Bioreactor Design and Analysis
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Lecture 02
Introduction to the course - Part 2

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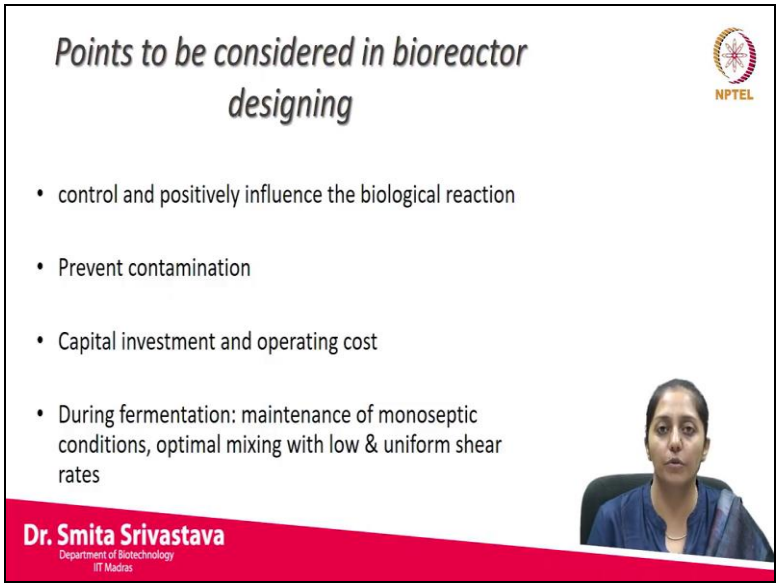

Welcome back students. So in the last class we stopped at the discussion on points which have to be considered when we are designing bioreactors. So, just to go through it once more time I am when we are designing bioreactors one should ensure that the bioreactor design should be able to control and positively influence the biological reaction. Further the bioreactor design should be able to prevent contamination.

It should also take care of the capital investment which is involved and the operating cost because this fermentations can be long drawn fermentations. Then during the fermentation the bioreactor design should enable maintenance of monoceptic conditions that has to be optimal mixing with low and uniform share rates.

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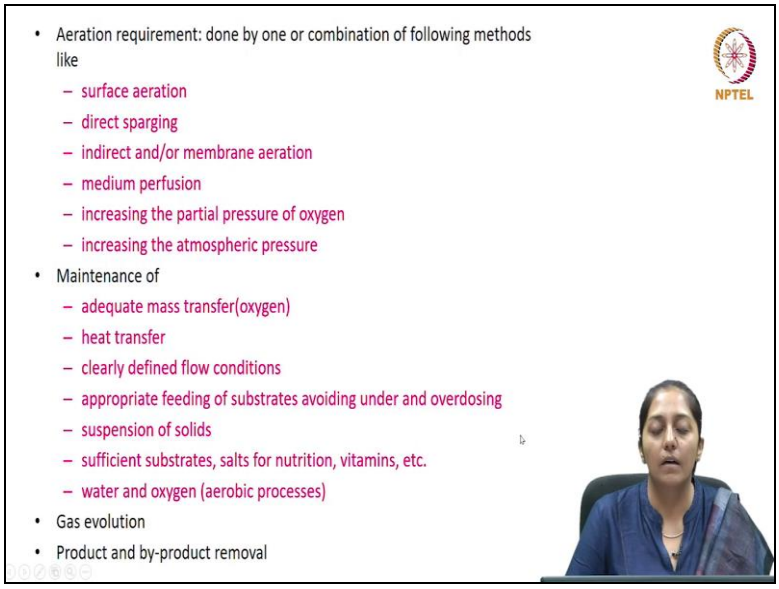

Further there are different ways in a bioreactors by which we can supply aeration to the organism like for example in some of the bioreactor designs aerations is done through the headspace which is called as surface aeration that can be direct passing. This is the most frequently used method of providing oxygen to the organisms, which is direct sparging inside the bulk medium or indirect sparging using membrane aerations this is generally used for Share sensitive cultures.

Then medium perfusion again for share sensitive cultures or if the turbulence can disturb the organisms which are sometimes for the growth to happen the prerequisite is to adhere to surfaces. So, one has to take care that there is not much turbulence cost even if it is due to the sparging. So in those cases sometimes medium perfusion is done and where the medium is circulated to the culture chamber and it is aerated in a separate system.

And it is brought again after being saturated with air it is recirculated to the culture chamber. Then the other ways by which dissolved oxygen can be improved includes enhancing the partial pressure of oxygen inside the reactor which may involve increasing the pressure or increasing the concentration of oxygen in the inlet air the fraction of oxygen in the inlet air which means manipulating the inlet gas composition.

But both these things can be costly and special in case of increase pressure that can be safety issues. When talking about maintenance of adequate mass transfer. So the reactor design which we choose should also ensure that there is adequate mass transfer which is being maintained as well as heat transfer that have to be clearly defined flow conditions inside the

reactor. And there have to be provisions for adequate feeding of nutrients to avoid under and overdosing.

The solids are the particulate matter present in the medium which involves the culture should be able to remain suspended inside the reactor. Otherwise it is settled down then this may cause mass transfer and heat transfer limitations and eventually affecting the Productivity and yield. Then sufficient substrates are salt, nutrition, vitamins they have to be provided inside the reactor. So, as to maximize the product and Biomass productivity.

The bioreactor system should have enough water activity and oxygen for the which is crucial specially for aerobic fermentation. Then there has to be provision for gas evolution because there is a continuous inlet of air and if there is no proper provision of gas outlet this may involve increase in pressure inside the reactor and finally busting. Then there has to be adequate provision for product and by-products removal from the reactor.

