Industrial Biotechnology
Professor Debabrata Das
Department of Biotechnology
Indian Institute of Technology Kharagpur
Module 2
Lecture No 22
Upstream Processing: Medium Sterilizer

Welcome to my course Industrial Biotechnology, today is shall discuss the medium sterilization. Now before I go for medium sterilization let me share what I covered in the last class. In the last class I discussed the air sterilization and I told you that biochemical industry that the sterility is a very important factor because we shall have to allow the grow of a deserved organism in the reactor so we should not allow any foreign organism so entered into the process.

And for that we find two important the source of contamination, one is here because most of the fermentation is carried out under the aerobic conditions and media that is required for the growth of the microorganism. So in the last class I tried to share that air sterilization and main objective was to remove the contaminants that present in the air. Now when we design any kind of air sterilization process that we always assume that that a basis is that out of how much of contaminants what is the how much organism we are going to remove

As for example whether you are removing 1 in 1000, 1 in 10,000, 1 in 1,000,00 like this so you have to the more your process is stringent your sterility is requirement is more, your you take more precaution and measure. And another issue I told you that again the type of organism we remove again it depends on the type of fermentation you are carrying out. I have given the example of citric acid fermentation process, we use the cane molasses as a raw material for citric acid production

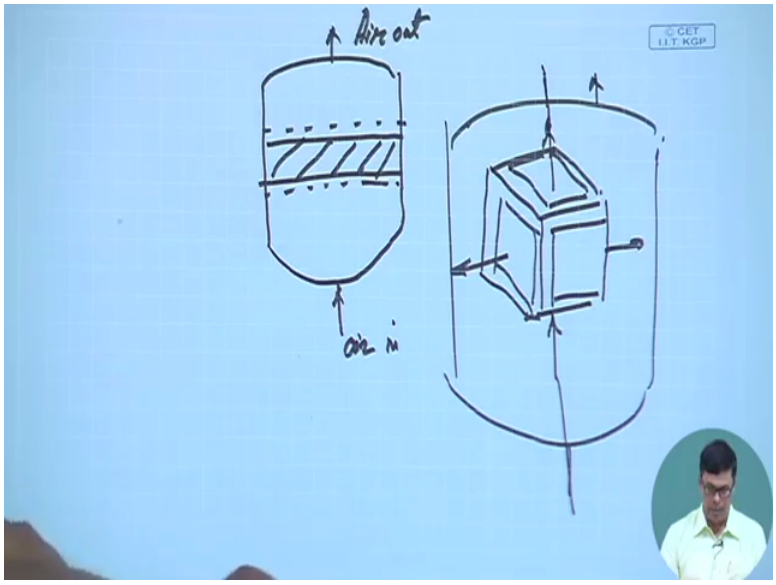
Now, cane molasses also very good raw materials for yeast fermentation process, now if you look at the doubling time of yeast is much less as compared to doubling time of that aspergillus nigar which produces citric acid. So naturally contamination of yeast is much more concerned as per the citric acid fermentation, so and usually the air in air filter we use the physical separation technique, we use some kind of depth filter to remove the contaminants

Now the it has been chosen that glass wool fibre is considered the best for the removal of the contaminants present in the air, the reason is that here that their coefficient is quite low and

not only that you can regenerate the glass wool fibre again and again because when after sometime when we use glass wool fibre as a filter after sometime your filter plate will be totally saturated with the contaminants, so in that case what you have to do, you have to remove the contaminants. Now question comes that the size of the air filter again depends on the quantity of air that we are going to sterilize

Suppose in case in told you that the size of the inoculum vessel is usually 5 to 10 per cent of the production fermenter, so naturally when we prepare the media we prepare the we design the air filter for the inoculum vessel. Our size of filter requirement will be very less, but when you go for production fermenter it will be very high because size will be 10 times higher than that of the inoculum vessel.

(Refer Slide Time: 4:48)

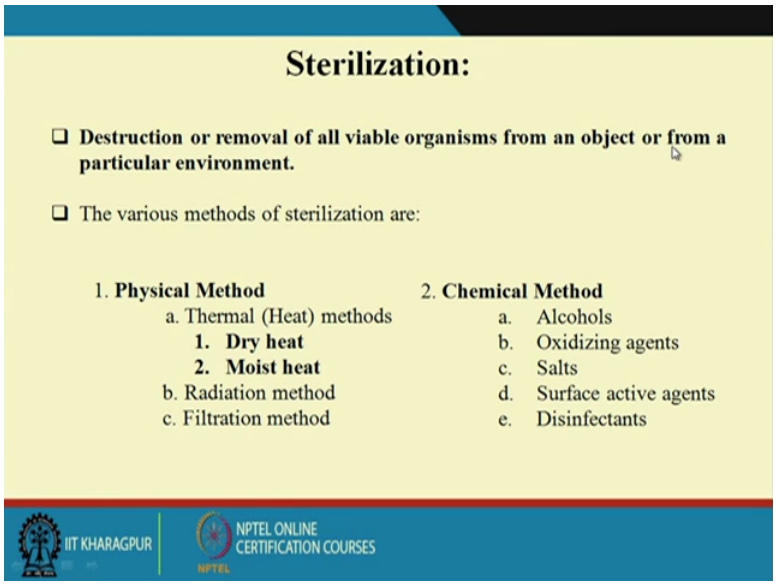


So now question comes that I can show you the very simple design of that air filter that is we have in practise in the industry that is like this we have we have this is the air filter we can have and here we should have some kind of stainless steel mesh just to protect the fibre. Now you pass your air like this and this is air in and this is air out. This is how we can do that, now this is the for inoculum vessel it is fine because this kind of small system we can use now when you go for the production fermenter so the size will be 10 times higher than that because the capacity of the production fermenter is more

