Analytical Technologies in Biotechnology
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Module - 5
Centrifugation Techniques
Lecture - 5
Types of rotors

In previous lecture, we were discussing about the preparative methods in ultracentrifugation. If you could be recall, we have discussed about different types of methods like differential centrifugation and density gradient centrifugation. In differential centrifugation, the separation of the analytes or the different types of particles in a sample is achieved by the differential sedimentation pattern of each particle. Now, in differential centrifugation, the sedimentation is based on mostly mass of the particle.

Now, we are in steps the centrifugation force is increased that is the centrifugation is performed at different centrifugal speeds or revolutions per minute, then like for example, you can start or one can start from say, 1000 g force or particular revolutions per minute. And as you applied this, for a particular period of time, certain analyte or certain particles in the samples will sediment. For example, many times the cell debris and other like heavy nuclei might sedimented that.

And then as you increase the centrifugal force in steps then subsequently different particles as per their sedimentation patterns will sediment. So, you can start from very low centrifugal force or we can say, centrifugal field to very high centrifugal field in ultra centrifugation, where you can achieve the separation or sedimentation of say, ribosomes or other macromolecular fractions. So, by performing centrifugation in steps or at different centrifugal force, one can achieve the separations of many kinds of particles like particularly, the subcellular components.

Different subcellular components can be separated from each other, membranes, ribosomes and other macromolecular fractions can be separated at very high centrifugal force. And likewise, this is a very popular method, it needs to be repeated like once you get supernatant and pellet, pellet might not be completely pure, so you might need to do it again and again. So that, you can get the best purification or homogeneity for a particular particle, there was another one, whose density gradient centrifugation.

In density gradient centrifugation, this one is based on the density one or size of the particle. Now, they were two kinds, one was rate zonal density gradient centrifugation, which was based mostly on size of the particle that is, if there are particular kind of say macromolecules, for example proteins, which have kind of same densities, but they have different size. So, if the proteins of different kinds of proteins, which have same density then if you are centrifuging them and they differ in say, size by three fourth then they will be easily separated.

So, rate zonal could be done for same density particles, same kind of particles in terms of density, but difference in the size of the particle. In rate zonal, one important thing was that, the density gradient which is formed the densest part of the density gradient that is, at the bottom, will be less denser than that densest particle in the sample. So, it is like time bound centrifugation, otherwise this pelleting might take place and to avoid pelleting, it should be completed in limited time.

On the other hand, there was isopycnic or isodensity separation, where it is based completely on the density of the particle, and where size might be same, but the densities are different from each other. And on basis of the density, the particle will move till it reaches it is isodensity and it will float on the cushion of the higher density material than itself.

So, that way there is no time boundation, you can run it for unlimited time, there could be possibility, where you can make the gradient with the density gradient maker beforehand, where you can have lighter density on the top and the heavier density at the bottom or it could be on the basis like caesium chloride or other, where they form density on centrifugation. So, mixture could be either mixed in there, the sample could be distributed homogenously or it could be layered on the top. So, either way it could be done then we were discussing about different criteria or parameters about the density gradient centrifugation like, how density gradient is formed, what kind of material, different kinds of density gradient patterns are there, all these things we were discussing.

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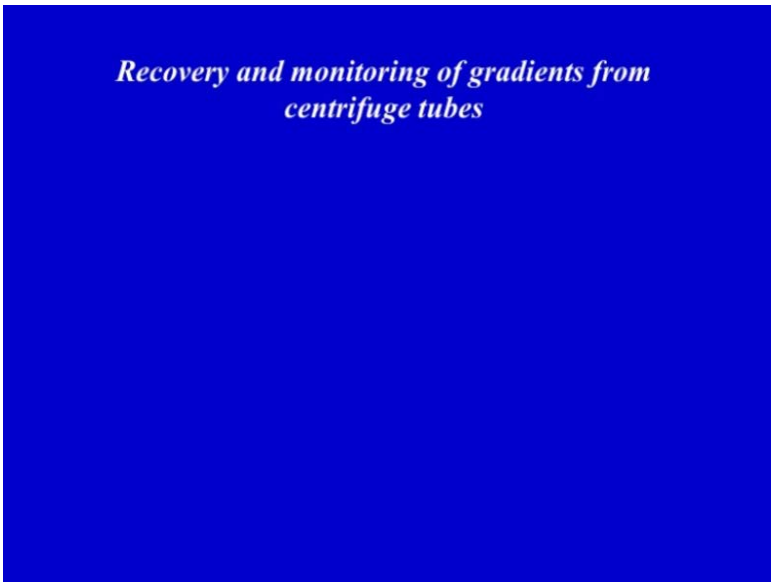

So, in this lecture, we will be going to extend our discussion on density gradient centrifugation. And we will start with one of the parameters that is, recovering and monitoring of gradients from centrifuge tubes. So, after the separation that is, particle separation is achieved, it is usually necessary to remove the gradient solution, in order to isolate the bands of separated material. So, like I said, in density gradient centrifugation, like once you have done it, the particle or particular analyte will float, will be like settle in it is isodensity and it will not move further.

So, once the separation is achieved then you have to recover the material for further analysis or any biochemical investigation. Now, in one case, it could be that bends are visible then by placing a pipette into the end, you can slowly withdraw the material from the bend. So, this is the case, where if certain material is or certain bends are visible, bends can also be removed using a hypodermic syringe, so they could be many different ways you can recover it.