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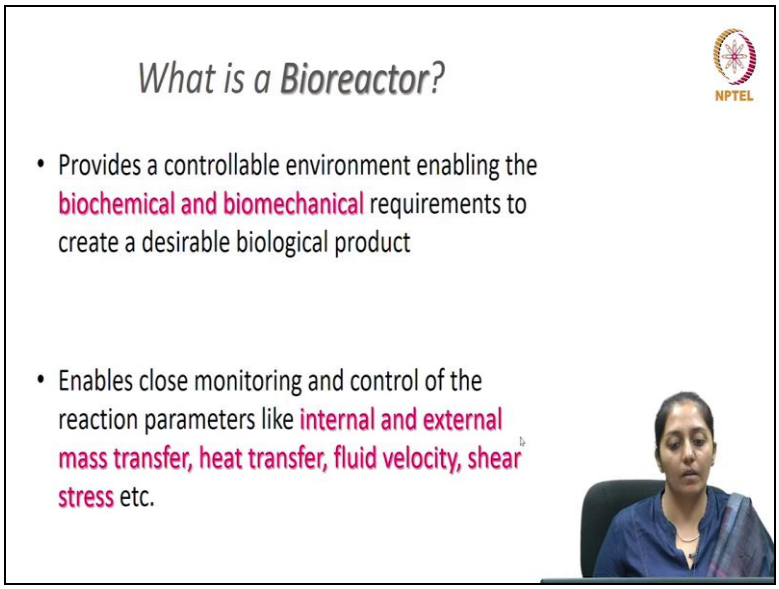

So in all a bioreactor is a device which can provide a controllable environment enabling biochemical and biomechanical required to create the desirable biological product. Further it should enable close monitoring and control of the reactor parameters like for example internal and external mass transfer, heat transfer, Fluid velocity, shear rates and other factors. So the bioreactor should be able to have devices or arrangements for the close monitoring of these operational parameters.

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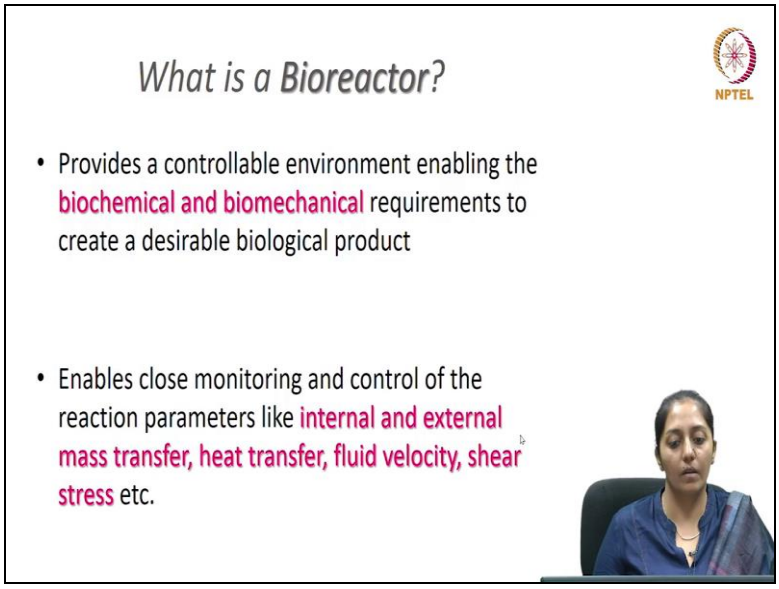

Coming onto the different features of a bioreactor; which can take care of these objectives; so, we spoke about the headspace Space. Head Space is all the volume of the reactor, which is above the working volume above the liquid broth. Agitator system it is involved in mixing. So agitator system includes the shaft and different types of impellers which are required for adequate mixing. And there is Oxygen delivery system which involves sparger the different designs of spargers which are used in fermentation.

Form control system this is mostly used in fermentation swear lot of forming in broth is observed. We will be talking in detail about what is a form and why it is caused in certain fermentations. So there has to be a provision to control forming inside the reactor excessive forming inside the reactor temperature and pH control systems in order to enable control of the parameters at the optimum values.

You need to have detecting systems and then control systems, so that involves temperature and pH as two crucial parameters which are measured and also controlled. Then sampling ports for sending the inoculum inside which is the starter culture inside the liquid broth and harvesting of the reactor through sample ports or intermittent Sampling and sample ports all this comes under sampling requirements, cleaning and sterilization system and then charging and empty lines after harvest.

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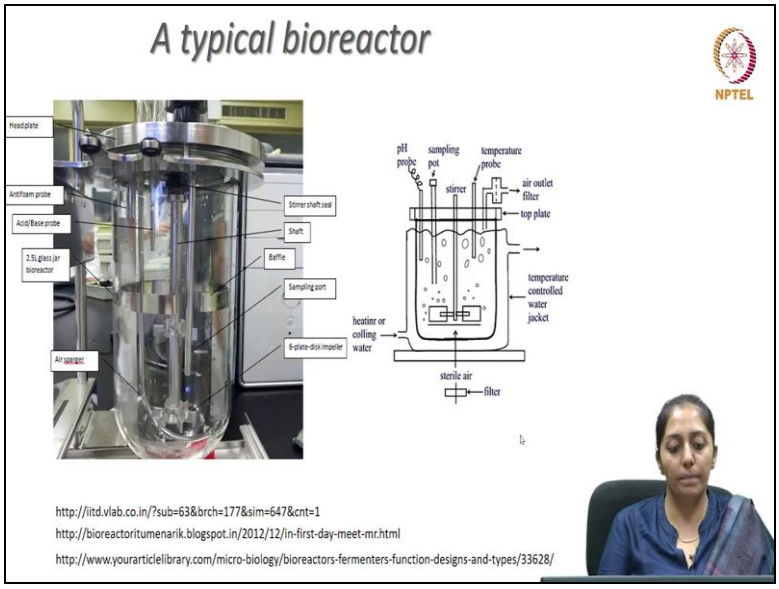

As you can see on the slide this is a schematic for a typical bioreactor with its different parts, which we discussed earlier. So there is a head plate frequency on the top then inside the glass vessel at the centre you will see the shaft which has impeller attached to it the photograph pictures soon has Sixth place disturb in impeller, which is mostly used for microbial fermentation. Then you will see a half u-shaped rod just ending beneath the impeller the bottommost impeller.

This is air spargers the different designs of air sparges like sintered air spargers or circular air spargers as which will have different dimensions of holes through which the air bubbles of different sizes can come out. Then near the glass body towards the inside wall you will see a circular attachment to which baffles are attached to it. So this is a baffle these are perpendicular plates on the surface of the on the inside surface of the reactor.

These are generally used to prevent vortex formation during bioreactor cultivation. Then from the headplate you will see the measuring system where you will have a pH probe, attachment a temperature probe attachment and antifoam probe attachment. There is a form probe there is a capacitor built, Spaced probe, your pH probe generally polara graphic or Galvanic probes and your temperature probe is based on the principle of Thermocouple. These are some of the crucial and major parts of the reactor.

