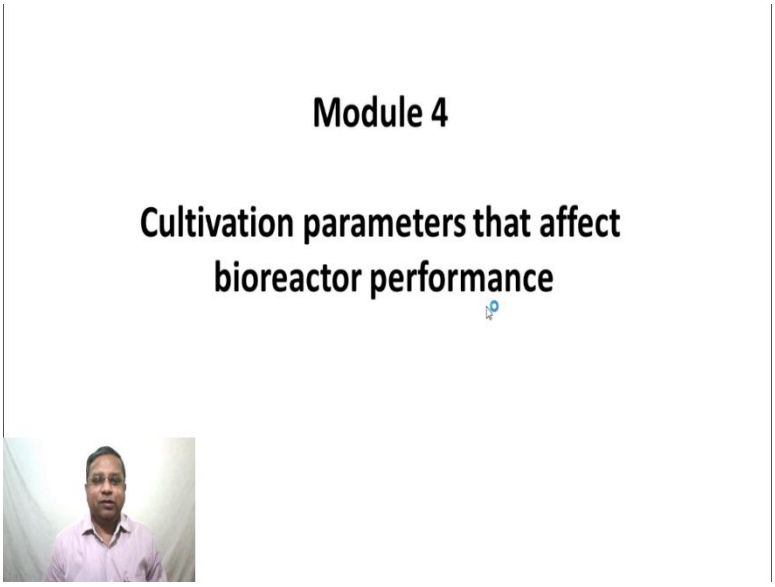
Bioreactors
Prof G. K. Suraishkumar
Department of Biotechnology
Indian Institute of Technology, Madras

Lecture – 14
Bioreactor environment parameters

Welcome to lecture 14, in the NPTEL online certification course on bioreactors. We will begin module 4 in this lecture.

(Refer Slide Time: 00:32)

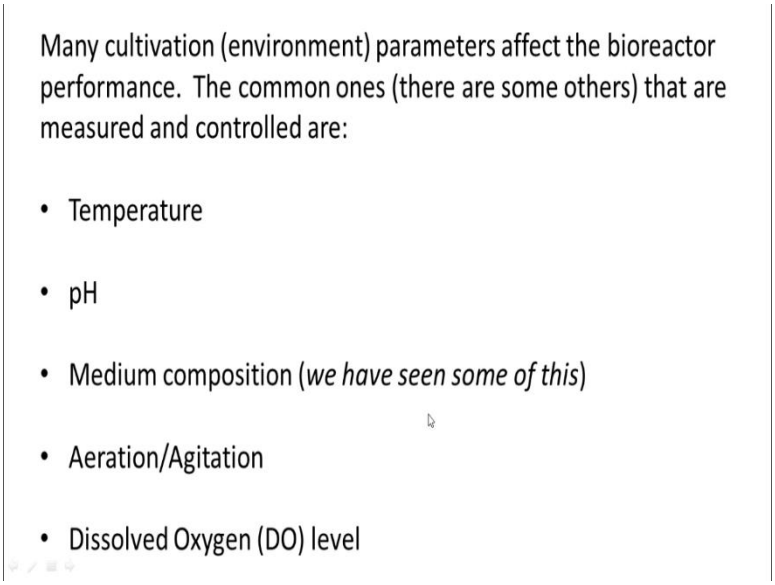


Title of Module 4 is Cultivation parameters that affect bioreactor performance. Now, we have a lot of cultivation parameters that we can measure and control. We had briefly seen some of these when we were introduced to bioreactors. In this module, we are going to see how they affect bioreactor performance and how we can optimize them. So, that we can get the best out of the bioreactor.

In the first module, we were introduced to bioreactors, we saw the common types of bioreactor and their common modes of operation and also some sterilization kinetics including the decimal reduction time. In the second module, we saw the outcomes of the bioreactor. The two major outcomes of the bioreactor, one is the biomass of cells and the product that is made by the cells or through an enzyme reaction. We saw a lot of details on the modeling of it, mathematical representation of them as well as enzyme kinetics,

enzyme inhibitions and measurement of important parameters such as cell concentration, substrate concentration, product concentration in bioreactors. In module 3, we looked at the analysis of the common modes of operation so that would be useful for some design decisions. In this module 4, we would look at the Cultivation parameters that affect bioreactor performance.

(Refer Slide Time: 02:17)



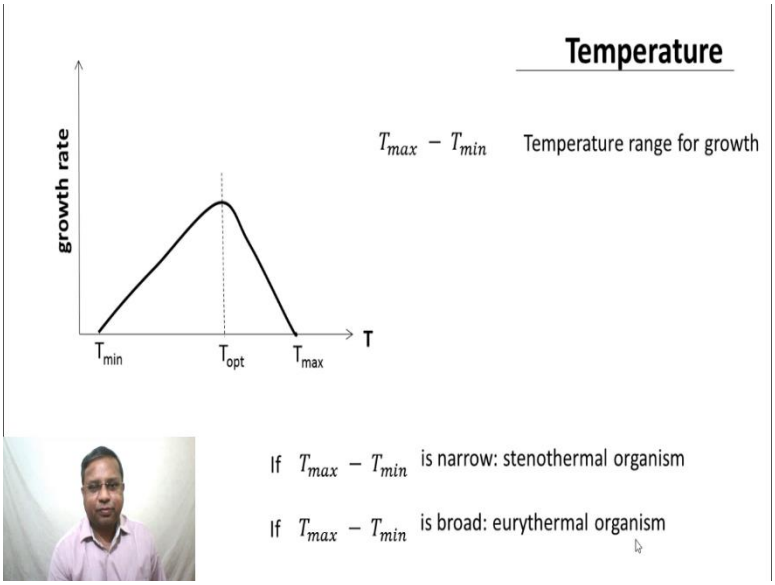
The Cultivation parameters are also called the Bioreactor Environment parameters. We said that the cell are the actual factories that produce the product in the bioreactor. Therefore, the parameters that affect the cellular environment or the environment in the bioreactor of the cells are called Environment parameters or Cultivation parameters. There are many cultivation parameters that one can think of. We will look at some common ones now. They are actually measured and controlled, as we mentioned this before.