Now question comes that if you increase the surface area like this 10 times it is very it will occupy lot of space and it is very difficult to design we difficult to control the the process. So what we have to do we shall have to make the process air filter very Compact. So question

comes how we can make this air filter Compaq. If we have some cubical things that you know like this air filter so we can have this like this inside, so if you pass air inside and air can comes out like this in different directions you can from the annular space you can you can keep whole the assembly inside the filter and you can increase the surface area

(Refer Slide Time: 7:00)



So like this you can take a you can take the air out from here the annular space we can make the system very compact in case you required higher surface area, so this I forgot to mentioned in the last class. Now today I want to discuss about the medium sterilization and in medium sterilization the purpose is same that we shall have to remove the contaminants that present in the in the media and this is the destruction or removal of viable organism which an object or from a particular environment

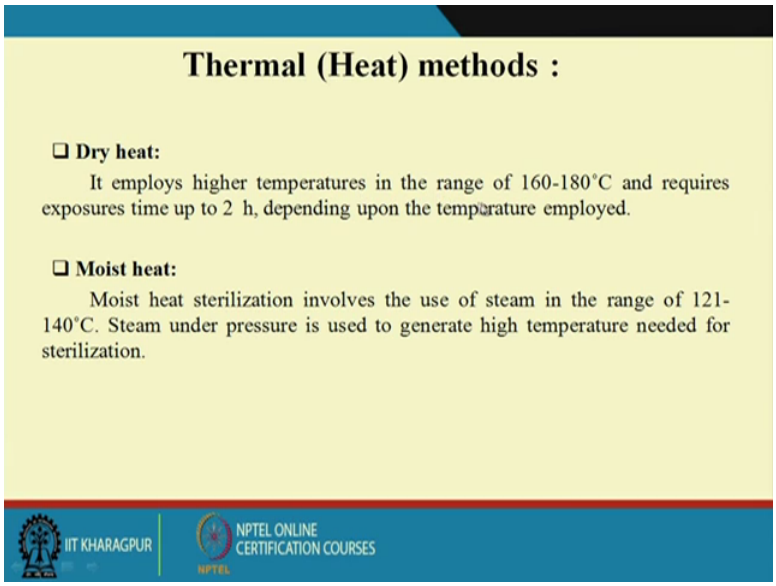
Now various methods of air sterilization is there, one is the heat is the heat is the best media for media sterilization because when we talk about media is the repeat media and heat is transferred by three different mechanisms, one is conduction, another is convection and radiation. So this is all three process should be very active in case of medium sterilization our we have two methods we have dry heat, we have moist heat we have seen moist heat much effective as compared to dry heat, I shall show you later, then radiation method then filtration method. Chemical methods will use for like alcohol, oxidizing agent, salts, surface active agents and this is chemical method is rarely used for medium sterilization mostly we use either heat or the filtration method.

Now again when we use the filtration method, I told you that heat as you know heat your media comprises of lot of nutritional components like vitamins and amino acids so this vitamins and amino acids they are very sensitive to temperature so as we increase the temperature, the quality of this media will be lost, so some fermentation process we require we have we shall have to maintain the quality of the that media so in that case we shall have to the vitamin and other things we can filter through the filter paper just to sterilisation purpose.

And as I told you when you filterd we use a membrane and membrane has some pore size and if you if you suppose I want to remove bacteria the smallest size of bacteria varies from 0.5 to 2 microns, so 2 microns, so suppose now if I keep the pore size less than 0.5 micron then we can when you pass your liquid then all the bacteria will be retain here and comes. Now only the problem with that I told you that there will be tremendous pressure drop across the membrane and the life of the membrane also quite less so it is very expensive as compared to heat.

Now when we consider heat as a sterilizing media the question comes, how heat is effective for sterilizing the media because we know that organism in the, when you heat the organism that contains lot of protein molecules and protein had high temperature, it undergo the denaturation as the denaturation of the enzymes take place inside the organism that the biochemical activity of the organism will be stopped and your organism will be killed

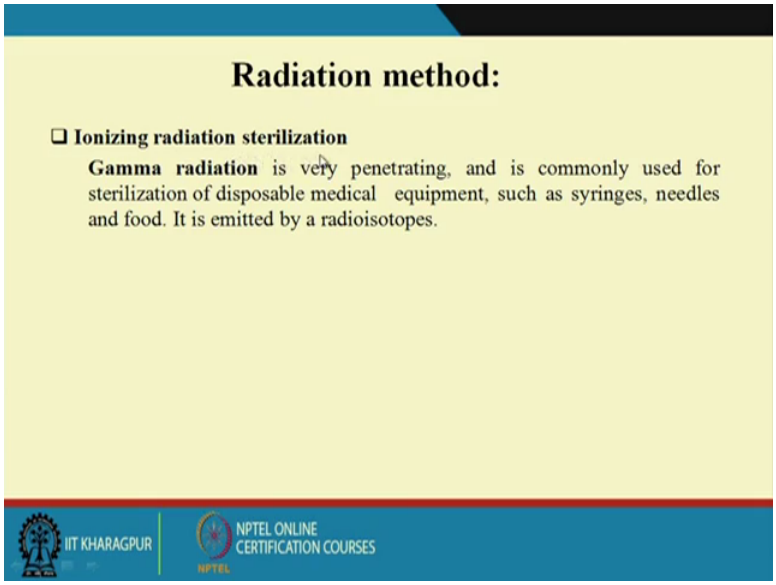
(Refer Slide Time: 10:21)



