Environmental Chemistry and Microbiology

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Module - 10
Lecture - 52
Microbial Growth and Control - I

Welcome everyone. This is a new topic. We are going to look at methods of controlling microbial growth. So, I am calling it Microbial Growth and Control. It is divided into 2 parts. And this is lecture number 52 of module 10.

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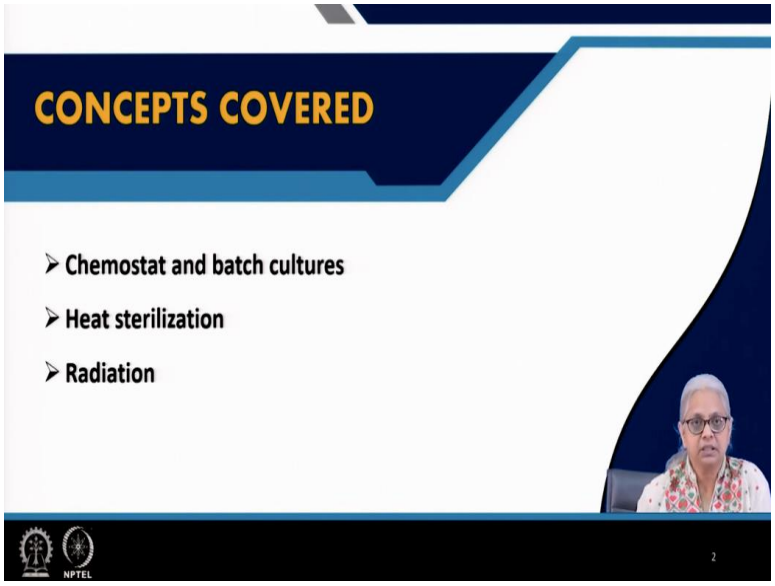

We are going to start with a chemostat and batch cultures. How do we grow microbes in the lab? How do we control their growth? And what are the major factors that need to be controlled in these cultures? Then we come to heat sterilisation and radiation. So, these are some of the topics that we are going to cover.

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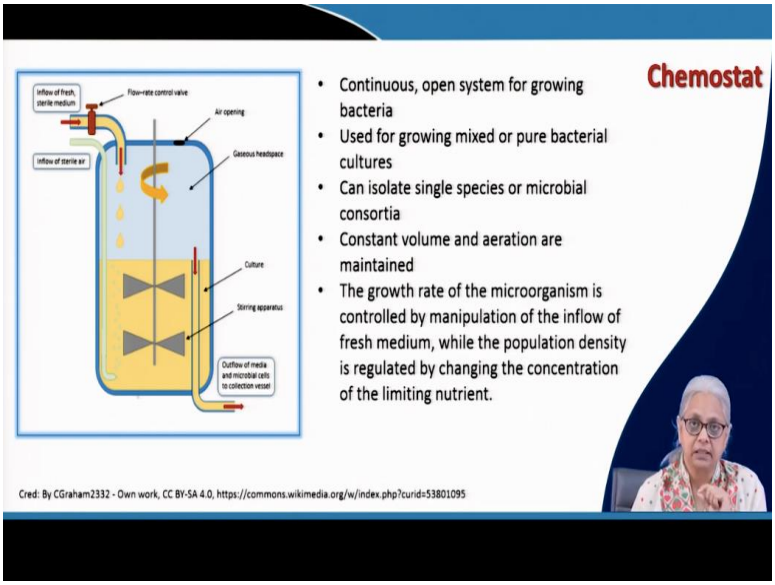

Let us start with a simple chemostat. Now, a chemostat, unlike a batch culture is a continuous flowing reactor. So, you have an inflow of fresh sterile medium coming in at one end; it is completely mixed inside the reactor; and you have an output of the media along with the microbial cells that are growing within the chemostat. So, therefore, it is a continuous open system for growing bacteria.

This is generally used in the lab for culturing bacteria on a continuous basis. You can use it for mixed cultures or for pure cultures. You can isolate single species or you can use microbial consortia. The main things are that it is generally constant volume and you have to provide aeration if you are running it for the growth of aerobic heterotrophic bacteria (correction: all aerobic bacteria). The growth rate of the microorganisms can be manipulated by manipulating the flow rate coming into the reactor.

So, this is the inflow of fresh media. And you can regulate the population of cells within the reactor by changing the concentration of the limiting nutrient. The limiting nutrient in this case, in all these derivations that we are going to go through in the next few slides, that is shown by the letter S. So, S stands for the growth limiting substrate.