The common ones, the first obvious one is the Temperature, pH of the medium, pH out side the cells - of the medium, the Medium composition - what the medium consists of. We have seen some aspects of this. Remember we looked at the effect of substrate concentration on growth rate, that kind of falls into this broad aspect. But the medium composition itself effects the product formation. There are many studies in the literature that look at media optimization - that is an entire field of study. There are various ways by which media are optimized, there are mathematical techniques that one can use. To

minimize the number of experiments that one does, to get at the optimum set of media parameters or medium composition, the Plackett-Burman technique and so on so forth. We will not look at those in this introductory lecture, it suffices to say that medium composition does effect, it is one of the environment parameters that effects the productivity of the bioreactor and a lot of work has been done on that.

Aeration and Agitation, we would kind of look at them together - they are 2 distinct aspects. Aeration is the rate at which air is provided into the bioreactor for oxygen supply or even a mixture of oxygen and something else say nitrogen, that is provided into the bioreactor for providing oxygen to the cells and Agitation is the rate at which the agitator moves. The agitator, what are its affects on the culture? They also determine other aspects which we will look at. In fact, both of them together are very important for what is called the Dissolved Oxygen level, DO for short this is not do, this is DO Dissolved Oxygen level. They are dependent on the Aeration and Agitation levels. This is so important that, it is considered as a separate parameter, it is measured separately and actually controlled separately through manipulating aeration and agitation.

(Refer Slide Time: 05:36)



Let us first look at Temperature. It is common knowledge that there is some kind of an optimum temperature at which the cells work well. You know our, the optimum temperature for a body as we know as the normal temperature, right. It is, you all know what the normal temperature is and beyond that temperature we are suppose to have

fever, the body causes fever for certain reasons and then it is brought back down to normal by many means and the body functions well. Similarly, the different cells function most optimally at temperatures which could be different. For example, there are certain cells which function optimally at 30 ºC. Our cells, mammalian cells function optimally usually at 37 ºC and so on. Within, the range is rather narrow, it is about let say 25 - 37 ºC is where most of the cells that we know of fall. There are of course, variations and that is what we are going to look at now, in some sort of complete lecture.

If we look at the variation in growth rate, with Temperature. Now, we are looking at the effect of growth rate. Remember we said how rates are important? And therefore, this would also focus on how the temperature affects growth rate. The variation is something like this, there is a temperature below which the growth cannot occur and then as the temperature increased the growth rate increases, reaches a maximum value and then falls down quite quickly. In fact, the rate of increase with increase in temperature is slower compared to the rate of decrease, if we take the negative of it the rate of decrease with increase in temperature. So, it is kind of squished or skewed to one side.

Tmin, or T minimum is the temperature below which growth does not occur. Tmax, the maximum temperature at which growth occurs, beyond which growth does not occur and T opt is the optimum temperature at which the growth rate is maximum and we would like to usually the operate at the T optimum unless we are looking at coming up with temperature based strategies by which the temperature is varied in a narrow range towards various ends. Can probably, briefly talk to you about it or maybe I can mention this, we have done some work on that.

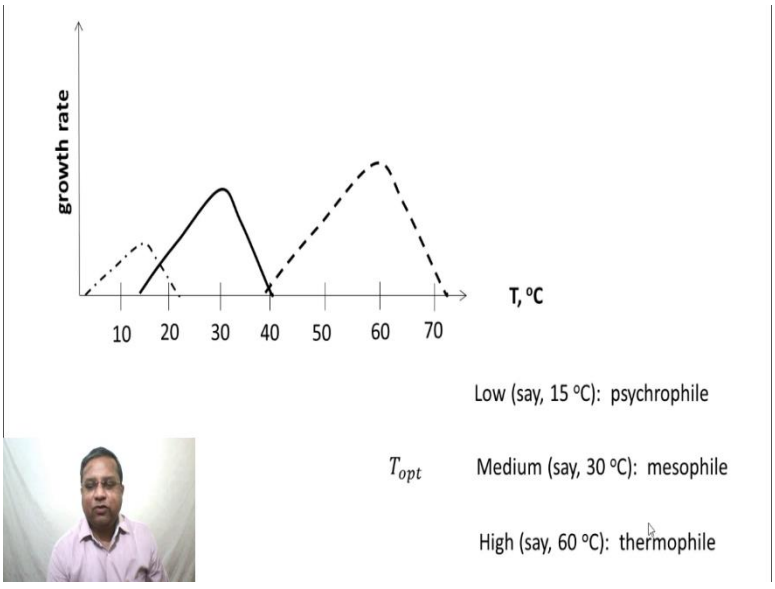
Let us say that the growth is optimum at a certain temperature whereas, the product formation is optimum at a different temperature, right. This is what we found when we did studies on hybridoma, the cells that produce monoclonal antibodies. They grow optimally at around 37 ºC whereas, their monoclonal antibody production is optimum at around 39 ºC. So, the straight forward strategy is to grow cells optimally first and then switch the temperature to 39 ºC so that the monoclonal antibody production takes place optimally. That can be done, because monoclonal antibody is a secondary metabolite, it is produced in the later phase of a batch growth, a secondary metabolite also goes with that. So, the idea is if you grow hybridoma at its optimum temperature for growth, when the growth is maximized. We get the maximum cell concentration, and then maximum

cell concentration is reached. If the temperature can be switched to the optimum for monoclonal antibody production, then the production goes up.