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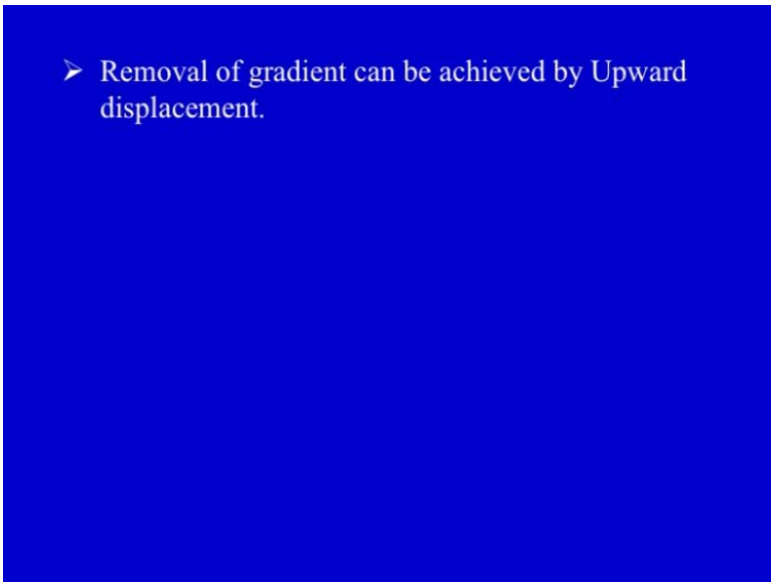

So, removal of gradient can be achieved by upward displacement, that is why using a denser solution of gradient medium or solution, that is marketed for displacement unloading of gradients. Example, there are lot of example, inert non viscous water immiscible organic liquids are utilized for this particular purpose.

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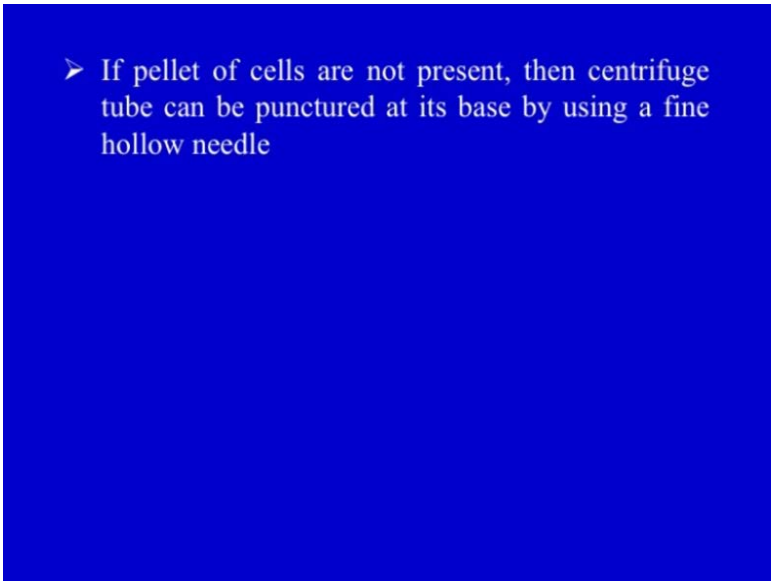

If pellet of cells are not present then centrifuge tube can be punctured at it is base by using a fine hollow needle. Now, analysis of the displaced gradient in order to identify and locate the separated components, can be achieved by different kinds of detached

methods like say, UV monitors or spectrophotometers, there could be refractive index measurements, may scintillation counting, enzymatic or chemical analysis. So, there could be lot of different ways, the isolated particles or samples could be analyzed or could be located.

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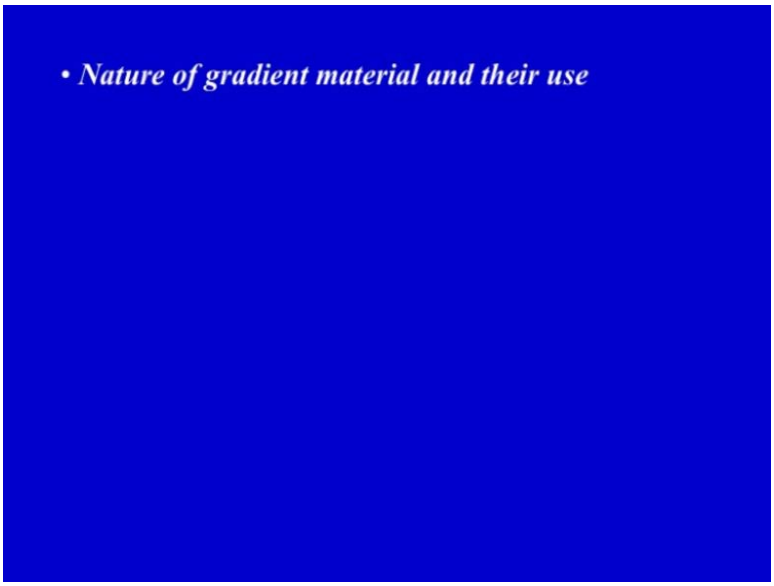

Now, let us discuss about nature of gradient materials and their use, the choice of solute depends up on the material to be fractionated. The gradient material should have certain properties, like it should permit the desired type of separation. So, gradient material should not obstruct or in any way hamper the particular type of separation. Gradient material should be stable in solution, it should be inert towards biological materials and not react with the centrifuge rotor tubes or caps.

That is very important that, the particular density gradient material should not be interacting with either biological materials or the different physical material like centrifuge or rotors or otherwise, so that is very important. Gradient material should not absorb light at wavelengths appropriate for spectrophotometric monitoring. For example, if you are going to monitor say, proteins at 280 nanometers or any other analyte at other wavelengths then gradient material should not be interfering in that, it should be sterilizable, it should be non toxic or flammable.

So, those are properties, which are very important for gradient material, also they should be having negligible osmotic pressure and cause minimum changes in ionic strength, PH

and viscosity. Gradient material should be also inexpensive and available in pure form, so that is also very important part of experiments. Gradient material should allow easy separation of the sample material from the gradient medium, without any loss of the sample or its activity. So, gradient material in anyway should not affect the activity or the amount of the sample, which you are trying to separate from the gradient material.

