Environmental Chemistry and Microbiology

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Module - 10
Lecture - 53
Microbial Growth and Control - II

Welcome to the second part of Microbial Growth and Control. This is lecture number 53 of module 10.

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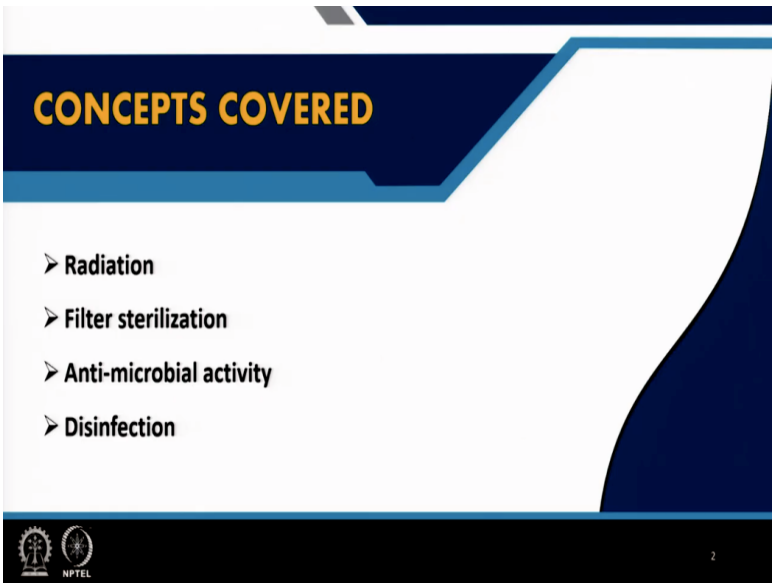

So, in this part, we are going to look at radiation, filter sterilisation, methods for measuring antimicrobial activity, and disinfection.

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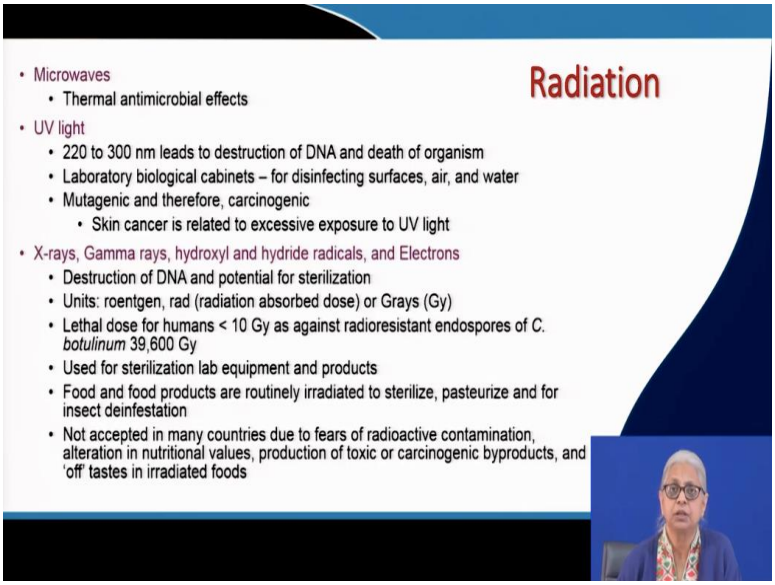

So, radiation is one of the most common methods of basically destroying or removing pathogens from either our food or our water. Many of you have used microwave ovens for heating food and water, but it can also be used under certain conditions for destroying microbes that are present either in food or water. But let me also add here that they are not very effective against microorganisms, because microbes have been found on the inner surfaces of these microwave ovens.

Now, if your food contains water and perhaps some pathogens in it, those pathogens can be killed by microwaving. Pathogens in solid foods cannot be destroyed by microwave. So, unless water is present, you do not get this antimicrobial effect of heating. So, I just finished in the previous lecture that heat is a very useful antimicrobial agent, but microwaves which do nothing else but provide heating; they are not sufficiently strong in terms of their antimicrobial effects. They do have some impact when there is water in the container and the food and so on.

Let us now come to UV light. UV light has become a standard part of what is called end of pipe treatment or home appliances that we use for water treatment. So, UV radiation or UV light has become part of it. And that is a standard physical method for disinfecting drinking water. So, the wavelength that we use is 220 to 300 nanometers. It can destroy the DNA and cause death of the organisms. So, it is a fairly effective way for ensuring that there are no pathogens in the water.

We also use it in laboratory biological cabinets. We have these laminar airflow hoods and they are the places where we do our biological or microbiological work and the surfaces, air and the

water that is placed in these cabinets can be disinfected using UV light. It is also important to remember that direct exposure to UV light can be mutagenic as well as carcinogenic. People have found that skin cancer is related to excessive exposure to UV light or sunlight which has, where there are no screens to protect you from UV light. So, that is, these things have been published in the literature.

So, we have X-rays. The other forms of radiation are X-rays, Gamma rays, hydroxyl and hydride radicals, and electrons. Now, all of these types of radiation can result in the destruction of DNA and have some potential for sterilising both food as well as other materials. The units are roentgens, rad (radiation absorbed dose) or grays (Gy). A lethal dose; I have already shown you in previous lectures, that lethal dose for humans is less than 10 Gy and we have endospores of Clostridium botulinum which can withstand 39,600 Gy. So, you can imagine that these endospores have enormous longevity, mainly because of their ability to withstand high radiation, heat, desiccation, all of these things are what gives endospores their longevity.

