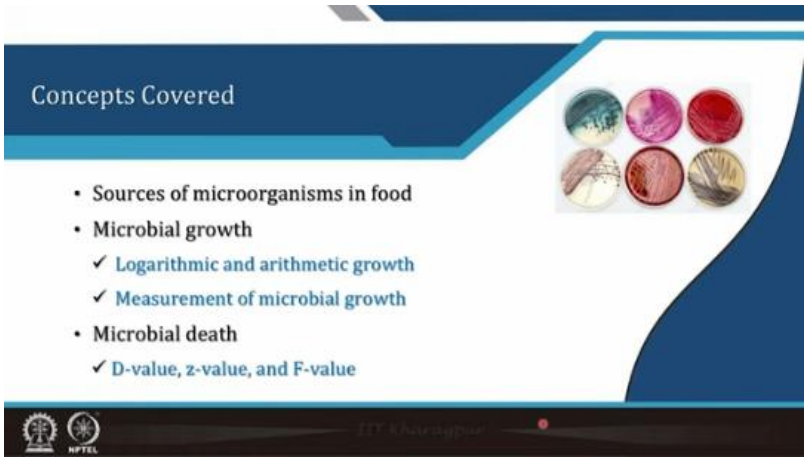
FOOD SCIENCE AND TECHNOLOGY

Lecture32

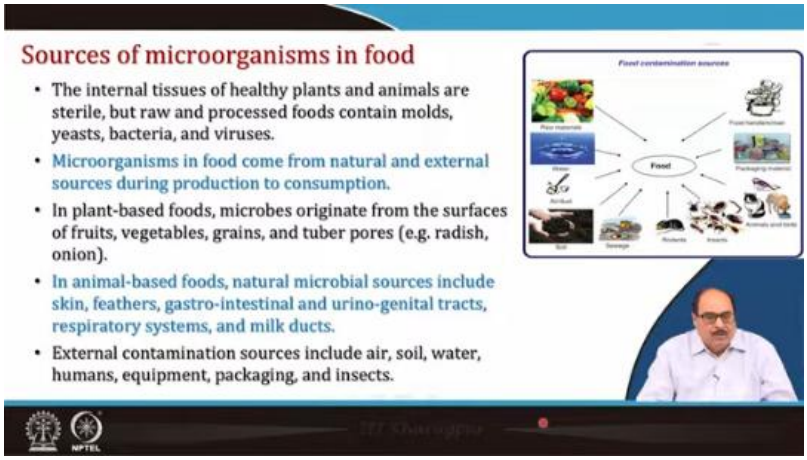
Lecture 32: Microbial Growth

Hello everyone, Namaskar.

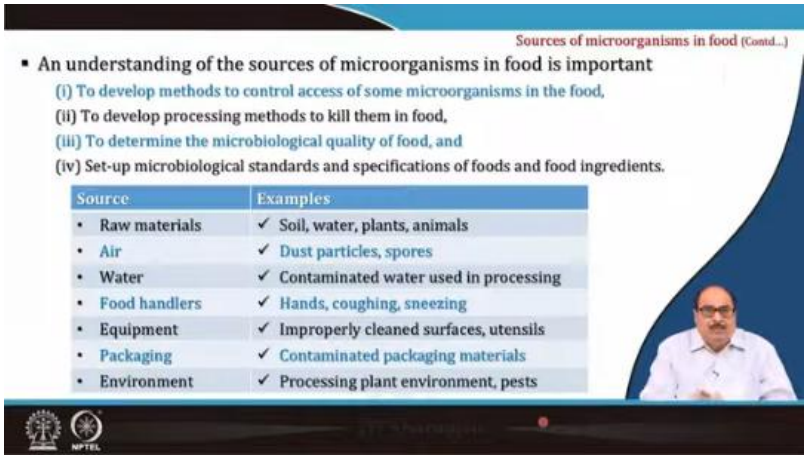
Now, in this lecture, in the next half an hour or so, we will talk about microbial growth and death.



The concept that we will discuss today is what the various sources of microorganisms in food are—that is, how food gets contaminated with microorganisms. Then, we will talk about microbial growth, both logarithmic as well as arithmetic growth, and we will discuss the measurement of microbial growth. and finally, we will also talk about microbial death, particularly in terms of D-value, Z-value, and F-value.

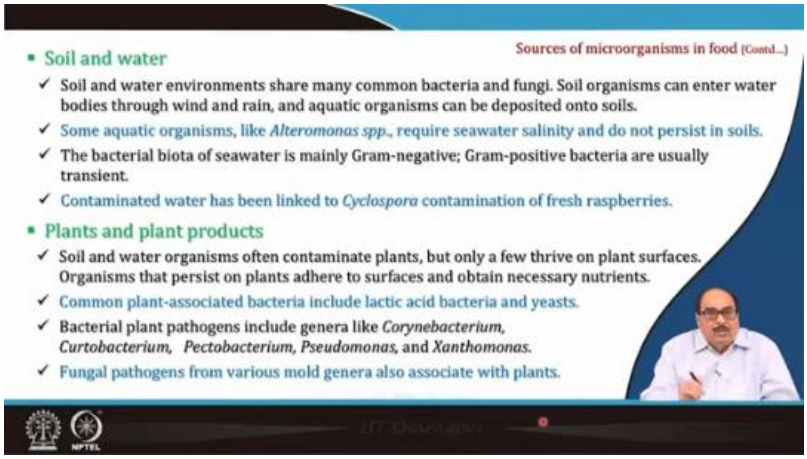


So now, let us talk about what the various sources of microorganisms in food are. You know, the internal tissues of healthy plants and animals are completely sterile. But raw and processed foods contain various microorganisms like moulds, yeasts, bacteria, and viruses. So, microorganisms in food come from natural and external sources, both during production as well as in the various stages in the value chain until consumption. At each and every stage, there is a lot of potential for contamination of the food with microorganisms if proper care is not taken. In plant-based foods, microbes originate from the surfaces of fruits, vegetables, grains, and tuber pores, like, for example, radish, onions, etc. In animal-based foods, natural microbial sources include skin, feathers, gastrointestinal tract, urinogenital tracts, respiratory systems, and milk ducts. So, internal or external contamination sources include air, soil, water, humans, equipment, packaging material, and, many times, various insects also cause the contamination of foods.