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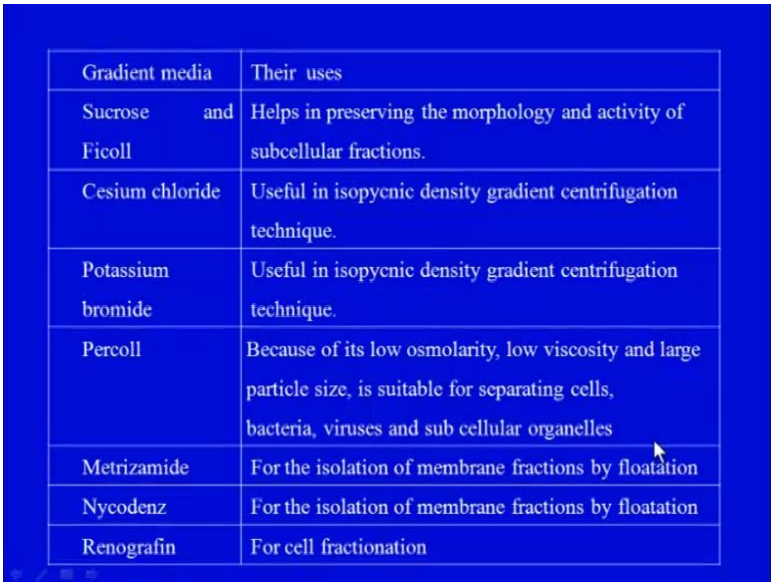

Gradient media	Their uses
Sucrose and Ficoll	Helps in preserving the morphology and activity of subcellular fractions.
Cesium chloride	Useful in isopycnic density gradient centrifugation technique.
Potassium bromide	Useful in isopycnic density gradient centrifugation technique.
Percoll	Because of its low osmolarity, low viscosity and large particle size, is suitable for separating cells, bacteria, viruses and sub cellular organelles
Metrizamide	For the isolation of membrane fractions by floatation
Nycodenz	For the isolation of membrane fractions by floatation
Renografin	For cell fractionation

Now, there could be lot of different types of gradient material or media and they are used for different purposes, on your screen there is a table here, which lists the some of the gradient material and their uses. So, we can go one by one, sucrose and ficoll, they are a gradient materials in terms of, the sucrose and ficoll helps in preserving the morphology and activity of subcellular fractions. Cesium chloride, it is useful in isopycnic density gradient centrifugation techniques, like if you could recall, nucleic acids are mostly done on cesium chloride density gradient material.

Then potassium bromide, it could be useful in again in isodensity gradient centrifugation technique. Percoll, because of it is low osmolarity, low viscosity and large particle size, it is very suitable for separating cells, bacteria, viruses and sub cellular organelles. Then metrizamide, it is used for the isolation of membrane fractions by floatation, nycodenz, it is for the isolation of membrane fractions by floatation and then renografin for cell fractionation.

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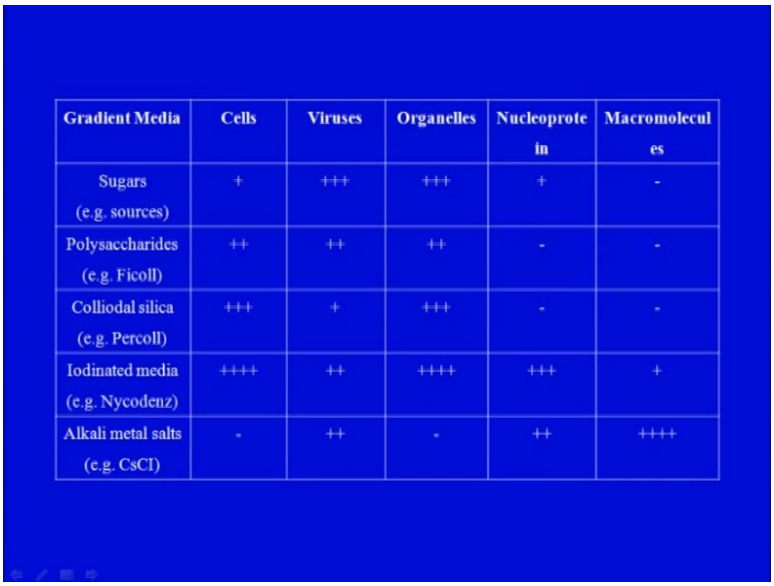

Gradient Media	Cells	Viruses	Organelles	Nucleoprotein	Macromolecules
Sugars (e.g. sources)	+	+++	+++	+	-
Polysaccharides (e.g. Ficoll)	++	++	++	-	-
Colliodal silica (e.g. Percoll)	+++	+	+++	-	-
Iodinated media (e.g. Nycodenz)	++++	++	++++	+++	+
Alkali metal salts (e.g. CsCI)	-	++	-	++	++++

So, there are lot of different, many more gradient material and they have different applications, different sugars like sucroses also comes in, but sucroses are disaccharide. Now, this table here gives a an idea of, where these applications could be there, it just summarizes what we were saying. For example, gradient media like sugars, is more useful say, viruses and organelles, here these signs tells that, highly favorable moderately favorable and low favorable.

Polysaccharides you can see, could be useful for isolating cells, viruses or organelles, colloidal silica is more favorable for isolating or separating cells and organelles. Iodinated media, much higher favorable for different types of cells, organelles and also for nucleoproteins. Likewise alkali metal salts, they are highly favorable for macromolecules, like we are talking about nucleic acids.

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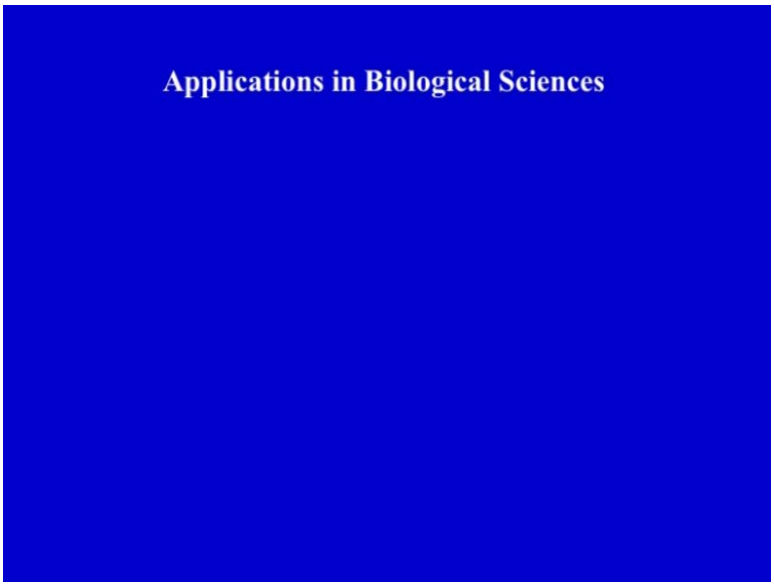

So, this is just summarizing different utilization patterns of these materials, there are lot of applications of the density gradient and other centrifugation methods in biological sciences. They are used for separations cellular and subcellular components as we have discussed, in differential and density gradients centrifugation, the centrifugation techniques are used for separating one cell type from another, they are used for removing cells or other suspended particles from their surrounding milieu, in either a batch or a continuous flow basis.