Now we have thermal heat method we have dry heat, it employs the higher temperature in the range of 160 to 180 degree centigrade and required exposure up to 2 hours depending upon the temperature employed. So this is very if you compare with the moisture heat. Moist heat is involved the use of steam in the range of 121 to 140 degree centigrade, steam under pressure is used to generate high temperature needed for the sterilization. So this moist is much effective than dry heat that is why dry heat we require lot of high temperature as compared to moist heat

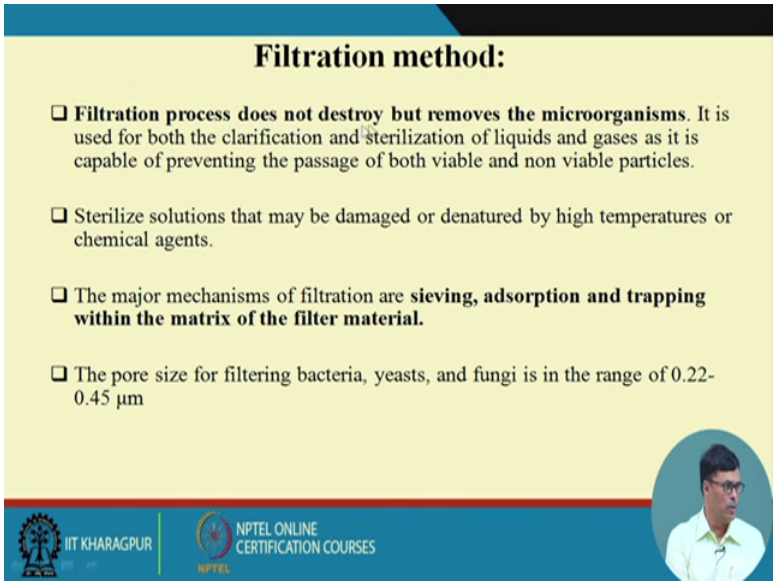
Now the that sterilisation by radiation can be achieved by the electromagnetic radiations such as the electron beam, x-rays, gamma rays or irradiation of subatomic particles. Electromagnetic or particulate radiation can be energetic enough to ionise the atom or molecule or less energetic so but non ionic radiation like UV rays radiation is useful to sterilise the surface some transparent objects, UV radiation is routinely used to sterilise the interiors of the biological safety cabinets because that is we know in the operation theatre also we use the UV rays for surface sterilisation purpose because in the biochemical industry also we have lamina flow where we do the we prepared our culture that inside the chamber we usually sterilize with the help of UV rays

(Refer Slide Time: 12:06)



Now the radiation method is the ionizing radiation sterilisation gamma radiation is very penetrating and is commonly used for sterilisation of disposable medical equipment such as syringes, needles and food, it is emitted by the radioisotopes.

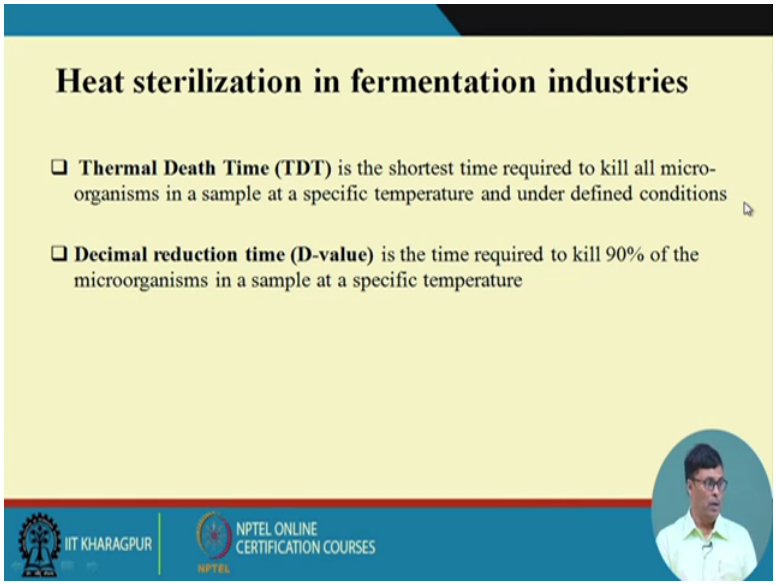
(Refer Slide Time: 12:28)



Now second is the filtration methods filtration process does not destroy but remove the microorganism that is why I told you that in case of heat sensitive material we use that, but this is very costly process. And you know when you talk about the biochemical industry we have two type of products; one is called high value product another is low value products. High value products means per unit cost of the product is very high (())(12:53) because since the per unit cost of the product is very high there we can think for this kind of technique where high cost involvement is there. But in case of low value product we cannot because low value low value and high value products we cannot think for this process would be no good

