Environmental Chemistry and Microbiology

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Module - 10
Lecture - 51
Microbial Growth - III

Welcome again, everyone. This is the third and final part of Microbial Growth. This is lecture number 51 of module 10.

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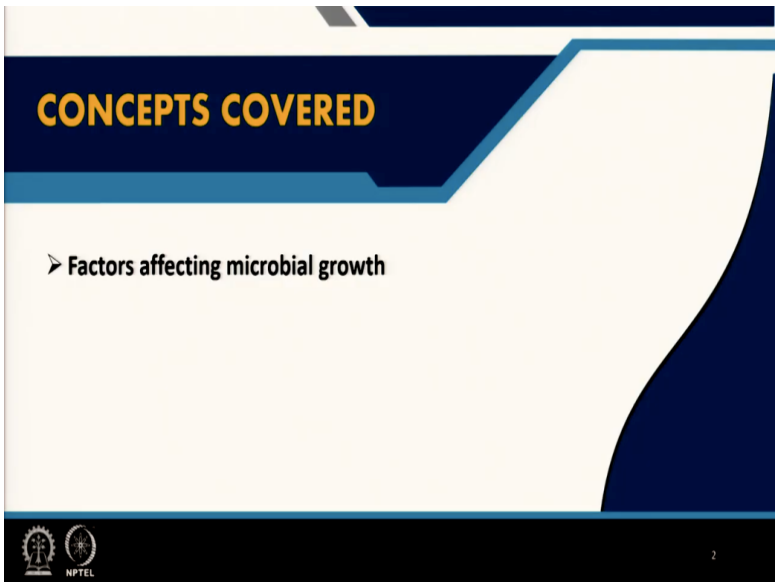

Here we are going to look at the factors that affect microbial growth.

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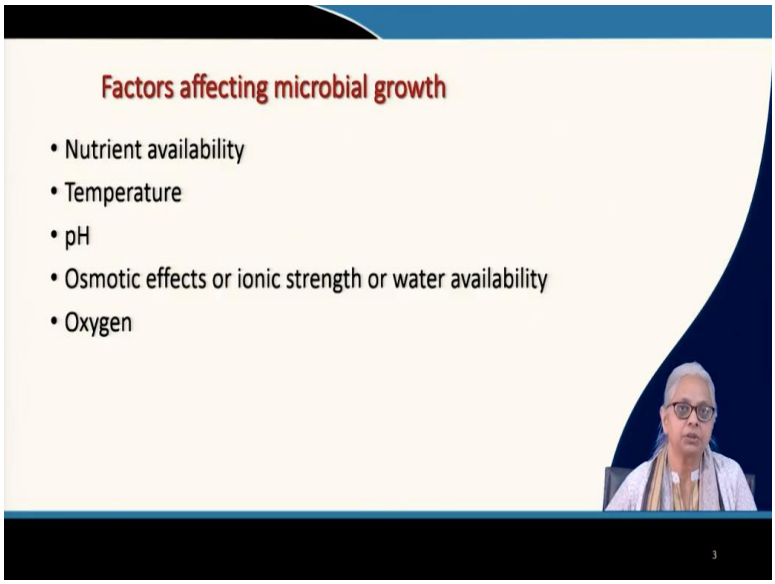

So, we are going to look at the factors that affect microbial growth. We have already seen the factor of nutrient availability. We have gone through the law of the minimum. So, we will now move to temperature, pH, osmotic effects, which can also be considered ionic strength or water availability or water activity, and finally, oxygen.

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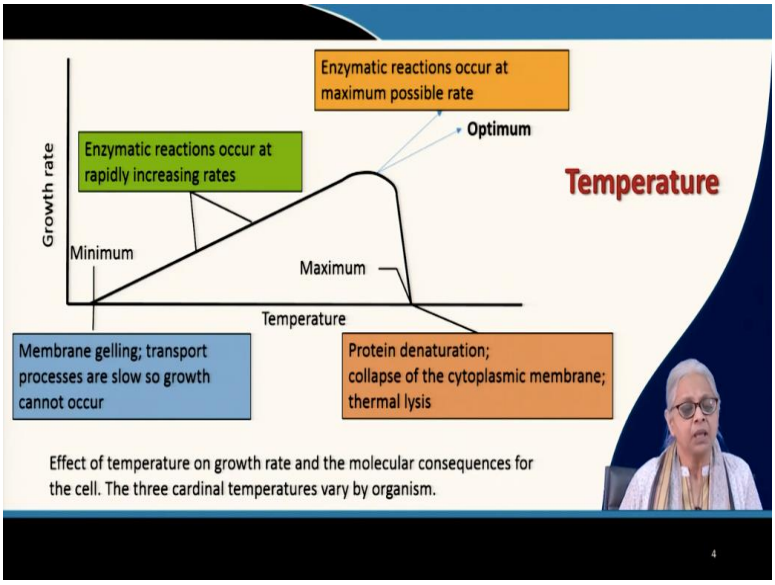

Let us take a look at temperature. How does it make a difference to the growth of bacteria or any other organism? Now, what you see over here is a schematic that shows you the relationship between temperature and growth rate. We know from experimental proof that when you increase the temperature, the rate of growth will increase up to a particular point. Now, you know from chemical reactions, chemical kinetics; you know from Arrhenius's law that when you increase the temperature, the rate of the chemical reaction increases.

So, what is biological reaction? Biological reactions are nothing but the sum total of various biochemical reactions. They are subject to the same principles or the same fundamental law that as the temperature increases, the kinetics of the biological reactions will increase. So, they will increase up to a certain point. If you go beyond that point, then there is damage to the cells. So, let us take a look at how it works for biological systems.