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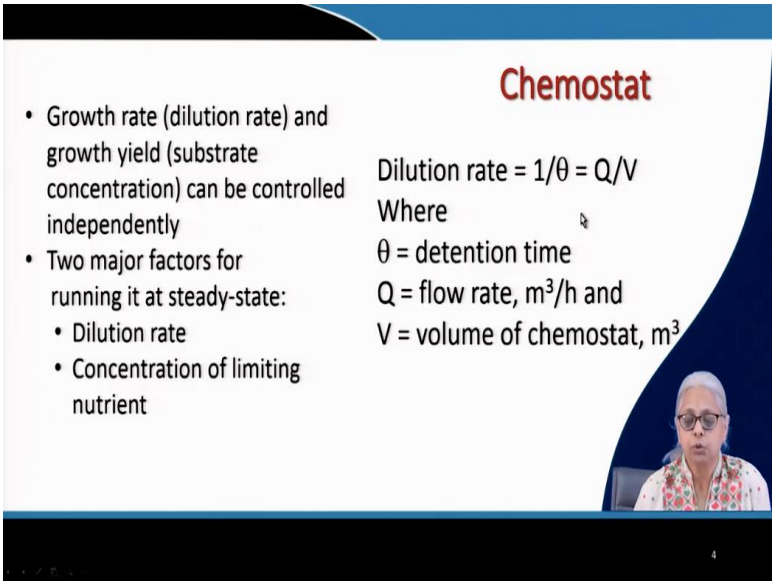

Before we come to the derivation, let us note a few other points. The growth rate is related to the dilution rate and the growth yield is related to the substrate concentration. So, you can control these parameters independently. And what do you need to do that? So, you need the dilution rate and the concentration of the substrate. What is the dilution rate? Dilution rate is defined as the flow rate divided by the volume of the reactor or the chemostat.

So, it can be in litres (L) or it can be in cubic metres (m^3). Flow rate is in cubic metres per hour ($\left(\frac{m^3}{hr}\right)$or litres per hour $\left(\frac{L}{hr}\right)$. And the detention time can be in minutes or hours. Detention time is the reciprocal of this. So, it is volume over flow rate $\frac{V}{Q}$. And therefore, dilution rate is $\frac{1}{\theta}$

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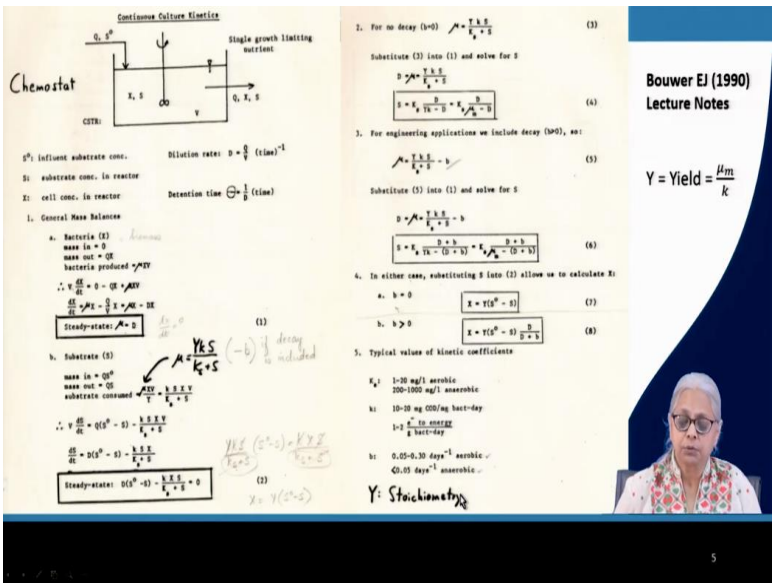

Here we have our continuous flow reactor. This is our chemostat. It has an input in terms of media of Q. The substrate concentration is S^0. So, the initial substrate concentration coming into the reactor or the chemostat is S^0. What is happening inside the reactor? There are 2 things that are happening inside the reactor. One is the growth of biomass and the second is the consumption of the substrate.

So, V is the volume of the chemostat, X is the concentration of biomass, and S is the concentration of substrate. We have a single growth limiting nutrient which is represented by S. What is coming out? For those of you who may be familiar with chemical reaction engineering, in a continuous flow reactor which is completely mixed, whatever is inside the reactor is what is coming out. So, Q, X and S are exactly what you see inside the reactor.

So, output is equal to whatever is inside the reactor. It may be common sense. So, let us just keep it that way. So, S^0 is our influence substrate concentration. S is our output substrate concentration as well as the substrate concentration inside the reactor. Same thing for X. X is the cell concentration leaving the reactor as well as whatever is inside the reactor. Dilution rate and detention time have already been defined.