So, an understanding of the sources of microorganisms in food is important to develop methods to control the access of microorganisms in food, particularly the favourable as well as unfavourable microorganisms. Also, understanding is required to develop

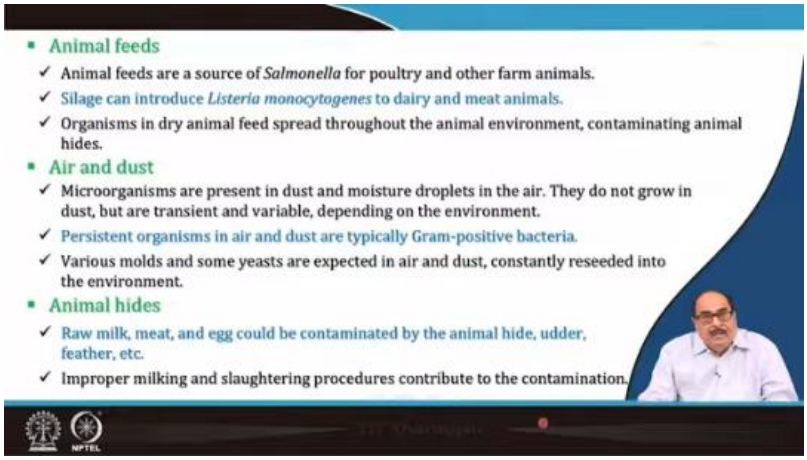
processing methods to kill microorganisms in food, particularly undesirable microorganisms. Also, we need to understand what the various sources of microorganisms in food are to determine the microbiological quality of food and set up microbiological standards and specifications for food and food ingredients. So, the various sources, as I told you, are, for example, raw materials, which might get contaminated with soil, water, plants, animals, etc. Even the air is another important source of contamination of food, like dust particles, spores, etc. Then water, if you use contaminated water in the processing, washing, etc. So, from there the food may get contaminated even the food handlers like hands if it is not properly the person who is handling the food if they are not using properly sanitary conditions there if their hands cough, if there is coughing, sneezing etcetera in the food areas, then that may be another source of contamination. Even the equipment, if it is not properly cleaned, surfaces, utensils, etc. In which foods are being handled, if they are not properly sanitised, they may also contaminate the food. Packaging material can contaminate food if the material itself is not sterilised, or if it is, the environment, such as the processing plant, etc., may contain pests and other insects. So, the environment again may be another source for the contamination of food.



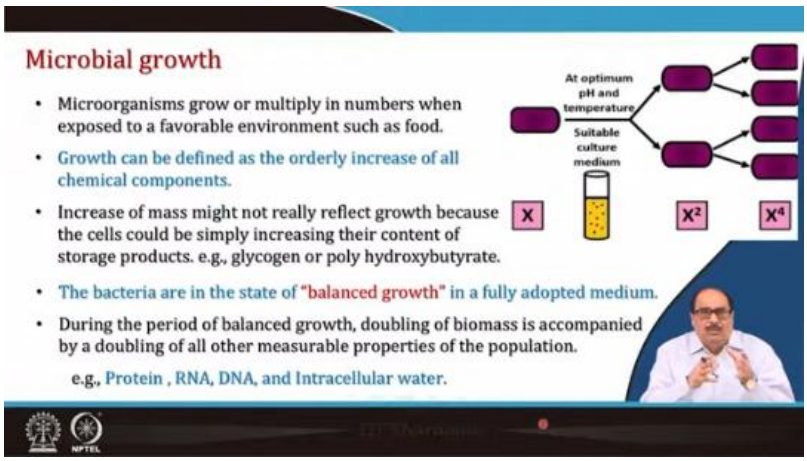
So, if you like to elaborate a little bit, like soil and water. In the soil and water environments, there are many common bacteria and fungi that are soil organisms. Water bodies are affected by wind and rain, and aquatic organisms can deposit onto the soils. Some aquatic organisms, like Alteromonas species, require seawater salinity and do not persist in soil. The bacterial biota of seawater is mainly gram-negative. Gram-positive bacteria are usually transient. Contaminated water has been linked to Cyclospora contamination of fresh raspberries. So, there are several examples of soil and water as sources of contamination of food materials. Similarly, plants and plant products, like soil and water organisms, often contaminate plants, but only a few thrive on plant surfaces.

Organisms that persist on plants adhere to surfaces and obtain necessary nutrients from those plants and in the process, they contaminate the surface of the plant. Common plant-associated bacteria include lactic acid bacteria and yeasts. Bacterial plant pathogens include genera like Corynebacterium, Curtobacterium, Bacterium, Pseudomonas, and Xanthomonas, etcetera. Fungal pathogens from various mold genera also associate themselves with the plant, and they contaminate the plant.

Food utensils, like surface organisms on vegetables, can contaminate containers and utensils during harvesting. A buildup of organisms occurs in utensils, leading to consistent contamination levels in food. So, cutting boards, knives, and grinders in the meat market, etc., are common sources of contamination. Similarly, there are several examples. Then, even the gastrointestinal tract—that is, if you take polluted water which is used to wash food products—can introduce gastrointestinal biota into the food. Then, intestinal biota includes organisms that do not persist long in water, like Salmonella. Enterobacteriaceae and intestinal pathogens, including five protozoal species, may be present in the fecal waste. An intestinal biota can also contaminate foods, and that is why even the coliform count, etc., may be increased. So, if you take the food count and you find that it has a higher amount of coliforms, it means that it is contaminated with fecal matter and other things. Then, food handlers' microbiota, or food handlers' hands and clothing, reflect their environment and habits. Sources of contamination include soil, water, dust, nasal cavities, mouth, skin, and, as I told you, the gastrointestinal tract as well. So, if personal hygiene is not maintained properly, poor personal hygiene can introduce gastrointestinal organisms into food. So, that is very important.

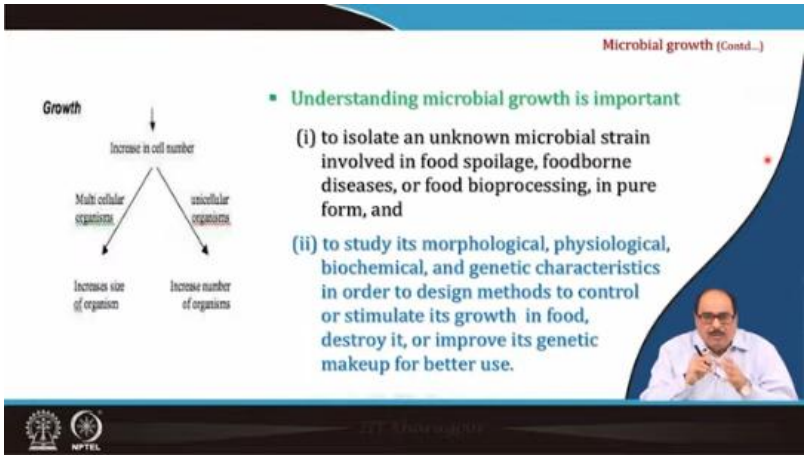


Animal feeds are also a source of S*almonella* for poultry and other farm animals. Silage can introduce Listeria monocytogenes to dairy and meat animals. Organisms in dry animal feed spread throughout the animal environment, contaminating animals. Also, you see microorganisms present in dust and moisture droplets in the air. They do not grow in the dust but are transient and variable depending on the environment. Persistent organisms in air and dust are typically gram-positive bacteria. Various moulds and some yeasts are also expected in air and dust, constantly re-entering the environment. Raw milk, meat, and eggs could be contaminated by animal hides, feathers, etcetera. So, improper milking and slaughtering procedures contribute to contamination.