We are going to discuss about these rotors in a little while then centrifugation techniques are utilized in isolating viruses and macromolecules including DNA, RNA, proteins, lipids or establishing physical parameters of these particles from their observed behavior during centrifugation. Affinity purification of membrane vesicles can be performed, it is also utilized for studying the facts of centrifugal forces on cells, developing embryos and protozoa.

There are lot of like analytical centrifuges, like we have discussed, is used for determining relative molecular mass, estimation of purity of macromolecules, detection of conformational changes in macromolecules. So likewise, there are whole lot of applications of this centrifugation and ultra centrifuge techniques, and they are utilized routinely in different labs for different applications. So, till now what we have discussed about is, different kinds of preparative and analytical techniques.

Like, we have discussed about analytical ultra centrifugation method and also we have discussed the preparative methods like differential method and density gradient method. Also we have discussed about applications of these methods in various areas for various kinds of different scientific experiments.

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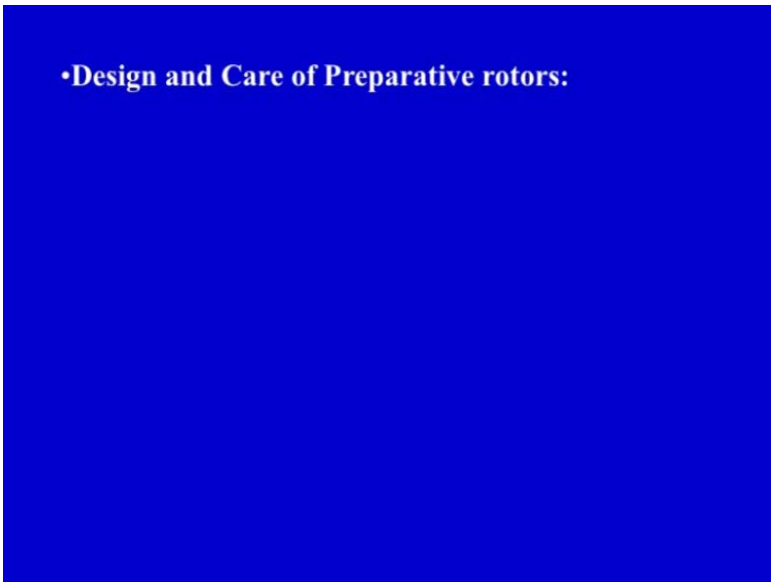

Now, let us extend our discussion to the rotors actually that is, the design and care of these rotors, which are utilized for different applications. So, at centrifuge, rotor is the rotating unit of the centrifuge, which has fixed holes drilled at an angle. Now, this is like, in particular, they could be different types, as we will discuss now. Now, in these drilled holes, test tubes or different containers are put in and the rotor is spins to 8 in the separation of the material.

So, the material is put in these tubes and these tubes are then put in to the rotor, so there are three types of centrifuge rotors mainly, swing bucket rotor, fixed angle rotor and vertical rotors. Also, we will be discussing about zonal rotors in a little while and continuous flow rotors. Now, centrifugal force created by a spinning rotor, generates loads of stress on the rotor material. So, when there is very high centrifugal force then the material of the rotor should be such that, it can bare that stress.

So, the materials used in rotor construction, one is that, many materials could be utilized most of the time. There are certain materials which are good for construction of rotors, like they could be made up or made of brass or steel or aluminium alloy and titanium

alloy. So, rotors for low speed like say, where they will experience lower degree of stress, will be made mostly of say brass or steel or prospects. So, these are rotors, which are not spun at very high speeds, there will be low speed rotors, as we have discussed in like certain low speed centrifuges.

But, rotors for high speed, will experience much higher degree of stress and so they are made up of the metals, which could take up that stress and so these high speed rotors are made up of mostly aluminum alloy or titanium alloy. For very high speed, titanium alloys are utilized and they are like much more inert stable and they can bear much higher stress also. So, titanium alloy having greater strength to weight ratio, are more robust, they experience less metal fatigue and they are resistant to corrosion.

Aluminum rotors are more susceptible to corrosion and being readily attacked by acids, alkalis or salt solutions, like high concentration salt solution. So, rotors are given protective coating to metal surfaces, either by anodizing in case of say, aluminum rotors or applying a black epoxy paints, so they should be protected and the protective coat is put on these rotors actually. For improvement in rotor safety, materials such as carbon fire are used for rotor construction. So, there are lot of various precautions and care is taken in construction of rotors so that, they can have a high strength and they can bear the stress, because of high centrifugal field.

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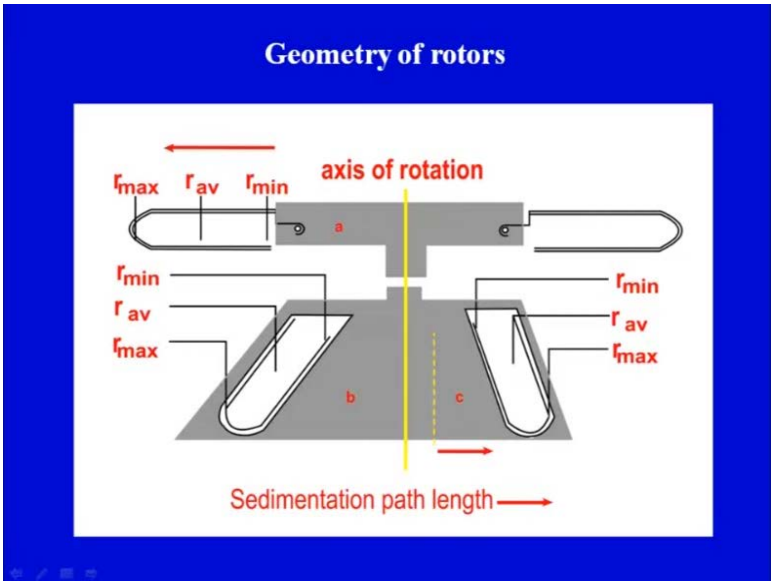

The general geometry of rotors could be like, as you see on screen, for swinging bucket rotors, it is like these buckets or these containers are. As the centrifuge is switched on, due to the centrifugal force, they will be extended outwards and this will be for swinging bucket rotor. Now, if you can observe here, there are r minimum sent, this is axis of rotation and the distance from the axis of rotation, which is, there is the three distances given r minimum, r average and r maximum.