So, here we have a minimum. Now, we know from refrigerating food, we know that we do not get too much bacterial growth in the freezer. So, if you put some food in the freezer, it will stay there for weeks or maybe even months without getting spoilt. So, why is bacterial growth not happening at those temperatures? For the simple reason that the membranes have gelled. The transport processes that are essential for bacterial growth and reproduction are slow. And they

are so slow that they are practically negligible and you get no spoilage of food at that point. As you increase the temperature; if you leave something out at room temperature, what will happen? Room temperature is where these bacteria which are present in your environment are going to enter the food and they will reproduce rapidly, because the nutrients are high in your food. And that is where the enzymatic reactions that are controlling these biochemical reactions will occur at very high rates. And that is what you see in this part of the curve. So, these are enzyme reactions. They are all; remember, all biochemical reactions are enzyme mediated reactions. They are all occurring at faster rates as the temperature increases.

We have also seen that one of the major factors that causes denaturation of proteins is high temperature. There comes a point at which the proteins that are part of the cell are going to be denatured. At this point, you will get protein denaturation, you will get collapse of the plasma membranes and you may get thermal lysis. So, when this optimum point has been crossed, optimum is defined as the point at which the growth rate is maximum. So, beyond that point the growth rate starts coming down, and that is because damage starts becoming apparent.

So, the cells are being damaged, the enzymes are being damaged, the cell wall may be damaged and so on. So, at that point, there will be a maximum. If you keep increasing the temperature, there will be a maximum at which the cells will be destroyed and that is thermal lysis. So, these are the 3 cardinal temperatures. So, you have minimum, optimum and maximum. You will find that these 3 cardinal points are there for practically every environmental factor that determines the growth conditions for any given organism.

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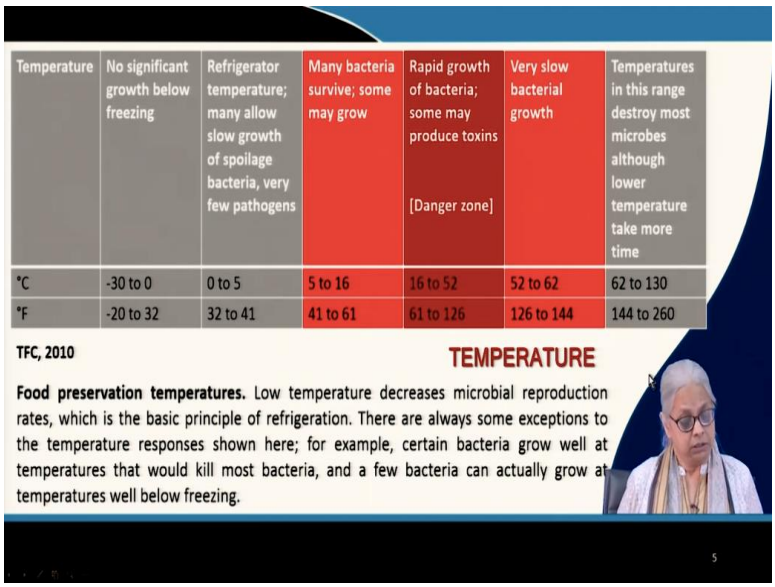

So, when we think about food preservation, the best example of, or rather I should say the most relevant application of microbiology is temperature. So, why do we use refrigerators? Why do we keep food at either refrigerated conditions or freezing or frozen conditions? So, when you have sub-zero conditions for the bacteria that are in our environment, in our normal environment, under room conditions, those bacteria are not capable of growing under sub-zero conditions. So, -30 to 0^{o}C, the food is safe. These bacteria cannot survive and the food will not spoil. 0 to 5 ^{o}C, this is perhaps close to your freezer temperature. Some bacteria may be present within the food, because not all food is sterilized. If they are present, they may grow and therefore they will spoil, but they will cause spoilage of food over a very long period of time. Now, the lower part of the fridge, which is not the freezer, which is not frozen, where you do not get ice; in that part, the temperature may be anywhere from 5 to 16 to 20 ^{o}C.

At this point, the bacteria are there in the food unless it has been sterilised. And if they are there, they will cause spoilage of food, but over a period of maybe a week or so, or even shorter; depends on the nature of the food; depends on the temperature in the refrigerated portion of your fridge; all that will determine what happens. If you leave it at room temperature, assuming your room temperature is anywhere from 25 to 35^{o}C, depending on where you are, you will get rapid growth of bacteria. In conditions in our country, we have room temperatures that vary anywhere from let us say 15^{o}C to 40^{o}C. This is dangerous in terms of food. So, food spoils very easily if it is not refrigerated. It will not even survive maybe 24 hours. I know that cooked food cannot survive even 24 hours under room temperature conditions. And the bacteria or the fungi and so many other organisms that can grow on the food under these conditions, they can be pathogenic.

In fact, pathogenic bacteria tend to grow most at under these conditions. Some of them may produce toxins. These can be endotoxins or exotoxins. They can cause food poisoning. So, this is the danger zone. So, leaving food out and then consuming it later is extremely dangerous from a health perspective. If you increase the temperature further, from 52 to 62^{o}C, you will find that the number of bacteria that are continuing to live in those samples; you can try it with milk, you can try it with any other liquid material that are food materials; you will find that the concentration of bacteria and other organisms will go down. And finally, if you take it past boiling point, you can be pretty certain that the pathogenic bacteria in your environment will not survive these conditions. So, you can boil milk, you can boil water and feel safe that pathogenic bacteria are not going to be able to survive these high temperatures, and therefore

consuming that material is safe. And that is what pasteurization is all about. We will come to that again.

