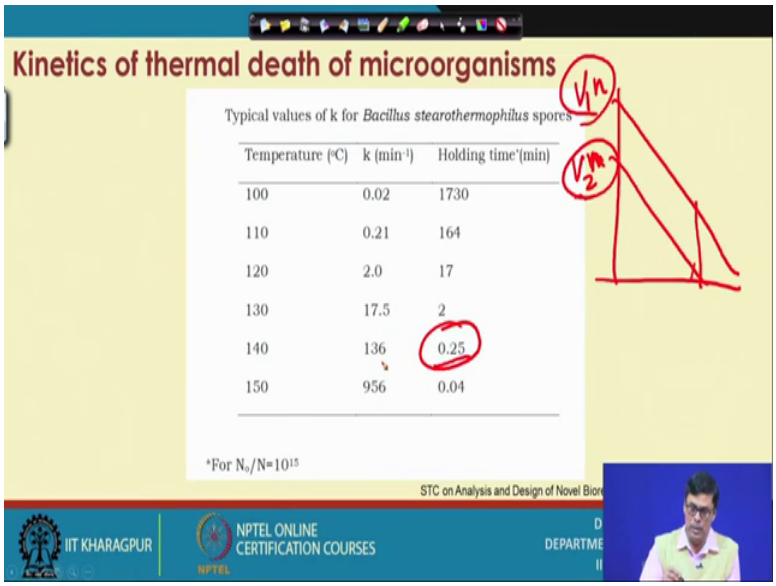


So, what I get I can tell this is this is 1 plot this is another plot. So, so, we have we have we can we can easily find out that the quality this is V 1 this is let as V 1 into n and this is V 2 into n. So, this is the smaller volume and this is the bigger volume. So, if you if this is the plot that you have.

Now, in case of in case of batch sterilization process, if you as you increase well to the you know that lab scale to the commercial scale you have to handle huge amount of liquid. Naturally you had that the time requirement for the sterilization very high as compared to so, this is one of the bottleneck and second bottleneck is the time up for this exposure time, and due to this 2 reason that our we do not use this in the industry we do not use the batch sterilization process for the sterilizing the media.

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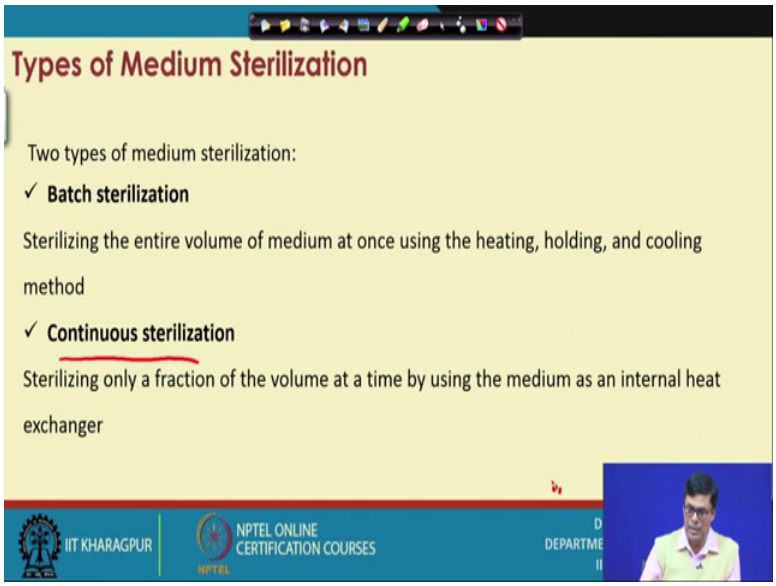


So, what we use we use the continuous sterilization. Now, batch sterilization I shall I shall discuss little bit in details that in the in the batch sterilization, what is happening?

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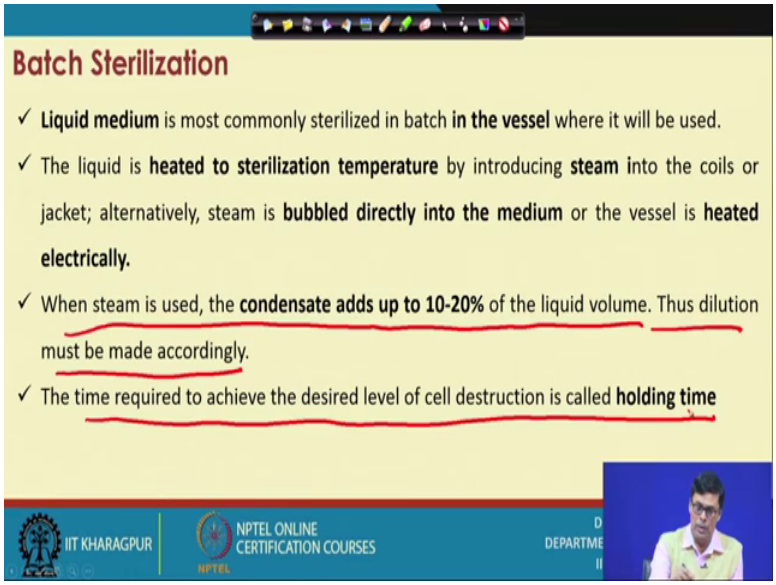


That, you know the liquid media is mostly is commonly this sterilized is sterilized in the batch vessel, where it will be used and the heated the sterilization temperature.