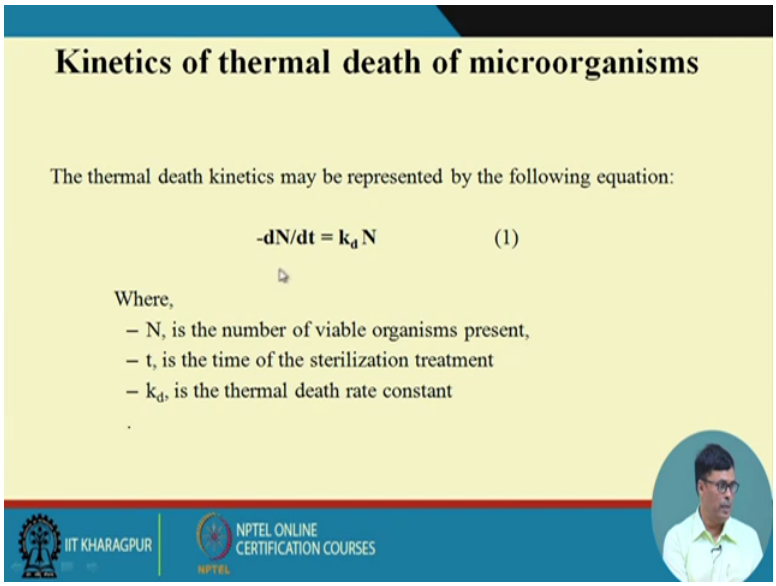
It is the used both for the clarification and sterilisation of liquid and gasses as it is capable of preventing the passage of both viable and non viable particles. Sterilize solutions that may be damaged or denatured by high temperatures and the chemical agents. The major mechanisms of filtrations are sieving, adsorption, trapping within the matrix of the filter material. The pore size of the filtering for filtering bacteria, yeasts and fungi is in the range of 0.22 to 0.45 microns, so this is the more we go to the lower size of the pore, the pressure drops across the membrane will be high.

(Refer Slide Time: 14:20)



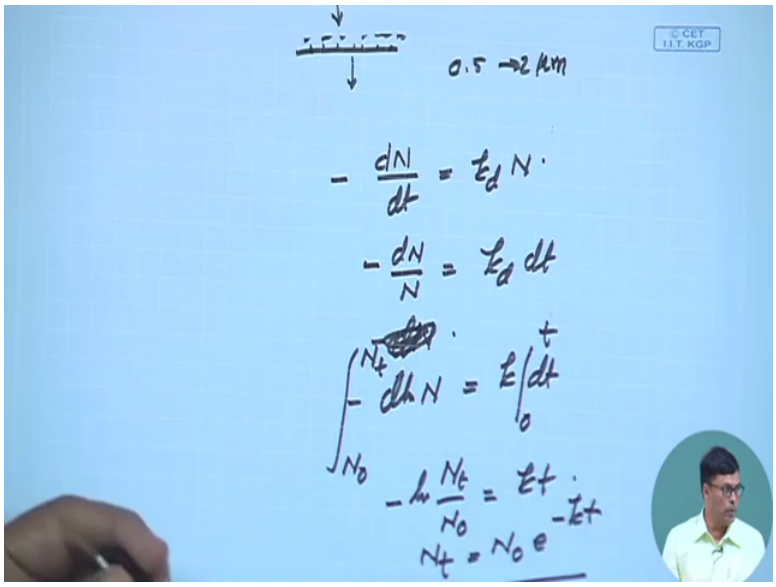
Now in case of heat sterilisation which is in practise in the industry, we have Thermal Death Time it is the shortest time required to kill all the microorganisms in a sample at a specific temperature and under defined condition. Now here I want to point out that whenever we design any kind of sterilisation process medium sterilisation process for the industry, we never go for the 100 per cent sterilisation we always assume one out of one million one out of ten million one out of ten thousand like this because as per the the whatever as per the requirement of the process. Now decimal reduction time that is also is a kind of term we related to the medium sterilization is the time required to kill 90 per cent of the microorganisms in a sample as a specific temperature

(Refer Slide Time: 15:21)



So the time required to kill the 90 per cent of the that means if 10 organisms is there it will be becoming one organism, so this is kinetics of thermal death rate death of the microorganism can be expressed like this dN – dN by dt equal to Kd into N where Kd is the thermal death rate constant and N is the number of viable organisms presence. Now this is like this I shall show you this. You see that if you if you if you look in, this is the plot of ln Nt Nt means to that number of viable organism present at time t and initial number of organism that is keep on decreasing the sharp plot with respect to time, this is the that means this follow the first order kinetics.

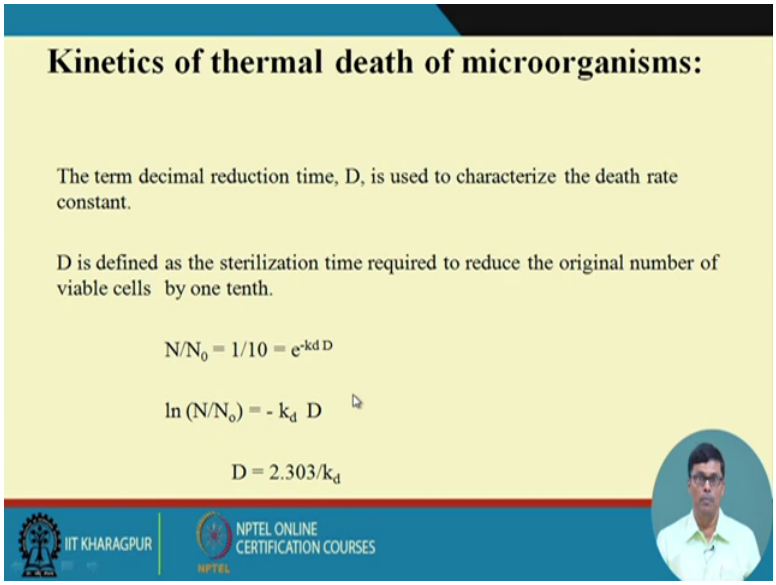
(Refer Slide Time: 16:26)



So this is like this so this is like this so so it is like this so we can we can we can write – dN by dt equal to Kd into N now we can we w first we can what we can do that, so we can write – dn by N equal to Kd into dt. Now this is equal to – dN sorry this is dlnN – equal to Kd into dt. Now if you if you if you integrate N0 to N whatever you have or Nt whatever you have this is 0 to t then what will be this – Ln Nt by N0 am I right this is equal to K into t. Now if you take this – this way this will be – Kt in that case Nt, I can write N0 into e to the power – Kt.