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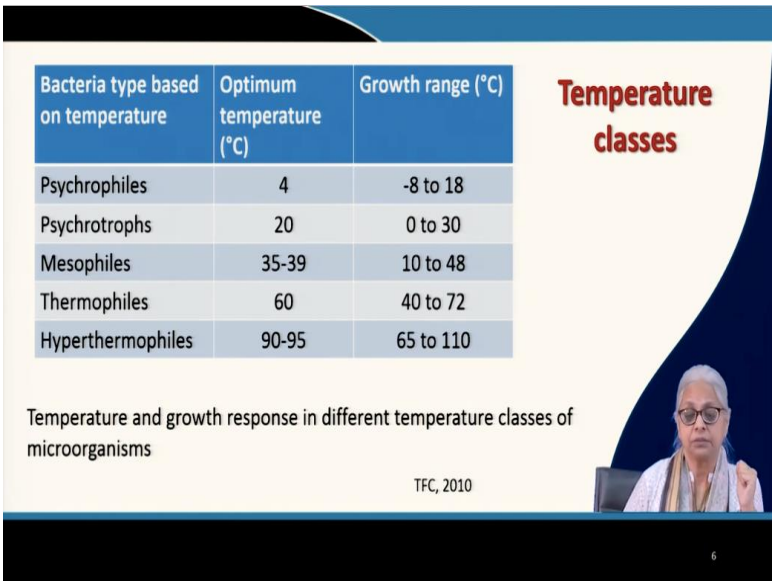

Now, we come to temperature classes. What we see in this table is that bacteria have been classified according to their optimum temperature. In the previous slide, I mentioned that bacteria that are in our environment, they are used to living in perhaps an optimum temperature of about 35 to 39^{o}C and a range of 10 to 48 ^{o}C. These are mesophilic bacteria. So, when your food is exposed to these mesophilic bacteria, it will spoil under normal conditions. Within these conditions; if these are the temperature conditions, your food will spoil. When you put it in the fridge, even if these bacteria are present in the food, they will not be able to multiply. That does not mean that bacteria do not exist in Arctic or Antarctic regions. Those are psychrophilic or psychrotrophic bacteria.

Psychrophilic bacteria have an optimum temperature of 4 ^{o}C and a growth range of -8 to 18 ^{o}C. Psychrotrophic bacteria have 20 ^{o}C optimum temperature and 0 to 30 ^{o}C range of growth temperatures. These are bacteria that can and do exist in Arctic as well as Antarctic regions or even high-altitude regions where there is very low temperature, like the Himalayas and so on. So, these are where you can find these bacteria. Now, these bacteria do not exist in your normal environment.

Then we come to thermophilic bacteria. Thermophilic bacteria have an optimum of 60 ^{o}C and a range of 40 to 72 ^{o}C. There is another group of bacteria, hyperthermophilic bacteria, which have an optimum of 90 to 95 ^{o}C and a growth range of 65 to 110 ^{o}C temperature.

So, if I were to ask you, can bacteria survive under boiling water conditions? The answer is, the mesophilic bacteria that live in our environment, cannot survive under those boiling water conditions. However, if you find bacteria in boiling water springs or in hot water springs, they may be thermophilic bacteria, and they are definitely capable of surviving under high temperature conditions. These are all archaebacteria. We have seen examples of that and they are not likely to be pathogenic in any conditions. Because their optimum growth conditions are very different from our body temperature condition, so they are unlikely to use us as hosts.

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Here we see lichen. You know that lichen is a mutualistic relationship between algae and fungi. And this is a photosynthetic lichen, which is growing at -24^{o}C. This is thick snow which is covered with algae. So, it is not just bacteria that can be psychrophilic. You can have algae growing in Arctic as well as Antarctic regions. And this is another graphic that shows you algae in Antarctic regions.

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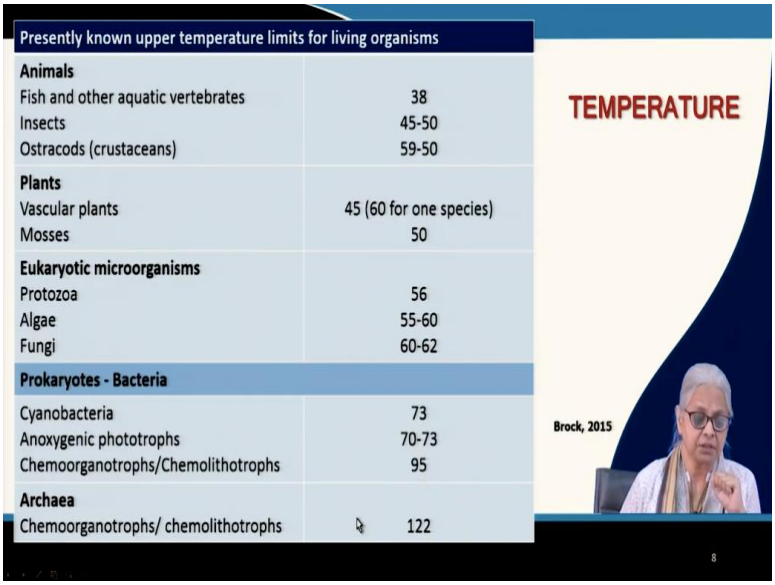

What is the upper limit for different living organisms? Now, let us take a look at that. Fish and aquatic vertebrates have an upper limit of 38°C. Insects can survive higher temperatures, 45 to 50°C. Crustaceans, the ones with shells can survive even higher temperatures, 50 to 59°C. Plants are generally more sensitive. Vascular plants need; they cannot survive beyond 45°C. For one species, it was 60°C. Moss, which is a type of plant, has an upper limit of 50 °C. Eukaryotic microorganisms: Protozoa, 56 °C; algae, 55 to 60°C; fungi, 60 to 62°C. Bacteria, as you can see, have much higher upper limits, upper temperature limits compared to all the other organisms. Cyanobacteria can survive 73 °C. Anoxygenic phototrophs can survive to the same extent. Chemoorganotrophs and chemolithotrophs are, they can survive up to 95 °C. Archaebacteria can survive far beyond that, they can tolerate even 122 °C.