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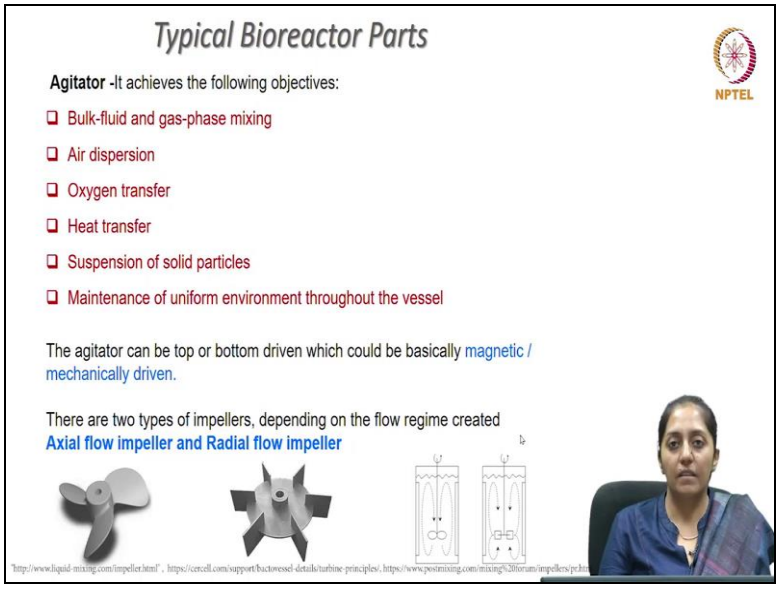

And talking about agitator it achieves objectives of bulk liquid and gas mixing provides dispersion of air, provides oxygen transfer from the gas bubble to the liquid broth. It facilitates heat transfer throughout the liquid broth and also from the liquid brought to the external coolant which is circulating to maintain the temperature of the reactor. Then it also helps in suspension of the cells aur solid particles inside the reactors during the cultivation does not allow them to settle.

Then it helps in general in the maintenance of uniform environment throughout the vessel. All these things play a crucial role in the bio process efficiency. Agitators can be top or bottom driven generally you find magnetically driven impellers or mechanically driven impellers. These days mostly magnetically driven impellers are used because they can avoid contamination due to leakage in the shop seals.

So on the slides two types of impellers are shown one provides axial flow and the other provides radial mixing. So both these claws have different purposes and both these kind of flows are sometimes required for the fermentation. So depending on the kind of load they generate with respect to the shaft like the axial impellers. They generate flow in the direction parallel to the shaft and radial impellers generate flow in the direction perpendicular to the shaft thereby creating high shear but better mixing.

And axial impellers are used when the suspension efficiency has to be enhanced or when suspension when you have when you are working with heavy or particles or large size aggregates, like plant cell cultures or tissue cultures where due to the weight. There is always

a high chance of them settling down at the bottom of the reactor thereby causing mass transfer and heat transfer limitations as the fermentation proceeds.

So their suspension requirements are higher and sensitive culture they may not be as high mixing requirements or oxygen transfer requirements. So depending on all these factors what kind of impellers you choose in a bioreactor design is crucial.

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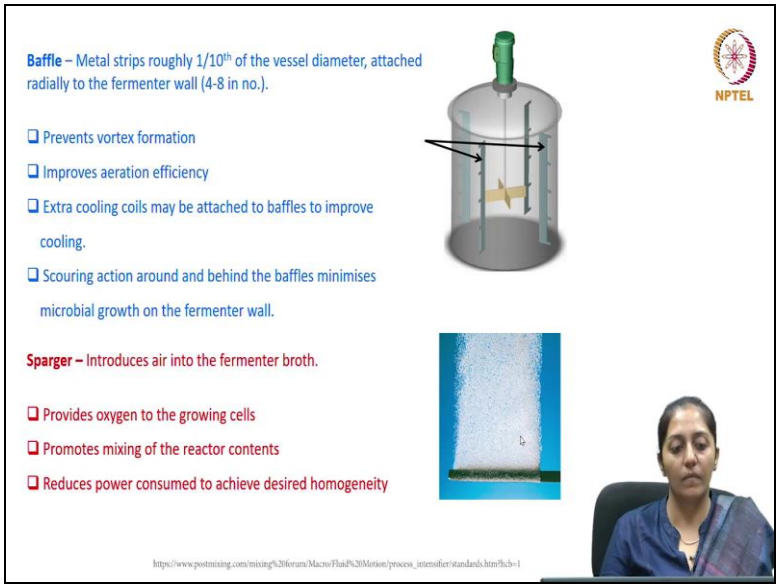

Baffles as I said earlier, baffles these are nothing but metal strip stainless steel strips roughly 110th of the vessel diameter attach radially to the fermenter walls. The function it helps in the vortex prevention of the vortex because if the vortex forms then it will lead to the appearance of dead zones at the corner of the reactors. So to avoid the dead zones which can hamper uniform mixing and uniform environment inside the reactor baffles are placed.

Then it also facilitates in improved aeration efficiency the reason being because of the increase turbulent which is caused by the small eddies which get formed when the water as it circulates and is resisted and the floor is resisted by the walls of the baffles as it is placed perpendicular to the liquid broth. Discovering actions which can produce when the eddies break behind the baffles around the baffles.

It also helps in minimising the adherence of the Biomass on the glass surface which may in turn cause mass transfer limitations as the cell layers grow one over the other on the fermenter wall. Then comes the sparger the picture shows a photo of a sintered sparger where

very fine bubbles can be introduced inside the liquid broth. So it can provide oxygen to the growing cells thereby promoting mixing of the reactant content.

So it also helps in promoting mixing of the reactor contents because of the sparging of the air bubbles causing turbulence in the liquid broth. And it also helps in reducing the power requirements which is consumed to achieve a desired level of homogeneity. As you already know are you must have read that our requirements for sparged system is less than on unjust power requirement to reach to a desired level of homogeneity.

As some of the mixing; is in turn caused by the turbulence of the liquid broth by the bubble dispersion.