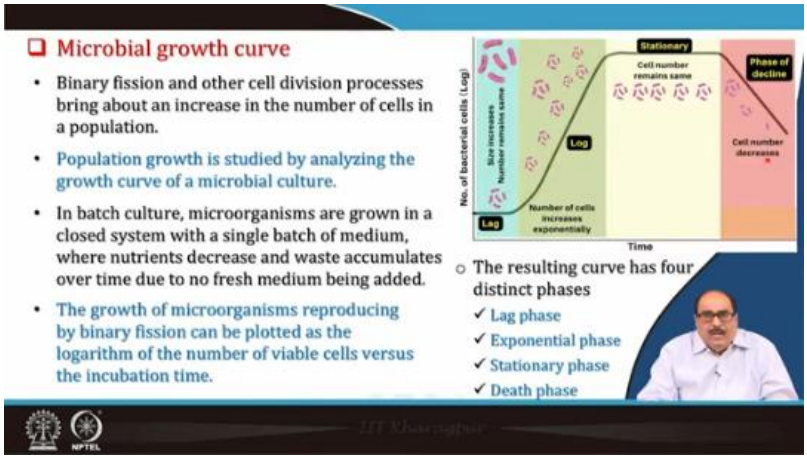
So, we have seen that there are various sources or chances of food contamination with microorganisms. So, once the food in the value chain is harvested, slaughtered, or handled, if proper care is not taken, it can get contaminated with food microorganisms, and then, when they find a suitable environment, they multiply; that is called microbial growth. There are microorganisms that grow or multiply in numbers when exposed to a favourable environment, such as food, because microorganisms also need food for their own survival,

and they take the nutrients from the food material or biomaterial to which they are contaminated. So, they take the food for their own survival and in the process, many a time they spoil the biomaterials or food.

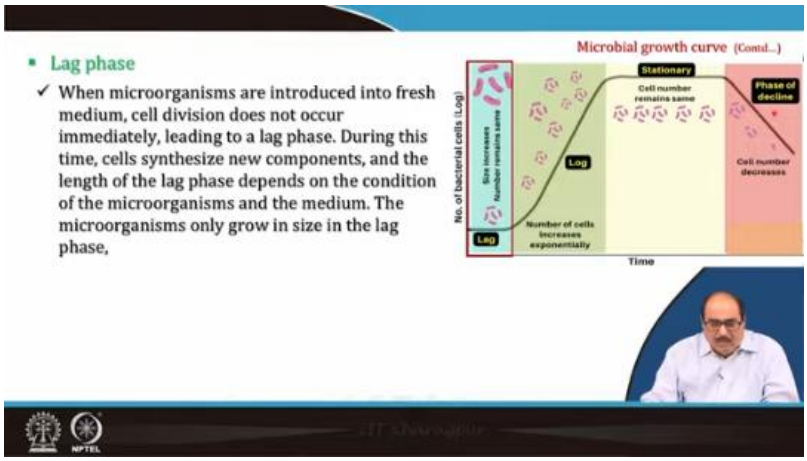


So, growth of the microorganism can be defined as the orderly increase of all chemical components, that is, an increase in mass. might really not reflect their growth, that is, the bacteria may not increase in mass, but still it might be growing because the cell could be simply increasing its content of storage products like glycogen or polyhydroxybutyrate, etc. So, the growth is basically an orderly increase of all chemical components. The bacteria are in a state of balanced growth in a fully adapted medium. And during the period of balanced growth, doubling of biomass is accompanied by a doubling of all other measurable properties of the population, like protein, RNA, DNA, intracellular water, etc., the quantity of all these is doubled, the biomass gets doubled, which is called balanced growth. So, in a fully adapted medium which has all the required nutrients for the bacteria to grow, and if the environment is totally favorable. So, it will remain in the period of viral growth. You can see here that if the initial number is X at optimum pH and temperature, if there is a suitable culture medium then it will multiply, that is, divide. It will become double X square, and then again it will double because one will go into two, this will go into two, and in the process, that is the doubling of biomass will take place, ok. So, understanding microbial growth is important to isolate an unknown microbial strain, involving food spoilage, foodborne disease, or food bioprocessing in pure form. It is also important to study its microbiological characteristics, that is, its morphological, physiological, biochemical, and genetic characteristics, in order to design methods to control or stimulate its growth in food. or improve its genetic makeup for better use. So, for these various purposes, if you understand properly how the microorganism is growing, what are the

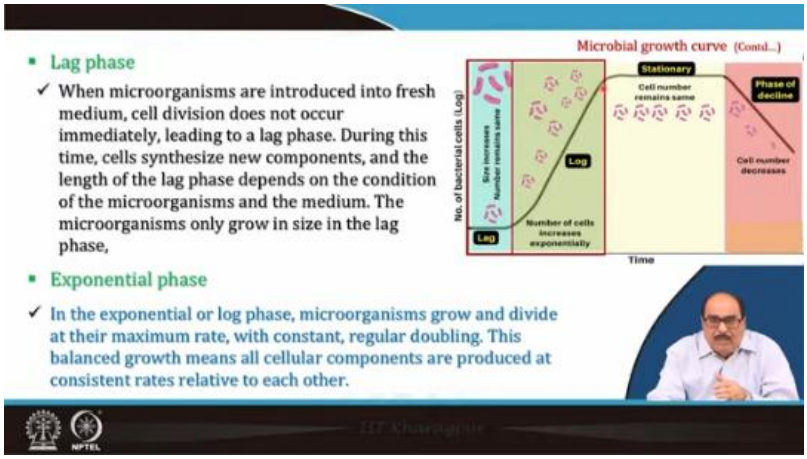
various factors that are affecting it. Control it, that is, you can create favourable conditions for its growth, or it will you can create undesirable conditions for its destruction, etc.



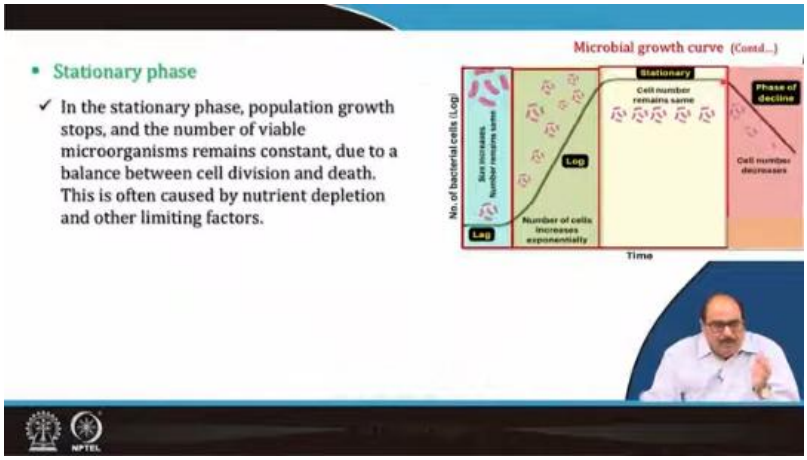
Let us see the microbial growth curve, as you have seen that it divides into two cells like binary fission. and other cell division processes bring about an increase in the number of cells in a population. That we have seen the growth. So, population growth is studied by analysing the growth curve of a microbial culture. That is, here it is shown—that is, the number of bacterial cells. When it is processed, that is particularly the log number versus the time, ok. So, if you see in a batch culture, the microorganisms are grown in a closed system with a single batch of medium. where nutrients decrease and waste accumulation increases—waste products, metabolites, etc., over time because there is no fresh medium being added in the batch culture, ok. The growth of microorganisms reproducing by binary fission can be plotted as the logarithm of the number of viable cells versus the incubation time, as I told you—it is shown here in this. The resulting curve has four distinct phases: lag phase, log phase, stationary phase, and phase of decline, which is popularly known as the death phase.