We already have a large number of cells or a larger number of cells compared to other temperatures and the total production is the amount produce by each cell times the total number of cells. So, we have maximized the total number of cells in a particular volume, in other words we have maximized the concentration of cells and then by increasing the temperature we are making each cell producer at the higher rate. Therefore, the overall productivity from the bioreactor will go up. If you are looking at these strategies, the temperature based strategies this information becomes rather crucial and these have already been done. They also have been used in the industry, the temperature shift strategies. Coming back to this, the range of temperatures over which the growth occurs, in other words mathematically, $T_{max} - T_{min}$, is called the temperature range for growth.

If this range, $T_{max} - T_{min}$, is narrow, relatively speaking these all are relative, we just cannot pin it down to very firm numbers. Let us say it is reasonably narrow a few degrees then or few degrees, let us say 10-20 degrees or so. Then the organism is called a Stenothermal organism. If the temperature range of growth $T_{max} - T_{min}$ is broad the growth occurs over a wide range of temperature let us say, then the organism is called a eurythermal organism.

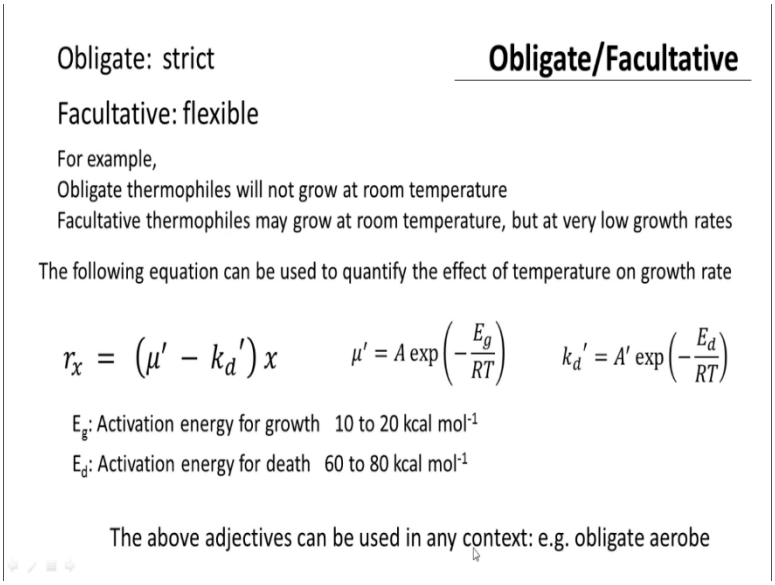
(Refer Slide Time: 11:53)



Let us look at the variation in growth rate with temperature again. The T optimum, the temperature at which the growth rate is maximum, can be medium let us say around 30 ºC which means the variation of growth rate in with temperature will be something like this. See it starts at around 15 or something like that, then goes up to reach an optimum of 30 and then falls down and the growth is not possible let us say after about 40. If this happens, if the T optimum is Medium around 30 ºC, then the organism is called a Mesophile. If it is low, you know the temperature optimum is around 15 ºC, then the organism is called a Psychrophile, phile is something that is loving. So, psychro low temperature loving, meso mid temperature loving.

And there is one more if it is high, let us say this is around 60 degrees, it is not 30 it is 60 degrees then it is called a thermophile. Let us probably correct it right now. So, that you will not get the wrong picture. Around 60 ºC, it is called a thermophile. Most organisms that we use in the industry are Mesophiles because it is much easier for temperature maintenance, you know there is lot of effort that goes into maintaining low temperatures. There is lot of effort that goes into maintaining high temperatures energy wise, effort wise and so on. So, unless the product is esoteric and has a huge market the Psychrophiles or thermophiles are not normally used, usually the Mesophiles are the once that are predominantly used in the industry.

(Refer Slide Time: 14:06)



There is another adjective that we should become familiar with, that is Obligate or Facultative. They go hand in hand, obligate on one side, facultative on the other side. What does that mean? Obligate means strict and Facultative means flexible. This can be used in any context, we will introduce it in the context of temperature.

For example, Obligate thermophiles, Strict thermophiles. Strict thermophiles will grow only at high temperatures, they will not grow at room temperature or they will not grow at temperatures that are not their optimum or that are not high. That is what obligate thermophiles mean. Whereas, Facultative thermophiles may grow at room temperature, but at very low growth rates. They will still grow but their growth rate of course, will not be optimum that will be much lower than the optimum, but they will at least grow. So, they are somewhat flexible, they can adapt to the lower temperature conditions or they can multiply under the lower temperature conditions, but at much slower rates. You could use these adjectives for very many different situations.

For example, an aerobe in oxygen requiring organism. If it is an obligate aerobe, then it will grow only in the presence of oxygen, it will be unable to grow in the absence of the oxygen. Whereas, a facultative aerobe can grow well in the presence of oxygen whereas, it can also grow in the absence of oxygen. So, it can be used in several different context.

Going forward, the following equation can be used to quantify the effect of temperature on growth rate:

$$r_x = (\mu' - k_d') x$$

Where r_x is x the volumetric rate of cell growth.