Because, what I want to mean here that you see that the it add up 10 to 20 percent of the liquid volume, because you know that is the that condensate because this is very important when steam is used the condensate added (Refer Time: 21:11) the 10 to 20

percent of the liquid thus the dilution will be will be made accordingly. The time required achieve desired level of destruction is called the holding time.

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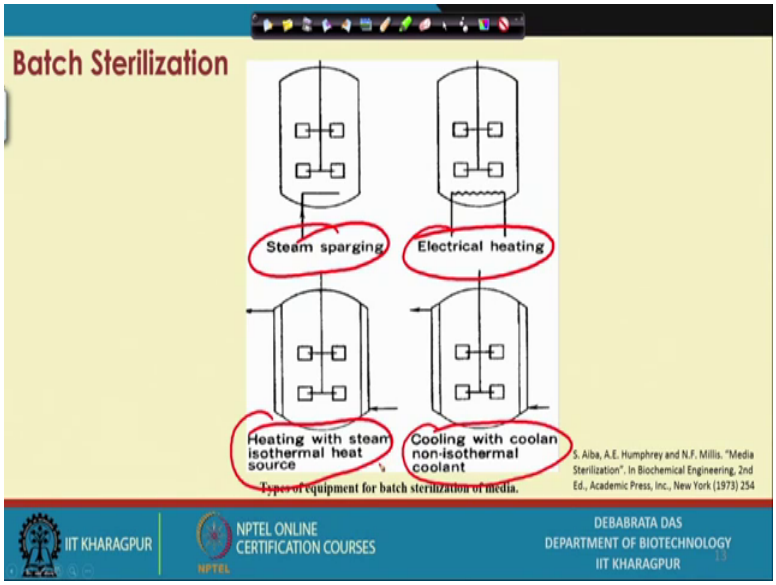


So, this is the major problem that we have I shall discuss more in details here and this is the device through, which we can we can do the batch sterilization one is steam and the electrical heating, and the heating will stream isolated heating source, and cooling with the coolant non thermal coolant, that we will different type of system we can use.

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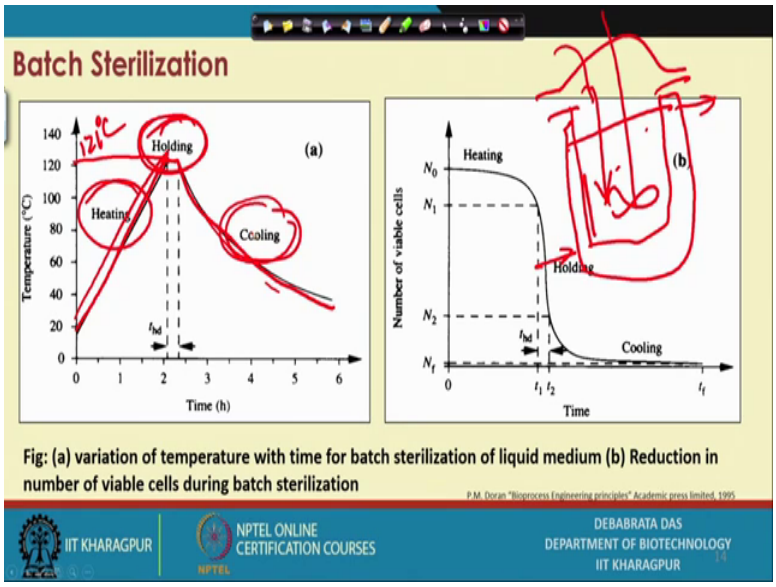

Now, this is the major problem that we have. Suppose this is the liquid am I right this is a liquid that present in the reactor and we want to heat it. Now, what we do that? So, suppose we can do it in two ways we can pass hot a hot a water here and increase the temperature this is the kind of started we have or we can inject the stream directly we can inject a we increase the temperature.

Now, when whenever we do that first your temperature will rise like this, then you have to hold this temperature because our sterilization temperature is 121 degree centigrade am I right.

And, then you hold for some time unit victories. Now, during heating the process the some organism will be get killed during the holding the process some most of the organism will be killed and cooling process also some organism will kill. So, it is total sterility not only depend on the holding section, it depends on the heating section as well as when the cooling section.