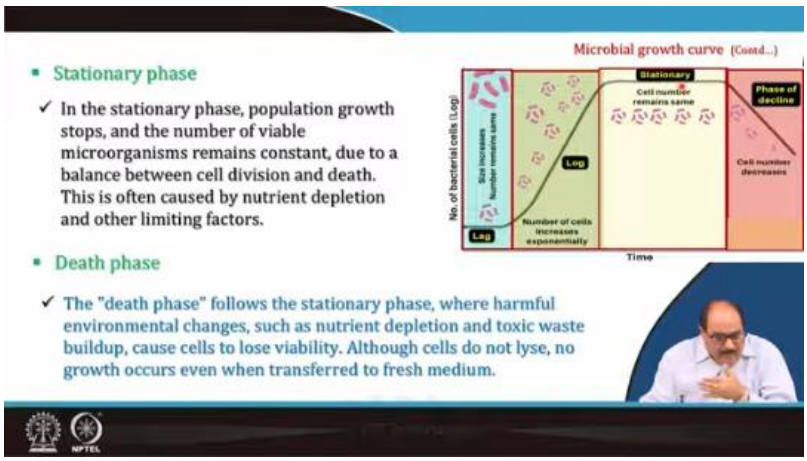
So, let us see one by one what is happening in these phases. So, lag phase, when microorganisms are introduced into fresh medium, it takes some period of adjustment. As you can see here, there is a cell division that does not occur immediately, and that leads to a lag phase period of adjustment. So, during this time, the cell synthesises new components and the length of the lag phase depends on this, which may be small, short or long. So, it depends on the condition of the microorganism and the medium. Before it was put into this medium, what was its previous history? what is the previous history, whether it was in an actively growing stage or in a dormant stage or which stage it was there? So, that will depend on whether the lag phase will be short or long.



So, the microorganisms in the lag phase only grow in size. They grow in size, but they do not multiply; they do not divide. The number of microorganisms remains constant in the lag phase. Then, once it is fully adjusted to a new environment, the next phase comes, which is called the exponential phase, and in this phase, it is also popularly known as the log phase. The microorganism grows and divides at its maximum rate and with a constant rate, which means it doubles in number regularly. And, this balanced growth means all cellular components are produced at consistent rates relative to each other. So, in the balanced log phase period, you will find that all the cells are of the same size, same number, it will double, and in all the cellular components, etc., it will be there. Then, there may be a few small periods of unbalanced growth, after which comes the stationary phase.

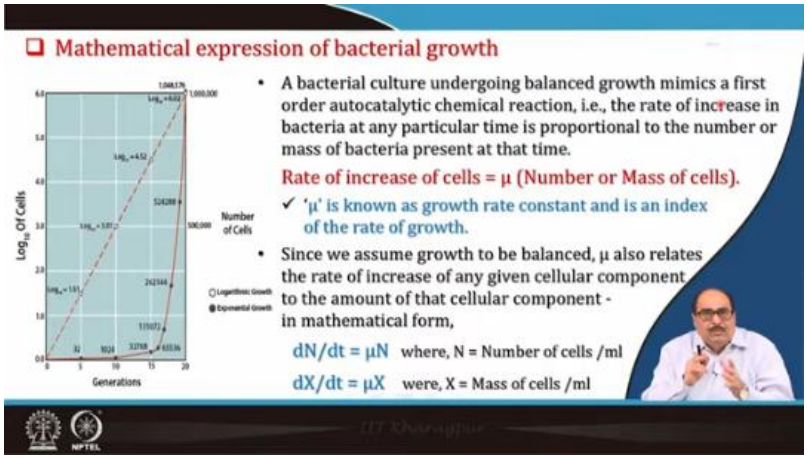


So, what happens in the stationary phase is that population growth stops, but cell number remains the same. This means that this stationary phase comes when the bacteria are growing actively. that it might be that in the batch culture, all the cell nutrients are depleted that bacteria has used all the nutrients are also in the medium that is when bacteria are growing there are some metabolites are excreted toxic metabolites So, either by exhaustion of the nutrients or by depletion of the toxic metabolites the growth stops and bacteria goes into the cell. dormant state, where cell division is not there. So, it remains in the dormant state. Then obviously, you will understand that if the cell remains for a longer period in the hard conditions in the stationary phase, eventually it results in death.



Then the death phase is followed by the stationary harmful environment changes, such as nutrient depletion and toxic waste buildup, cells lose their viability, and although cells do not die, no growth occurs when they are transferred to a fresh medium. So, they become

totally unviable; they are dead cells, might be there, but they are dead cells. So, that is the bacterial growth curve.



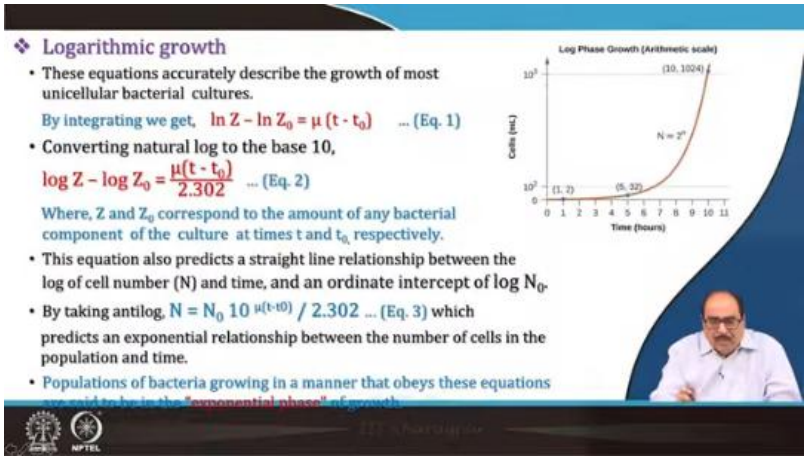
So, now let us talk about how the growth of bacteria or microorganisms can be expressed in mathematical terms. That is a bacterial culture we have seen undergoing balanced growth, mimicking a first-order autocatalytic chemical reaction. What does it mean? The rate of increase in the bacteria at any particular time, that is, the rate of increase in the bacterial cell, bacterial mass or any other component, is proportional to the number or mass of the bacteria present at that time, that is, the rate of the increase of cells is equal to mu multiplied by the number or mass of the cells. where mu is known as the growth rate constant, and it is an index of the rate of growth. So, since we assume that growth is balanced, you can also relate the rate of increase of any given cellular component to the amount of that cellular component. In mathematical form, you can see