The constants $\mu' \; and \; k_d'$ are given by the Arrhenius equation

$$\mu' = A \exp\left(-\frac{E_g}{RT}\right)$$

$$k_d' = A' \exp\left(-\frac{E_d}{RT}\right)$$

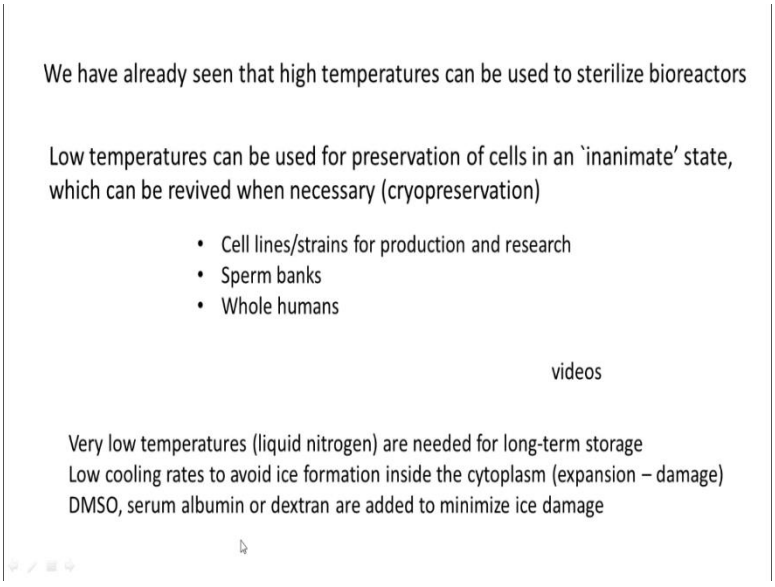
Where E_g is the activation energy for growth, around 10 – 20 kcal mol^{-1}

And E_d is the activation energy for death, around 60 – 80 kcal mol^{-1}

So, the temperature dependence has been brought in here. So, μ' is a function of temperature and if E_g is known, one can even predict what its specific growth rate will be at a different temperature.

So, this is some sort of a mathematical model which gives us the effect of temperature, on the growth rate of the organisms.

(Refer Slide Time: 17:57).



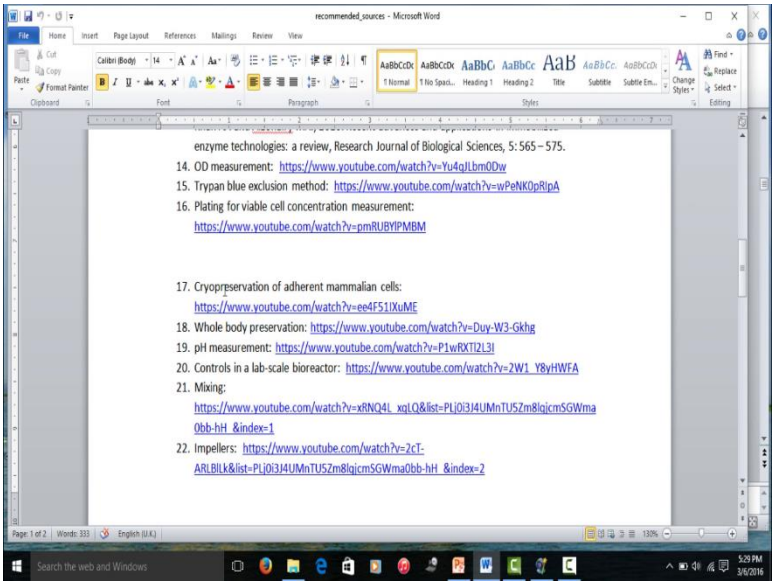
We have already seen that high temperatures can be used to sterilize bioreactors. We did go through quite a bit of analysis on that, we did a problem on that, to better understand how the temperature effects the cells, in the context of killing all the cells. Low temperatures, that was high temperature. Low temperatures can be used for preservation of cells in an 'inanimate' state, which can be revived when necessary. Such a process is actually called cryopreservation. You might ask, where is it really relevant? Why do you want to preserve cells? Its a very common question. And if you think about it becomes very necessary.

There are so many cell lines and strains, that are used for production and research, some which have been modified for a certain purpose and so on. And, how do you ensure that goes on? Right. So, what is done is, some cells are, in a good amount of some cells with desirable properties, after they have been modified to give those desirable properties, are actually stored in an inanimate state at low temperatures, at liquid nitrogen temperatures. And, low temperature is used for maintaining those cells in an inanimate state and when needed they can be revived for a production also, as well as for research. A lot of research depends on the availability of cell lines, at a certain state. In fact, that becomes very crucial for the, even maintaining the research in some labs. Whenever, the power goes down, one of the biggest concerns is whether the -80 ºC freezer is that going to get affected.

When we designed our buildings, we had to ensure that we had dedicated power lines which were supported by the UPS's and so on. So, that there would be continuous power to those -80 ºC freezes. So, it is rather crucial and it is done all the time. Sperm banks, this is an example that many can relate to, quite easily. There, the sperms are maintained in an inanimate state at, using low temperatures, to be used when needed. And even, whole humans have been kept in some sort of in an animated state immediately after their death and so on so forth. There are various ways in which they go about doing that. The idea is that probably somebody is just about dying from a disease so which there is no cure now, maybe there will be a cure in the future and therefore, if the person is maintained in an inanimate state very carefully, the procedure is very carefully done and so on. And it is a hugely expensive kind of a proposition and hopefully when a cure is available, probably the person can be brought back to life and then the disease state

cured is some of the justification given for such process, but they are all real. In fact, I am going to give you some videos, let me tell you what videos they are.