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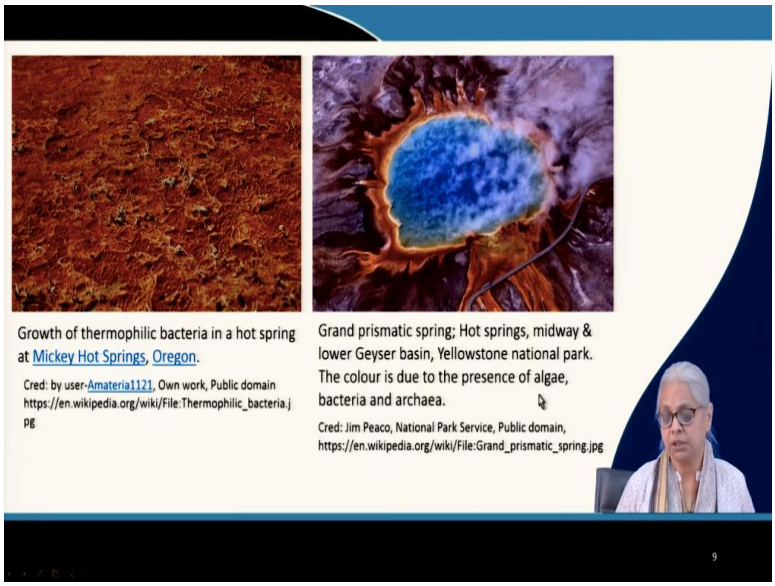

So, here are some examples of thermophilic bacteria in a hot water spring. This is a hot water spring from the Yellowstone National Park. The colours are due to the combination of algae, bacteria and archaea.

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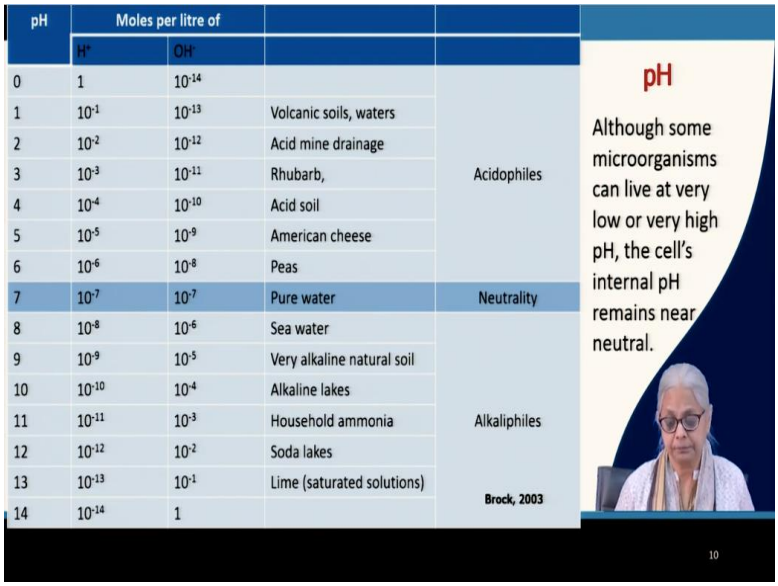

Let us now come to the next environmental factor and that is pH. So, in the table, you see the pH scale all the way from 0 to 14. You know what that means in terms of the concentration of protons and hydroxide ions, and in terms of the classes of bacteria or other microorganisms that can survive. So, if the organism can tolerate acidic conditions, it is called an acidophilic organism or a bacteria.

If it can survive basic conditions or high pH conditions, it is called an alkaliphile. So, from the environmental perspective, some of the things that are very important for us are the acidophilic bacteria. These are found in volcanic soils, in acid mine drainage, in certain types of food, in certain types of soil, in cheese, in peas. These are some of the foods as well as other types of environments where you are likely to find acidophilic bacteria. Also keep in mind that generally we pickle food. And the reason for pickling food is that again the normal species that we see around us are not going to withstand the low pH of acidic, the acidic pH of pickles and so on. And that is why pickling food helps to preserve the food for a long period of time. So, we know neutral is 7, and that is what we try to do most of our work at.

And very often we see deviations in this pH in the environment, just like acid mine drainage or soda lakes and alkaline lakes and so on; seawater, which is on the basic, the alkaline side of the scale. So, these are areas where you are likely to find deviations from neutral conditions.

Under those conditions, it does not mean that bacteria and other organisms cannot survive. You will find certain species that are capable of surviving under those conditions.

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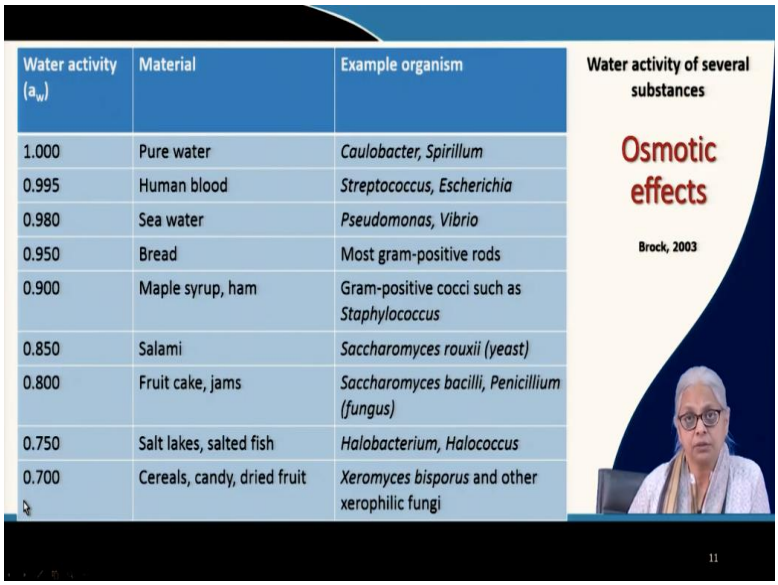

Then we come to the next one, and that is osmotic effects. These osmotic effects, like I said to you in a previous lecture that they are related to the ionic strength in the solution versus the ionic strength within the cell. So, let us take a look at another parameter that is used to define the activity or ionic strength. I have used the word ionic strength in a previous lecture. Another parameter that is used in the textbook is water activity.