So, let us do a simple mass balance for the biomass as well as the substrate. If I do a mass balance for the biomass; biomass meaning bacteria; so, mass coming in. Let us assume that the bacterial concentration coming in is 0. You are inoculating it with a small amount of bacteria; and whatever is coming in with the flow is 0. $Q \times X$ is the mass of the bacteria going out of the chemostat.

What is being produced within the chemostat is $\mu \times X \times V$. μ is the growth rate; X is the biomass concentration; that is proportionate; so, whatever is happening inside the reactor is proportionate to the biomass concentration; and V is the volume. So, that is the total mass that is being produced inside the chemostat. The $V \times \frac{dX}{dt}$; you can write it in this way.

$$\boldsymbol{V \times \frac{dX}{dt} = 0 - QX + \mu XV}$$

Dividing both sides by Volume (V)

$$\boldsymbol{\frac{dX}{dt} = \mu X - \frac{Q}{V}X = \mu X - DX}$$

At steady state $\frac{dX}{dt} = 0$; $\boldsymbol{\mu X = DX}$

$$\boldsymbol{or, \mu = D}\ (Eq.1)$$

So, you have accumulation on the left-hand side; mass in is 0; mass out is QX; and whatever is being generated inside the reactor is μXV. This is our first point. Then we know that we are running it at steady state. So, at steady state, $\frac{dX}{dt} = 0$. Let us do 2 things. First is divide the entire equation by volume. And second, let us take dX by dt = 0. So, after dividing it by V, we then put a 0 on this side and $\mu X - \frac{Q}{V}X$ is what we get here.

And then, when that is equal to 0, $\mu = D$. D stands for dilution rate. Let us now look at substrate. Mass in of the substrate is QS^0; mass out is QS. How is the substrate being consumed? We have already gone through Monod kinetics. Substrate consumed is $\frac{\mu XV}{Y}$. Y is yield and that is $\frac{\mu_{max}}{k}$. So, we have already gone through Monod kinetics and we have already seen the derivation for yield.

So, this can also be expressed in terms of substrate. And like I said, we have already done this; so, I would not repeat it here. This is equal to $\frac{k\ S\ X\ V}{K_S + S}$, K_s is the half-velocity constant. So, $V\frac{dS}{dt} = Q\ (S^0 - S) - \frac{k\ S\ X\ V}{K_S + S}$. And again, at steady state, we say $\frac{dS}{dt} = 0$, and you get the final form, $\boldsymbol{D\ (S^0 - S) = \frac{kSX}{K_s + S}}$ That is equal to 0.

$$\boldsymbol{V\frac{dS}{dt} = Q\ (S^0 - S) - \frac{k\ S\ X\ V}{K_s + S}}$$

Dividing by Volume on both sides;

$$\boldsymbol{\frac{dS}{dt} = \frac{Q}{V}\ (S^0 - S) - \frac{kSX}{K_s + S}}$$

$$\boldsymbol{\frac{dS}{dt} = D(S^0 - S) - \frac{kSX}{K_s + S}}$$

At steady state $\frac{dS}{dt} = 0$

$$\boldsymbol{D\ (S^0 - S) = \frac{kSX}{K_s + S}}\quad (Eq.2)$$

Now, there is another term that needs to be included, and that is decay. So, if you want to assume that decay is not important, then b = 0, and this is our equation for μ. So, $\mu = \frac{YkS}{K_S + S}$.

And when you substitute this particular term into our first equation, then you get these 2 terms. So, you get the equation for D and the equation for S, without accounting for decay.

$$\boldsymbol{b = 0\ ;\ \mu = \frac{YkS}{K_S + S}}\ \ (Eq.3)$$

Substitute (*Eq. 3)* into (*Eq. 1)* and solving for S

$$\boldsymbol{D = \mu = \frac{YkS}{K_S + S}}$$

$$\boldsymbol{S = K_s \frac{D}{Yk - D}}$$

$$\boldsymbol{S = K_s \frac{D}{\mu_{max} - D}}\ \ (Eq.4)$$

If you want to account for decay, then this equation number 3 has to be modified. b stands for the decay term, that is the endogenous decay rate. And you can modify equations 1 and 2 again similarly, to get equation 6.

$$\boldsymbol{b > 0\ so;\ \ \mu = \frac{YkS}{K_s + S} = b}\ \ (Eq.5)$$

Substitute (*Eq.5)* into *(Eq. 1)* and solve for S

$$\boldsymbol{S = K_s \frac{D + b}{Yk - (D + b)} = K_s \frac{D + b}{\mu_{max} - (D + b)}}\ \ (Eq.6)$$

Now, if b = 0, then $X = Y\ (S^0 - S)$; and if b > 0, which means you are accounting for decay, then you get this form $X = Y\ (S^0 - S)\frac{D}{D+b}$. Typical values for these coefficients are noted over here. So, you have K_s, k, b and Y. Y can also be determined from the stoichiometry, depending on the nature of the substrate.