And this is because, at different parts of the tube, the particle here will experience different centrifugal field and particle here will experience a higher centrifugal field, because of the distance. So, this particular one, many times r average is taken for calculations, but the particle at different places due to the distance from the axis of rotation, will experience varying applied centrifugal field. Likewise, in angled rotor, this is like, there are drilled holes and the tube is fixed, so it will not extend like the swinging bucket here. But, here also you will have r minimum, r average and r maximum, and r average will be taken for this particular purpose for calculations. The vertical rotors, third one will have vertical like, there will no angle here, it will be totally vertical and they will be another type of rotors.

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Different types of rotor head, corresponding RPM and RCF

Rotor	RPM	RCF *g
12*1.5 ml Angle	20,000	26,600
10*8 ml Angle	16,000	21,750
6*30 ml Angle	12,000	12,880
20*5 ml Angle	17,000	22,620
9*1.5 ml Swing out	10,000	9,620
24*1.5 ml Angle	17,500	23,970
4*30 ml Angle	16,500	20,100
4*50 ml Angle	13,000	14,200
24*05 ml Angle	20,000	26,600

There are different types of rotor heads and corresponding to particular RPM and RCF value, there whole lot of different types, we have listed some of them here on your screen. So, if you can see, say one rotor could be contain, can take 12 tubes or 12

containers and like say centrifuge tubes, here 1.5 ml angle rotor, now it can go upto 20 000 RPM and RCF value will be around 26600 g. Likewise, there could be other, like say it could 10 slots for 8 ml material, it is a angle rotor and RPM is 16000, where RCF is 21750.

Likewise you can see, there are whole lot of different centrifuges, which we have listed in here and you can see that, depending on the type of centrifuge and rotations, RCF will be done. So, like if you know what RCF has to be calculated then from nomogram, you can calculate or you can put the revolutions per minute for a particular rotor and then it could be operated. So, these are different rotors, but they could be many more different types of rotors, which are swinging bucket, angled or vertical, and they will have particular kind of RCF and RPM value. Remember that, these rotors for a like higher RCF value, they will be much stronger rotor in terms of, what metal or alloys they are made of. If they have low speed rotors then one does not have to really go for alloys like aluminium alloy or titanium alloy, because these will become expensive as the rotor material is different.

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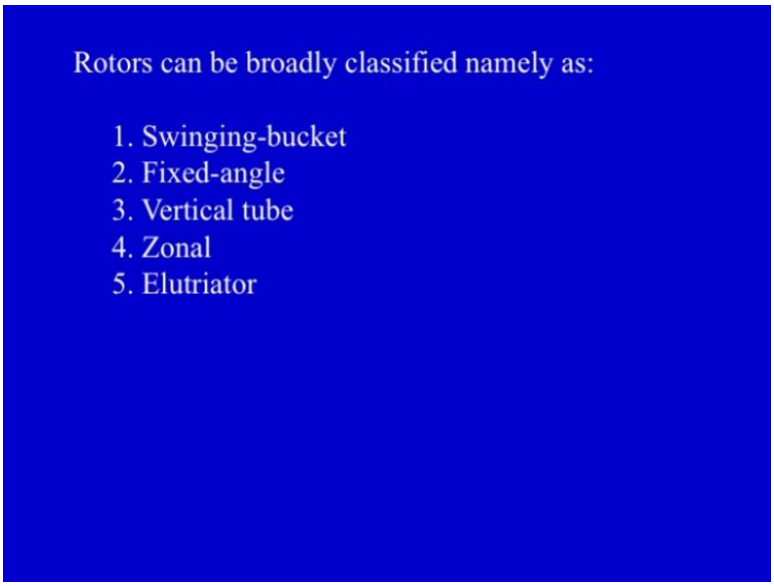

Now, let us move on to like different types of rotors, like we were saying there could be swinging bucket, essentially like for routine purposes. There could be three kinds of rotors swinging bucket, fixed angle and vertical tube rotors, but like I said, there are other rotors for special purposes, like they are utilized in say, density gradient

centrifugation or other applications like zonal and elutriator or you can say continuous flow rotors. Each type of rotor has strengths and limitations, depending on the type of separation, so let us discuss each of them one by one.

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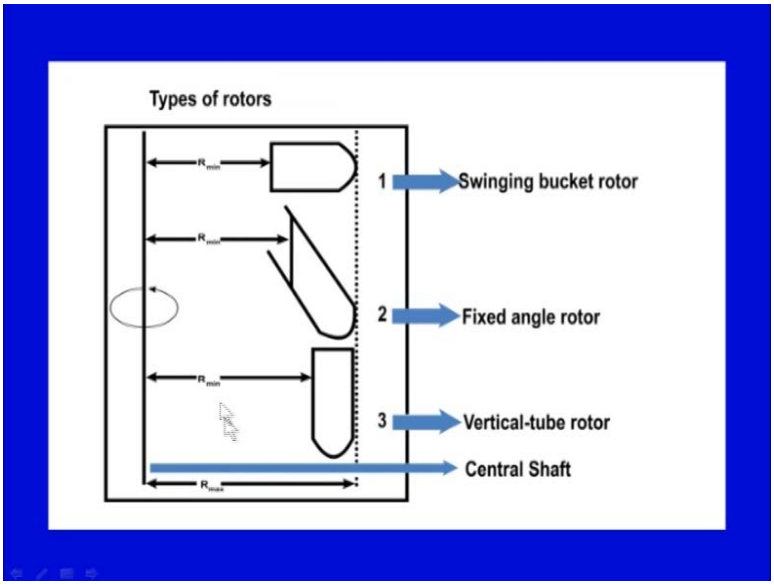

On your screen, you can see that, there are three rotors we have shown you here, this is the position, where it is swinging bucket rotor, this is angled or fixed angle rotor and this is vertical tube rotor, where tube is in vertical position. This is the central axis of rotation, now if you see here that, how this whole thing when a spinning takes place occurs.