So the number of organism present at any time t we can easily and this is K is nothing but Kd, so this is the Kd Kd is the thermal death rate constant. The unit of this since it follow the first order kinetics so it is it is the unit is time inverse. This Kd unit is time inverse

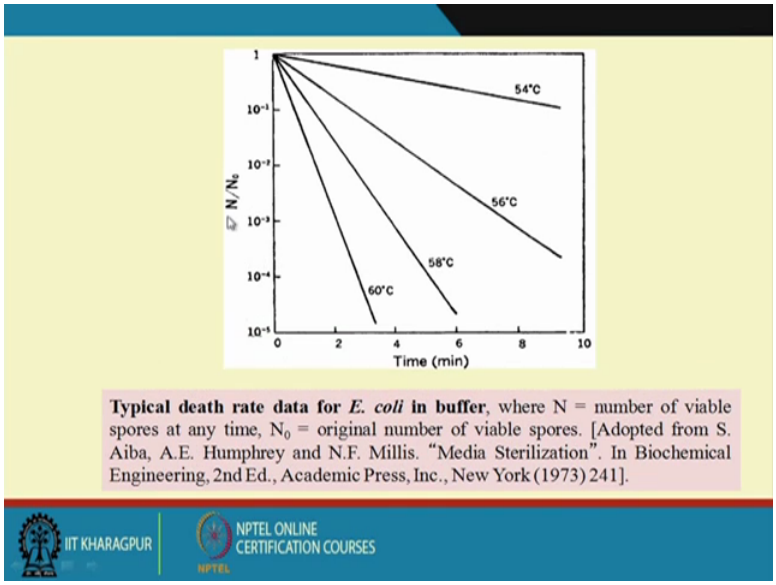
(Refer Slide Time: 17:56)



Now this Kd we can determine experimentally because there is some technique because we have to stake in small capillary if you have hot and cold bath and you take the culture initially if you take the instantaneous to cool and hot this material then what will happen you can find out that that how much viable cell is present by exposing a particular temperature for a particular time, so you can easily calculate the Kd value. Now how to calculate the decimal reduction time, decimal reduction time means 90 per cent removal of the initial microorganisms.

So N by N0 will be 1 by 10 and if you write it so will be coming this D equal to 2.303 by Kd that means your decimal reduction time is inversely proportional with thermal death rate constant this is Kd inversely proportional. So as the time increases, the Kd value will increase because I can show you here that here you can see this is like as the as the time increases the this is becoming more steeper and like this.

(Refer Slide Time: 19:23)

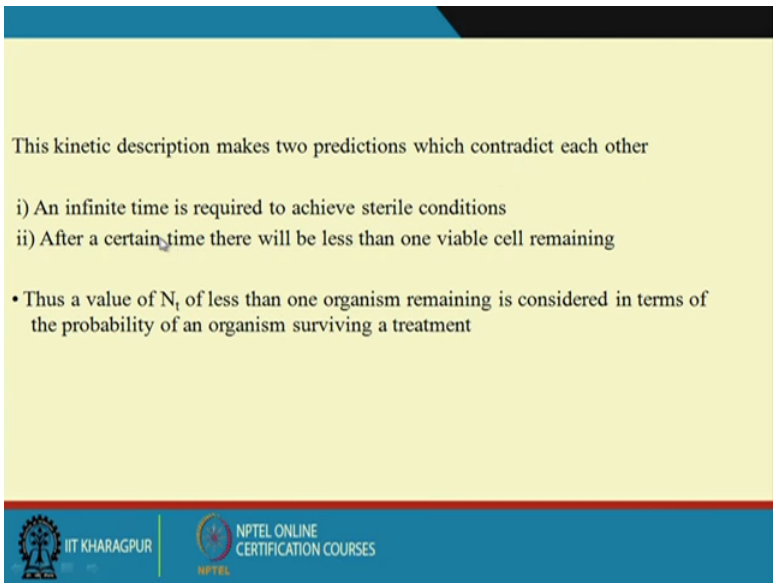


Typical death rate data for *E. coli* in buffer, where N = number of viable spores at any time, N_0 = original number of viable spores. [Adopted from S. Aiba, A.E. Humphrey and N.F. Millis. "Media Sterilization". In Biochemical Engineering, 2nd Ed., Academic Press, Inc., New York (1973) 241].

IIT KHARAGPUR | NPTEL ONLINE CERTIFICATION COURSES

Now if you plot this log graph paper then we have this kind of plot, this is in case of 60 degree centigrade and 54 degree centigrade, more increased the temperature it will be more steeper so Kd value will increases. So what are you going to means that as you increase the temperature Kd value will increase to a great extent and your D value will decrease.