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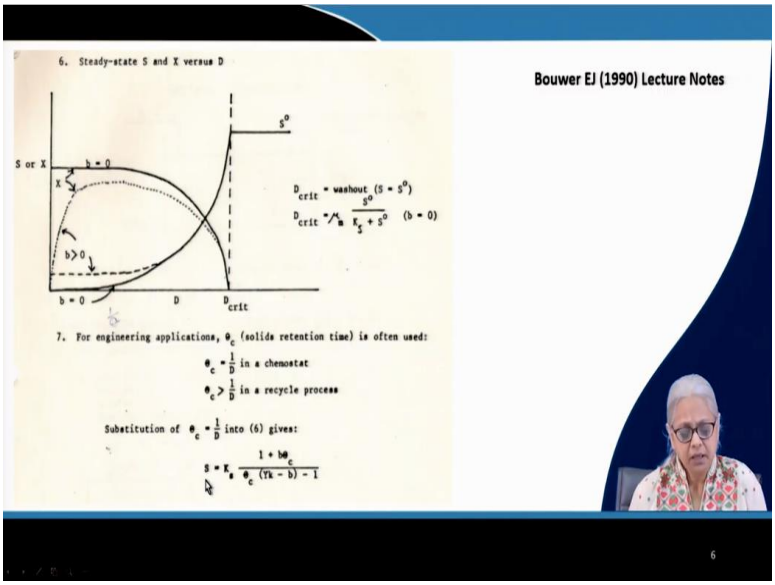

We come to steady state, substrate and biomass concentration versus the dilution rate. D_{crit} means, if the substrate concentration coming out of the chemostat is equal to S^0; it means, nothing is being consumed; and that means that all of the biomass is being washed out. So, that is D_{crit}. And that is what is shown in this graph that this is the dilution rate at which all the biomass will be washed out. So, that is a critical point literally.

And if you do not account for decay (b =0), then D_{crit} can be written as,

$$\boldsymbol{D_{crit} = \mu_{max} \frac{S^0}{K_s + S^0}}$$

So, this is the point beyond which you do not want to increase the dilution rate. And so, for a chemostat, we do not have recycling (correction: you can have recycling in a chemostat as well). So, θ_c is this solid's retention time, which is also called the biomass retention time or you can say mean cell retention time.

So, if there is no recycle, $\theta_c = \frac{1}{D}$; or, if you have recycling, then $\theta_c > \frac{1}{D}$. $If\ \theta_c = \frac{1}{D}$; and S can be modified from all these equations to derive as:

$$\boldsymbol{S = K_s \frac{1 + b\theta_c}{\theta_c(Yk - b) - 1}}$$

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Let us come to the next point and that is, how do we control microbial growth? There are several different methods for controlling microbial growth. We will take a look at all of them. The first one is heat sterilization, followed by radiation, filter sterilization and chemical control. Then we have within chemical control we have antiseptics; we have disinfectants; therapeutic agents; medicines and so on; and growth factor analogs.

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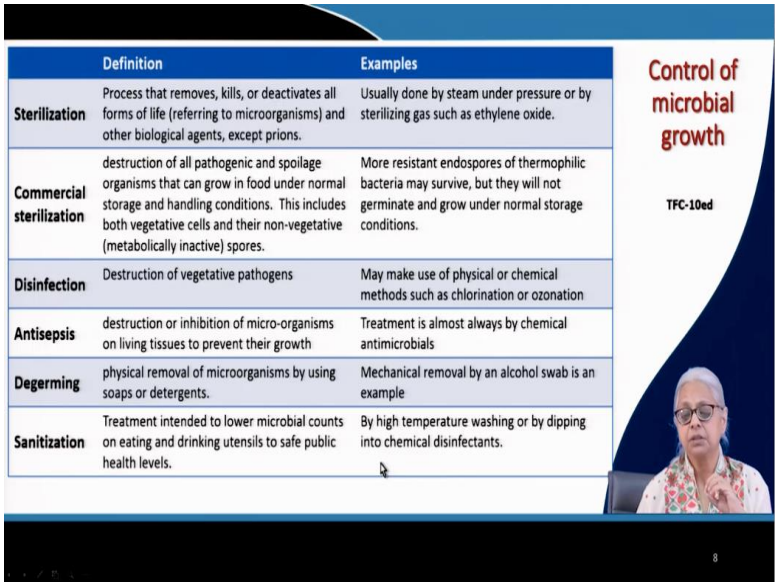