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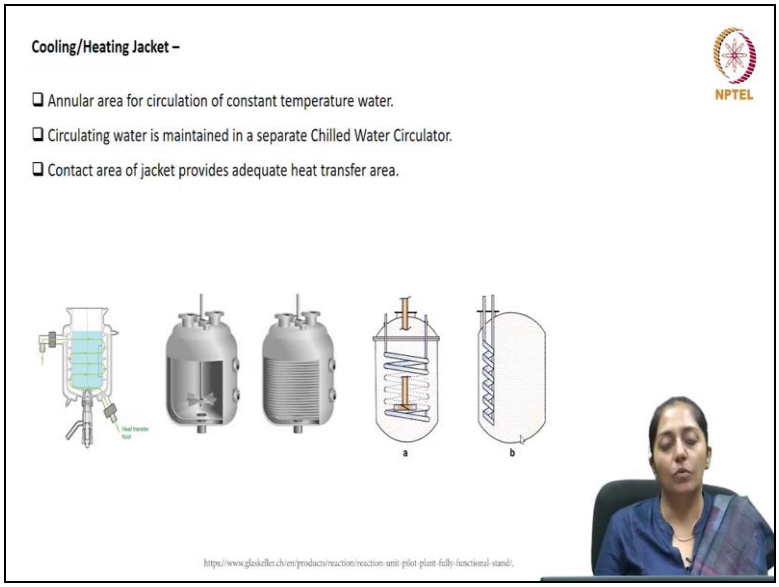

Then come the heating and cooling jacket arrangement. An annular area of circulation of constant temperature water depending on the size of the reactor you can see different ways in which the heating and cooling jackets are arranged that can be water coming from the chilling unit which is circulated around the entire reactor body or if the reactor volume is very small then you can have a heating or cooling fingers inserted inside the reactor through which the chilling water on the chilled water from the chilling unit is circulated for maintenance of the temperature of the liquid broth inside the reactor.

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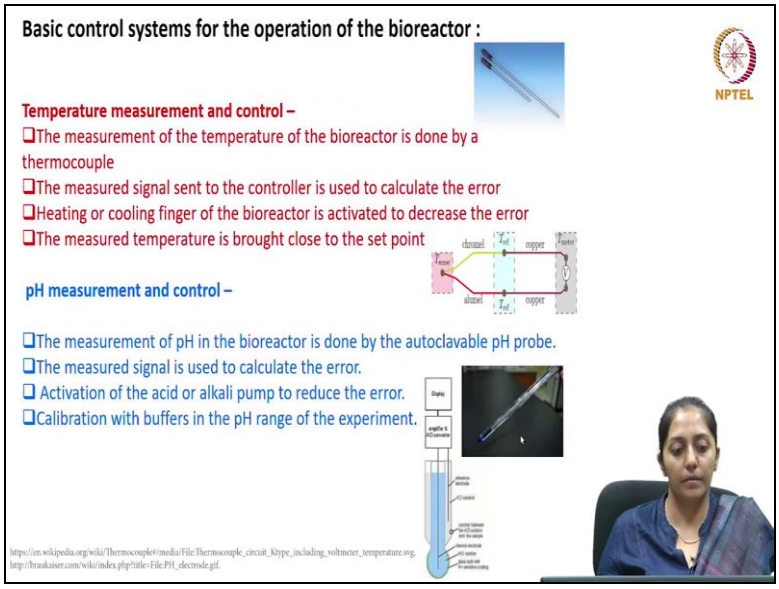

And talking about the control systems present inside the reactor for temperature control and measurement generally we have temperature probe. This probe is based on the principle of a Thermocouple so what happens that the temperature of the reactor is measured buy this probe and the measured signal is sent to the bio controller, which is used to calculate the error. Now this error is the difference between the measured temperatures of the liquid broth inside the reactor versus the set point.

This difference set point is the desired temperature or the optimum temperature required. So, depending on this error between the set point and the current temperature of the broth water from the chilling unit is circulated through the heating of the cooling jacket or the heating element of the cooling element is switched on or off accordingly. The measure temperature this happens until the measure temperature is brought close to the set point.

Similarly pH measurement and control again for pH measurement we have pH probe these are generally glass body probes the efficiency the picture on the slide the glass bulb. The bottom surface is selectively permeable to hydrogen ions and the potential difference created between the reference electrode and the second electrode. Is directly measured as the pH of the broth again per pH control the measured signal is used to calculate the error. The activation of the acid or base pump is then carried out automatically to reduce this error. So, the pH probe requires calibration regularly with acid and alkali.

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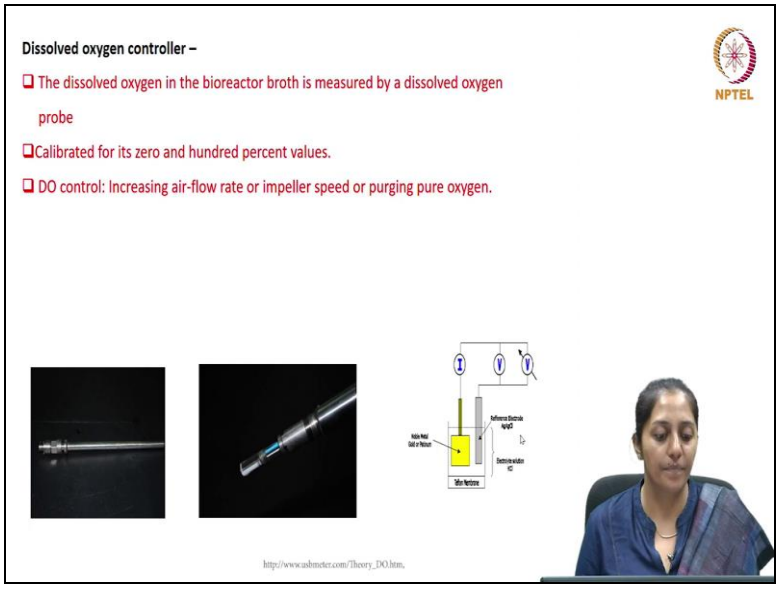

Then talking about the dissolved oxygen probe: The dissolved oxygen in the reactor broth is measured by dissolved oxygen probe it is based on the principle of Galvanic or polarographic electrodes. The schematics hear the schematically shows a polarographic electrode where the membrane at the bottom. It is selectively permeable to oxygen and the oxygen get reduced at the noble metal electrode due to which the current flows and it is measured as proportional to the oxygen tension of the broth.

Again for control the measured signal is compared with the set point and the difference which is called as error is then minimised by either increasing the RPM or increasing the air flow rate till the difference is brought close to the set point.