This water activity can be compared relative to pure water. So, there are certain organisms which can survive in pure water. In general, most organisms do not want to survive in pure water, because they need the salts and minerals that are present in water. So, the ionic strength generally is higher, and therefore the water activity is lower. So, pure water which has an activity of 1, does allow certain bacteria to survive, Caulobacter and Spirillum.

Human blood does not have very low water activity compared to pure water, it is 0.995; and *Streptococcus*, *E. coli* and several pathogenic organisms are capable of surviving under those conditions. Seawater is much higher in ionic strength and much lower in water activity; it is 0.98. *Pseudomonas, Vibrio, Vibrio cholerae,* famous for causing cholera can survive under high ionic strength or low water activity.

Our foods, breads, maple syrup, fruit cakes and so on; all these are very low water activity and high ionic strength, ranging from 0.95 all the way to 0.8; and several different types of pathogenic as well as other bacteria can be found.

Salt pans; we have seen that you can have bacterial growth under salt pan like conditions. So, the water activity in salt lakes, in salted fish, in salt pans can be very low, which means the ionic strength or the salt concentration is very high.

So, that is 0.75. You get *Halobacterium*, *Halococcus.* I will come back to this point again. And you can see dry fruits. Dry fruits, cereals, candies, they have very low moisture content. The water activity is as low as 0.7. And there are fungi and bacteria that can survive under these conditions as well.

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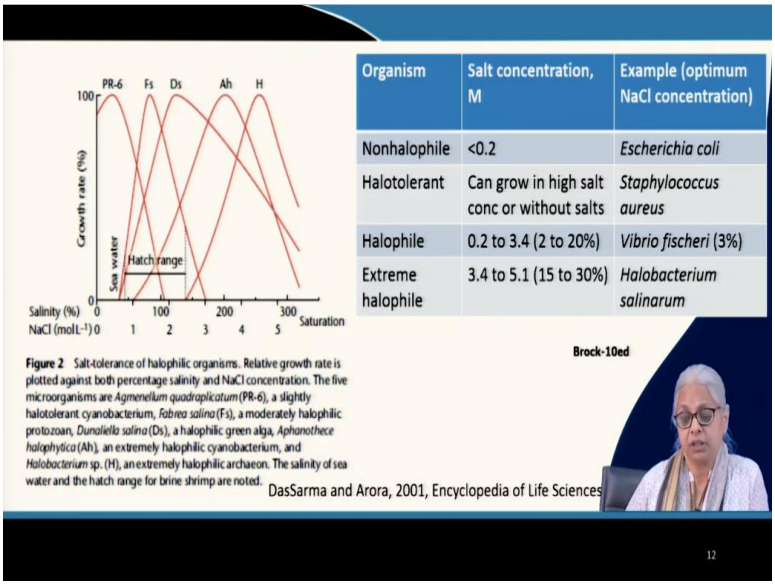

In terms of Halophilic organisms, salt-tolerance of certain organisms is very high. So, when we think about saltwater, we generally think of seawater. Seawater has a salt concentration of 3.5 percent NaCl. So, when we think about *E. coli* and *Pseudomonas*, which are common bacteria found in our environment, these are non-halophilic bacteria. In terms of salt concentration, they want a salt concentration less than 0.2 molar salt concentration.

Halotolerant bacteria are the ones that can grow in high salt concentration or without salts. So, that is *Staphylococcus aureus*. Halophilic bacteria are the ones that can withstand seawater concentrations, which is 2 to 20%. Seawater is 3.5%. So, these halophilic bacteria are likely to

be found in marine environment. So, you have *Vibrio fischeri* and so many other types of *Vibrio sp*.

Then we have extreme halophilic bacteria. They can withstand 15 to 30% salt concentration. Now, this kind of salt concentration is found only in salt pans. Several textbooks have photos of salt pans which are coloured, and that shows the presence of certain types of bacteria and algae that are salt-tolerant. They are extremely halophilic organisms. This is an example of *Halobacterium salinarum*. So, there are organisms on the planet that can withstand extremely high salt concentrations. And you can see another graph from a paper. And you can see the salinity levels as well as the examples of halotolerant as well as halophilic bacteria, algae and protozoa.

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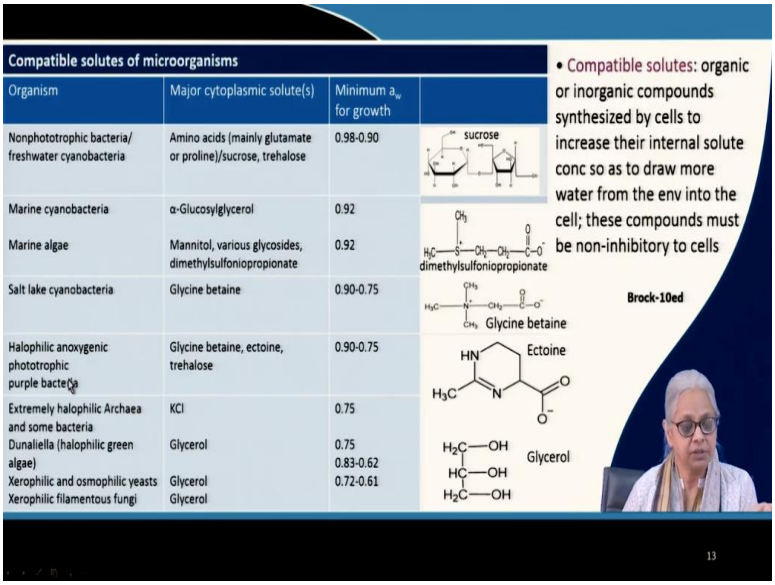

So, this is a slide that shows you compatible solutes. Compatible solutes are organic or inorganic compounds that are synthesized by cells to increase the amount of water that they can withdraw from the environment. Now, by increasing the salt concentration within the cell, they can cause the concentration gradient of water from the environment to be higher, and that will allow them to bring in water from the environment.