	Definition	Examples
Sterilization	Process that removes, kills, or deactivates all forms of life (referring to microorganisms) and other biological agents, except prions.	Usually done by steam under pressure or by sterilizing gas such as ethylene oxide.
Commercial sterilization	destruction of all pathogenic and spoilage organisms that can grow in food under normal storage and handling conditions. This includes both vegetative cells and their non-vegetative (metabolically inactive) spores.	More resistant endospores of thermophilic bacteria may survive, but they will not germinate and grow under normal storage conditions.
Disinfection	Destruction of vegetative pathogens	May make use of physical or chemical methods such as chlorination or ozonation
Antisepsis	destruction or inhibition of micro-organisms on living tissues to prevent their growth	Treatment is almost always by chemical antimicrobials
Degerming	physical removal of microorganisms by using soaps or detergents.	Mechanical removal by an alcohol swab is an example
Sanitization	Treatment intended to lower microbial counts on eating and drinking utensils to safe public health levels.	By high temperature washing or by dipping into chemical disinfectants.

So, let us take a look at some definitions, before we go into the methods. So, the first one is sterilisation. What is sterilisation? It is a process that removes, kills or deactivates all life forms. So, whenever you go for getting an injection or if you happen to be in hospital for any major surgery or anything; minor or major or even a dental procedure; all of the equipment that is used has to be completely sterilised, because you do not want to pick up any infections.

So, using steam under pressure or by using a sterilising gas like ethylene oxide, you get complete sterilisation of whatever materials are being used. Then we come to commercial sterilisation. Now, food which is packaged is often sterilised on a commercial basis. So, you want to destroy all life forms within the food, and prior to packaging it. So, destruction of all pathogenic and spoilage organisms that grow in food under normal storage and handling conditions is called commercial sterilisation.

It includes destruction of both vegetative as well as non-vegetative spores and cells. So, resistant endospores of thermophilic bacteria may survive, but they are unlikely to be present in normal conditions. Under normal conditions, you are not going to have thermophilic bacteria. But if their spores are there, they will also, they may survive under these conditions. But they are not likely to be pathogenic. We do not know any examples of thermophilic bacteria that are pathogenic. They will not germinate or grow under normal storage conditions.

Then we come to disinfection. Disinfection is very important for us from a water treatment point of view. By now, you should be clear about the fact that anytime you have drinking water that is going to be stored, it should be disinfected. So, destruction of vegetative pathogens is the goal of disinfection. That is the definition and the goal. And we can use physical or chemical methods. Chemical methods are the use of chlorine, the use of ozone. Physical methods will be membrane filtration. That is a simple example of different methods that are used for disinfection.

Then we come to antisepsis. Antisepsis is where, let us say most of us have experienced this when you get a cut or a bruise or some injury, minor injury. What do you do? You reach for an antiseptic solution. So, destruction and inhibition of microorganisms that are likely to grow on living tissue is to be prevented; and antisepsis is the way to do that. So, the chemical is almost; chemical antimicrobial solutions are generally what is used.

Degerming is the physical removal of microorganisms using soap or detergent. Mechanical removal by an alcohol swab is also considered to be degerming. So, anytime you go for an injection or for getting a blood test done, they will always use an alcohol containing swab to clean the area, prior to inserting the needle. So, these are examples of degerming.

And then we have sanitisation. We want to ensure that any public places, door handles, desks, all these things, utensils that we use in public areas or even at home, you want to ensure that the microbial count on these surfaces is very, is as low as possible, close to zero.

So, we use all kinds of sanitisation methods. There can be several different examples; and we will go into the chemical disinfectants that can be used for sanitising public or private spaces. So, high temperature washing is one method; and dipping or wiping the surfaces with chemical disinfectants is another method.