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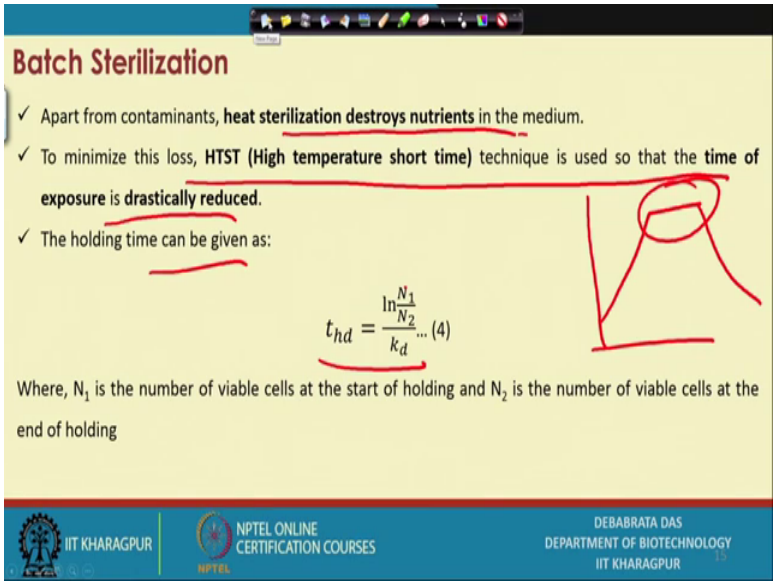


Now, this is the apart from the contamination the heat of sterilization destroyed the nutrients in the media, high temperature short time techniques is used. So, that the time exposure is drastically reduced the holding time we can holding time what is the specialty of that in the holding time you are melting the temperature constant.

So, your k d value is constant. So, we can write the time required holding time is the N 1 to N t N 2 that is reduced is divided by where we can find out what is the holding time required here?

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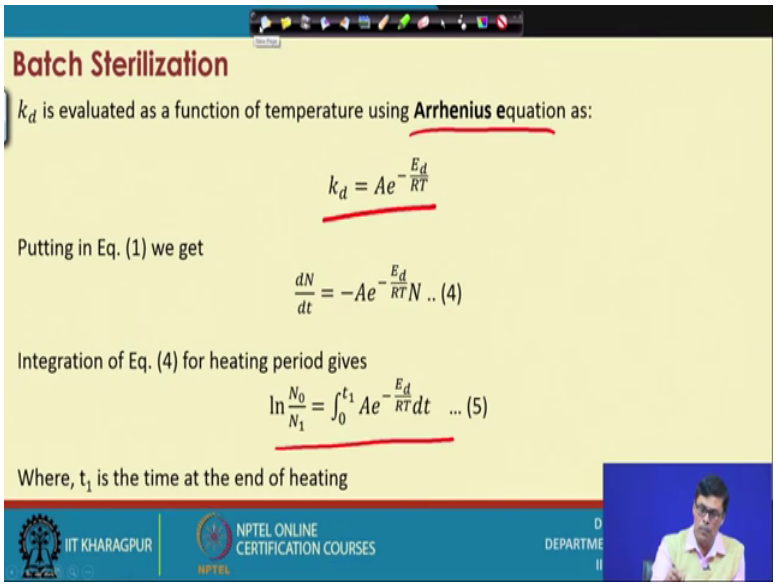


Now, k d can be evaluated like this. So, that you know this is Arrhenius equation that we know k d equal to a into a is the Arrhenius constant and e d it is the deactivation energy required R d is the gas content is the absolute temperature. So, this can be correlated like this is equal to d N by d t equal to k d into N am I right. So, if it is. So, we can we can we can derive this equation this equation from this equation for 4.

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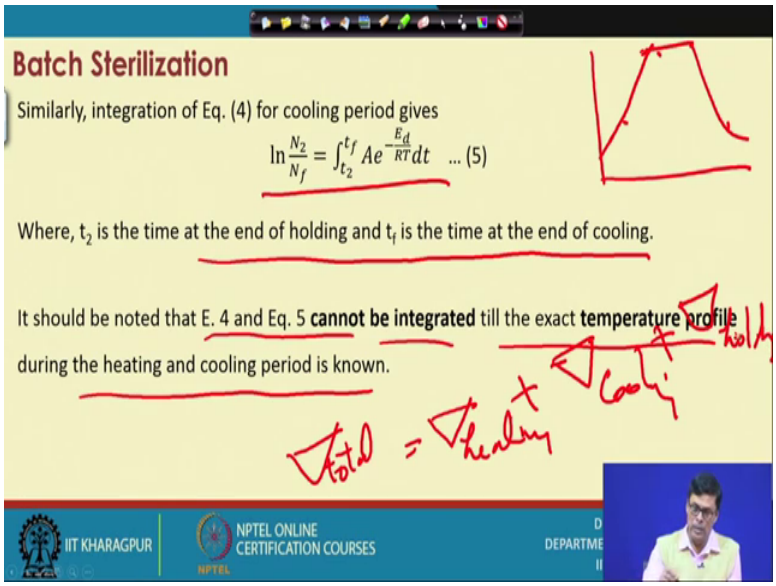


Now, finally, we have this equation that is l N 2 by l N f that is final this is the equation we will get, that t 2 is the time at the end of the holding and t f is the at the end of cooling. So, the equation 4 and 5 cannot be integrated till the exact time of profile during the heating and cooling period is known. So, what I what that is exactly I am saying that the amount of organism killed during heating amount of organism killed cooling and total sterility depends on the summation of this plus this plus this. So, it will be like this, this is for heating this is a total removal total removal of the organism is the this is heating, then this is cooling and plus this holding.

So, all this if you if you play if you, if we add together we will get that.