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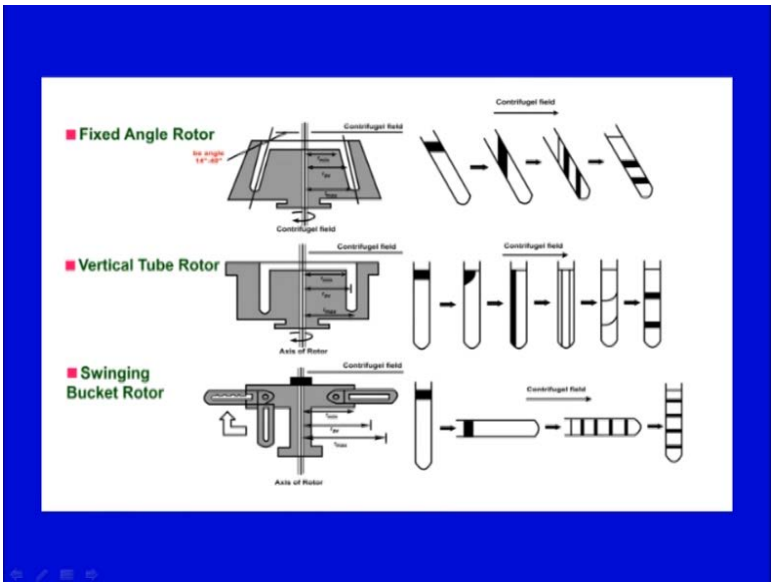

If you see on your screen, the fixed angle rotor like these tubes are put at a angle, which could be from 15 degrees to 40 degree or 45 degrees and depending on that, they will have different r minimum, r maximum or r average. Now, if you see here, these are the positions, which are being given when rotor is at rest. So, first tube here, when it is put into this rotor and this rotor is placed in the centrifuge on the central drive shaft.

And when the rotor is switched on and program for a particular revolutions per minute or particular RCF then as the centrifuge begins at the resting position, this will be the position of the your meniscus here or the liquid, which is placed in the tube. As the rotor starts and centrifugal force is applied, the position of the, from here the position due to the centrifugal force that is, outward centrifugal force, which is in this direction will be like this.

The separation will take place of this sample and different layers will be formed if it is a density gradient or otherwise and then after a period of time, the rotor will be slowed down and finally, it will come to rest and then it will return to this particular position. So, these layers which were in this particular formation due to the centrifugal force, they will be in the resting position in here. So, you can see that, the series of representations for, while it is at rest, while it is spinning and then again it comes at rest with the separated analytes.

In vertical tube rotors, if you can see here, in vertical tube rotors, these are in tubes or particularly in vertical position, there is no angle as in angle rotors or fixed angle rotor. So, you have layered your sample on the top and this is the resting position here, now as the spinning starts, since like I said it is outwards force, centrifugal force is in this direction. So, everything is oriented in this direction that is, there all like vertical way, this is distributed in vertical fashion then separation takes place in their direction.

And after sometime, when it again comes to rest, it again reorients to the resting position with the separation achieved. In swinging bucket rotor, like here these are the resting position, this is the resting position of the tube. Now, here these are not restricted actually, this tube here which is hanging in here, is not restricted in here and as soon as the centrifugal force is applied, these buckets or these containers, they extend outward, because of the centrifugal force.

The centrifugal force is in outward direction from the center of rotation or axis of rotation, so both tubes extend outwards. And so if you see the resting position, this is this and then when it is a extended outward, this layer will be distributed here, separation will take place. And again when the centrifuge is switched off or when it is slowed down, it comes to rest then this position will be achieved. So, you can see that, pelleting happening in here, if pelleting has to happen then it has to happen on in fixed angle rotor on this wall here, outer wall.

Likewise here, if pelleting have to occur, it will occur on outer wall here, in vertical tube rotors also and then it will slide down somewhere. But, in this case, they will be, directly pelleting will be not to on wall, but it will be a directed to the bottom actually. So, this is the difference between swinging bucket rotor and angle rotors, all these three, mostly vertical tube rotor is not so much used, but fixed angle rotor and swinging bucket rotors are widely used for different applications.

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Type of Rotor	Pelleting	Rate-zonal Sedimentation	Isopycnic
Fixed-angle	Excellent	Limited	Variable*
Swinging –Bucket	Inefficient	Good	Good**
Vertical	NS	Good	Excellent
Zonal	NS	Excellent	Good

NS = Not suitable
*Good for macromolecules, poor for cells, and organelles
**Good for cells and organelles, caution needed if used with CsCI

So, we summarize like different types of rotors and their use here, now fixed angle rotor is excellent for pelleting, but it is limited for rate zonal sedimentation and isopycnic also it could be utilized, it is like not compulsory. Swinging bucket rotors is not very efficient for pelleting as such, but it is good for rate zonal sedimentation or isopycnic sedimentation that is, density gradient centrifugation.

Then, vertical and vertical rotor is not suitable for pelleting, swinging bucket could be utilized for pelleting, it is not that it could not be utilized. Then rate zonal, just this is quite good vertical for this particular one and it is quite good for isopycnic. Likewise, zonal rotors are excellent for density gradient centrifugation and not so much for pelleting.

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So, this is like, how these rotors could be applied or could be used for different applications here, right from pelleting to say rate zonal or isopycnic or other separation methods. Now, little bit about swinging bucket rotor, so swinging bucket rotors, sample tubes are loaded into an individual buckets that hang vertically, while the rotor is at rest, like I have shown you here. When the rotor begins to rotate, the bucket swings out to a horizontal position, so that during centrifugation, the tube and it is solution is aligned perpendicular to the axis of rotation and parallel to the applied centrifugal field.

Now, the tube returns to it is original position during deacceleration of the rotor, so I have shown you all these positions and how the liquid is or the material is oriented in there. Now, as centrifugal field is exceed, particles in centrifugal field fan out radially from the center of rotation, rather than sedimenting in parallel lines. So, longer distance of travel may allow better separation such as, in density gradient centrifugation. Now, some particles may strike against the wall of the tube and then travel along the walls causing convection current.