$$\frac{dN}{dt} = \mu N$$

where n is the number of cells per mL or

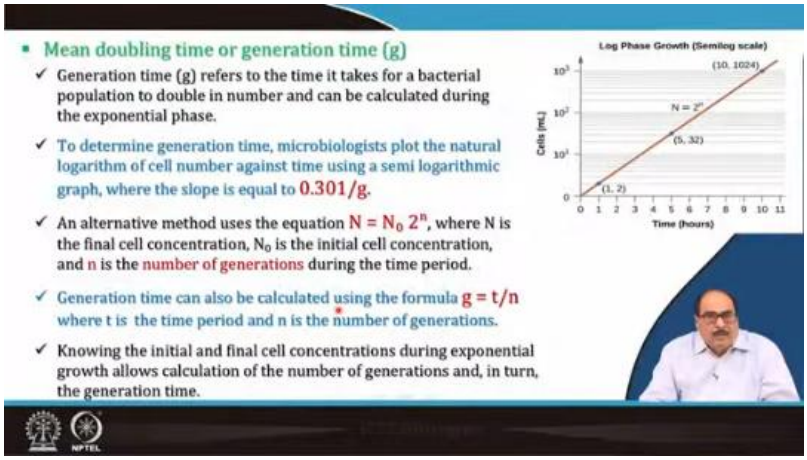
$$\frac{dx}{dt} = \mu X$$

where x is the mass of cells per ml.

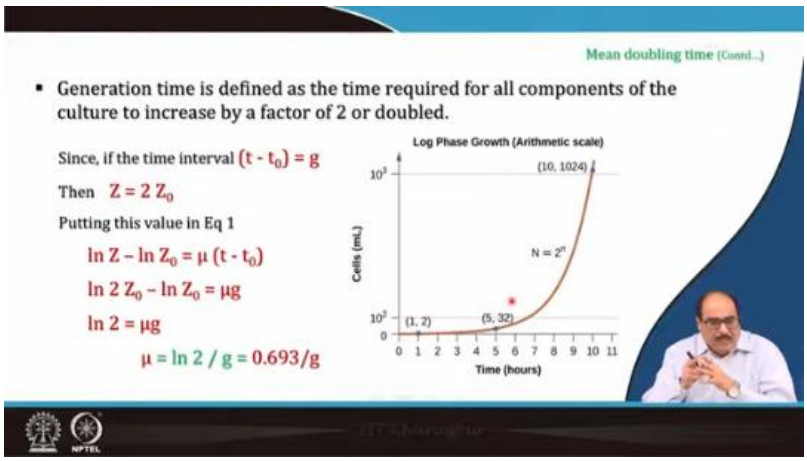


So, now the logarithmic growth, that is, you see that lag phase or exponential growth, and these equations accurately describe the growth of most unicellular bacterial cultures. And that earlier equation, which we have seen, all right, that if we integrate, we can get $\ln z - \ln z_0 = \mu(t - t_0)$, where Z we are, that is, any cellular component, it may be DNA, it may be RNA, it may be mass, it may be number, it may. So, it is a wider, a little wider representation. So, Z and converting, if you convert this to natural log, that is, a natural log to log to the base 10, then you get $\log Z - \log Z_0 = \mu \frac{t-t_0}{2.303}$.

As I told you, Z and Z_0 correspond to the amount of any bacterial component of the culture at times T and T 0, respectively. Now, this equation also predicts a straight line relationship if you plot the log number, n and the time on the ordinate intercept of log n0. So, semi-log curve, if you take the anti-log of that, we get the exponential equation like $N = N_0 10^{\mu(t-t_0)}/2.302$. This equation predicts an exponential relationship between the number of cells in the population and time. So, populations of bacteria growing in a manner that always follows these equations are said to be in the exponential phase of growth. So, in the exponential process mu you have seen that there is a rate constant growth rate constant. So, there is another very important term that is the g, which is generation time or mean doubling time.

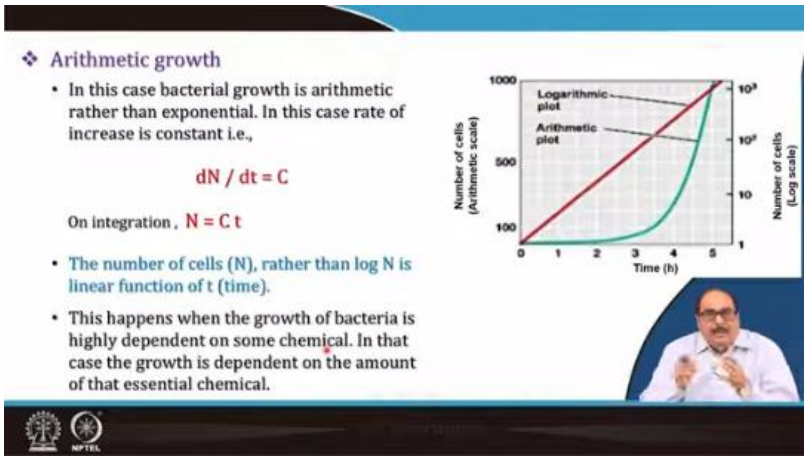


So, this generation time refers to the time it takes for a bacterial population to double in number. and can be calculated during the exponential phase. Then, to determine generation time, microbiologists plot the natural logarithm of cell numbers against time using a semi-log paper, where the slope of this is equal to 0.301/g. An alternative method uses the equation $N = N_0 2^n$, where n is the final cell concentration, n_0 is the initial concentration, and n is the number of generations during the period. So, generation time can also be calculated using the formula $g = \frac{t}{n}$, where T is the time period and n is the number of generations. So, knowing the initial and final concentrations during exponential growth allows calculation of the number of generations and, in turn, the generation time as well.

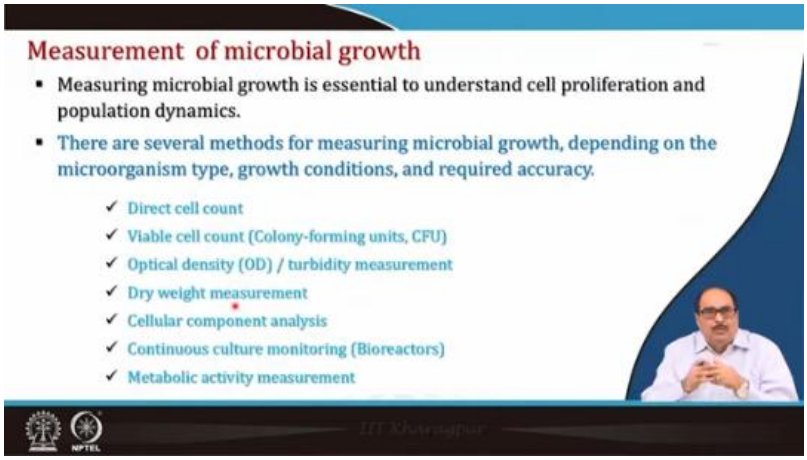


So, generation time is defined as the time required for all components of the culture to increase by a factor of 2, or it is doubled, as you can see here in the figure. So, if the time interval $t - t_0 = g$, then you can take $Z = 2Z_0$ and put this value in equation 1 earlier, which we have seen $\ln Z - \ln Z_0 = \mu(t - t_0)$ or if this Z you put 2 Z_0. So, $\ln 2Z_0 - \ln Z_0 = \mu g$, or $\ln 2 = \mu g$, and then $\mu = \frac{\ln 2}{g} = \frac{0.693}{g}$

means that if you know the growth rate μ, you can calculate the generation time.