These types of radiation can be used for sterilising lab equipment as well as products, all types of products. They are used for sterilising food as well as food products. They can be used for pasteurisation as well as for insect de-infestation. One of the problems in food preservation is when you have a large amount of dry grains and legumes and so on. These types of grain type foods are often infested by the larvae of insects. So, sterilising with these types of radiations has been found effective for preserving these types of foods and food products for a longer period of time. However, it has not been accepted in many countries because of fears of radioactivity.

So, these types of radiation are also; they have a certain amount of radioactivity that is associated with them. There can be alteration in nutritional values of the food. There can be production of toxic or carcinogenic byproducts. And some people have complained that the taste of the food is altered. So, for all these reasons, many of these types of radiation methods have not been accepted.

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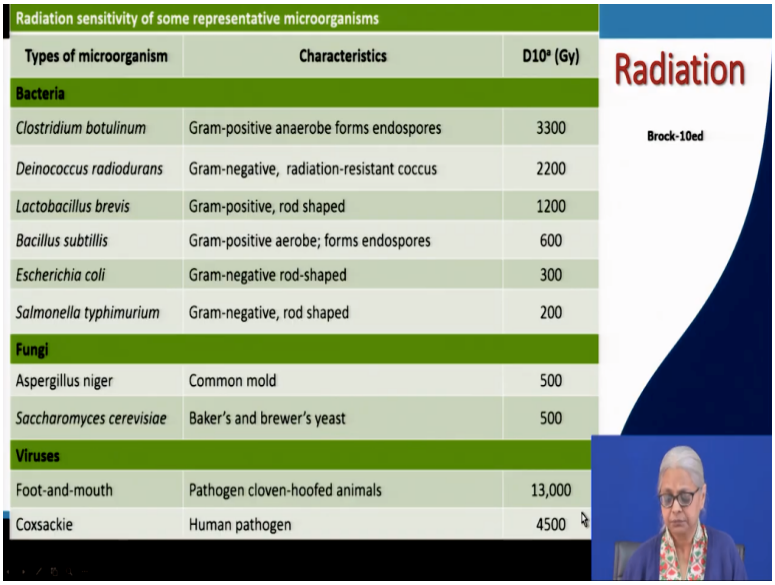

Let us now look at the sensitivity of certain microorganisms to radiation. So, what we have over here are bacteria, fungi and viruses. So, several types of bacteria, both gram-positive, gram-negative, aerobes, anaerobes, all of them have the ability to withstand fair amounts of radiation. So, D10 is again decimal reduction and 1log reduction in the population of these particular bacteria, bacterial species, in terms of Gy.

So, how many Gy does it take to get 1log reduction. And you can see that the endospores are much more resistant to the, compared to the vegetative cells. Then we have fungi. These fungi, we have *Aspergillus* which is the common mold. We have yeast. They have about 500 Gy, which is again much higher than any human being. Then we have viruses; foot and mouth disease causing viruses, *Coxsackie* viruses; you can see the range that they can withstand.

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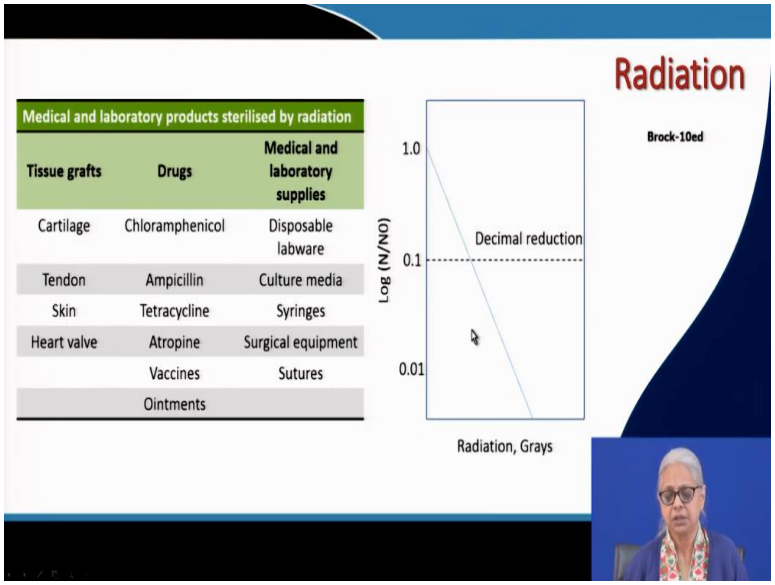

I said also that radiation is a method of sterilisation. So, many medical as well as laboratory products are sterilised by radiation. So, you have tissue grafts; you have cartilage, tendon, skin, heart valves; all these can be irradiated. You have drugs; pharmaceutical materials; chloramphenicol, ampicillin, tetracycline, atropine, vaccines, ointments; all of these are sterilised by radiation.

Then you have medical and laboratory supplies. All these can also be sterilised by radiation. And this is the decimal reduction in the number of cells. So, N is the number of cells; N_0 is the initial number on a relative scale starting from 1. If you get 1log removal, that is decimal reduction. And the point at which the number of Gys at which you get 1log reduction is what is shown over here.