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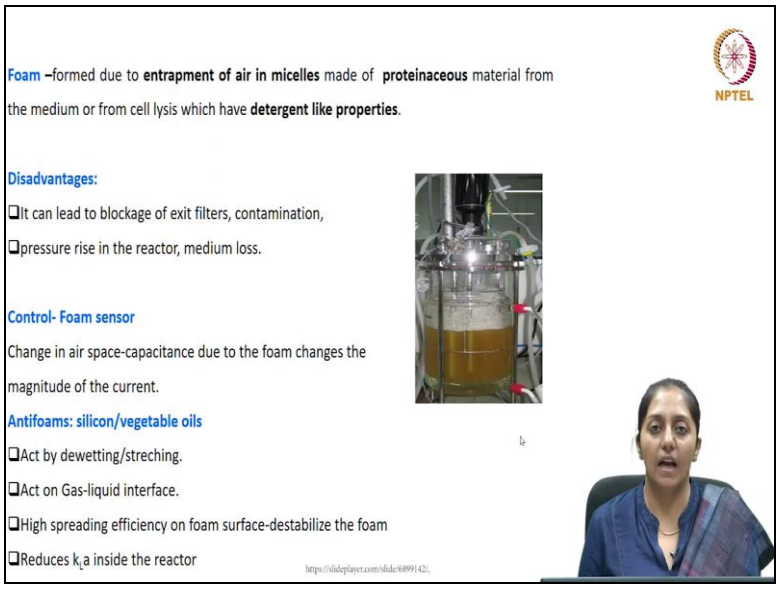

Talking about the anti foam: Now foam is formed due to entrapment of air as my seals which are made of the proteinaceous material from the medium or due to the cell lysis which have detergent like properties. That mostly happens in plant cell fomentation or animal fermentations where sometimes the medium components required proteins or amino acids or hormones or when the cell lysis happens and proteinaceous material components come out from the life cell into the broth.

So because they will have detergent like properties entrapped air in the micelles and the air is not able to escape. So they keep collecting in the form of foam on the surface of the liquid broth. Now the disadvantage is that it can lead to blockage of the exit filters and thereby contamination. And because of the blockage of the exit filters, it can eventually lead to pressurize inside the reactor which can be dangerous.

So to control forming inside the reactor, there is a anti foam or there is a probe which is based on the air capacitance. As the foam rises from the surface the capacitance changes because the air is replaced by the foam and this change is measured by the probe and accordingly the anti foam can be added. The signal is sent to the bio controller and from the bio controller the pump which is connected to the anti foam addition bottle gets switched ON and the anti foam is added in side reactor.

Now, what is the mechanism of action of these antifoams what they basically 2 dibetic agents this spread on the surface of the foam and disrupt the barrier between the air and the form so which is the gas liquid interface. They have very highest spreading efficiency which can destabilize the foams office surface. However, they can lead to reduction in the mass transfer efficiency of the liquid broth if they are added in very high quantities.

So on the bases of the agents are the cells which are used inside the reactors. Can be broadly classified into two groups? One which is based on living cells and the other which are based on the cellular components; which are mostly enzymes. In terms of processor requirements they can be classed as aerobic anaerobic solid state or immobilized cell bioreactors.

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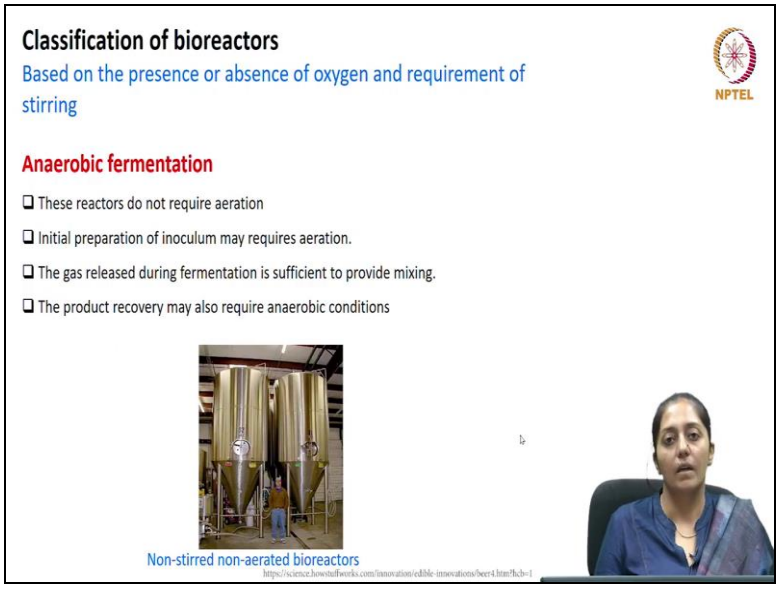

Give brief Idea on these different classifications basically the presence of oxygen or the absence of oxygen requirements, the fermentations can be classed as aerobic or anaerobic. In anaerobic fermentation the reactor they do not need large aerations initial preparation of inoculum all the might require little aeration. But the production does not need aerations. And the gas, which is released during the fermentation is sufficient enough to create adequate fixing.

The product recovery may also require anaerobic condition. One of the biggest example of anaerobic fermentation is beer formation.

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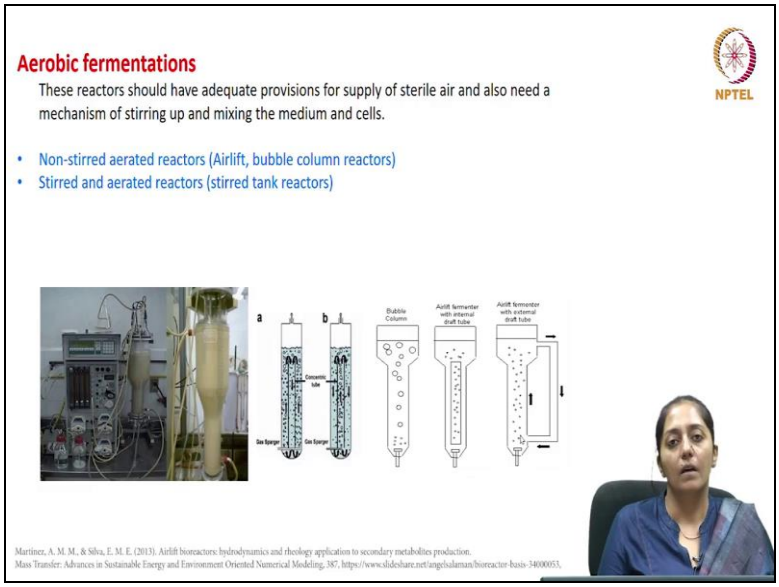

Talking about aerobic fermentation: In aerobic fermentation the different types of reactors. They can be classed as non stirred aerated reactors stirred aerated reactors. These reactors

should have adequate provisions for supply of sterile air and also need a mechanism for starting up and mixing of the medium and cells. So under non stirred aerated reactor some of the examples are like airlift reactors or bubble column reactor.

As you can see the schematic on the slide of the airlift and the bubble column reactor was and I hope looking at the schematic you are able to make out the difference between the two types of reactors. Can you see how it is different right? So if you can notice There is a presence of draught tube which is an internal circulation glue inside the columnar structure. So these are airlift reactors while in bubble column reactor. There is absence of any part inside the reactor or such columnar loop which is called as draught tube.