(Refer Slide Time: 21:44)



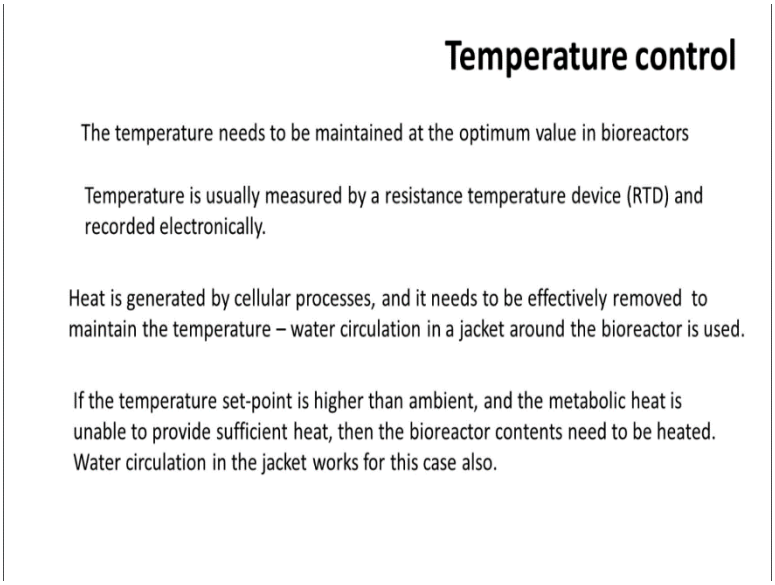
Number 17 here, in your file cryopreservation of adherent mammalian cells. This is a nice video to look at, how to maintain our cryopreserved mammalians cells that are used for research production and so on. Number 18 is, Whole body preservation, right. You can click on these links and watch these short videos to gain a better understanding as to these processes, they are very interesting. Those are the videos that we talked about. The liquid nitrogen temperature, very low temperatures are needed for long term storage and it is absolutely crucial that those temperatures are maintained throughout.

Also, the rates of cooling, when the cells are taken from let us say room temperature down to liquid nitrogen temperature. The rates of cooling, the rate at which you reach those temperatures, needs to be very carefully, it is difficult to monitor them but needs to be carefully done according to set procedures. So as to avoid ice formation inside the cytoplasm. We all know that ice is or water is a strange chemical in the sense that, the density of the solid is less than the density of liquid. In fact, the maximum temperature of H_2O is at around 4 ºC. So, since the density is smaller of ice compared to the density of liquid, the volume of ice is larger than the volume of liquid and if ice forms inside the cell due to the water freezing inside the cell, it can even break open the cells. So, that is real danger and therefore, the rate of cooling needs to be kept in mind and set procedures

used and some other methods used to prevent ice break, ice damage when cooling is done.

In fact, an interesting outcome of the lower density of ice is that, ice forms on top of lakes and so on in winter in cold countries and you can, people skate on ice and so on. In fact, the solid is on top, the water below is actually liquid and that actually allows for life to exists even at low temperatures because ice is formed, that prevents further cooling and so on and it is actually, a solid which floats on top of water. There are these very interesting things that happen in nature biology so on. To minimize ice damage some chemicals are used such as DMSO (Dimethyl Sulfoxide), Serum Albumin or Dextran and so on.

(Refer Slide Time: 24:59)



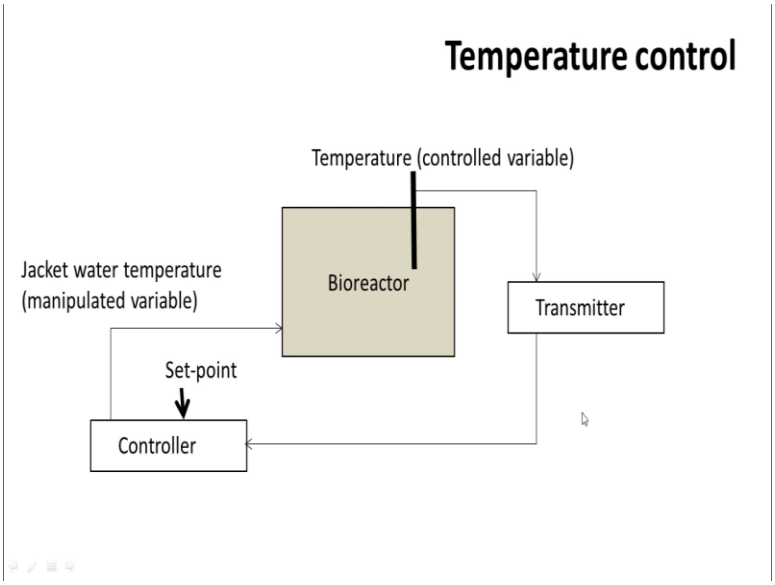
So, if temperature is so important, it needs to be controlled in the bioreactor at the optimum point which is should be the set point. For example, if somebody is growing mammalians cells in a bioreactor, the set point would be 37 ºC, if somebody is growing yeast cell in a bioreactor may be 30 ºC. Some bacteria grow at 37 ºC, some bacteria at say at 30 ºC, 28 ºC and so on. Some algae, micro algae at 25 ºC. So, the Temperature could vary. In any case, once the organism is chosen the temperature needs to be controlled at its optimum level, at the optimum level for its growth in the bioreactor or at the level that is desired in the bioreactor, if you are doing the temperature shift strategies and so on.

Temperature, if you need to control you first need to measure it, right. The Temperature is usually measured by a resistance temperature device, RTD device and recorded electronically. Thermometer can be used for a small-scale kind of a thing and so on. If for manual read out but, here you need to continuously work on the temperature control and that is typically done electronically through a control loop and so on. For that, there should, the manual intervention would be difficult and in such cases, the measurement is done by a resistance temperature device, the RTD device.