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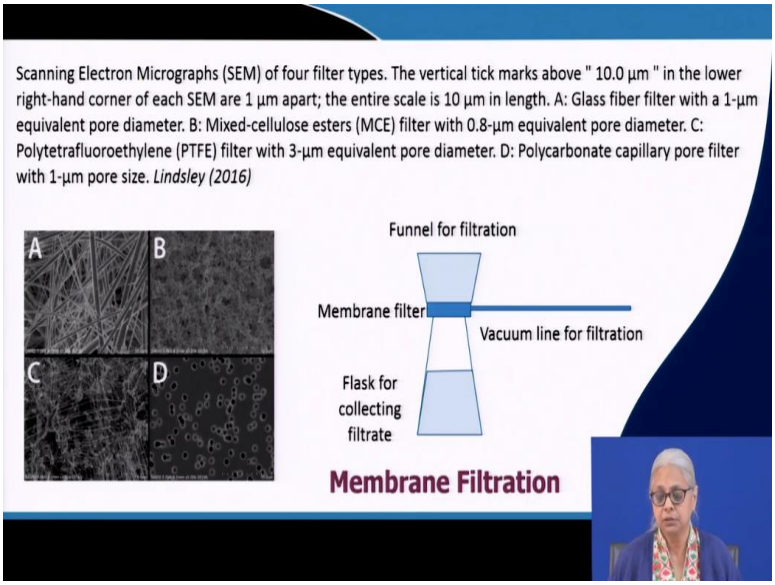

Then we come to filter sterilisation. Now, filter sterilisation has become a very important point in water treatment. Since that is the area that I work in, so, this is something that we do very frequently. So, when we take water samples, we want to filter sterilise them for various other applications. So, these are filter sterilisation units. They have 3 major parts to them. There is a cup at the bottom which can be connected to a vacuum line.

So, a negative pressure or a suction pressure is applied over here. This cup has another module; these are all detachable parts. So, there are 3 major parts to it. So, this is the cup which is attached to the vacuum line. That has a membrane holding fixture over here. So, the membrane is mounted on this particular part. And then there is another cup which does not have a bottom, that is fixed to this entire setup. And we normally have a clamp. If it is a glass-based apparatus,

then we have a clamp for holding all 3 parts together. Or in this pre-moulded plastic type of filter sterilisation unit, there may be pre-packaged and so on. So, you do not have; they are all moulded together. In any case, you have a membrane over here. The sample, whether it is a water sample or any other sample that is to be filtered, is passed under negative pressure or vacuum pressure through this membrane. Because, all membranes, especially 0.2µ membranes are very difficult to filter (through) without applying a vacuum pressure. So, that becomes very essential. So, here is the water sample, let us say, that is being passed through the membrane filter. So, this is how we use filter sterilisation units, which may be pre-moulded. These pre-moulded units are actually disposable units. And you can also have reusable units made out of glass.

So, here are scanning electron microscopes of the different types of membrane filters that can be used. So, the first one is glass fibre filter. This is very common. So, we have paper filters, asbestos fibre filters and glass fibre filters. The advantage of using glass fibre filters is that when you do suspended solids, in quantification of suspended solids, you want to know the volatiles suspended solids, then you burn these glass fibre filters and they burn without ash. If you use a paper or a cellulose type filter, then it will leave a remainder of ash; and you would not know the weight difference between the ash from the filter versus the solids. So, glass fibre filters are used for volatile suspended solids measurements. This one has a pore diameter of 1µ. They are generally more than that. They are 1.5, 2 µ pore diameter, average pore size.

Then we come to mixed cellulose ester filters. This one in particular has 0.8 µ pore diameter. Now, equivalent pore diameter; I will come to this idea again. None of the pores in these filters is uniform. They all have a large variation in terms of pore sizes. So, there used to be a standard practice of using protein solutions, pure protein solutions with a defined molecular weight to determine the pore sizes. So, those were called effective pore sizes. Here we have equivalent pore sizes.

Then we come to polytetrafluoroethylene filters which has a 3µ pore size or a pore diameter. And the final one is polycarbonate capillary pore filter with a 1µ size. These are done by etching with a laser beam. They are all exactly uniform.

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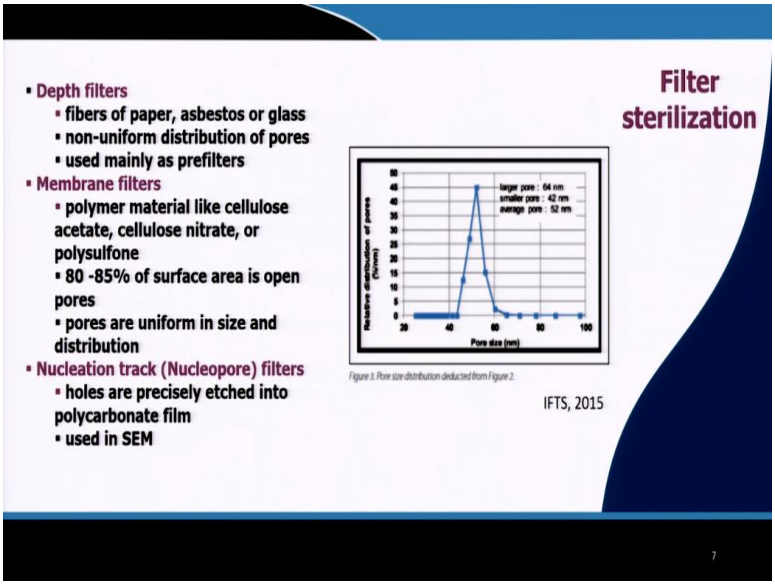

And I will come to a little more about that. So, in depth filters, there are several different types of depth filters that are available. They are made out of fibres of paper, asbestos or glass. The advantage of using glass fibre filters is that they do not leave any ash behind. So, we normally prefer to use glass fibre filters for measuring suspended solids. And this is a very common application in both Civil and Environmental Engineering, basically in wastewater treatment, where we are trying to measure the suspended solids and separate them into volatile and fixed solids.

They have a non-uniform distribution of pores and they are generally used as pre-filters. Then we come to membrane filters. You have polymeric materials just like the ones I have shown over here.