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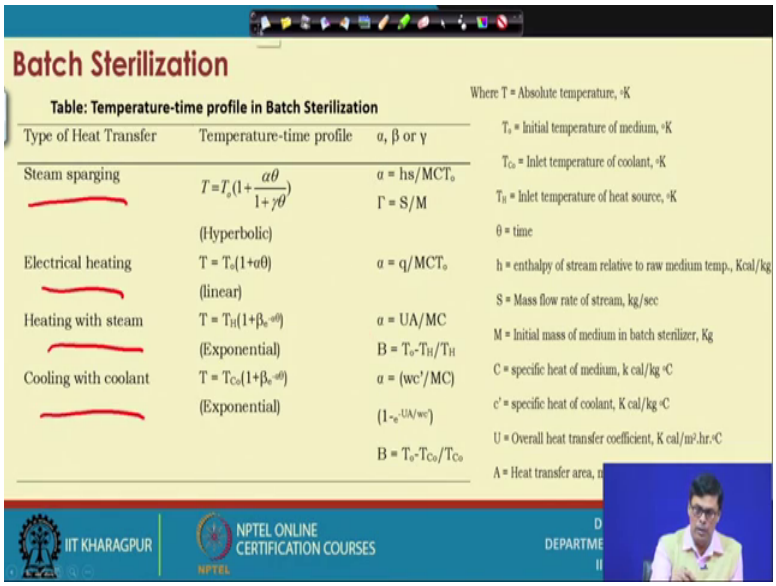


Now, in case of batch process we have different device we have already shown, one is steam sparging, electrical heating with stream cooling with coolant that this is different equation, we can we can we can use just to calculate.

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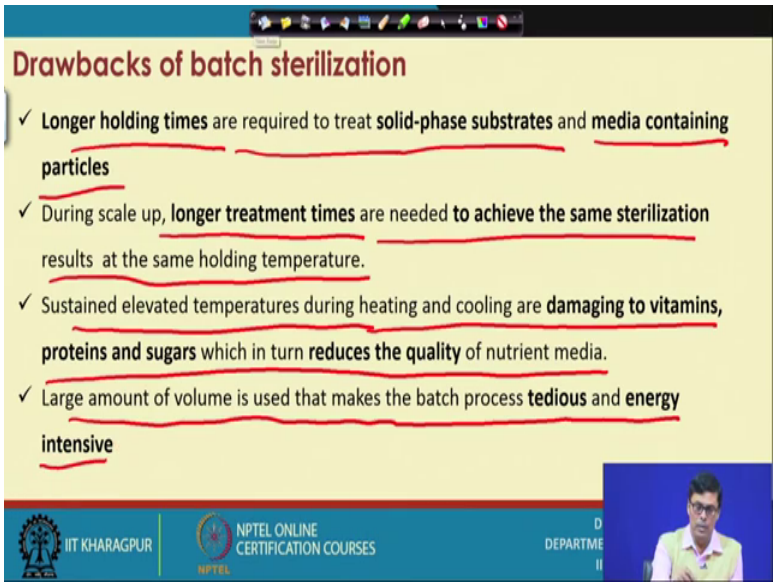


Now, longer that what is the major drawback the longer holding time is required to treat the solid phase substrate and media containing the particles? During the scale up the longer treatment time are added to achieve the same sterilization I just know I mentioned resulting the same holding temperature.

Sustained the elevated temperature during the heating and cooling damage the vitamin proteins and sugar which done the reduce the quality of the nutrient. And large amount of volume is used to make the batch process tedious and energy intensive.

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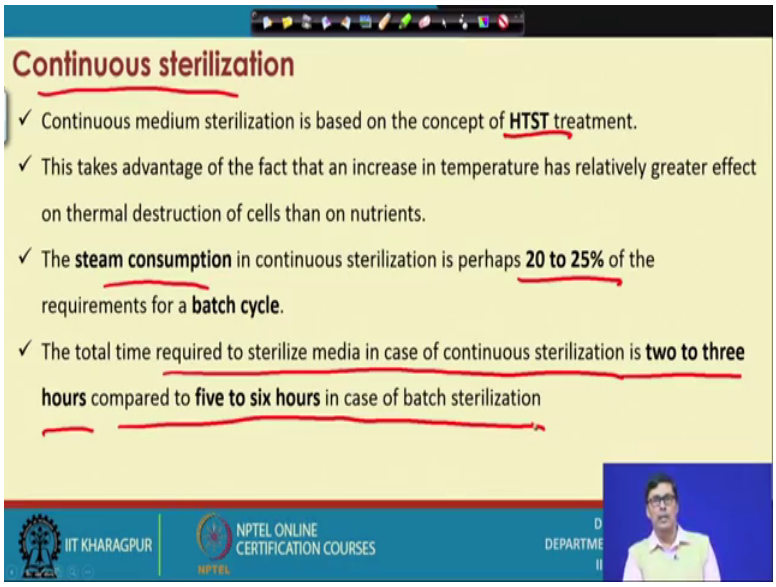


So, this is the reason this is a couple of reason , why the batch sterilization process that all is not that they are not followed in the industry. In the industry we follow the continued sterilization process. And one of the major and technique as I told you that we use that HTST technique and the amount the major important factor is the steam consumption is reduced to 20 to 20 5 percent, and that total time required for the sterilization of the continuous steri system is 2 to 3 hours as compared to 5 to 6 hours in case of batch sterilization.