So, these compounds have to be non-inhibitory to the cells; obviously, otherwise they would not be able to survive. So, we have cyanobacteria, we have algae, we have bacteria, archaebacteria, yeast, fungi. All of them are capable of doing, of creating these types of solutes.

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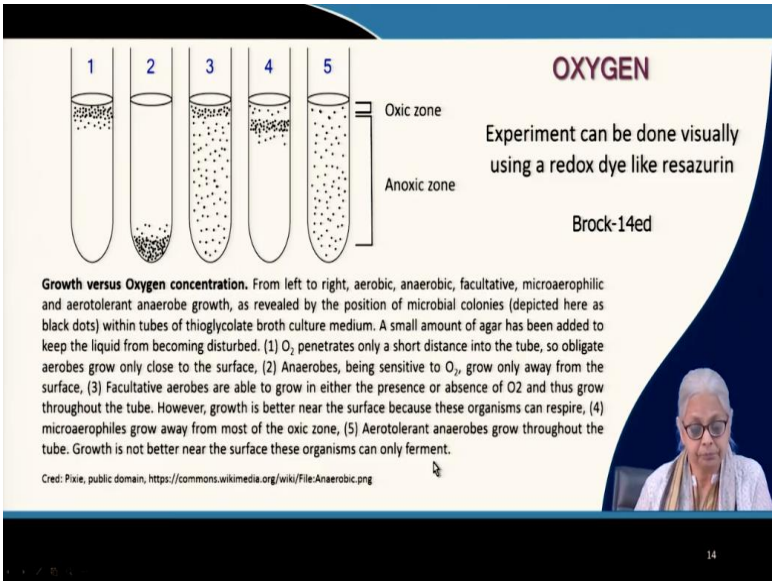

We then come to the last part, and that is oxygen. Here we see the clustering of different types of bacteria in response to an oxygen gradient, oxygen concentration gradient. So, you have 5 different test tubes. The oxygen concentration is going to be highest at the surface. So, aerobic bacteria are likely to be clustering at the surface. So, whatever you see at the surface or closest to the surface are aerobic bacteria.

Then in the second one, you have bacteria that are as far away from oxygen as possible. Oxygen being highest at the surface; and as you go further down in the test tube, you are likely to have lower and lower oxygen concentrations; as long as it is not mixed. If it is mixed, then obviously it is uniformly distributed. So, if it is a not-mixed test tube, then anaerobic bacteria are likely to cluster at the bottom.

Then we come to a uniform distribution of bacteria. If they are uniformly distributed throughout the test tube, they are likely to be facultative; which means, it does not matter what the oxygen concentration is, they are capable of surviving under both aerobic as well as anaerobic conditions.

We then come to a very interesting phenomenon, shown in tube number 4. Here, the bacteria have clustered a few layers, let us say below the surface; they are not at the surface; it is not like tube 1. Here, they are clustering not at the bottom, but somewhere in between, just below the surface. These are microaerophilic bacteria, which means they want extremely low levels of oxygen. They do want oxygen, but very low levels.

And then you have aerotolerant anaerobic bacteria. Now, in the second case, these are anaerobic bacteria because they are going far away from oxygen. They are strictly anaerobic or what we call obligate anaerobes. In the last case, in number 5, these are aerotolerant anaerobes which are capable of growing regardless of whether there is oxygen or not. They do not utilize the oxygen. They will not utilize the oxygen, but they can tolerate the presence of oxygen in their environment.

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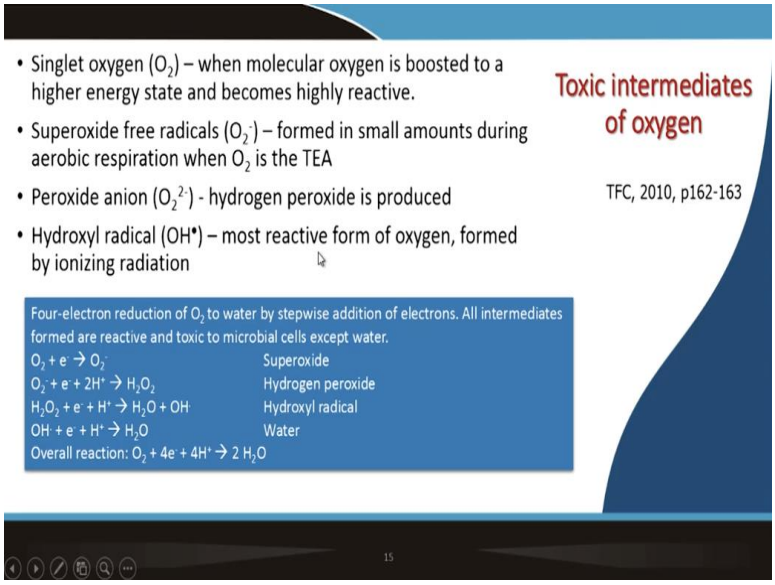

Now, having seen that there are several different groups of bacteria and they all respond to the presence of oxygen in very different ways, it is also important to realize that in aerobic; so, there are many different issues over here. One is that in aerobic respiration, there is a four-electron reduction of oxygen to water in a stepwise fashion. So, there are several intermediates that are formed. They are all highly reactive and toxic to microbial cells.

It is only in the last reaction that you have an end product like water, which is non-toxic to the microbes. So, if we look at the first reaction, $[\boldsymbol{O_2 + e^- \rightarrow O_2^-}]$ molecular oxygen plus an electron will result in O_2^-. This is called superoxide (O_2^-). This superoxide plus 1 electron and 2 protons will result in H_2O_2 (hydrogen peroxide) $[\boldsymbol{O_2^- + e^- + 2H^+ \rightarrow H_2O_2}]$. Hydrogen peroxide plus an electron and a proton will result in hydroxyl radical and H_2O $[\boldsymbol{H_2O_2 + e^- + H^+ \rightarrow H_2O + OH^-}]$. This hydroxyl radical plus an electron and a proton will result in water $[\boldsymbol{OH^- + e^- + H^+ \rightarrow H_2O}]$.