Heat is generated by cellular processes, because of the metabolism that occurs in the cell many processes that occur in the cell and therefore, the bioreactor can generate heat and because it is generating heat, it needs to be removed effectively to maintain the temperature and that is usually done by circulating water in a jacket around the bioreactor. You have a bioreactor vessel you say cylindrical vessel, concentrate to that cylindrical vessel you have another container with, through which water can be circulated at appropriate temperatures to maintain the temperature of the bioreactor context, that is the way the temperature is maintained. It is a little difficult to have heating by an element, by an electric element or something like that because the heating element would reach very high temperatures and cells that come in contact with that heating element could be dead if such a system is used to maintain temperature, it is not normally done. Usually it is done through control of the jacket water temperature.

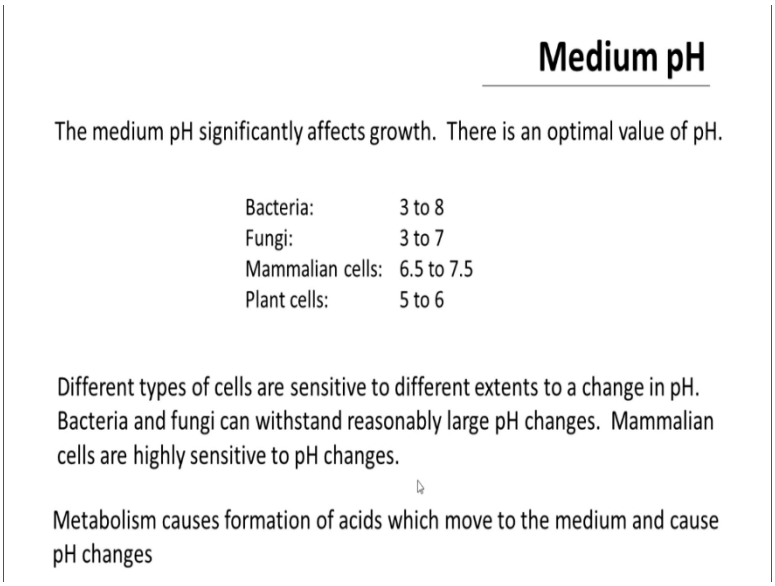
If the temperature set point is higher than the ambient, let us say 37 ºC is definitely higher than the ambient and the metabolic heat is unable to provide sufficient heat to maintain temperature then, the bioreactor contents need to be heated. Water circulation in the jacket, again works with this very effectively.

(Refer Slide Time: 28:20)



Schematically, from a control point of view the bioreactor shown here the temperature is measured through RTD device. RTD - resistance Temperature device and this is measured and controlled and therefore, this is the controlled variable, Temperature is the controlled variable. Whatever is measured is transmitted through a transmitter to a controller which has in it the set point set, another words it is 37 ºC is set here, that is the represented by the set point. Therefore, the controller takes action so as the set point is maintained in the bioreactor. It takes action in this particular case by changing the Jacket water temperature that is circulated in the jacket of the bioreactor to control the temperature and therefore, the jacket water temperature is the manipulated variable. This is a straight forward temperature control, this is a fundamental or basic control that one looks in to if somebody is interested in control of systems. This is a classic way of representing the control variable and manipulated variable, which are terms that are used in control literature.

(Refer Slide Time: 29:47)



That, I think is good information on temperature for us. Let us look at the second variable which is the pH of the Medium. Now, pH as a first approximation is the negative of the logarithm of the hydrogen ion concentration. The hydrogen ion concentration in the medium could be measured and that gives us the pH. The Medium pH significantly effects growth and there is an optimal value of pH, that we can expect. For bacteria, the optimal pH, the pH over which growth can occur without much effect is actually very broad. It can grow well between 3 and 8, the pH of 3 and 8.

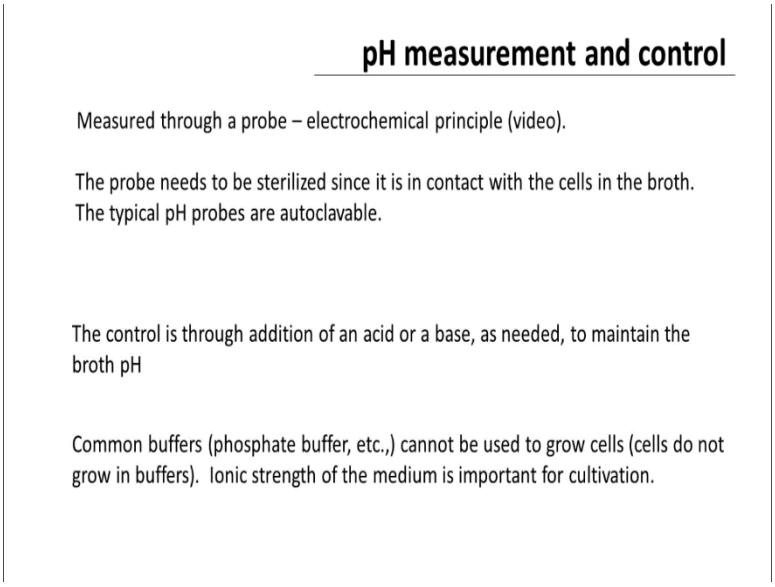
For fungi, it is let us say between 3 and 7 whereas, mammalians cells are very strict there they can grow only if the Medium pH is between 6.5 to 7.5. In fact, if it goes below 6.5 it does not grow, if goes about 7.5 it does not grow. So, the medium has some special dyes added to it phenol red, that indicates the pH by its color. In fact, if the medium had become pink then we know that the temperature has, the pH has become too high possibly the cells are dead. When it becomes orange due to acid formation, it is gone below let us say 6.8 and so on and so forth. And, so we need to worry about it, even in the lab we use this. phenol red is normally added to many mammalians cells media such as DMBM and so on. To give a visual indication of the pH.