Now, as we as you know that industry time is very important because every every time they count money because how much money is spent. So, since the time required for the batch sterilization process is more. So, money involvement also will be more.

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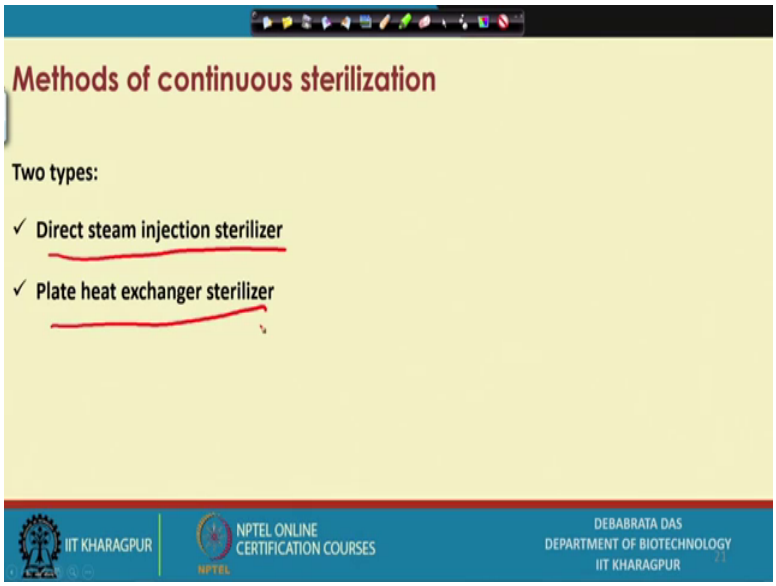


Now, there are two type of continuous sterilization process one is direct steam injection another is the plate heat exchanger let me explain.

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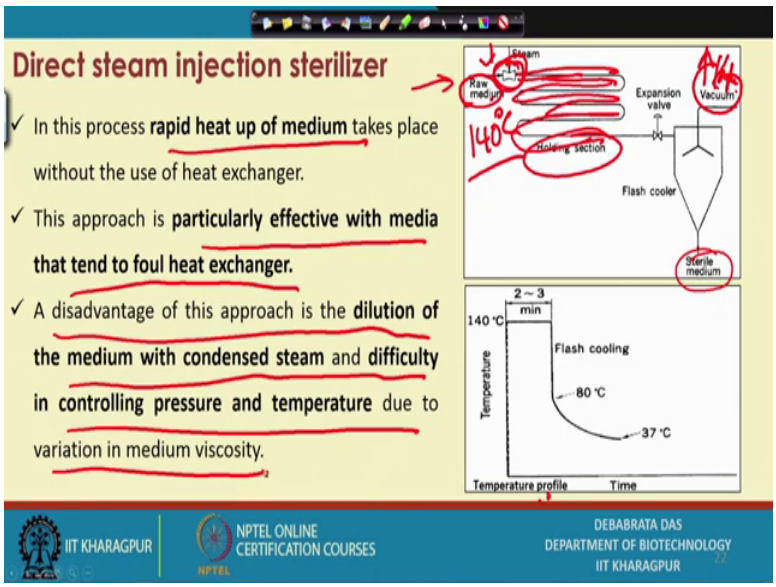


Now, direct steam injection because how it is done suppose this is the raw media am I right this is coming in this direction and this the steam is coming in this direction steam is coming this way and this is the mixing thing. And then this is this is called holding section, this is the holding and this is this is insulated this pipe is totally insulated so, that no heat loss take place.

So, the beauty the main advantage of this process, that raw media and steam they instantaneously they mix together and raise the temperature. Suppose the sterilization temperature is 140, because the industry we use the 140 degree centigrade, because we keep it very short time not like our lab 121 degree centigrade. So, this is the rapid heat up of the media that take place am I right.

Then, here we have the expansion valve through which we put it in the flash just you throw it out, then you know the flash cooling will take place and here we will get the sterilized media and here at the top the vapor will go out. And so, you might have seen that lot of industry they are discharging lot of white flues and white flues are nothing, but it is vapor that is coming out from the that from the from the industry. And, this approach is particularly effective with media that tend to fall the heat exchanger and disadvantage is that this approach is the dilution of the media with condensed steam and difficult to control pressure and temperature due to variation of viscosity (Refer Time: 29:15) way let me let me explain that.