So, overall, when you have oxygen as your terminal electron acceptor, there are several steps in the process. All of the intermediate compounds that are produced are toxic to microbial cells.

Now, all the different aerobic species of bacteria, as well as some of the others have different ways of dealing with it. We will come back to that later.

So, these are the toxic intermediates of oxygen that the aerobic as well as facultative bacteria have to deal with. And we will come to the other groups of bacteria in a little bit. So, we have singlet oxygen, which I said is molecular oxygen. Basically, the molecule is boosted to a higher energy state; it becomes highly reactive; and that is one of the toxic intermediates. Then we have superoxide free radicals, O_2^- which is formed in very small amounts during aerobic respiration when molecular oxygen is the terminal electron acceptor.

Next one is peroxide anion. So, O_2^{2-} which is not shown in this stepwise reduction. That is where hydrogen peroxide (H_2O_2) is produced. And finally, we have hydroxyl radical (OH^-), which is the most reactive form of oxygen and that is formed by ionizing radiation.

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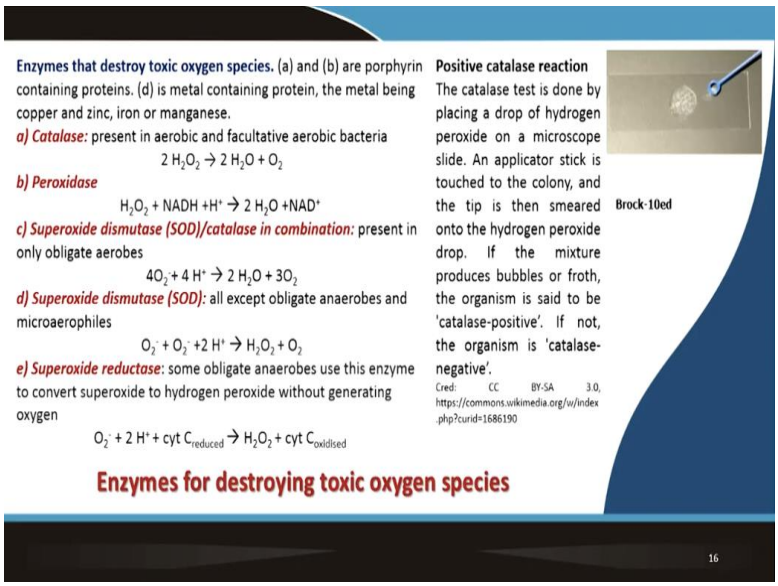

Now, the fact that these toxic intermediate species are formed whenever oxygen is present in the environment, those microorganisms including bacteria that are capable of utilizing oxygen have enzymes for destroying the toxic oxygen species. So, let us take a look at a few of them. So, we have catalase and peroxidase. These are porphyrin containing proteins. Catalase is present in aerobic as well as facultative bacteria; and it has the ability to convert hydrogen peroxide to oxygen and water.

Then we have peroxidase. Again, hydrogen peroxide along with NADH and a proton will get, will give you water and NAD^+.

The next one is superoxide dismutase. And this one, in combination with catalase is present only in obligately aerobic bacteria. So, it will convert superoxide; O_2^- plus protons will be converted to water and molecular oxygen. So, you can see that the end products have to be non-toxic species of either water and/or molecular oxygen.

The next one is superoxide dismutase. Superoxide dismutase is common to all bacteria except obligate anaerobes and microaerophilic bacteria. So, you have, 2 molecules of O_2^- plus 2 protons will result in hydrogen peroxide and molecular oxygen. And the last one is superoxide reductase. This is present in some obligate anaerobes.

They use this particular enzyme to convert superoxide (O_2^-) to hydrogen peroxide (H_2O_2). But since they do not tolerate oxygen, this superoxide reductase is able to convert (O_2^-) to hydrogen peroxide (H_2O_2) without generating oxygen. So, it gives you, cytochrome c which is in the reduced form is oxidized; and the end product is hydrogen peroxide, which can then be further detoxified by other enzymes.

So, here is an example of the positive catalase reaction. If you want to know whether a particular organism have catalase enzyme or not, there is a very simple test that is done for it. It is done by placing a drop of hydrogen peroxide on a microscopic slide. An applicator which has been touched to the colony of the microorganism that you want to test for; a little bit of that colony is picked up on the applicator stick. This is the applicator stick. And the tip is then smeared into the hydrogen peroxide drop. If there are bubbles or froth, then the organism is considered to be catalase-positive. If there are no bubbles or froth, the organism is considered to be catalase-negative.

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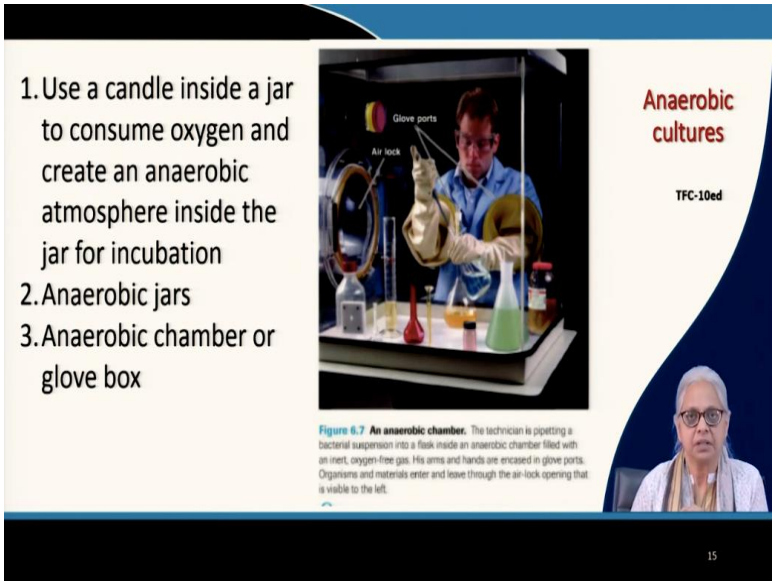

How do we culture anaerobic bacteria? Now, that becomes a major challenge because our conditions, our environmental conditions are fully aerobic. We have ample oxygen in our environment. And therefore, whatever we culture here is generally aerobic bacteria and other microorganisms. If I want to cultivate anaerobic microorganisms, I need a different kind of setup.