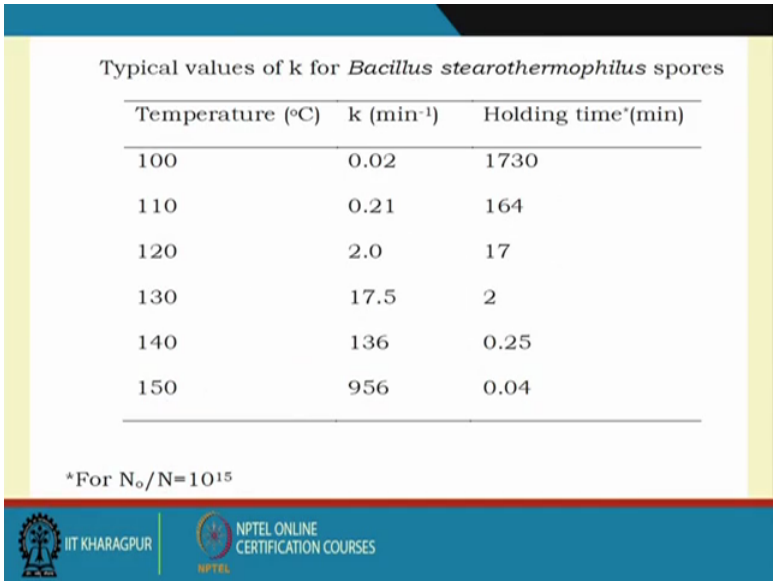
(Refer Slide Time: 20:04)



Now so this kinetic description makes two predictions which contradicts with each other at definite time is required to achieve the sterile condition and after certain time there will be less than one viable cells remaining. So you know that this is thus a value of Nt less than one microorganism remaining is considered in terms of probability of an organism surviving a treatment, so I told you that that when you when you design any kind of sterilization process

always we assume one in thousand or one in ten thousand, one in hundred thousand, what sterility factor you are going to have in your system.

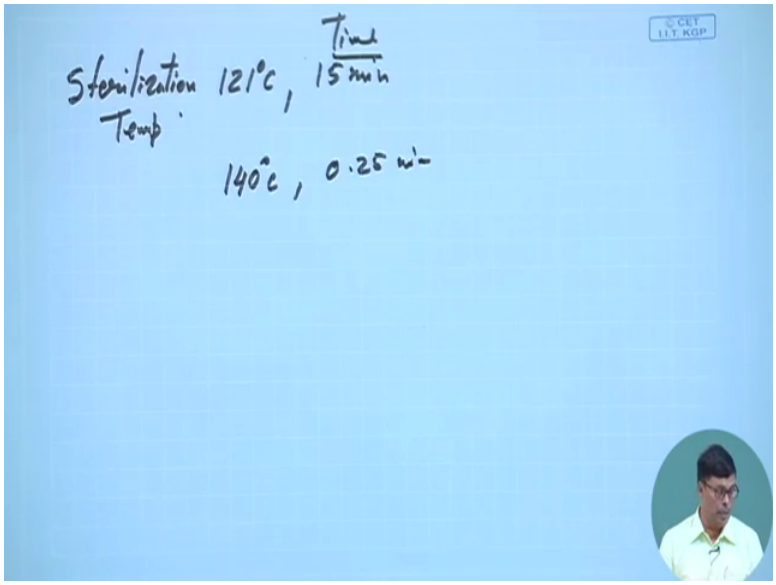
(Refer Slide Time: 20:42)



Now here is a interesting table that shows the how temperature affect the value of kd value, Kd is thermal death rate constant as we know that as we increase the temperature 100, 110, 120, 130, 140 and 150, the Kd value drastically changes this unit is minute inverse. The holding time means this is the time required for sterilization, I shall explain the what is called when I shall discuss the continuous sterilisation process.

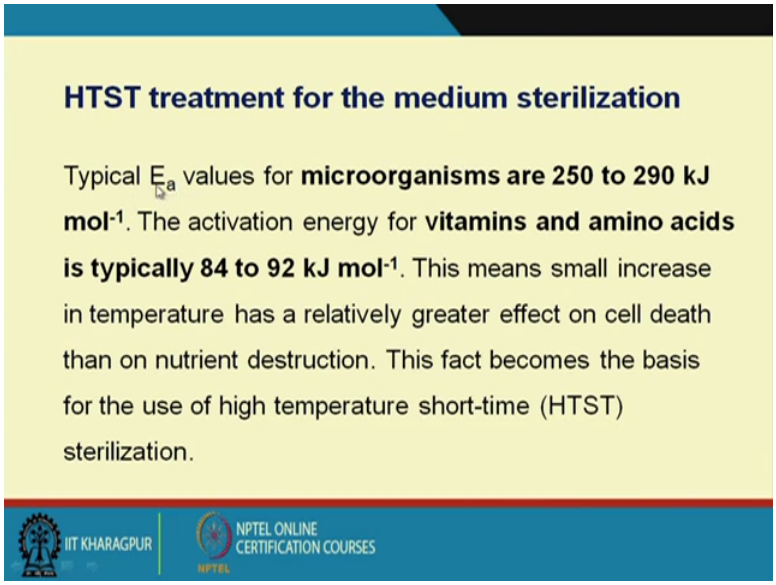
Now as you as you see when you have we have the 100 degree centigrade, we heat the media 100 degree centigrade then kd value is 0.02 we have to sterilisation time is 1730 minutes, but 110 degree centigrade the K value the Kd value is 0.21 then it is reduced to 164 minutes. 120 degree centigrade Kd value is for 2 that your the sterilisation time is reduced to 17 minutes, now 130 degree centigrade it is 17.5 and this is the 2 minutes which is sterilization time.

(Refer Slide Time: 22:32)



140 degree centigrade is the 136 is a Kd value and holding time is 0.25, now in the industry that you know that in the laboratory we use our sterilisation temperature is 121 degree centigrade for 15 minutes, this is the sterilisation temperature and time this is time requirement is this. So now in the industry that we use actually 140 degree centigrade because now and usually that you see that your sterilisation time is reduce to 0.25 minutes the holding time is 0.25 minutes.