Plant cells are again reasonably strict 5 to 6. And different types of cells are sensitive to different extents to a change in pH. Bacteria and fungi can withstand reasonably large pH

changes as can shown here. Mammalian cells are highly sensitive to pH changes as we just mentioned, they just will not grow.

The metabolism in the cell, the set of reactions in the cell causes acids to form. Lactic acid is a very common byproduct of the mammalian cell metabolism. And that gets secreted out of the cells and that can reduce the Medium pH. And, if this happens the medium acidifies. And, even alkalinization can happen in many different situations due to related but different phenomena.

(Refer Slide Time: 32:37)



You know, the carbonic acid is used while controlling pH and so on. Even for cultivation of micro algae, you know the photosynthetic micro algae, you have carbon dioxide in the air that dissolves, produces carbonic acid and that carbonic acid buffer system is used for, is the part of the process while growing micro algae in bioreactors. pH measurement and control, it is measured through a probe the principle is electro chemical. In fact, the principle is very nice I have given you a video for that, it might take some time in this lecture. Let me just point you out to a video, this is a nice video which you gives you the principle of pH measurement through a pH probe. The pH measurement number 19 you can go and take a look at that.

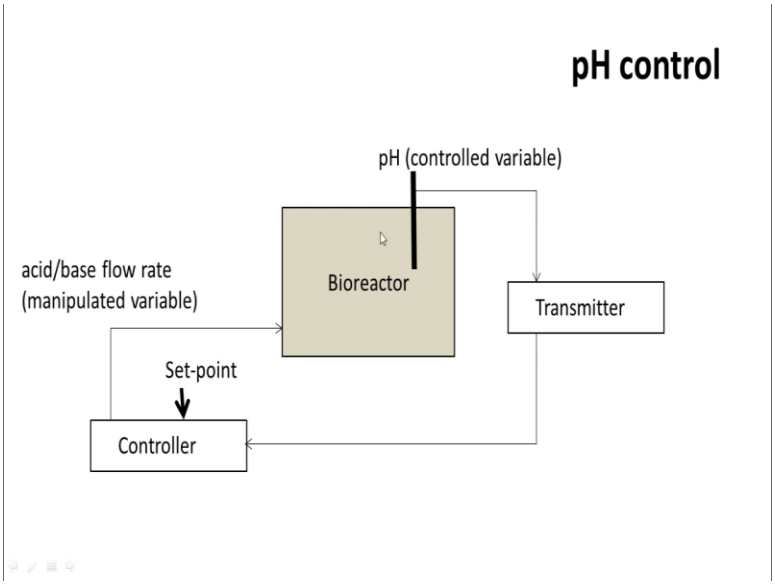
Probe, will of course be in contact with the medium and therefore, it needs to be sterile it cannot have organisms on it otherwise, the medium will get affected. Therefore, the probe is sterilized before use, along with the medium itself it is sterilized whenever the

bioreactor, whenever it is used in a bioreactor. And usually the probes that are associated with the bioreactors are autoclavable. Many probes are not. For example, the pH probe that we use in the lab for measuring the pH for solution,s that is not usually autoclavable. The control of pH, i.e. if the pH goes up then needs to be brought down, when the pH goes down it needs to be brought up, happens through addition of an acid or a base as needed to maintain the broth pH so, it is controlled.

Then there are 2 vessels, one containing an acid, the other containing a base. And, what to add? That decision is made and acid or a base is added to maintain the pH, at a particular set point. One can always ask, you know there are these so called buffers; phosphate buffers, acetate buffers and so on, citric buffers and so on. Which are designed to withstand huge changes, huge assaults to their pH value and they maintain the pH value at that set point, through the chemical mechanisms. You know, they contain substances which can counteract the effect of either acid addition or base addition to maintain the pH the same. They are used in very, very many different analysis.

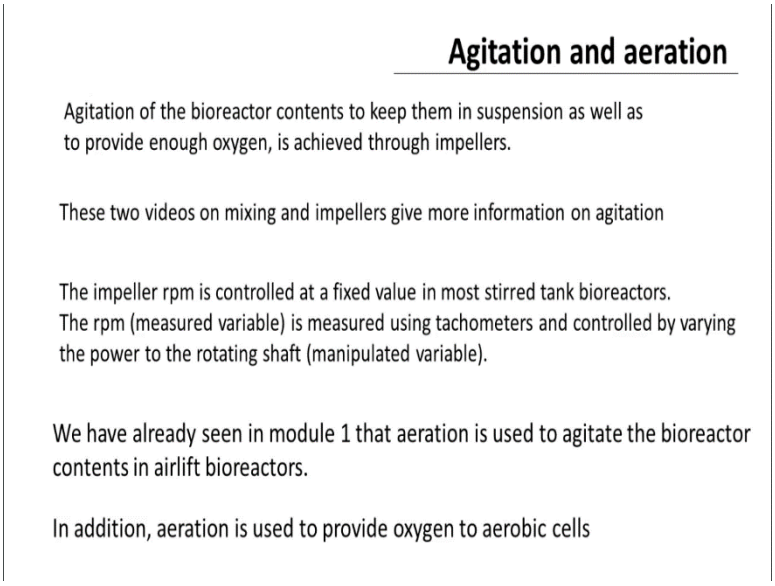
Why cannot the cells be grown in buffers and so the pH control becomes obviated you know, you do not need pH control if you grow them in buffers, that is one of the questions can come up. In fact, we have done some work on that we have tried growing cells in buffer, we did not find literature so we did not know whether it had been done. So, we went ahead and grew cells in buffers and to our surprise we found that they do not grow in buffers at all. So, they do not grow because ionic strength of the medium is important environmental variable or an environmental variable for cultivation ionic strength, there is very different in buffers. It is probably the reason, why they do not grow. So, you cannot use common buffers in the medium to grow cells.