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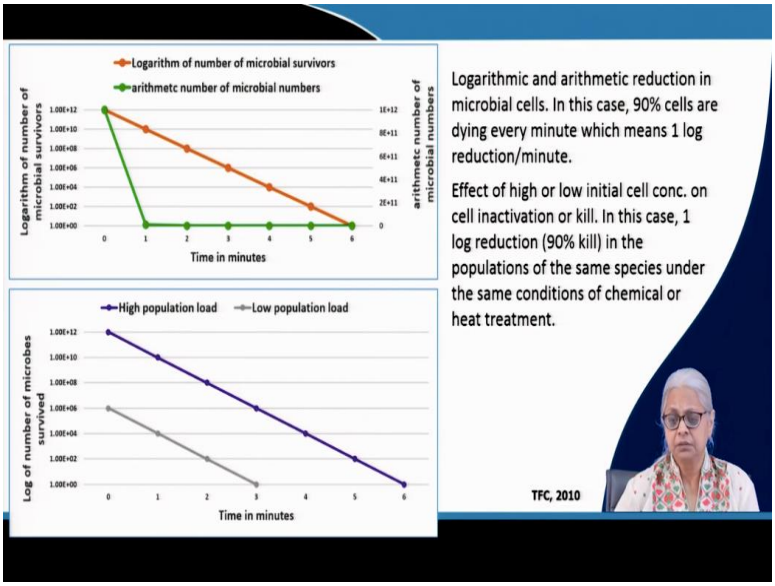

Let me also talk about how we measure the destruction of microbes. So, what you see over here is the reverse of what we saw in the growth lectures. And here we are looking at the log reduction in the cell count. Now, one of the parameters that is very common in the literature is either decimal reduction time; how much time does it take to destroy 90% of the population? So, that is one parameter.

In water treatment, we use another parameter and that is called log reduction. So, I want to know how many logs of cells have been destroyed or removed in a particular process. So, the rating of a membrane filter for example is in terms of 2log reduction, 3log reduction, 4log reduction. These are the ways in which we quantify how many cells are being removed from the water sample or any other sample.

So, what you see over here is a logarithm of the number of microbial survivors. And on a log scale, you see the orange line. So, on a log scale, this is log linear; time is in minutes; it is a

linear scale; and the y-axis is a log scale. So, you have a line, straight line. If you were to do it in terms of arithmetic numbers, you would get exponential decay; and that is what you see with the green line. In terms of the effect of high or low initial concentrations.

Now, let us say I have 2 samples. One has 1 million cells, or in this case, 10^{12} cells / ml. And in the second case, I have 1 million cells. And I want to know how much time it takes to reduce it by 1log or 2logs or 3logs, whatever it is. So, if we were to look at the 1 million sample, I am getting 6log reduction in 3 minutes. In the case of the 10^{12} cells / ml or whatever the unit is, it takes how much time to get the same log reduction. So, 6log reduction, from 12 to 6 is again in 3 minutes. So, under the same conditions, temperature conditions, chemical concentration conditions, under the same conditions, the same species should give you the same result. So, regardless of what the starting point is, regardless of what the initial population of cells is, whether it is high population load or low population load, in terms of log reduction and the time it takes to get that log reduction, the time should be the same. That is the assumption.

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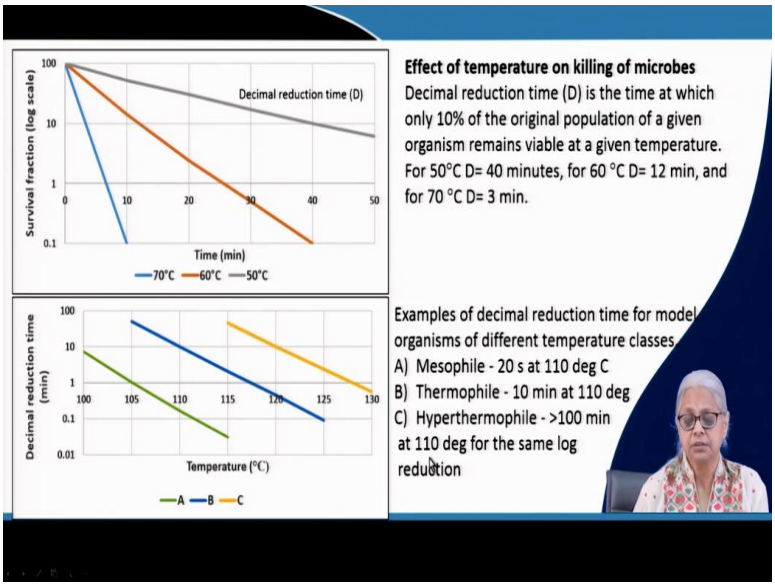

Then let us come to the effect of temperature. We know that chemical reactions, when you increase the temperature what will happen. As the temperature increases, the reaction rate increases; and therefore, the time to achieve the same outcome is going to be less. So, the same thing happens with bacteria. So, we have decimal reduction in, let us say at 70℃, 1log; so, from 100 to 10 will happen in 3 minutes. At 60℃, 1log removal in 12 minutes. And at 50℃, 1log removal in 40 minutes. So, this is what you are looking at.