(Refer Slide Time: 23:19)

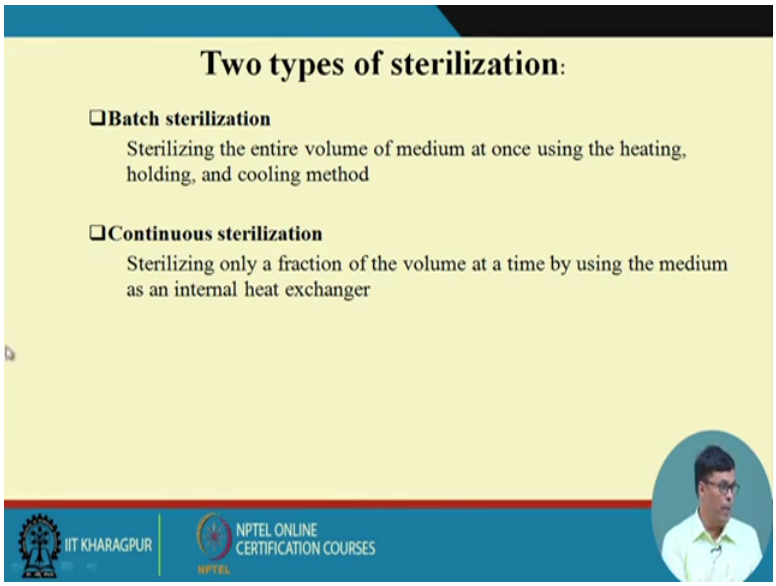


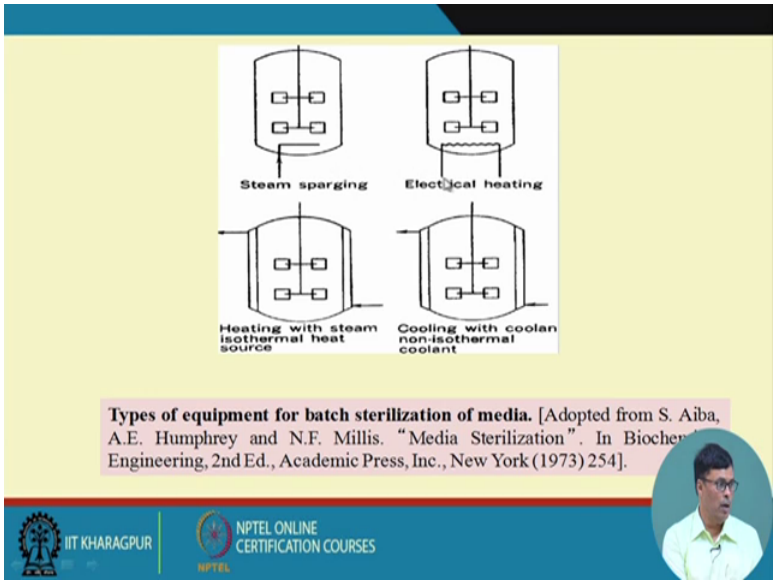
Now question comes why we go for this high temperature sterilisation, in the industry we usually follow the technique what we call high temperature short time techniques. Now why we follow the high temperature short time techniques, the reason is that that that if you if you

look at the Ea value Ea value means activation energy value of the microorganisms are 250 to 290 kilo Joules per mole and the activation energy for vitamins and amino acid is typically 84 to 92 kilo Joules per mole that is that means if you heat the media at a low temperature it is more effective for destroy the vitamins and amino acids that is why you know that in the in the house hold purpose when we purchase some kind of medicinal tonic it is always prescribed that you should keep it at low temperature so that your those tonic mostly comprises of some vitamins and amino acids and they are very sensitive to temperature.

So even at low temperature the denaturation may take place, but if you look at the higher temperature that you have Ea value is we can have higher that is activation and the requirement high so this means the small increase in temperature has the relatively greater effect on cell death than the nutritional destruction. This fact becomes the basis for the use of high temperature short time technique in the industry, this is why that industry will follow the high temperature short time technique for the sterilisation of the medium.

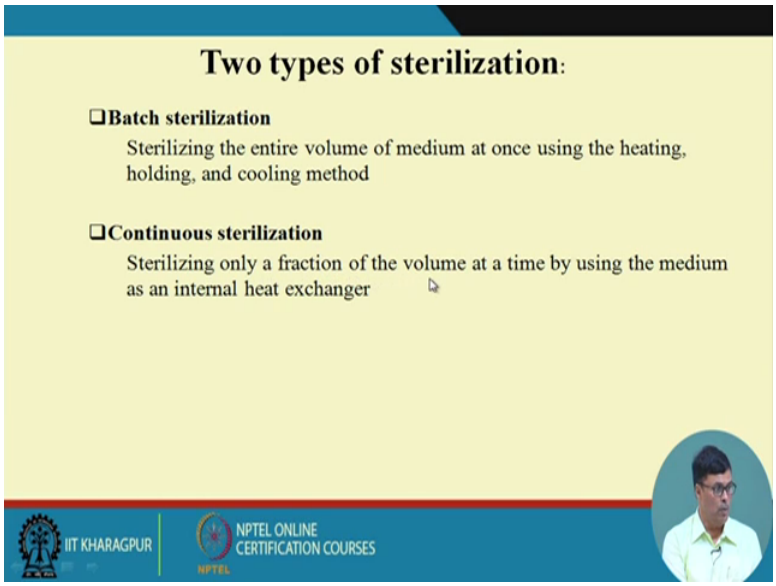
(Refer Slide Time: 24:52)