(Refer Slide Time: 36:30)



These schematic for pH control is something like this, pH is the controlled variable which is measured through the pH probe. The pH value which is measured electrically electro chemically is sent to the transmitter, the transmitter to the controller, there is a set point which is optimal for the cultivation that is set here. Actions are taken to manipulate the acid or the base flow rate that is what is the manipulated variable here, into the bioreactor to maintain the pH at a preset value.

(Refer Slide Time: 37:03)



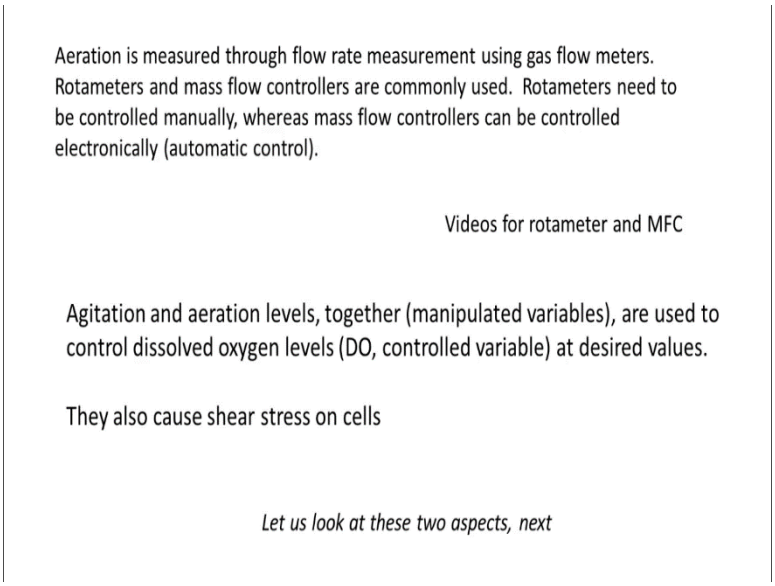
Let us very briefly see Agitation and Aeration in this lecture. And then we can look at the details in the next lecture.

Agitation of the bioreactor contents is needed to keep the contents in suspension as well as to provide enough oxygen and which is achieved through the impellers and so on. There are 2 videos on mixing and the impellers, which give more information on agitation. Let me point them out to you. You can go and see them yourself. The mixing. This is controls in the lab scale bioreactor this is also useful video. This goes along with the earlier thing; this gives you all the controls in a lab scale bioreactors. It is not a very high quality video, but it gives you an idea of what they does.

Number 21 is on Mixing and number 22 is on the various types of Impellers. You may want to see these videos to get an idea, better idea of mixing to get details on the mixing phenomenon and details on the impellers and so on.

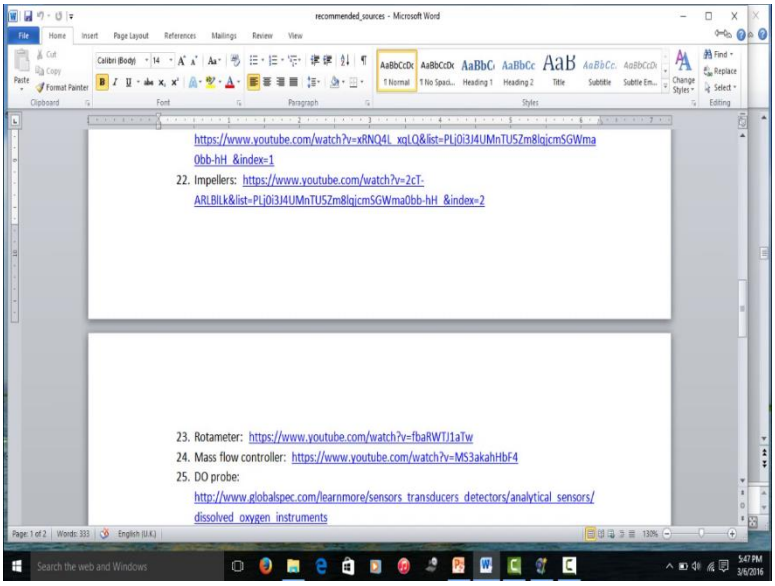
The impeller rpm is controlled at a fixed value in most stirred tank bioreactors. The rpm which is the measured variable is measured using what are called tachometers and controlled by varying the power to the rotating shaft, so the power to the rotating shaft is the manipulated variable. And we have already seen in module 1 that aeration is used to agitate bioreactor contents in airlift bioreactors, not just agitation not just agitators you could also use aeration to agitate to keep to mix the contents of the bioreactors to keep them in suspension and so on. In addition, aeration is used to provide oxygen to aerobic cells.

(Refer Slide Time: 39:10)



Aeration is measured through flow rate measurement using gas flow meters as they are called. Rotameters and mass flow controllers are commonly used. Rotameters need to be controlled manually, whereas mass flow controllers can be controlled electronically, through automatic control. There are a couple of videos that I would like you to see on the Rotameters and the mass flow controller. The mass flow controller principle is very interesting. Let me point out those videos to you.

(Refer Slide Time: 39:45)



In a number 23 gives you the video for the Rotameter, number 24 for the mass flow controller, you may want to see them.

And Agitation and Aeration levels, together both are manipulated variables, are used to control the dissolved oxygen levels, at certain desired values. So, when you are looking at DO control, then agitation and aeration levels become the manipulated variables. They themselves are controlled and monitored and controlled, but they take in together also effect, also determine the DO value. They, agitation and aeration can also cause shear on cells, shear stress on cells. We will look at that in some details, later. And when we meet the next time or yeah the next lecture we will look at the details of DO control, DO first what it does and then control of Do and also shear stress on the cells.