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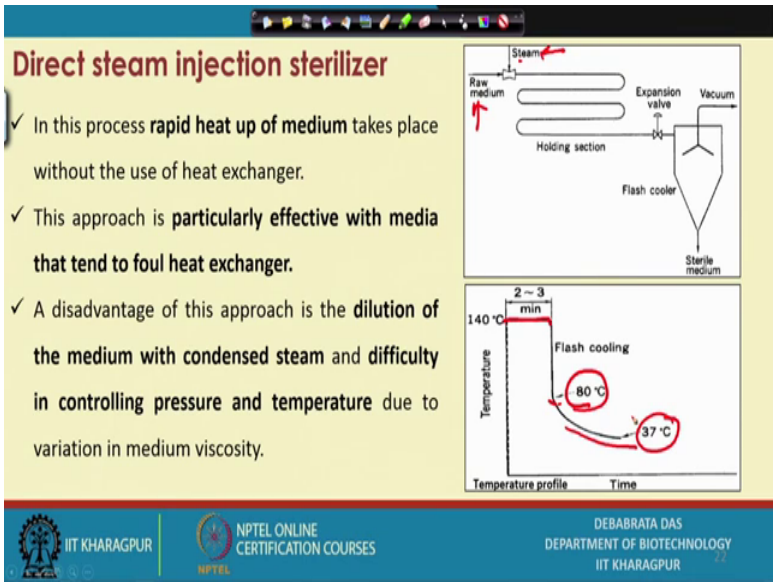


Because, if you look at here that you are what you are doing you are mixing media and, steam am I right as soon as you mix together the steam will undergo condensation as soon as the steam undergo condensation your media will be diluted and viscosity will be changed density will be changed as the, we know the flow characteristics of the fluid depends on the viscosity and the density. So, as they are changing. So, what will happen

the flow characteristics will change and temperature control will be a problem the operational problem we have. This is the major problem we have for operating this direct steam injection streaming.

Now, this is the if you have temperature type diagram of this particular system is like this the temperature instantaneously increase 140 degree centigrade and hold it and then your flash cooling flash cooling to 80 degree centigrade, then it slowly slowly is cooled down to 37 degree centigrade.

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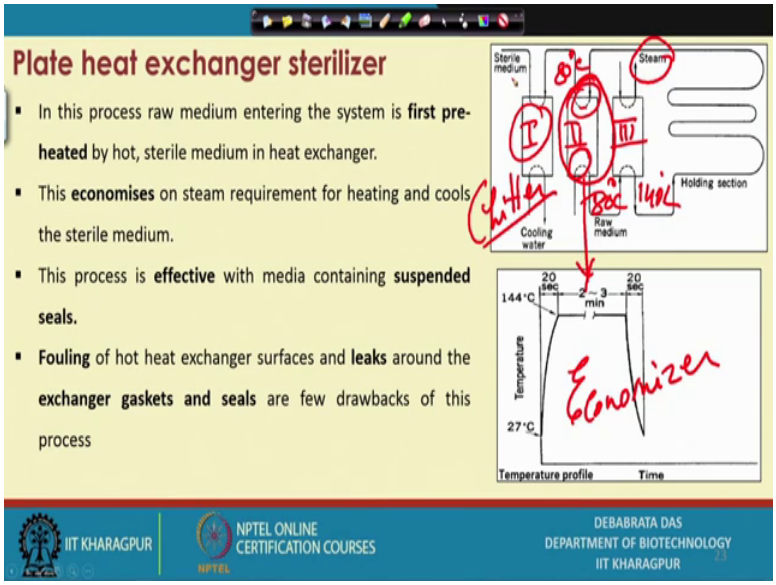


Now, plate heat exchanger that is mostly used by the industry this is what is this is they have different heat exchanger. This is suppose this is 1, this is 2, this is 3, am I right 3 exchanger they use. Now, here this two they call it the economizer eco why they call it the economizer, because what they do that here that here your preheating the media. We how we are preheating the media the media after holding section when the media is going out that heat we are using for preheating the media.

So, that we can save lot of energy and that is why we call it economy that and then we may raise the temperature at as high as 80 degree centigrade, then we pass through this and here might be 140 degree centigrade, then we keep it in the holding section or whatever time is required for the sterilization here we use steam for heating purpose.

And then we can we can we can pass it here we might be having 80 degree centigrade or you know more than that. So, here you will pass this is a chiller this is called Chiller. Chiller means we passed cold water and to reduce the temperature of the this is this is how this is operated and temperature time diagram is like this temperature increases like this, then we hold it and then we cool cool it like this.

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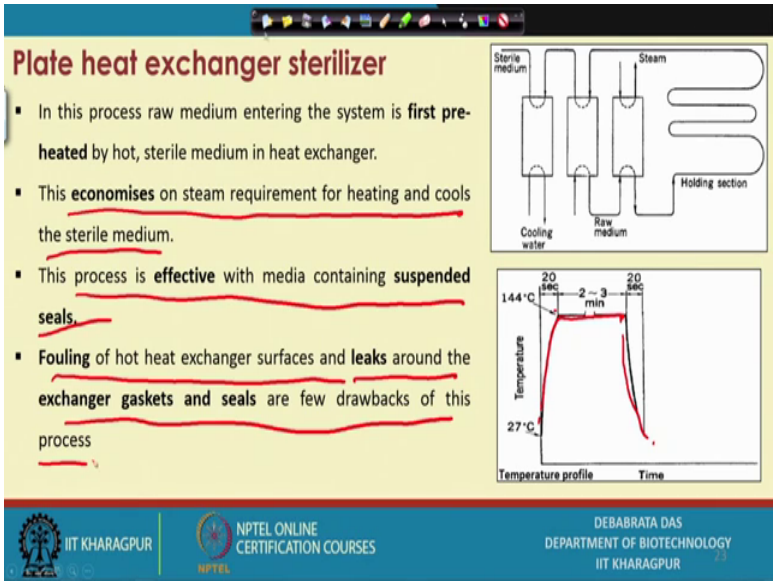


The, economy this is this economy this the steam requirement of heating and cooling the sterile is the media this process is effective with media, containing the suspended seals the seal fouling of the heat exchanger surface leaks around the heat exchanger gasket and seal fewer drawbacks of the process.