Now, do all species react to the same disinfectant at the same temperature in the same way? The answer is, no. Different species will react in a very different way. So, a mesophilic species; the green line is a mesophile. You get 1log reduction in 20 seconds at 110℃. It is mesophilic bacteria; it has no ability to withstand high temperature like 110℃. A thermophile on the other hand is resistant to, it is fairly resistant to high temperatures. So, it takes 10 minutes to get 1log reduction at 110℃. Then we have hyperthermophilic bacteria. Greater than 100 minutes are required to get 1log reduction at 110℃. So, that is all of it.

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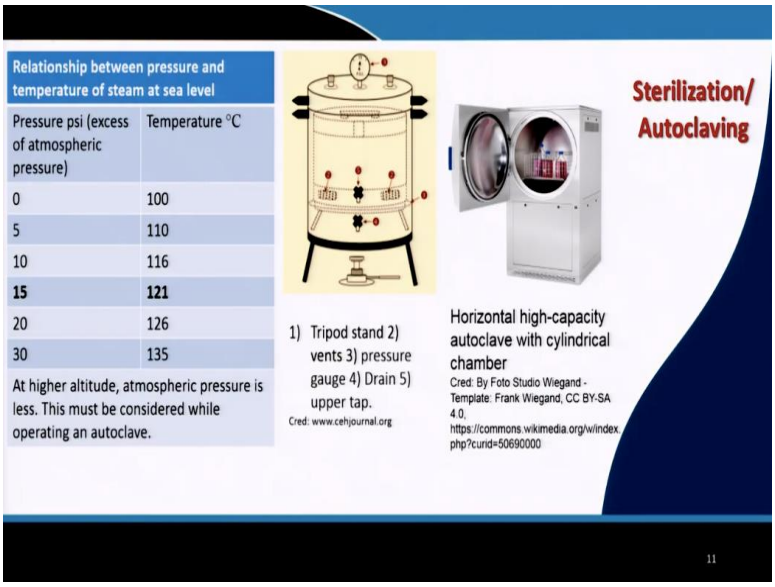

How do we achieve sterilisation? For example, so, when we do microbiological experiments in the lab, we need to make sure that all our equipment, our media, our water, everything is fully sterilised. And in general, autoclaving is a method that we use for sterilising our equipment and the media and so on. So, these are the conditions.

15 *psi* atmospheric pressure and a temperature of 121℃ is used for standard autoclaving or standard sterilising procedure. There are several types of autoclaves that are available in the market. This one is a tripod. And you can see it has 3 feet; it has vents; pressure gauges; a drain for the water and the steam; and an upper tap. So, this is very common. This is exactly what we have.

So, there are horizontal loading autoclaves. You can have room size autoclaves; you can have cabinet sized or refrigerator sized autoclaves. They come from very small autoclaves to very large ones. Your pressure cooker is also just like an autoclave. You can use it for sterilising

small amounts of, small objects and small amounts of media and so on. So, this is a horizontal loading autoclave, which means, all the material is put in, in the horizontal direction.

In the first case, it is a top-down loading autoclave. So, all types are available in the market. So, we are going to look at some of the details regarding how an autoclave works, the principles; and you can even see some of the details. I would ask you to refer to figure 7.2 in the textbook. So, TFC 2010 edition, it is figure 7.22. So, please refer to that for the remaining part.

What is the autoclave principle? So, here is the lock. This is horizontal loading autoclave. So, you have the lid. The lid of the autoclave is a very heavy lid, because it has to withstand all that steam pressure that is going to build up over the autoclaving cycle. So, this is the door and depending on the size of the autoclave, you will have several safety features that will prevent any escaping of gas, steam during the autoclave cycle.

So, all this material; whether it is dry material, whether it is wet material, whether it has caps or no caps, whatever it is; you load it into the autoclave; you seal the door. The door has several features that allow you to seal it safely, just like your pressure cooker. And you set the temperature as well as pressure to the standard conditions; allow the steam to come in; the steam will build up.

Now, a very important point in autoclaving is that the entire cycle, in practical terms, from the point of closing the door to the point when it is safe to reopen the door, can be anywhere from one and a half hour to 3 hours or so. But the actual autoclaving time is actually 15 minutes, because it takes a fair amount of time for the steam to build up. You have to maintain the steam and pressure for 15 minutes to ensure that you get complete sterilisation of the equipment, both the dry goods and the wet goods. And then, like I said, a fair amount of time to bring that high temperature and pressure back to normal room temperature conditions. Only then is it safe to open the door; otherwise, you can have problems. So, there can be accidents, there can be problems if you try to open an autoclave before it has come back to room condition, so, room temperature and pressure conditions.