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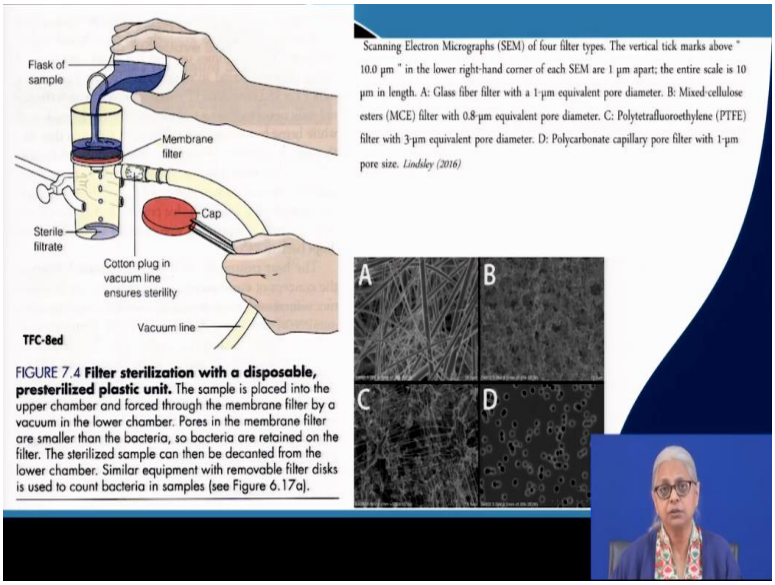

You have the mixed cellulose esters and the PTFE filters. So, these are the membrane filters made out of synthetic polymeric material. Cellulose acetate, cellulose nitrate, polysulphone; these are examples of membrane filters that are available in the market. And you can see over here, the surface of the filters about 80 to 85% is pores. So, the amount of open space on the filter is enormous. So, 80 to 85% of the surface area is open pores.

These pores can be uniform in size and distribution, compared to the depth filters. And finally, we come to what are called nucleation track filters; and the brand name is nucleopores. These are holes or pores that are precisely etched into the polycarbonate film. So, you can see the etching of the pores into the film. It gives you a uniform size distribution of the pores.

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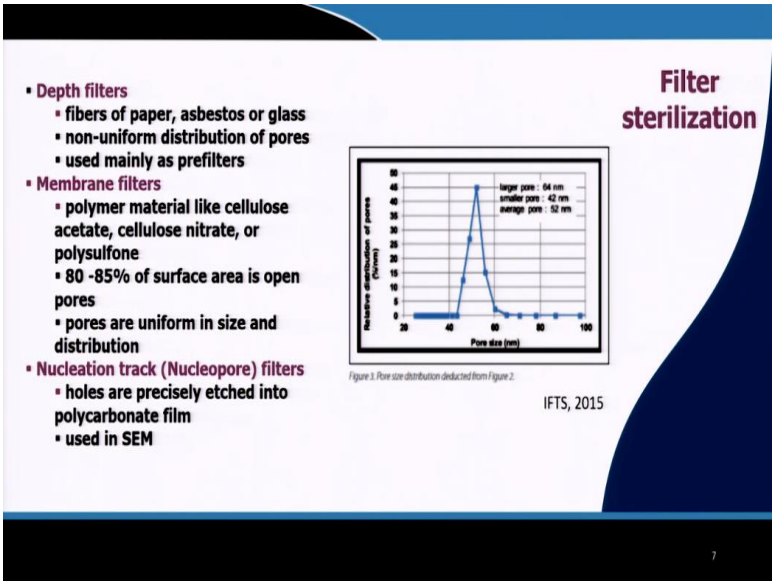

What you see here is the pore size distribution for any given filter. This is for a particular brand. And you can see the pore sizes are in the nanometer range. This is actually a very uniform pore size distribution, because the smallest one is 42 nanometers and the largest one is 64 nanometers with an average of 52 nanometers. Now, this is a very tight pore size distribution. But if you think about depth filters, you will get a much wider distribution of pore sizes.

And when I say I am using a 0.45 µ filter, that is the average pore size of the filter; and that can mean $\pm$ 0.45 or even more. So, it entirely depends on the type of membrane filter that you are using and the nature of the material that is used in the membrane.

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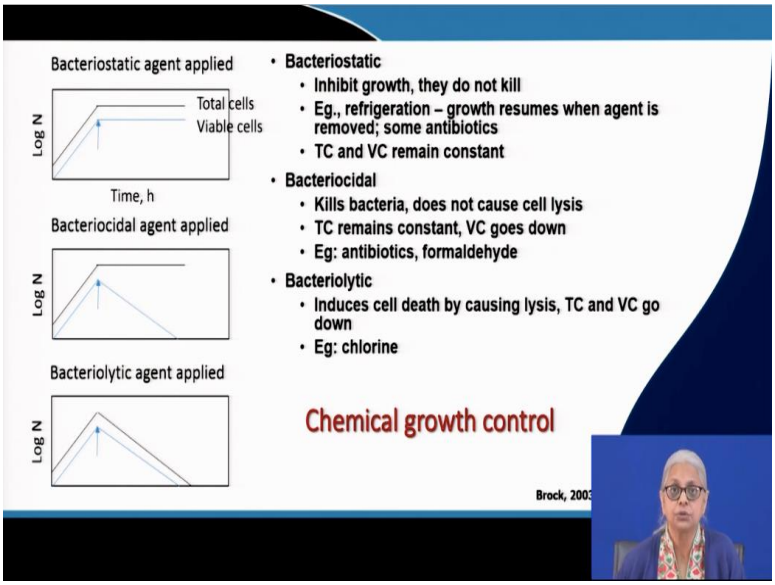

When we think about chemical growth control; so, up to this point, I was talking about physical growth control, now we look at chemical growth control. So, you can use various types of chemicals to control the growth of bacteria. In this case, we are using bacteria as our microorganisms. So, the first one is bacteriostatic. Now, I have already said a little bit about the fact that when you take any sample, it has a total number of cells.