In air lift reactor depending on the position of spargers. They are further class does inner loop or outer loop reactors. And depending on the position of the draught tube whether it is inside the reactor body or it is outside their further called as external draught tube airlift reactors or internal draught tube airlift reactors.

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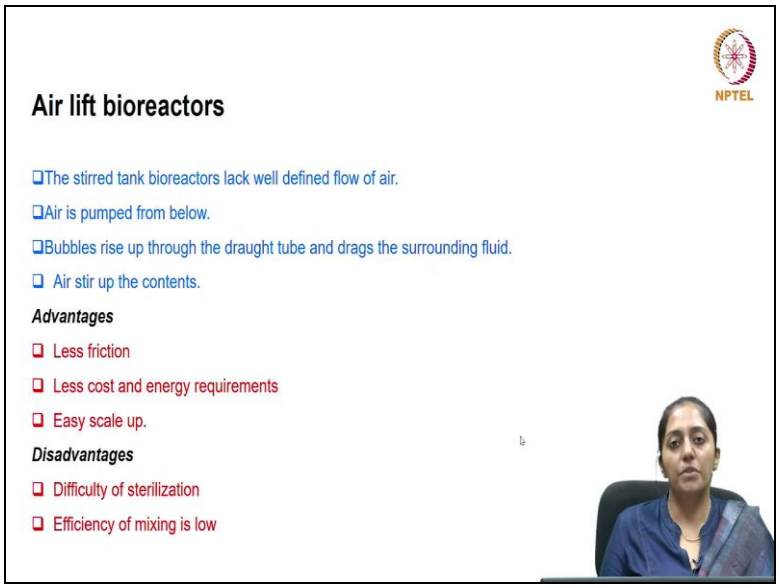

Most frequently used reactor design. Involves stirred tank reactor configurations, but depending on certain requirements airlift reactors have been introduced. Like for example the stirred tank bioreactor still AC well-defined flow of air the air is pumped from below and the bubbles as they arise up through the draught tube it drags the surrounding fluid and on the way it stirrups the contents.

Reactors include less fiction less cost and energy requirements and easier scale-up. There are certain demerits also attached to these kinds of actors. It involves difficulty of sterilization and the efficiency of mixing is low obviously because there are no moving parts inside these reactors, but the mixing is happening because of the liquid current which are formed as the air bubbles drag the liquid along with them.

So, depending on the placement of the sparger, liquid circulation can be clockwise or anticlockwise direction to facilitate mixing.

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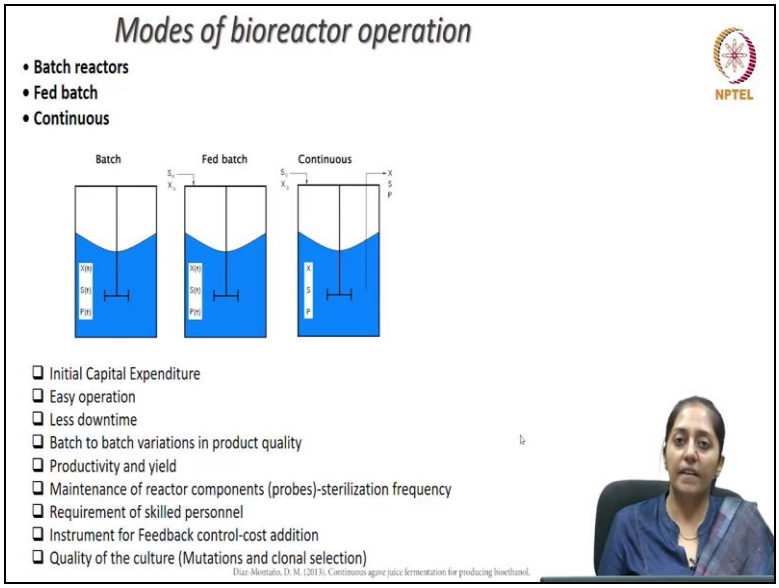

Even in a given reactor configuration thare can be different ways of cultivation. So there are basically three different ways cultivations are carried out. One is a Batch Mode of cultivation, then fed Batch Mode and the other is continuous mode. So these are three basic ways for modes of cultivation in bioreactors. Now batch reactor as you can see on the left most side of the slide. It is a closed system.

There is no entry of any feed during the cultivation. So, one time added at the beginning and then finally the reactor is harvested after the cultivation period. While the metal schematic is a fed Batch Mode of cultivation in which you can clearly see a feed coming inside the reactor from left hand side. Now this but nothing is going outside the reactor. So this is class under fed Batch Mode of cultivation.

So because there is no continuous stream coming outside the reactor the volume keeps on changing with time. In the third diagram you can see that there is one stream which is going

inside the reactor and there is another stream which is also coming out from the reactor. So there is continuous inlet and outlet from the reactor. This mode of cultivation is termed as continuous mode of cultivation. We will be talking about these in details when we will study the designing of these different modes of cultivation.

Now by choosing a particular mode of cultivation one should keep in mind the following factors. The initial capital expenditure, the ease operation, a mode which can provide minimum downtime. Downtime is the time period spent between two consecutive runs then batch to batch variation in product quality that factor has to be kept in mind while choosing the right mode of cultivation.

Maximum productivity and yield maintenance of reactor components which means the number of sterilization cycles which these different parts of the reactors will have to go through. So every part is not indefinitely going to work. So one has to take care especially the probes. The roundly set number of cycles of sterilization till then it is recommended to be used for its most efficient working.

Then the requirement of skilled personnel there can be variation in the skill of people which will be required to run these different modes of cultivation. So, one has to take care keep that in mind as well. Then instrument for feedback control specially in the open systems like fed batch and continuous. There will be additional requirement of pumps these pumps have to be calibrated and depending on the duration of the fermentation one has to ensure that the pumps are running in proper conditions.

Because of the pump requirements and running it can be an additional cost to fermentation. Then quality of the culture: If these are very long drawn fomentation on the number of cycles involved. Then there is always a chance of genetic mutation happening or contamination. So sometimes depending on the purpose also the; different modes of cultivation chosen. Like for example, if clonal selection of cell line selection has to happen then containers cultivations are preferred. We will be talking about more in detail men will be discussing containers fermentations.