The major drawbacks is the suppose if you if you look at that the heat exchanger this is usually the parallel plate heat exchanger it looks like this, this is the parallel plate heat exchanger. So, one plate we are passing hot media another plate we are passing that you know your media. So, the it will show happen the hot surface the media comprises a lot of lot of organic material lot of dissolved solid that may deposited on the surface of the that one and that that you know that scale when there is a scale formation of the that wall that is the less conductor of heat.

Initially your heat requirement will be more that is the undesirable that is the major problem and another is the leak this you know plate and plate they bounce together with

the help of some gasket. And since you are how you maintain the 100 and 40 degree centigrade by increasing the steam pressure at very high.

So, naturally that there is every possibility that there is a kind of leakage that that take place inside in the in the gasket. So, that you have to open it again to rectify this problem.

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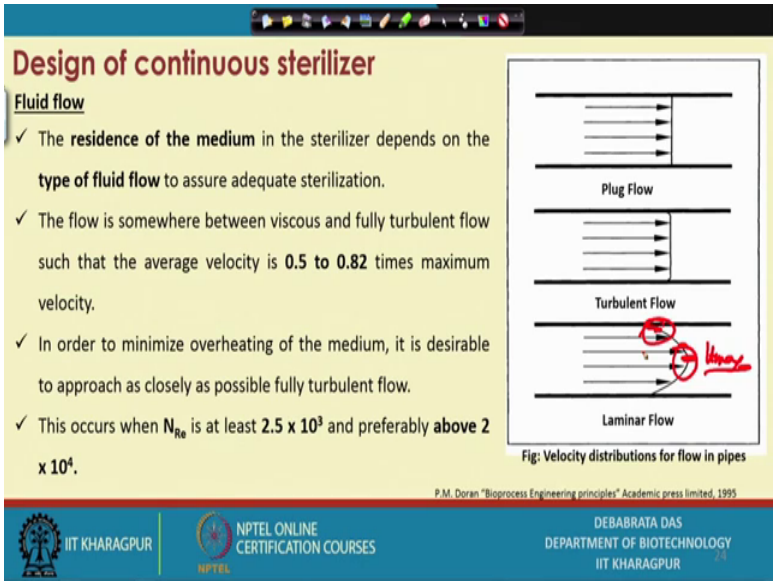


Now, we find that in the industry that up to up to 140 degree centigrade, we can usually use for the sterilization of the media. Now, flow characteristics of the media plays very important role, because if you know that there we have you know we have this is the plug flow, this is the turbulent flow, this is the laminar flow. Now, if you have this laminar flow you can see the velocity gradient act across the cross section of the tube.

Now, if you take this velocity this velocity at the middle of the tube then it is the very high this is u max am I right. Naturally then on the basis of that if you take a if you if you consider that time required for the sterilization, that here the media will be under heated. Now, here if you take this time here time for the for determining the length of the that sterilizer, then you will find that yours media here this will be overrated.

Now, overheating and underheating 2 problem that we have due to the, that flow characteristics of the fluid there, usually we prefer that plug flow is the ideal flow very difficult to achieve, but usually we use the turbulent flow. So, that temperature gradient

across, the cross section of the tube should be minimum the residence of the media, in the sterilization depends on the type of fluid flow to assured that you get sterilization flow is somewhat between the viscous and fully turbulent flow such that the average velocity 0.5 to 0.8 2 time not the maximum velocity.

In order to maximize the overheating of the media it is desirable to approach closely fully turbulent flow and this occurs the energy value at least 2.5 into 10 to the power 3 preferably above 2 into 10 to the power 4 that. This is the desirable characteristics that we have of the fluid for the proposed sterilization of the media.

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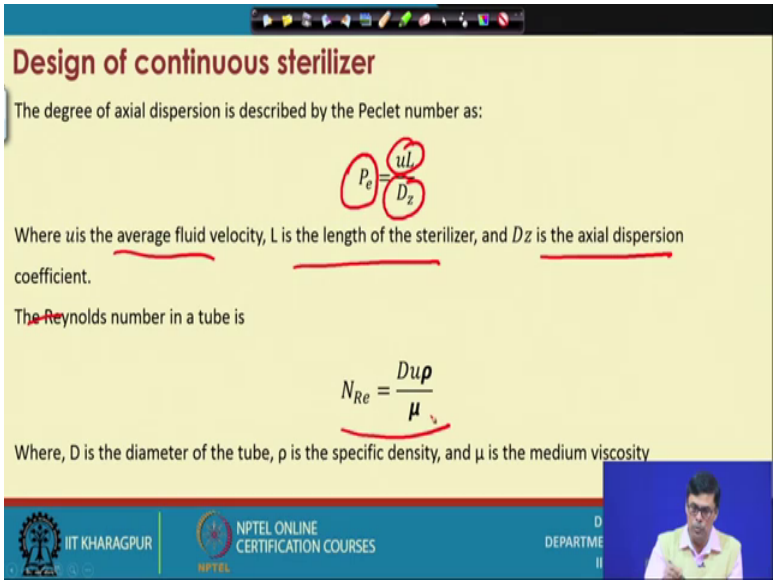


Now, then other part for finding out that you know that that sterilization temperature or design the sterilizer, we come across different other parameters like peclet number which is equal to u l D z and D z is the axial dispersion coefficient one is the length of the sterilization and u is the velocity of the fluid and Reynolds number we know d u rho by mu.