So, that is how, wall effects is another problem with these rotors, undesirable swirling effects cause mixing of tube contents and also doing rotor acceleration and deacceleration, this could occur. Control of swirling effects and convection can be achieve by slowly accelerating and deaccelerating the rotor, that could be done like, this occurs mostly during acceleration and deacceleration. Now, just usually support samples ranging and volume from say, few ml to very, like liters actually.

Now, this depends on, like how big the sample bottle size is and accordingly, this could be done, it could be used for like large volumes are utilized for pelleting also for many times or for other applications and different amount of volumes could be taken for different application. Swinging buckets can support two types of separation, they could be rate zonal or isopycnic and rotor is relatively inefficient for pelleting. But, it does not mean that, they are not utilized for pelleting, like for many times, if you are harvesting say bacterial cells in your having large amount of material, the swinging bucket rotors can be utilized like large buckets could be utilized for pelleting purpose also.

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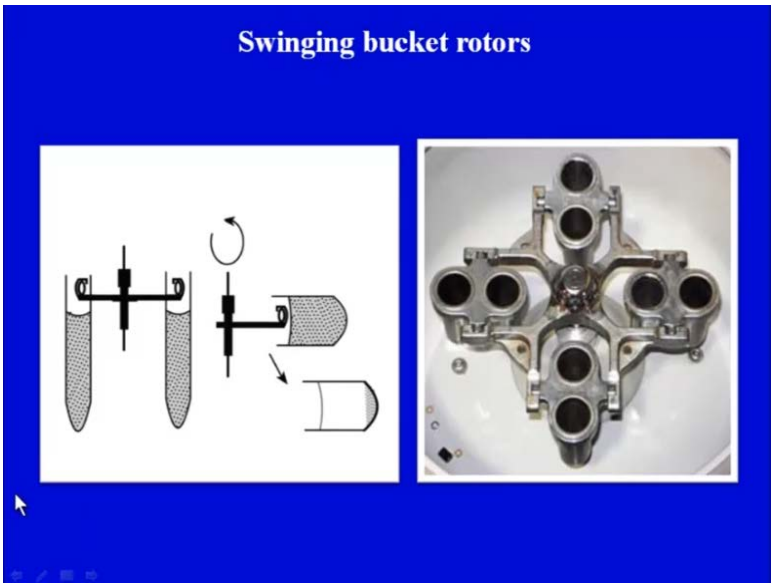

So, here you can see, these are the tubes and they like hang in here and then they extend outward, when this is in this fashion. These are like large buckets, here tubes could be placed, here swinging bucket rotors. Remember to balance them like, if you are putting one two here then two balance has to be here and large samples could be achieved, two could be put in here, so these are swinging bucket rotors.

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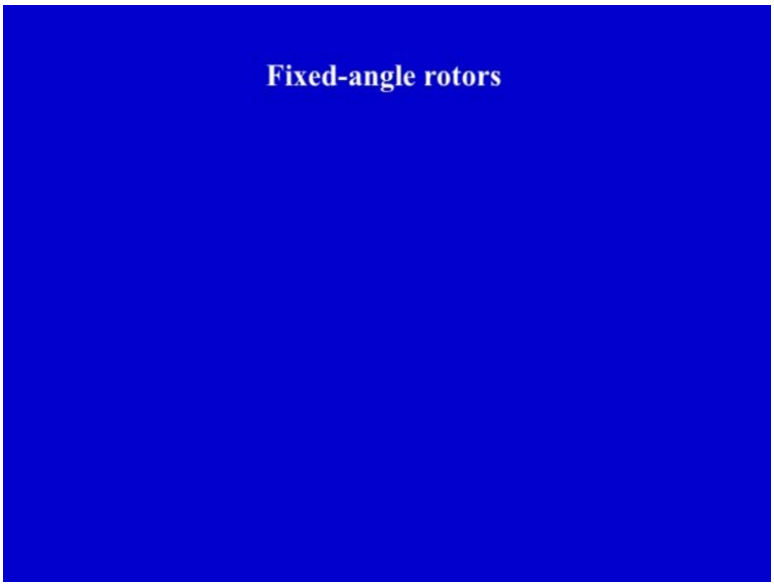

Now, fixed angle rotors, in fixed angle rotors, sample tubes are held at fixed angle and these could be ranging from say, 20 or 15 degrees to 45 degree in the rotor cavity. And when the rotor begins to rotate in this case, the centrifugal field is oriented at an angle to the tube wall, so the solutions in the tubes reorients actually. Fixed angle rotors are usually used for pelleting applications to either pellet particles from a suspension and remove the excess debris or to collect the pellet.

Now, examples include pelleting say bacteria, yeast and other mammalian cells, it is also useful for isopycnic separation of macromolecules such as, nucleic acids. Rotor cavities range from like say, from 0.2 ml to 1 ml or may be higher, the most important aspects in deciding to use the fixed angle rotor is a k factor. The k factor indicates, how efficient the rotor can pellet at maximum speed, the lower the k factor, the higher the pelleting efficiency.

Remember here also, different types of rotors are available, which can range from few like centrifuge tube rotors or micro fuses kinds of rotors to very high volume rotors like say, 50 ml rotors or more are available. So, and with different tube capacities like say, for example, they could be 6 tube rotors or may be higher, depending on the volumes which are taken, more is the volume, less number of tubes could be accommodated in there.

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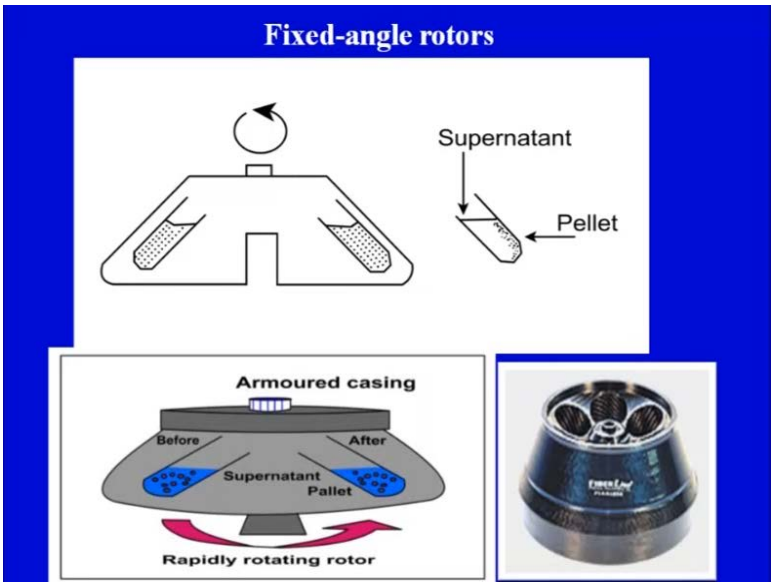

This is the representation of a fixed angle rotor, now if you can see here, the tube here is placed at a particular angle in a slot and the axis of rotation, which rotates in this particular direction. As the rotation occurs, if it is a pelleting application, pellet will be settling or pallet will occur at the outer wall of the tube. So, the reorientation of the liquid material like, you can see here reorientation will take place, as I have shown you earlier, as the centrifugation starts.