The simplest, crudest way of doing it is you take a glass jar; put a candle along with the media, whether it is solid media or liquid media; you put all of them inside a glass jar; put a candle along with it; burn the candle; seal the jar; and after some time, all the oxygen inside the jar will be consumed by the flame; and you will get anaerobic conditions. So, it is a basic, very crude way of doing it, but it is neat and perfectly clear. That is the first one.

The second one is an anaerobic jar. I will come to that later. What we see here is an anaerobic chamber or an anaerobic glove box. In the anaerobic glove; this is an inflatable device, so it is generally made out of transparent plastic. It can be much bigger than what is seen in the photo over here. Regardless, it is mounted on a table, on a very large table. It is connected to an airlock, which is connected to gas cylinders. Different gases are used; I will come to that, one point after another. So, the first thing is that you open the airlock and place whatever materials that you need for culturing and incubating in the glove box. So, this is the glove box or chamber in which all the materials have been placed. The airlock is; all the air from the glove box is then withdrawn through the airlock. As the air is withdrawn, nitrogen gas is added to the glove box.

So, it remains inflated, but now it is a nitrogen containing atmosphere. So, there is no oxygen, because anaerobic means no oxygen. And nitrogen gas is inert. So, this is our inert oxygen free atmosphere that has been created within the glove box. And these gloves; there are gloves on the outside, where the person who is going to work in the anaerobic glove box can put in his or her hands and manipulate all the objects that are inside the glove box. So, this is a very, it is kind of difficult and it can be done; and this is how you can do large-scale experiments with anaerobic conditions.

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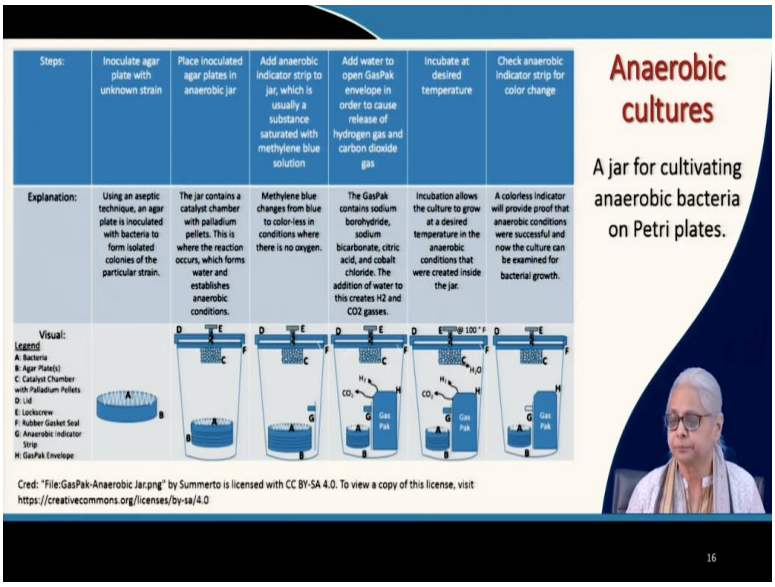

I then come to anaerobic jars, which is for small scale experiments using jars. So, in the first case, you inoculate the agar plate with whatever anaerobic bacteria you have; place them in an anaerobic jar and an indicator strip is part of the jar. So, these are commercially available setups and they are generally saturated with methylene blue. You add water to open the gas pack that is part of this package, the entire thing; and it will cause the release of hydrogen gas and carbon dioxide. And that has to replace the original air that may be inside the anaerobic jar. You can then incubate it at a particular temperature and check the indicator strip for any colour change.

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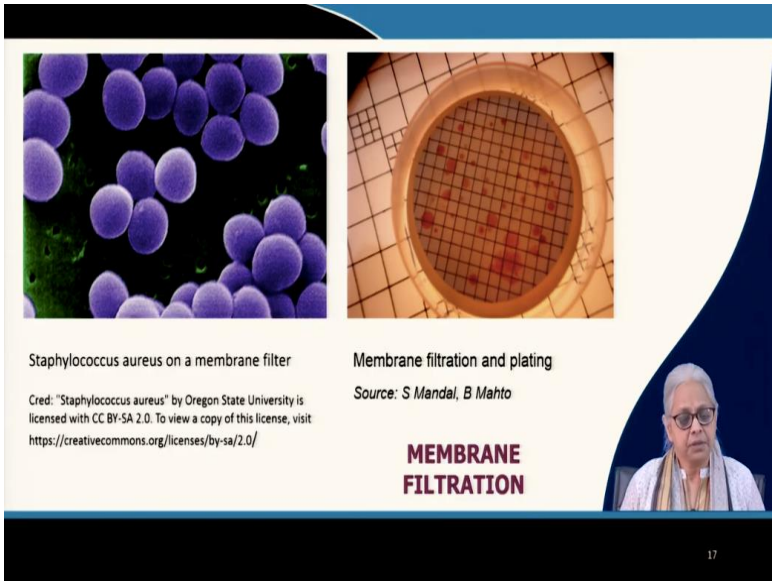

These are examples of membrane filtration. I think I have already shown these to you.

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And I will stop at this point. Thank you.