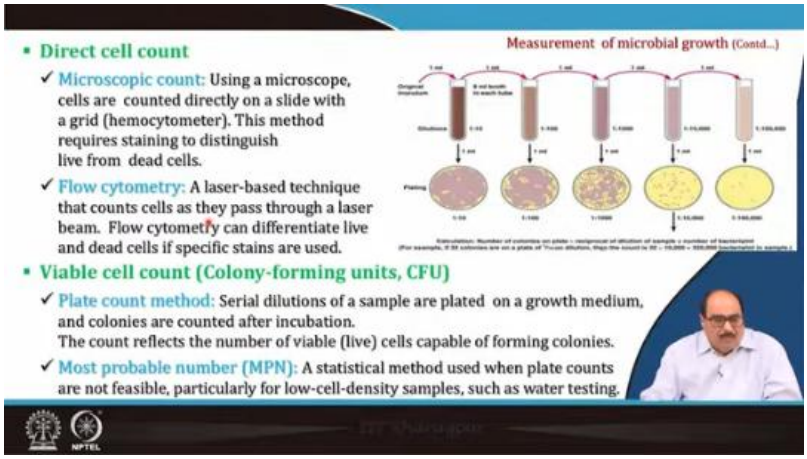


Then comes the arithmetic growth. In this case, bacterial growth is arithmetic rather than exponential. So, here the rate of increase of the microbial population is constant, like the equation: $\frac{dN}{dt} = c$, the rate of growth. It was earlier μ, growing exponentially, but here it becomes constant. Upon integration, you can get $N = C\ t$. So, the number of cells N, rather than the log number—not the log number, but rather a simple number of the cells—is a linear function of time t. And this happens particularly when the bacteria are highly dependent on some chemicals, like, for example, they require certain nutrients that are essential for their growth.

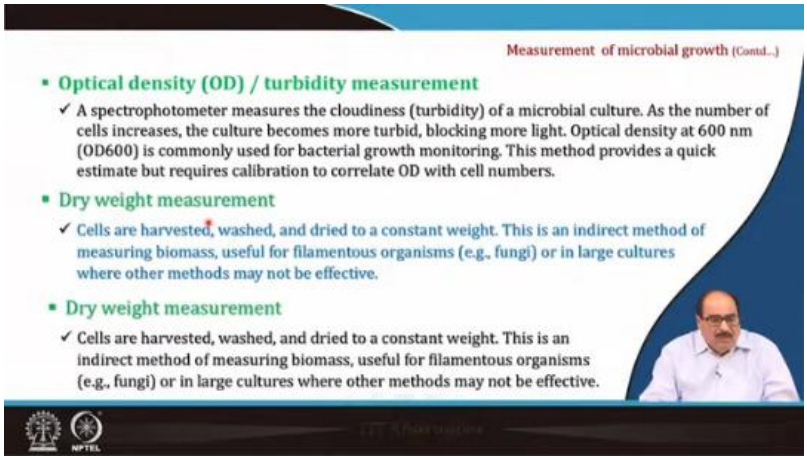


So, the growth of that bacteria will depend upon the amount of that nutrient present in the culture. That is, suppose after a certain time, the growth will depend upon the rate of the amount of addition of that essential growth factor or essential chemicals, etcetera. That is the arithmetic growth. So, to measure microbial growth—measuring microbial growth is essential to understand cell proliferation and population dynamics. There are several

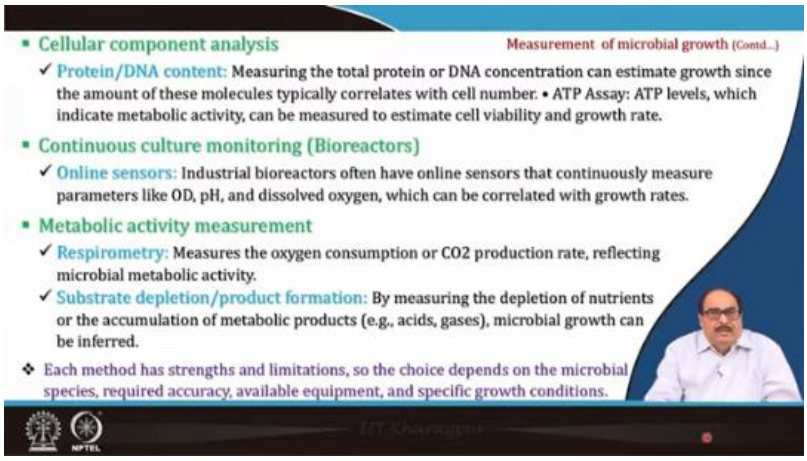
methods of measuring microbial growth, depending on the microorganism type, growth conditions, and required accuracy. Like, one can measure microbial growth in terms of direct cell count, viable cell count, like colony-forming units, one can also find out optical density or measure the turbidity. Even dry weight measurement, cellular component analysis. Continuous culture monitoring or metabolic activity measurements, etcetera. These are the various methods by which one can measure microbial growth.



So, in the direct cell count, there are methods like microscopic count, where one can use a microscope to count cells directly on a slide with a grid, that is, a hemocytometer. This method requires staining to distinguish live cells from dead cells. Then, there is also flow cytometry, a laser-based technique that counts cells as they pass through a laser beam. Flow cytometry can differentiate live and dead cells if specific stains are used. Then, viable cell count: colony-forming units. Here, one can use the plate count method, where serial dilutions of samples, as shown in this figure, are plated on a growth medium, and colonies are counted after incubation under proper conditions for a certain period of time. So, the count reflects the number of viable cells capable of forming colonies. Then, there is also the most probable number. That is a statistical method used when plate counts are not feasible, particularly for low cell density samples, such as water testing, etc., most probable numbers are calculated.

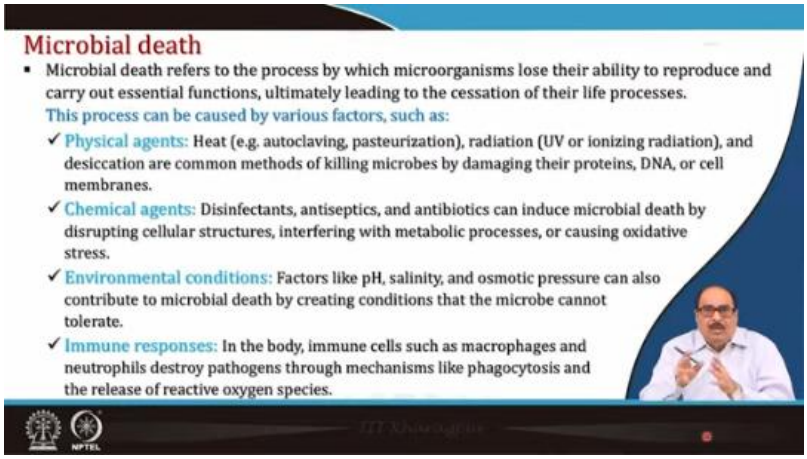


The optical density or turbidity density measurement, where a spectrophotometer is used to measure the cloudiness of a microbial culture. As the number of cells increases, the culture becomes more turbid, blocking more light and optical density at 600 nanometers is commonly used for bacterial growth monitoring. This method provides a quick estimate but requires calibration to correlate optical density with cell number. In the dry weight measurements, cells are harvested, washed and dried to a constant weight, and this is an indirect method of measuring biomass. It is useful for filamentous organisms like fungi, etc. or even in large cultures where other methods may not be effective. Then dry weight measurements in these cells are harvested, washed and dried to a constant weight. This is an indirect method again measuring the biomass, it is useful for filamentous organisms like filamentous fungi or in large cultures where other methods may not be effective.