And the number of viable cells in that total population of cells is going to be either equal or less than the total concentration. Now, if I apply a chemical at some point; while the culture is growing, at some point, we apply a particular chemical; or in this case, a physical agent like refrigeration. What will happen is that the total cells and the viable cells, both concentrations will level off. And that is because, under refrigeration conditions, there will be no growth and there will be no death of the cells either. So, the cell concentration will be constant; viable cells will remain constant. Total cells will also remain constant, because there is no death for the cells; it will inhibit growth, but they will not die. Therefore, you get these types of curves. Then we have agents that are considered bacteriocidal. So, bacteriocidal agents are the ones that can kill the bacteria, but they do not cause cell lysis. So, when the agent is applied; let us assume antibiotics or formaldehyde; these types of agents will cause the viable cells to be destroyed. So, the viable cell concentration will go down, but the total cell concentration will remain the same, because the cell is intact. So, the dead cells will be part of the total population, but the viable cells will go down.

Then we go to bacteriolytic agents. So, let us say we use chlorine or ozone. What will happen? They will damage the cell; they will call lysis of the cell; and both TC and VC, both will go down.

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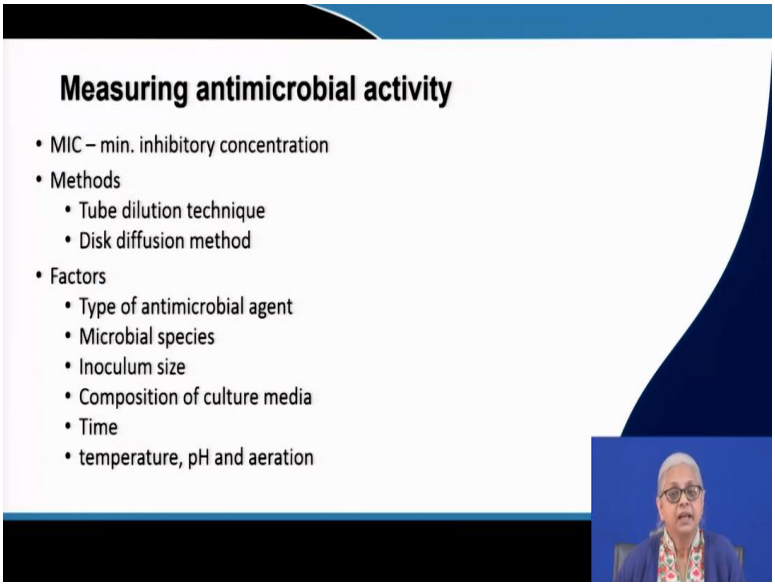

Then we come to measuring antimicrobial activity. So, we have minimum inhibitory concentration (MIC) that we need to find out. So, whenever we are using a particular antimicrobial agent, we want to find out what is the MIC essential for achieving our objective. There are 2 methods of doing it. One is tube dilution and the second is disk diffusion method.

So, here we have the tube dilution technique and the disk diffusion method. And there are several factors that will impact the results with these methods. So, we have the type of antimicrobial agent; we have microbial species; inoculum size, the nature of the culture media; time; temperature, pH, aeration; all these conditions, all these factors will have an impact on the result.

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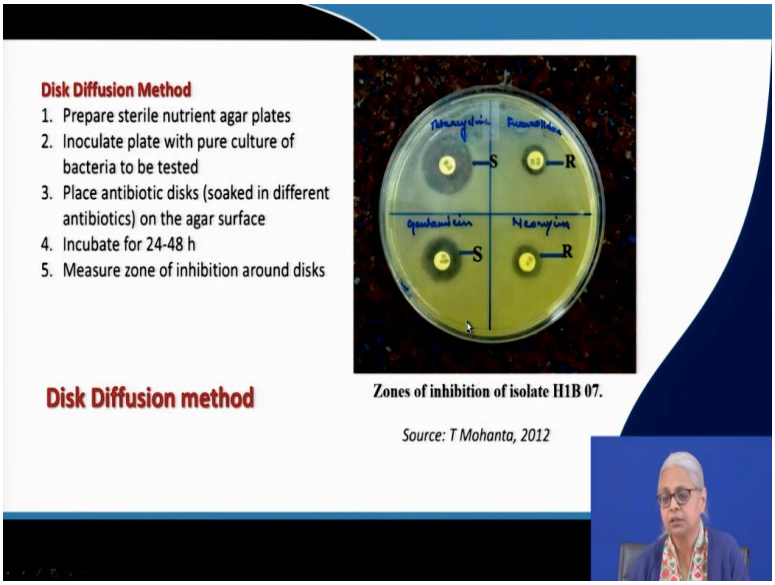

So, let us take a look at the disk diffusion method. In disk diffusion method, it is very simple. The nutrient agar Petri-plates are prepared with the appropriate nutrient agar or media, and they are inoculated; the plates are inoculated with pure cultures of the bacteria that needs to be tested. Now, there will be 4 antibiotic disks that are soaked in different antibiotics with a prescribed concentration. These antibiotic disks which are basically absorbent pads which are soaked in these antibiotics, will be placed in the 4 quadrants of the plate. So, you can see here, tetracycline, gentamicin, neomycin and so on. And these plates, after this has been done, they will be incubated for 24 to 48 hours. After the incubation period, there may be a zone of inhibition around the disks. This zone of inhibition may be very small or very large.

So, if it is very small, it means that this bacteria that is on the entire plate is resistant to that antibiotic agent. So, for example, in tetracycline, this particular bacteria was not resistant; it is sensitive; S for sensitive. It was extremely sensitive compared to the other antibiotic agents. So, this is what you can do with disk diffusion method. It has been around for a long time.