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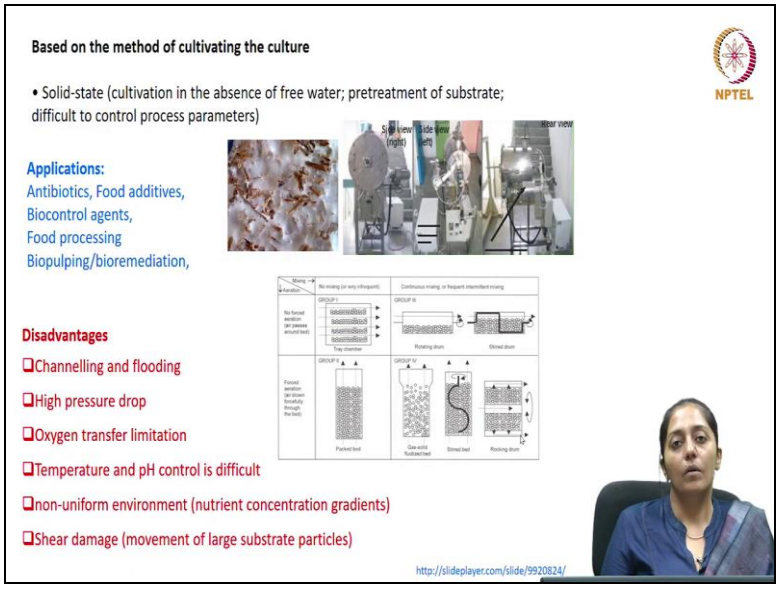

Now apart from different modes of cultivations so we have classified bioreactors, depending on the different modes of cultivation at batch reactors, fed batch reactors and containers reactors. Now reactors can further be classified based on the method of cultivating the culture which means that depending on the water activity solid state fermentation or suspension cultures. Solid state fermentation is the involved cultivation in the absence of free water this may require pre-treatment of substrates.

And because there is less water activity it might be difficult to control the parameters with the help of the measuring systems like PH and temperature probes. Because they cannot ensure a uniform or homogeneous reactor environment but still depending on the applications and the culture requirements solid state fermentation are in use commercially like for example an antibiotic fomentation is used in filamentous, fungi or in food additives production or biocontrol agents.

Specially fungal fermentations filamentous fungi there are ample examples of solid state fermentation being used. The disadvantage as I spoke about involves channelling and flooding thereby causing non-homogeneous or non-uniform environment. Then there can be high pressure drop due to large amount of resistance to flow leading to oxygen transfer limitations. Difficulty in the control of temperature and pH because It is not a homogeneous environment so what the probes will measure may not be the right representation at different points inside the reactor.

Then because of the movement different configuration is available for solid state fermentation where some movement inside the reactor of the substrate is specially treated by rotating the body of the reactor like rotating drum bioreactors. But this can also lead to high shear when large particulate matter rubs along the culture or cells present inside the reactor.

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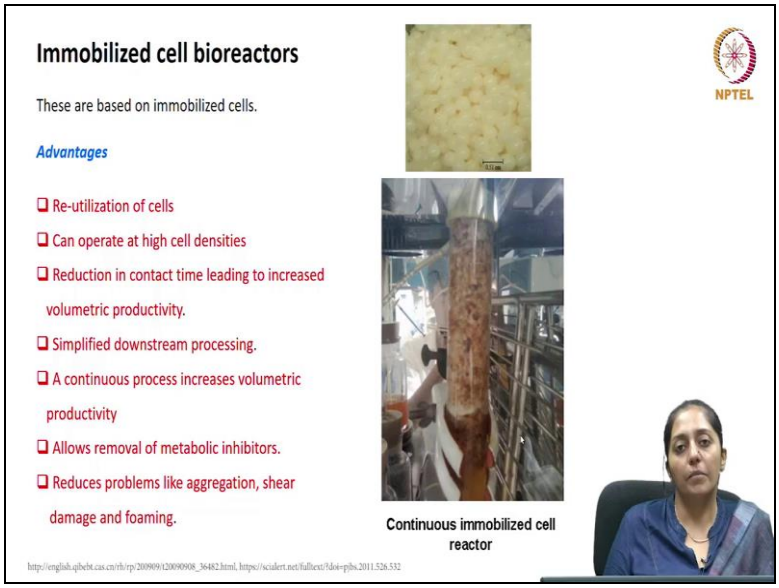

Then another type of reactors are called as immobilized cell bioreactors. You can see the pictures. These are alginate beads inside which the cell suspension has been encapsulated. So immobilize bioreactors they generally used to enable Re utilisation of cells till the viability is intact one can run high cell density cultivations. It enables reduction in the contact time leading to higher volumetric productivity.

If the process is extracellular then it is simpler downstream processing. Continuous process can be maintained which further enhances the productivity which is volumetric productivity. It allows the removal of metabolic inhibitors which can further improve product productivity and there are less problems like in case of free cell bioreactors in terms of aggregation share damage or foaming.

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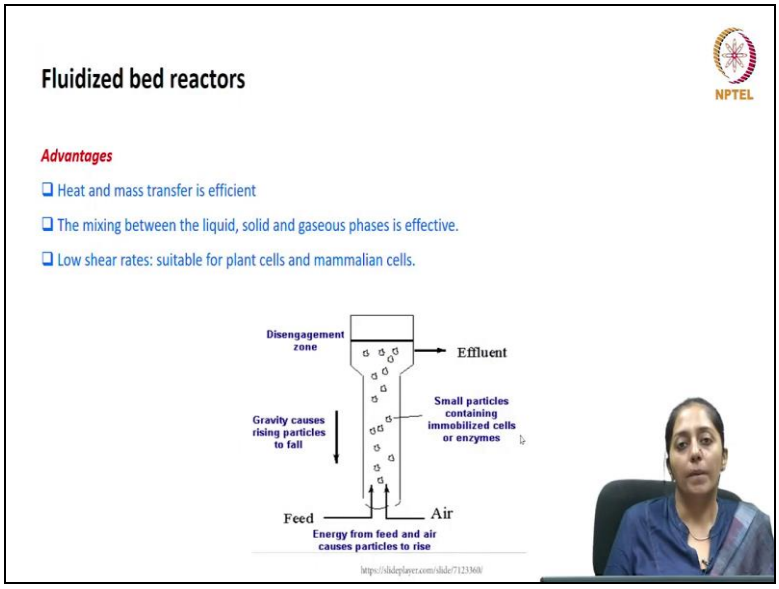

Nw immobilized cell bioreactors are further modified to overcome certain limitations of mixing in these reactors they are modified for better mixing by making them as fluidized bed bioreactors. The advantage is that there are better heat and mass transfer maintenance the mixing between the liquid solid and the gaseous phase is more effective and there are low shear rates in comparison to flee cell bioreactors.

So cells which are sensitive can be cultivated in fluidized bed reactors or which are self mobilizable.