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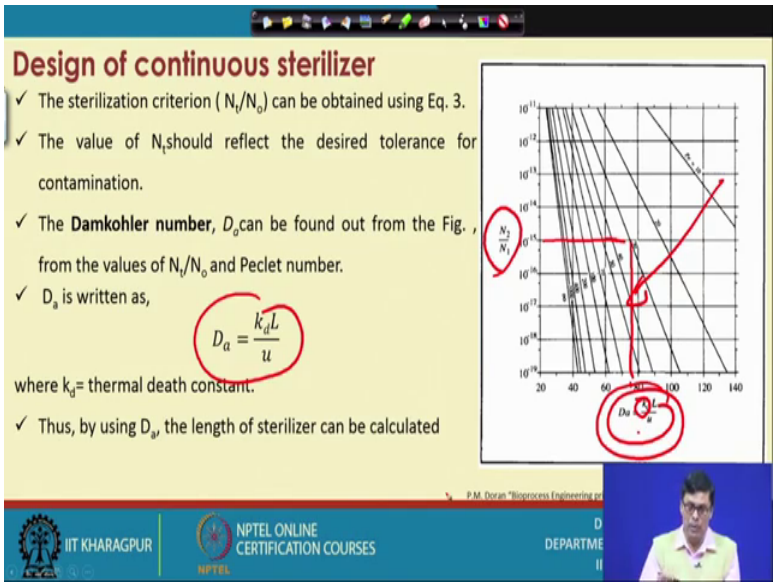


And, this is how we can find out that Damkohler number this is equal to k d into l by u this we can we can we have the correlation this N by N by N n 2 by N 1 and this is the peclet number as the peclet number increases the correlation is like this. So, if you know this value how much how much sterility degree of sterility you want to maintain? Then you can easily find out that what is the what is that damkohler number k d l by u and if you know that at particular peclet number then you can easily find out the k d value. And k d value as you know it depends on temperature you can easily calculate the temperature of sterilization.

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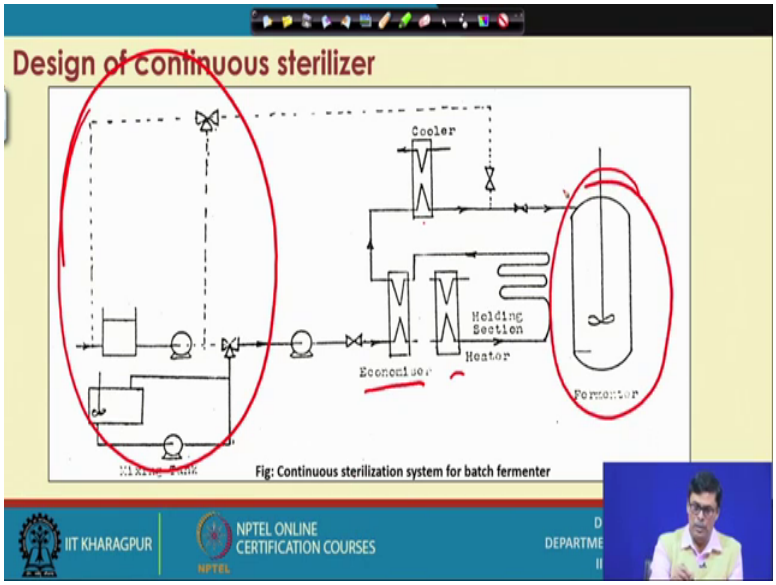

And, this is the kind of schematic diagram of the continuous sterilization process that you know this is the mixing tank, where we here we mix the media mixer we should be thoroughly mixed and then we pass through this that you know sterilization process, passed the economizer then him we put it in this section then holding section if the heater is there, then we put it that heating section, then we this is pre heating the media, then cooler and then ultimately comes to the fermenter this is how we it is operated in the industry?

So, in this particular lecture I try to give you how the medium sterilization is carried out in the fermentation industry and which is which plays very important role. And, we observed that that continuous sterilization process is appears to be the better as compared to the batch sterilization process particularly when we do it in a commercial scale, but another thing we observed that that field the sterilization with the help of filtration material immaterial filter is not recommended.

In case of low value products, but for a high value products it is typical cases where the media quality plays the important role in the in that particular biochemical process. There we can think for it otherwise most of the cases we use heat as is sterilizing media for the medium sterilization. And in the for continuous sterilization process I mentioned that HTST technique is the use the reason is that at the high temperature the that the it is more effective to kill the microorganism rather than to lose the vitamin order you know quality of the media, because they exposed a time will be very less.

So, thank you very much.