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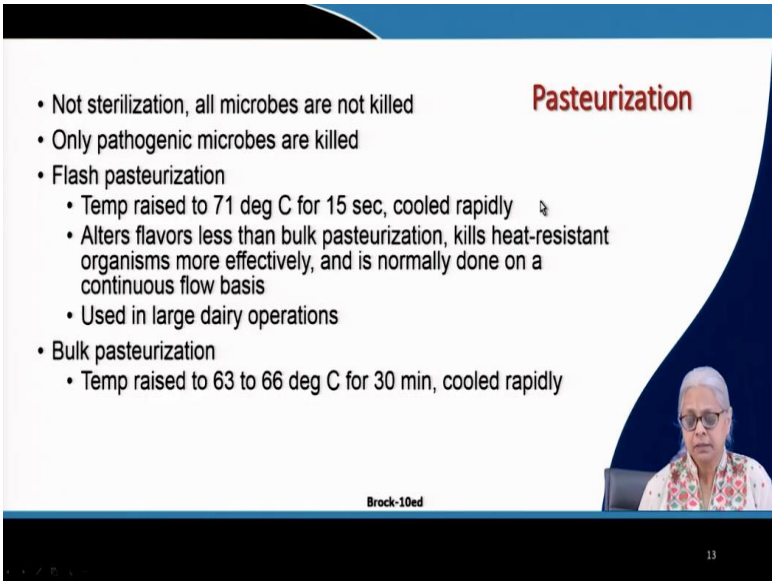

Then let us take a look at another method which is fairly common, and that is pasteurisation. Now, pasteurisation is not the same thing as sterilisation. In this case, all microbes are not going to be killed; our objective is to kill only pathogenic microbes. And remember what I said previously as well, and that is that most pathogens are mesophilic species. And these mesophilic bacteria are easily killed once the temperature is taken to higher than 70℃.

So, we have flash pasteurisation, where the temperature is raised to 71℃ for just 15 seconds, and then allowed to cool rapidly. The benefit of doing this for a very short period of time is that it does not alter the flavour of products like milk and so many other things where you use flash pasteurisation. So, the benefit of using flash pasteurisation is that it does not alter the flavour to the same extent as bulk pasteurisation.

It kills heat resistant organisms very effectively, and it can be done on a continuous flow basis. This is used in large dairy operations. And when you have bulk pasteurisation, the temperature is raised from 63 to 66 ℃ for 30 minutes and then allowed to cool. So, this is done, like I said, again in large operations to pasteurise large amounts of material. And that is about it.

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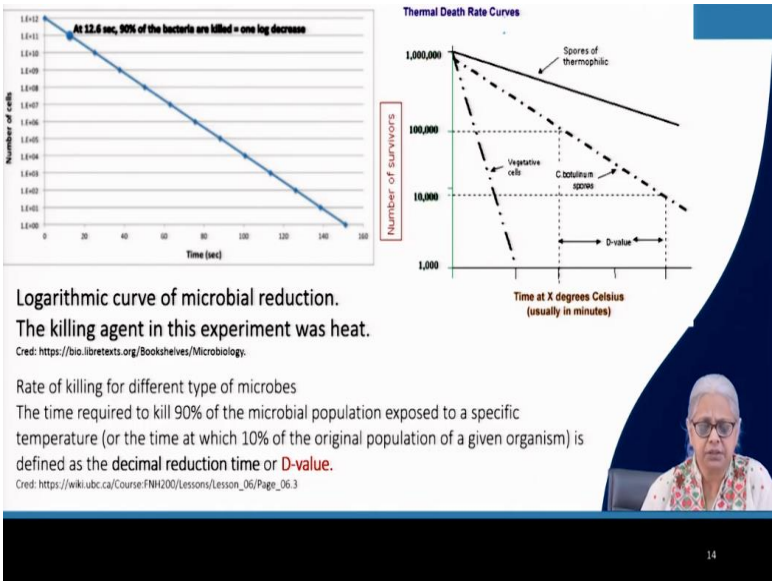

So, in this particular slide, what is shown is the difference in decimal reduction time for vegetative cells and spores. So, you can see, it takes much less time to get 1log reduction with vegetative cells. And for *Clostridium botulinum* spores, you can see how much longer it takes. So, this is generally clear. And the same thing for thermophilic spores in this case. So, in the other graph that you see over here, 1log decrease or 1log reduction in the bacterial count was obtained in 12.6 seconds.

So, this is our 1log reduction. So, these kinds of logarithmic curves can be obtained and they are generally done in the lab. You subject the sample to different temperatures and measure the concentration of the surviving bacteria. So, that is our decimal reduction time, which I have already mentioned.

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That brings me to the end of this lecture. Thank you.