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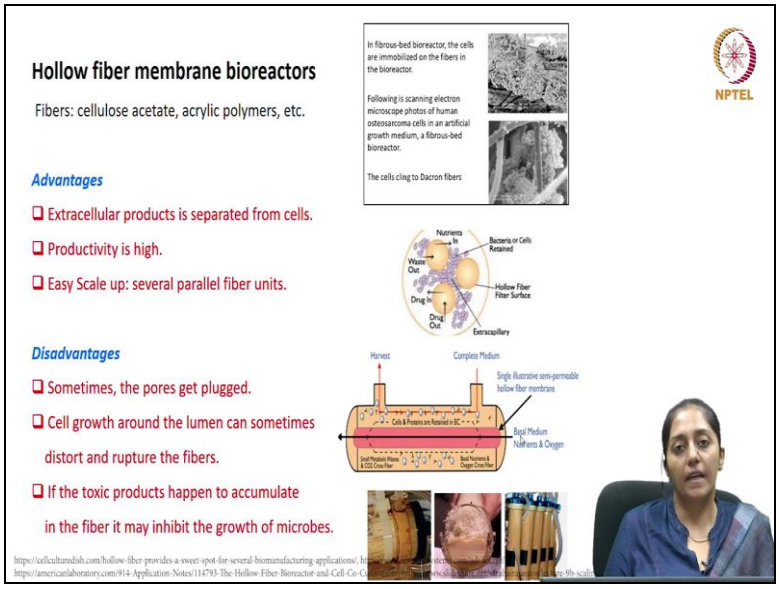

These are certain kind of reactors which are generally used in industry for animal cell fermentations? These are called as hollow fibre membrane bioreactors. The advantages that the extracellular product can be easily separated from the cells these are similar to immobilize

cell bioreactors. The productivity is very high therefore. And they are easy to scale up because there are many parallel fibre units which are inserted in one column. The disadvantage is that sometimes the pore of these fibres can get blocked because of the excessive cell growth around these fibres.

The cell growth around the lumen can distort or after the fibres even and if there are toxic by-products formations. Then they can get accumulated in the fibre which may further enabled the growth of the microbes. So they can suffer from mass transfer limitations as the cultivation time proceeds.

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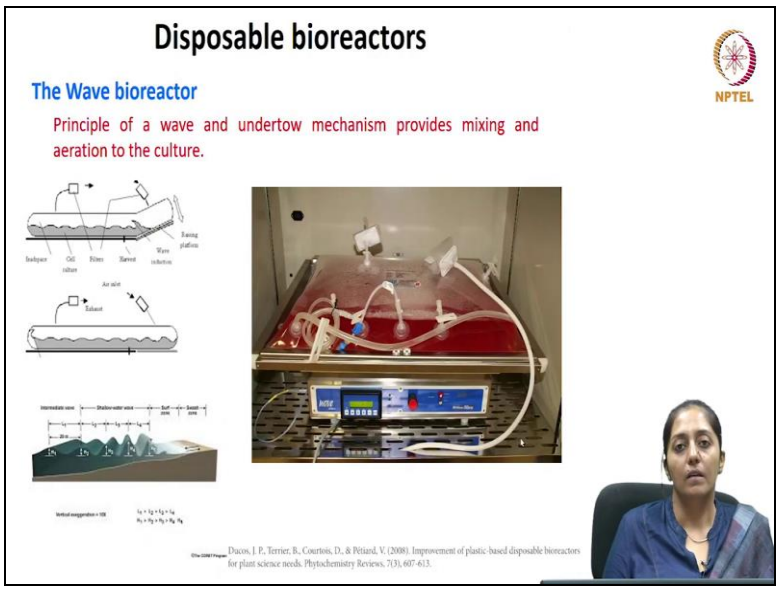

There are new set of reactor designs which are now been commercially utilised which come under disposable bioreactor classification where the reactor body is use and throw types. The mixing it is majorly meant for fermentation which do not require large amount of mixing or aeration where the specific oxygen demands are low like in case of animal cell fermentation and plant cell fomentation.

Where mixing requirements are less and they are self in mobilizing in nature and they are shear sensitive. So in those cases disposable bioreactors or wave bioreactor is one such example of disposable bag bioreactors, which is currently in use commercially for antibody production for phytochemicals production. So, it is based on the principle of wave and under two mechanism, which is enough to provide adequate mixing to these cultures and oxygen requirements are also adequately met by the wave when under two mechanism.

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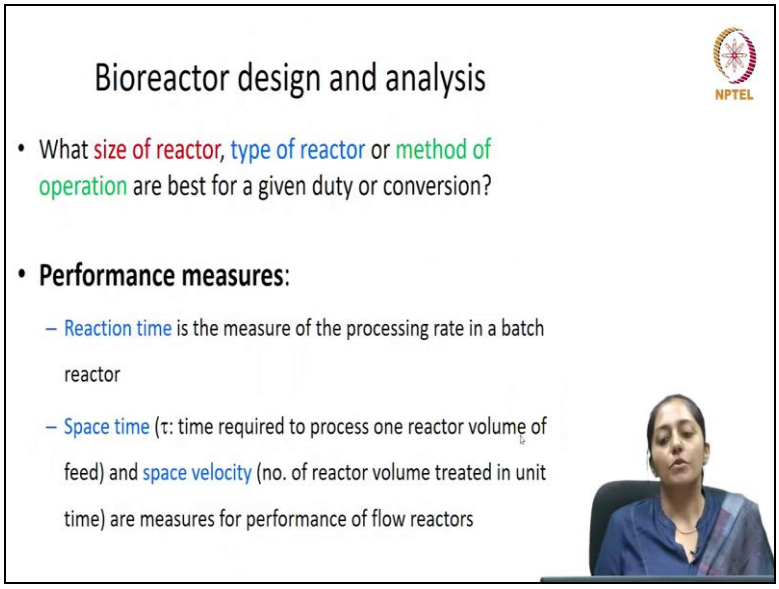

For bioreactor design and analysis is basically to determine what size of the reactor which type of the reactor and method of operation would be best for a given substrate duty or conversion where the performance measures are generally based on the reaction time which is nothing but a measure of the processing rate in a batch reactor or in terms of space time. Which is time required to process 1 reactor volume of the feed or space velocity which is termed as the number of reactor volumes which can be treated in unit time.

So these are the different measures now space time and space velocities are generally used to measure the efficiency of flow bioreactors while reaction time is generally used as a performance measure for batch bioreactors. Thank you students will begin with batch reactor design in the next class.