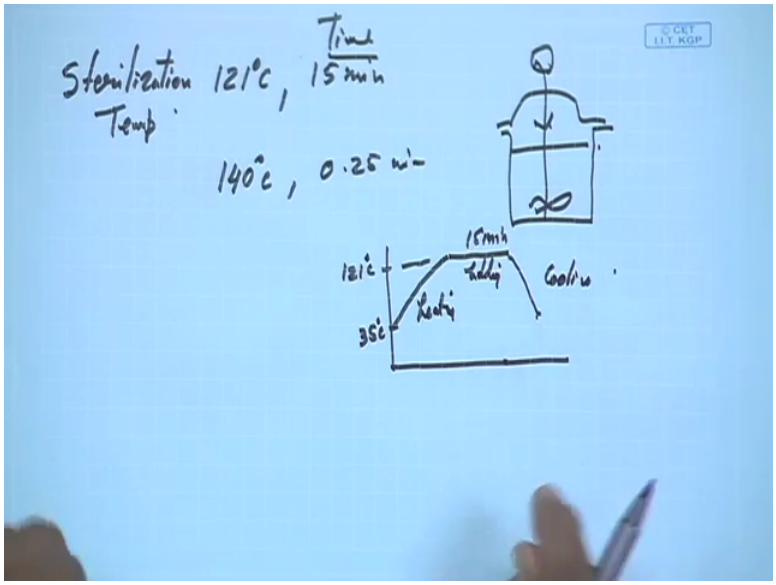
Now we have two type of sterilisation in the industry that is we have batch sterilisation, batch sterilisation sterilising the entire volume of the medium at once using the heating and holding the cooling methods. So you can you can have this, this is the media and you can pass your steam like this or you can you can electrically heat it or you can have jacketed whatever you can, so you can you can do the sterilisation like that this is called the batch sterilisation

(Refer Slide Time: 25:27)



And continuous sterilisation means sterilising only a fraction of the volume at a time by using the temperature as a internal heat exchanger, now basic difference between the batch sterilisation and continuous sterilisation, in case of batch sterilisation the inter volume of the media at once the heating, holding and cooling so what does it mean what does it mean suppose in the, this is the fermenter and you have media now you want to sterilise this media

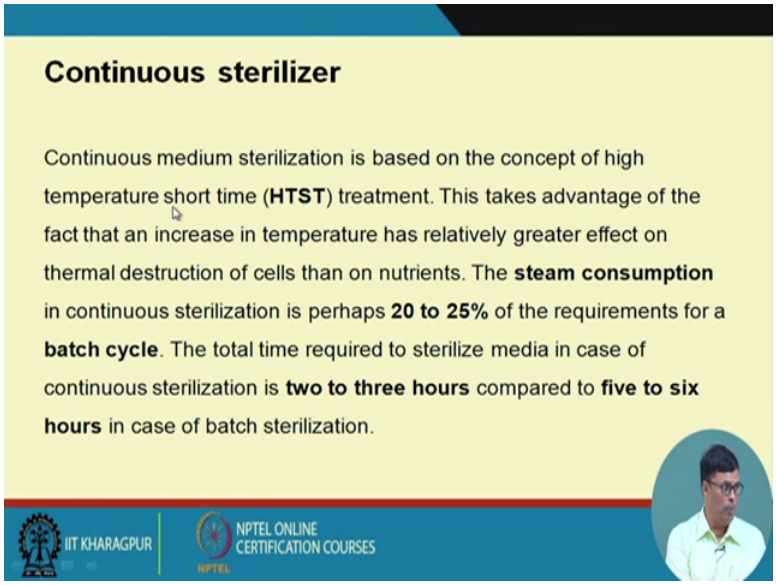
(Refer Slide Time: 26:47)



So this is the starrer we have and we want to sterilise the media so first you have to heat it so you require some time to suppose this is our ambient temperature is about 35 degree centigrade, so you have to increase the temperature and then it attains 121 degree centigrade then you have to hold it for 15 minutes that is like this then you have to cool it am I right. This is cool to the again 35 degree or 40 degree centigrade whatever you have. So this is so this is called heating then this is holding you are holding the temperature and this is called cooling.

Now we have seen that we have seen that different organism has different depends the death of the organism depends on the temperature, we have seen just now that as we increase the temperature Kd value changes drastically so that means that different temperature you have different Kd value, so there will be death of the organism during heating, there is the death of the organism during holding the temperature, there is death of organism during the cooling

(Refer Slide Time: 27:48)



So you know that the what the total stability is a combination all the three things both heating, holding and cooling that that is the storage effect we have that is why if you look at that that you know that the basic difference between the continuous sterilisation and the batch sterilisation. In the continuous medium sterilisation is based on the concept of high temperature short time. This take the advantage of the fact that and increase in temperature has relatively greater effect on this thermal destruction of the cells than on nutrients.

The steam consumption of continuous sterilisation is perhaps 20 to 25 percent of the requirement for a batch cycle. So this is a very crucial factor because whatever steam we require for sterilising the media in a batch system that is very high as compared to that of continuous sterilisation process. The total time required to sterilise the temperature in case of continuous sterilisation is 2 to 3 hours compared to 5 to 6 hours in case of batch sterilisation process, so time requirement time is a very crucial factor as we know in the industry so this is very effective that you know that that the continuous sterilisation process is very effective. One point I forget to mention that when I showed you effect of temperature on the killing of microorganism even if you increase the temperature from 140 to 150 degree centigrade your sterilisation time again further reduced.

Now question may be asked why you are not going for very high temperature now you might be aware in the industry we increase the temperature by increasing the steam pressure, now if we have very high temperature the equivalent design is a problem because some kind of stringent we have with respect to equivalent design that is why we in the industry we fulfil

140 degree centigrade is most suitable for the sterilisation of the media, so I want to stop here now and next class I am going to discuss about the continuous sterilisation process. Thank you.