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More examples of the disk diffusion method. So, we have 3 different bacteria on these plates, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. 4 different disinfectants have been used; disinfectants or antibiotic agents. So, you have chlorine, hexachlorophene, o-phenylphenol and quat or quaternary ammonia. And you can see the results that chlorine is highly effective against *Staphylococcus aureus*. It is somewhat effective against *E. coli* and not effective against *Pseudomonas aeruginosa*. You can see the zone of inhibition becoming smaller and smaller. The same thing for the other antibiotics. Pseudomonas aeruginosa is completely resistant to the other 3 antibiotics. *E.coli* is only somewhat sensitive to quaternary ammonia and o-phenylphenol. Same thing for *Staphylococcus*. So, this is the way to determine whether a particular antibiotic or a particular disinfectant will be effective against any bacterial species.

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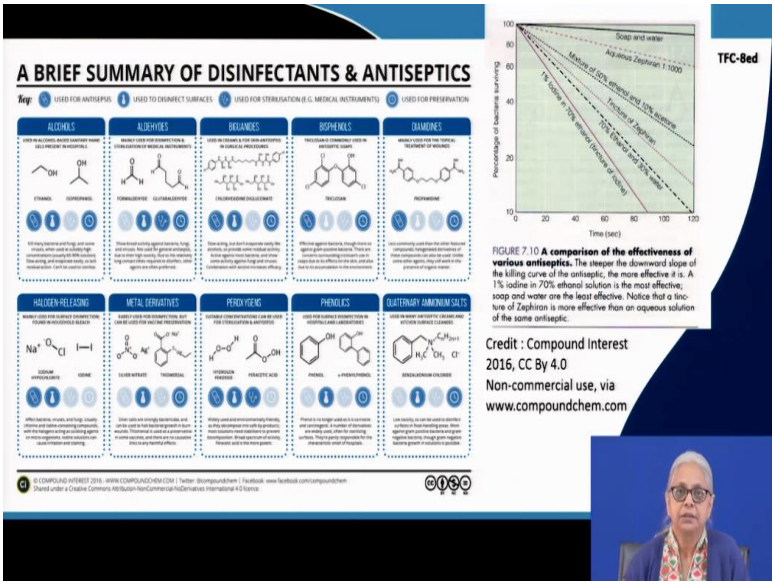

This is another way to quantify the effectiveness of different antiseptics. I am going to rerecord this one as well; I am going to modify this one. So, I will just skip it for now.

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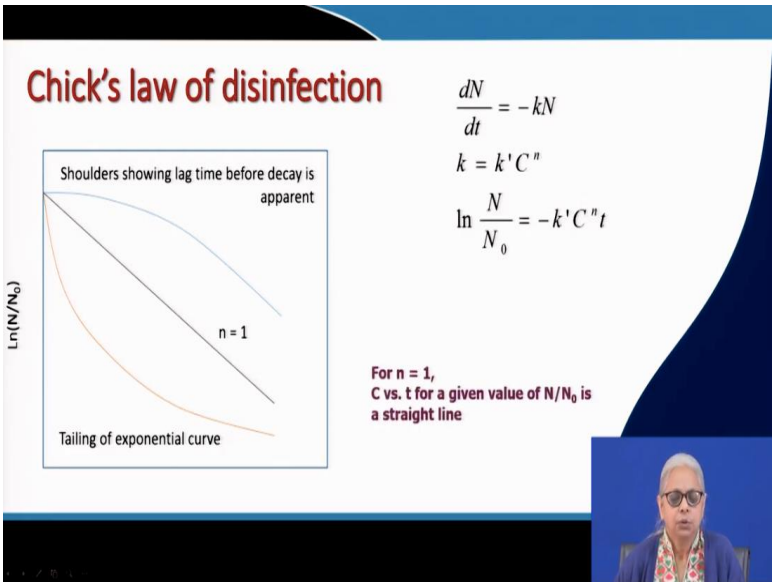

Let us come to another one. Now, let us take a look at how to quantify the disinfection effectiveness of any disinfectant. So, this is the Chick's law of disinfection. The first part is simple enough. So, $\frac{dN}{dt}$, N is the number of bacterial cells. The change in the bacterial cell population is $\frac{dN}{dt} = -kN$. And k is your proportionality constant; N remains the number of bacterial cells.

So, Chick-Watson's law is what we use to describe the reduction in the number of cells when they are exposed to a particular disinfectant. That disinfectant can be chlorine, it can be chloramine, it can be ozone, whatever it is. So, here we have $ln\frac{N}{N_0}$. N_0 is the initial bacterial population; N is the population at any time t; X-axis is also time. You can have these 3 types of curves. Now, the curve in the middle is n = 1, based on this particular equation; $\frac{dN}{dt} = -kN$. Now, this k is assumed to be a constant, but we know it is proportional to the disinfectant concentration.

$$\frac{dN}{dt} = -kN$$

$$\ln\frac{N}{N_0} = k'C^n t$$

So, k can be also written as $k'C^n$. And the straight line is for n = 1. So, when we integrate this, $ln\frac{N}{N_0}$ is going to be $-k'C^nt$. Now, if we assume that n = 1, Ct is the design parameter that we use for disinfection. So, when we do water treatment, we normally say, what is the Ct value required for 1log reduction, 2log reduction, 3, 4, whatever number of log reduction we want?