There is another representation here, where you can see the rotor and the two tubes put in here. Now, this can carry more tubes as per the rotor capacity and the volumes each tube carries, now you can see here that, the material is spread in here and when pelleting will occur, they will pellet on the outer side of the wall, on this wall here. So, this is how, the angle rotor will look like and this is like typical angle rotor, you can see the tubes can go here, these are larger tubes can go in here. This is placed into the centrifuge and on the central drive shaft and then it is spun at a particular RCF value, as per the specification of a particular rotor.

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So, we come to the third one that is, vertical rotors, now vertical rotors are highly specialized, these are 0 angle fixed rotors. Now, here there is no angle, like in fixed angle rotors, if you could recall, there was like from 15 or 20 degree to 45 degree angle could be there, but here there is no angle, as I have shown you earlier. Sample tubes are held in vertical position during rotation.

So, during operation of the rotor, solution in tube reorients through 90 degrees during rotor acceleration, to lie perpendicular to the axis of rotation and parallel to the applied centrifugal field and this will again return to it is original position during deacceleration of the rotor. So, like here, you have seen that, the reorientation takes place at 90 degrees, now sedimentation can be achieved more quickly than fixed angle or swinging bucket rotor, because the path which has to be travelled here is very small.

If you see in swinging bucket rotor, since the tubes extend outwards, the path is the highest, like for pelleting much higher path has to be travelled to reach to the bottom of the tube. But, in case of vertical rotors, the path traveled like, it is a very small tube and since it has to just travel a little only so then this here pelleting is much or sedimentation is much quicker and then vertical or then fixed angle or swinging bucket rotor and vertical rotor takes the most time.

Vertical rotors have very low k factor, which is useful if the particle must only move a short distance, until it pellets. So, run time on vertical rotors are very short, but it

sometimes, if you require that, particle should move larger distance then this might not be very appropriate. Now, this type of rotor is not suitable for pelleting applications, but is most efficient for say, density gradient centrifugations, isopycnic separations, due to the short path length and applications could include like isolation of say, plasma, DNA, RNA, lypoproteins, etcetera.

These are typically used to bend DNA in cesium chloride also and so vertical rotors also have certain applications, may be limited though, as compared to fixed angle or swinging bucket rotors, which are widely used for many different types of applications. Now so this was about three kinds of rotors, which are kind of very common rotors. Now, there are other rotors, which we were talking about, like zonal rotors and the continuous flow rotors, which also comes in zonal rotor only.

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So, zonal rotors, these are you can say, spherical or cylindrical shape, which should be flat end sphere or certain cylindrical kind of shape could be there and the interior of these rotors might be divided into sectors, may be four sectors by septa. Zonal rotors are designed to minimize the wall effects and that is why, their shape is for minimizing the wall effect, as we will see in the next lecture. These are like, these wall effect which are encountered in swinging bucket and fixed angle rotors are minimized quite a lot here and also these rotors are utilized for large sample size, which could not be done in other rotors, very routine rotors.

Batch type rotors, it could be like, zonal rotors could be of two kinds, one is batch type, another could be continuous flow rotors. Now, as name suggest, batch type rotors will be one, where you do a particular thing in batch format and another rotor would be, where in continuous flow rotors, there very large volumes are utilized. The sample is applied continuously, the cells pellet or the material particle pellet, rest of the solution or liquid is driven out in these rotors, we are going to discuss about one of these rotors in the next lecture.

Now, batch type rotors are most extensively used, they have less wall effects, they have more sample size could be accommodated in there and based on sample loading and unloading, there could be different batch type rotors. So, there could be batch type rotors, which are low speed batch type rotors, there could be rotors which are high speed batch type rotors then there could be on differences in unloading and loading, like in batch type rotors, there could be dynamic or static type of rotors.

The dynamic rotors, the loading is done while rotor is spinning at a slow speed and then unloading also done when it is brought to a lower speed. But, in static rotors, they are at rest when loading or unloading is done, but they are slowly accelerated first to a particular speed then they are accelerated further to operating speed. So, there are differences in their operation and the way they are loading and unloading is performed in these rotors.

Likewise continuous rotors, there is a single rotor, rather than or you can say, there is single slot in there and they are designed in such a way that, there is an inlet and there is an outlet. From inlet, continuously the solution or the liquid containing the material, let us say for example, large volumes containing cells, could be pumped in and then sedimentation of the particles occur and the rest of the liquid could be going out from the outlet so that, large volumes could be worked up on here.

So, in this lecture to summarize, we have discussed about different types of rotors, particularly we have discussed in this lecture about the swinging bucket rotor, fixed angle rotor and vertical type of rotor. We have also seen about, we also discussed about the types of materials, which are used for making the rotor and the care taken for these rotors, like they should have capacity to bare the stress, which is generated due to the high centrifugal fields.

They should be inert or they should not be effected by various types of solutions, which are used in these rotors like for say, acidic, alkaline or high salt concentrations, so all these things are important for maintenance and long life of the rotors. In this lecture, we have introduced the zonal rotors and in the next lecture, we are going to extend our discussion to the zonal rotors and also the care. In detail we are going to discuss about the various aspects for the rotor various parameters and other aspects for taking care of the different types of rotors and their use. So, we will be discussing in the next lecture, about zonal rotors, batch type rotors and continuous flow rotors, as well as the care of the rotors.

Thank you.