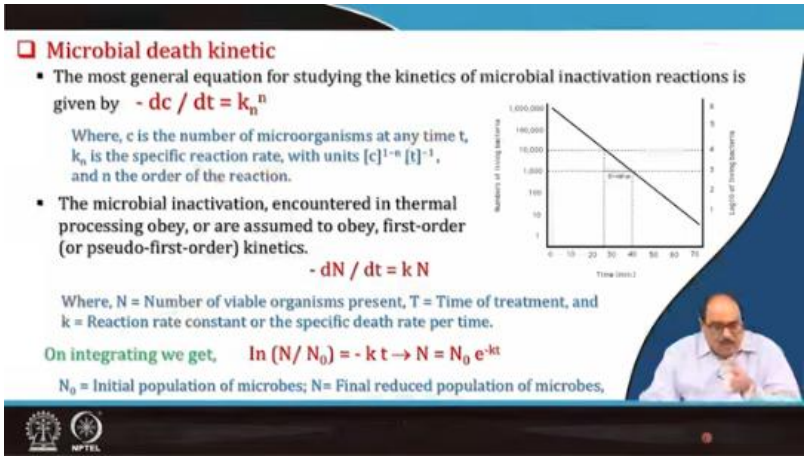


Then, cellular component analysis—the proteins or DNA content— that measures the total protein or DNA concentration—can estimate growth rate. And the amount of these molecules typically correlates with cell number, like ATP assays. ATP levels, which indicate metabolic activity, can be measured to estimate cell viability and growth rate.

Then, continuous culture monitoring—bioreactor, etc., where online sensors can be used. Industrial biosensors often have online sensors that continuously measure parameters like optical density, pH, and dissolved oxygen. These can be correlated with growth rates. Even metabolic activity measurement, where respiratory measures the oxygen consumption or carbon dioxide production rate, reflects microbial metabolic activity. Substrate depletion, like product formation, by the depletion of nutrients or accumulation of metabolic products, microbial growth can be inferred. So, here I have briefly told you, but let us understand that each method has strengths and limitations. So, the choice depends on the microbial species, required accuracy, available equipment, and specific growth conditions. Accordingly, one should use the method.



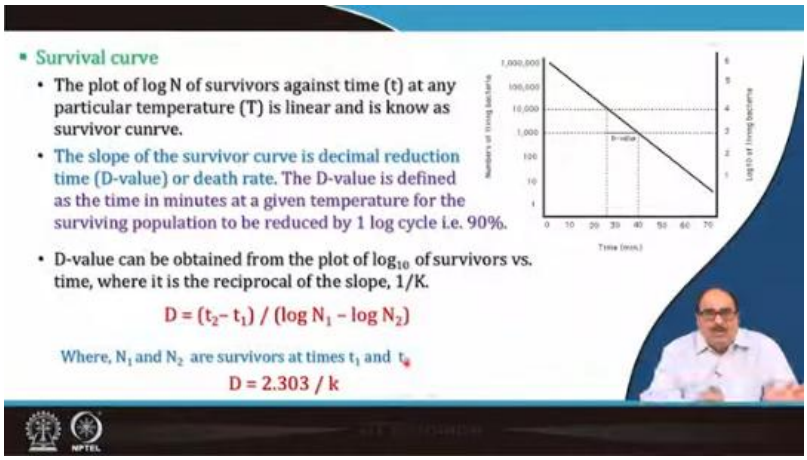
Now, let us briefly talk about microbial death. Microbial death, as you have seen in the growth curve, occurs after the stationary phase in the death phase curve. So, death refers to the process by which microorganisms lose their ability to reproduce and carry out essential functions, ultimately leading to the cessation of their life processes. This process can be caused by various factors, such as physical agents: heat, radiation, desiccation, which are common methods of killing microbes by damaging their proteins, DNA, cells, etcetera. Even chemical agents like disinfectants, antiseptics, antibiotics, etc., can induce microbial death by disrupting cellular structures, interfering with metabolic processes, or causing oxidative stress. Even environmental conditions like pH, salinity, and osmotic pressure can also contribute to microbial death by creating conditions that microorganisms cannot tolerate, creating more stressful conditions for the microbes. Then, the immune responses in the body, such as Macrophages and neutrophils, destroy pathogens. through mechanisms like phagocytes and the release of reactive oxygen species, etc.



So, the microbial death kinetics if you see like the growth kinetics here also the most general equation for studying the kinetics of microbial inactivation is given by $-\frac{dc}{dt} = k_n^n$

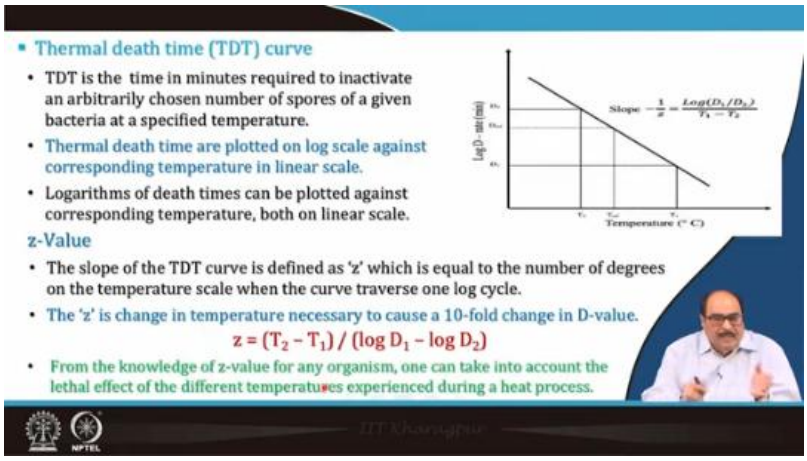
where c is a constant number c is the number of microorganisms, K_n is the specific reaction rate of the units becomes $[c]^{1-n}[t]^{-1}$, n is the order of reaction. So, the microbial inactivation encountered in thermal processing obeys or is assumed to obey first-order or pseudo-first-order kinetics, as expressed by the equation $-\frac{dN}{dt} = kN$.