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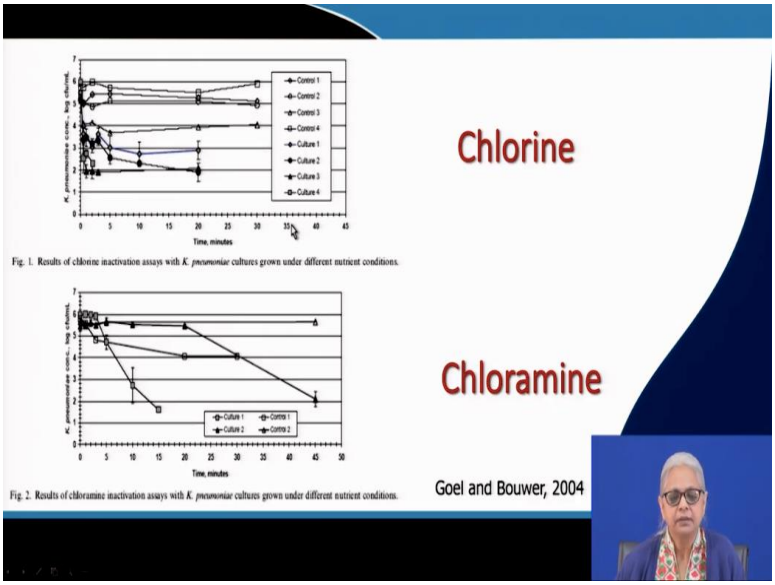

So, these are examples of the types of inactivation curves. So, these are laboratory assays that are done to determine how particular species of bacteria or any other microorganism will respond to the presence of a disinfectant. So, in this case, it is chlorine and chloramine. The concentrations of chlorine were close to 0.5 mg/L in the first case and the chloramine concentration was about 2 mg/L in the second case.

So, these are different cultures that were cultivated under different nutrient conditions and what is important to see here is the nature of the curves. So, the culture is what I am going to focus on. You can see, there is an exponential decay. So, from an initial concentration of 10^6, it fell to 10^2 in a very short period of time. So, you can see that all of the cultures that were exposed to chlorine, were at least 4log reduction to about 2log reduction within less than 5 minutes.

So, that is the first part of the curve. That is, there is an exponential drop in the cell population. But then, what is interesting is that in 3 out of 4 cultures, you get these long tails. And these long tails are similar to what you see here. So, you have an exponential curve, and then there is a long tail. Now, this long tail can be for various reasons. I will come to that later.

For chloramine, what you see is a different kind of phenomenon. In chloramine, you see this kind of shoulder, and then an exponential decay. So, again, you can see for different types of cultures 1 and 2, it takes a long time before the decay period starts. In culture 1, it takes about 5 minutes before the cell population starts declining. In culture 2, it takes more than 20 minutes before the cell population starts declining. So, this is what we call shoulders. These shoulders show a lag time before the decay is apparent.

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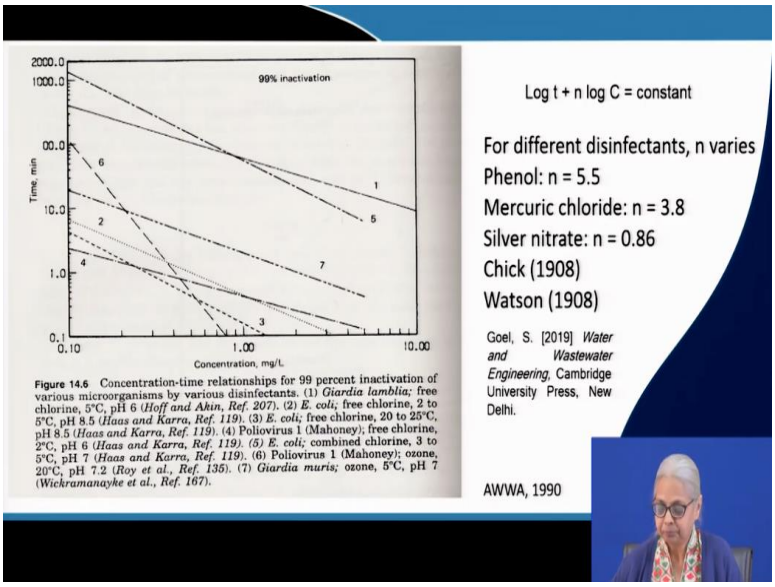

Figure 14.6 Concentration-time relationships for 99 percent inactivation of various microorganisms by various disinfectants. (1) *Giardia lamblia;* free chlorine, 5°C, pH 6 (*Hoff and Akin, Ref. 207*). (2) *E. coli;* free chlorine, 2 to 5°C, pH 9.5 (*Haas and Karra, Ref. 119*). (3) *E. coli;* free chlorine, 20 to 25°C, pH 8.5 (*Haas and Karra, Ref. 119*). (4) Poliovirus 1 (Mahoney); free chlorine, 2°C, pH 6 (*Haas and Karra, Ref. 119*). (5) *E. coli;* combined chlorine, 3 to 5°C, pH 7 (*Haas and Karra, Ref. 119*). (6) Poliovirus 1 (Mahoney); ozone, 20°C, pH 7.2 (*Roy et al., Ref. 135*). (7) *Giardia muris;* ozone, 5°C, pH 7 (*Wickramanayke et al., Ref. 167*).

As part of the Chick-Watson law of disinfection, the same equation that is written over here can also be written in another way. This can be written in another form. So, if I take the log. So, log k will be constant, some constant. And then you have log k' + n log C. So, I will modify all of this. So, this is the equation that you get. Now, if I have concentration versus time data for different species of bacteria; and in this case, 99% inactivation means, 2log reduction, because I have only 1 survivor in 100. So, that is 2 log reduction, yes.

And this is the kind of graph that you can generate for different species. So, this is a 2log generation for different species, *Giardia, E. coli, Poliovirus*. So, this kind of work has been done over a long period of time by various researchers in the field. So, this is kind of common; and this is based on this equation. So, these are the things that one can do. And now, from the original Chick-Watson paper where different disinfectants were tried. So, here we have phenol, where the n value was found to be 5.5. Mercuric chloride, n value was 3.8; silver nitrate n values were 0.8 for a single species.

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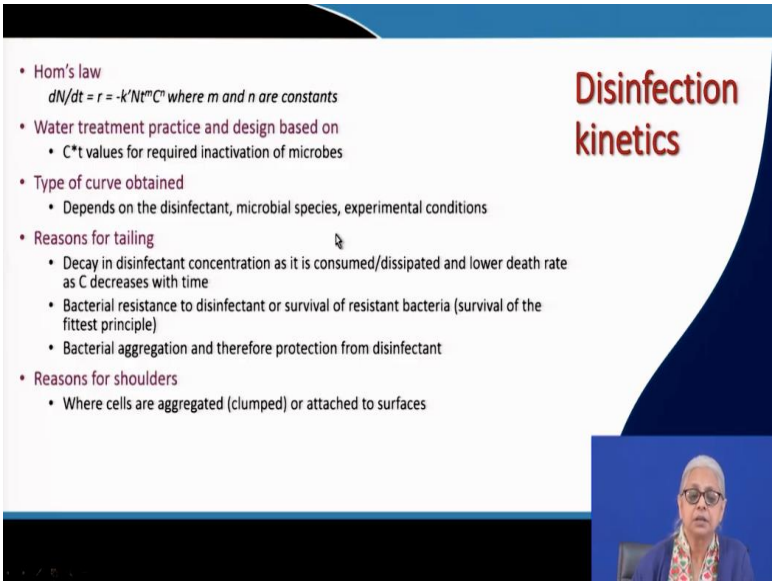

So, that is about the nature of the curves that you may see for different species and different disinfectants. So, you have Hom's law. So, you have $\frac{dN}{dt}$, which is another way; this is different from the Chick-Watson law. So, here is $\frac{dN}{dt} = -k'Nt^mC^n$. So, we know that, in water treatment practice, we use Ct values for a required log reduction in the microbial concentration.

The nature of the curve, as I have already shown you, depends on the nature of the disinfectant, the nature of the microbial species and the experimental conditions. Now, I also said that you see tails and shoulders. So, chlorine, as you see, has a tail; it provides a tail in the inactivation assay. And in chloramine, you see shoulders. Now, what is the reason for that? We think that the tails are because of several reasons. One is that bacteria, despite the fact that they are defined as free living independent organisms, they tend to remain in clusters. So, they keep multiplying, but they remain in clusters, which we call aggregates. So, when these aggregates are exposed to the disinfectant, the top level of cells will be exposed to the disinfectant. But the internal cells that are part of the aggregate are not going to be exposed to the disinfectant, because they are protected by the other cells.

So, that is one reason that you get survivors. That is, like I said, one reason. The second reason is bacterial resistance to the disinfectant or the survival of resistant bacteria. So, the Darwinian principle, survival of the fittest means that some bacteria will die based on exposure, but the others will survive. The third reason is the disinfectant concentration. The disinfectant concentration is being dissipated; it is being consumed. In the process of inactivating the microbes, the disinfectant is being consumed. Now, in this particular Chick-Watson law, we

are assuming that the disinfectant concentration is remaining constant, which is not true. We know for a fact that it gets consumed or dissipated. And because the concentration is going down, obviously, the number of cells that are being exposed to this concentration is also going to be able to survive the lower concentration.

So, the death rate will decrease as the disinfectant concentration decreases over time. So, these are some of the reasons that may be responsible for these long tails. What about the shoulders with chloramine? Again, we think it is because of aggregation. So, when the cells are aggregated or attached to surfaces, these disinfectants, especially chloramine which is a very slow or a poor oxidising agent, it has a very slow reaction time. It will take a long time to penetrate through biofilms, because when bacteria are attached to surfaces, they are in the form of biofilms. I have already shown you biofilms. So, it takes a long time for chloramine to penetrate into the cells when they are attached to surfaces or when they are in the form of aggregates.

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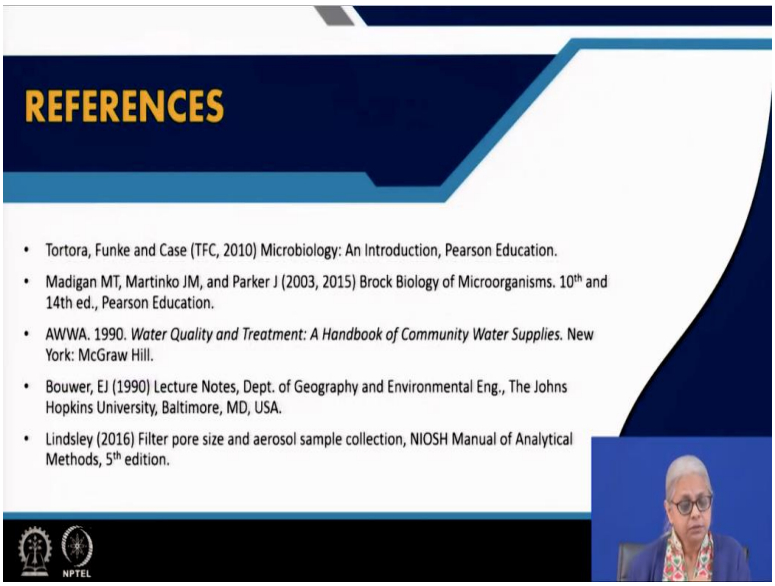

That brings me to the end of this lecture. Thank you.