Here again, n is the number of organisms present, t is the time of treatment, and k is the reaction rate constant or specific death rate per time. So, on integrating this equation again you can get $\ln(N/N_0) = -kt \rightarrow N = N_0 e^{-kt}$ this equation, where n_0 is the initial population of the microbes and n is the final population of the microbes. So, from this data, one can plot a survivor curve.

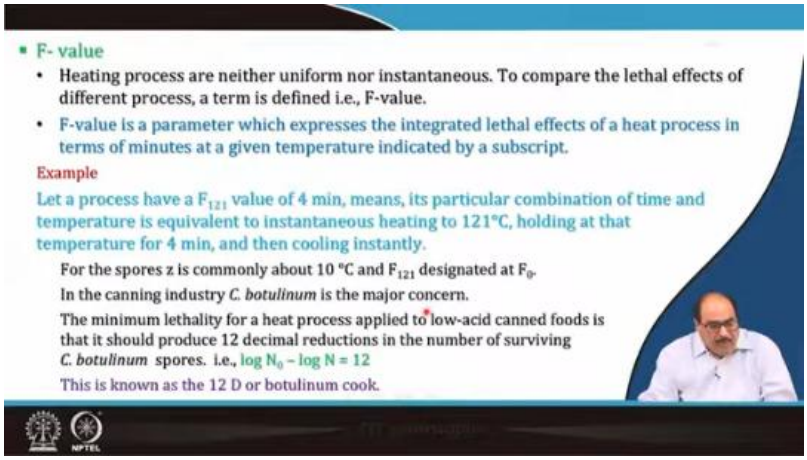


The survivor curve is the plot of the log number of surviving organisms against time t at any particular temperature, and this is linear, and it is known as the survivor curve. So, the slope of the survivor curve is known as the D value. You can see here that this is the

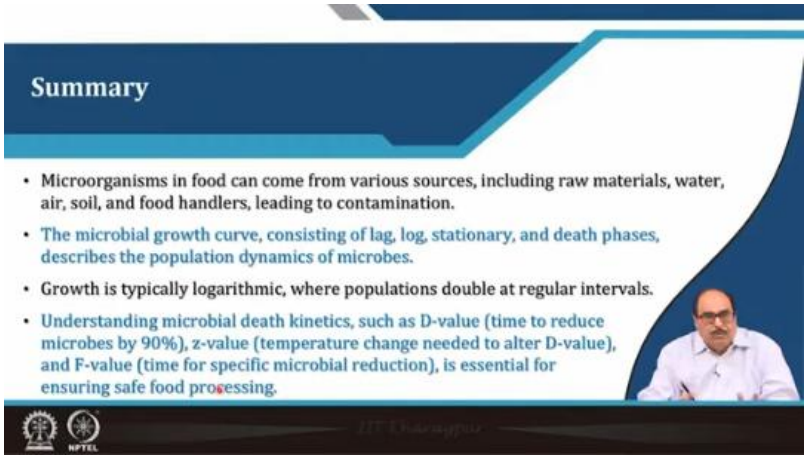
survivor curve. If the slope is the D value, it means that they are also the death rate. So, the D value from this curve can be defined as the time in minutes at a given temperature for the surviving population to be reduced by 1 log cycle or the time taken to reduce the bacterial population by 90 percent is the D value. The D value can be obtained from the plot of log10 of survivors versus time, where D is the reciprocal of the slope $1/K$, like $D = (t_2 - t_1)/(\log N_1 - \log N_2)$, where N_1 and N_2 are the survivors at time T_1 and T_2. And therefore, from this equation, you can find out that $D = \frac{2.303}{k}$.



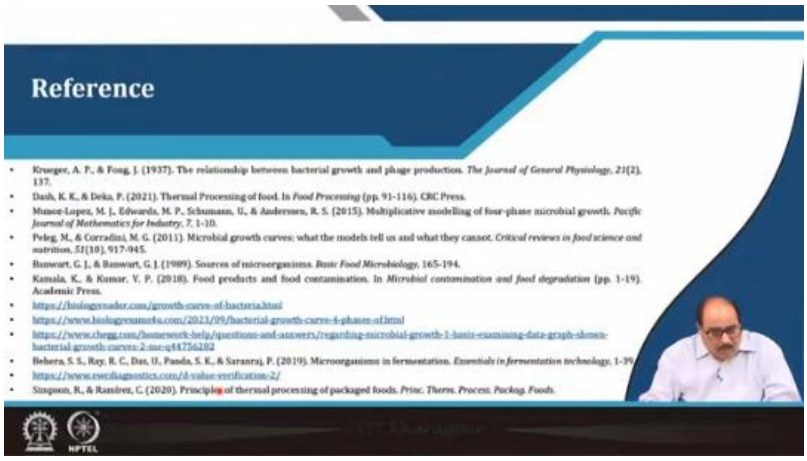
Then comes another thermal death time curve, where TDT is the time in minutes required to inactivate an arbitrarily chosen number of spores of a given bacterium at a specified temperature. So, thermal death times are plotted on a log scale against the corresponding temperature on a linear scale, either the death time or the log D. It is plotted against the temperature, and so the logarithm of the death time can be plotted and from here, the slope, like in the survivor curve, the slope was the D value and here the $slope\ of\ the\ TDT\ curve = \frac{1}{z} = \frac{\log D_1 - \log D_2}{T_2 - T_1}$ or the Z from this, you can say that it is the change in temperature necessary to cause a 10-fold change in the D value. That is the D value or the time in a way, whereas the Z value is the temperature by which the D value of the microorganism is reduced by a factor of 10.



It is also the time F value that is reduced by a factor of 10, or by 90 per cent. It is also the time F value that is reduced by a factor of 10, by 90 per cent. So, from the knowledge of the Z value of any organism, one can take into account the little effects of the different temperatures experienced during a heat process. F value is the parameter which expresses the integrated lethal effects of a heat process in terms of minutes at a given temperature, indicated by a subscript, for example. F value is the parameter which expresses the integrated lethal effects of a heat process in terms of minutes at a given temperature, indicated by a subscript, that is, for example, that particular combination of time and temperature is equivalent to instantaneous heating at 121 degrees Celsius, holding at that temperature for 4 minutes, and then cooling instantly. So, for the spores, Z is commonly about 10 degrees Celsius, and F 121 is designated as F0 in the canning industry. C for the Clostridium botulinum is the major concern. So, the minimum lethality of a heat process applied to a low-acid kind of food is that it should produce a 12-decimal reduction of the surviving Clostridium botulinum spores, that is, $\log N_0 - \log N = 12$. N is equal to 12, and this is known as the Bartoloneum cook or 12-day process.



So, finally, we summarise this lecture as the microorganisms in the food can come from various sources, the microbial death curve consisting of lag, log, stationary, and death phases, and it describes the population dynamics of microorganisms. And, growth is typically logarithmic when populations double at regular intervals. So, understanding microbial growth and death kinetics is essential for ensuring safe food processing. Where is the other? If it is a beneficial microorganism, you have to understand its growth. If the undesirable microorganism, we must consider the death kinetics, etc., so as to control this as the case may be.



These are the references for this.

Thank you very much for your patience. Thank you.