Aspects of Biochemical Engineering
Prof. Debabrata Das
Department of Biotechnology
Indian Institute of Technology, Kharagpur

Lecture – 57
Downstream Processing – I

Welcome back to my course aspects of biochemical engineering. I was discussing last couple of lectures on the different biochemical processes and we try to understand that how the chemical reaction kinetics can be applied in the biological system for better understand on the process like enzymatic reaction kinetics and the microbial growth kinetics and after that I try to discuss the transport phenomena how different transport mechanism that involve in the bioprocess.

Then finally, I also discuss some kind of upstream processing like ah like we have air sterilization and medium sterilization, which is appears to be the very important as per biochemical industry is concerned. Now downstream processing in other way it plays very important role in the biochemical industry because ah because the purity purification of the product is essential for increases market value.

And if you look at the type of chemical that is available in the market that is of two type, one is a of analytical grade, another is commercial grade. Analytical grade the purity of the chemicals is more than 99.9 percent and that commercial grade about 90-95 percent.

So, the cost difference is huge because the analytical grade may be as I high as four times as compared to commercial grade. So, purification processes we required lot of steps involvement that that I want to give a typical example of the citric acid industry where we find the citric acid is produced by aerobic fermentation process using aspergillus from cane molasses and after citric acid formation what do you do?

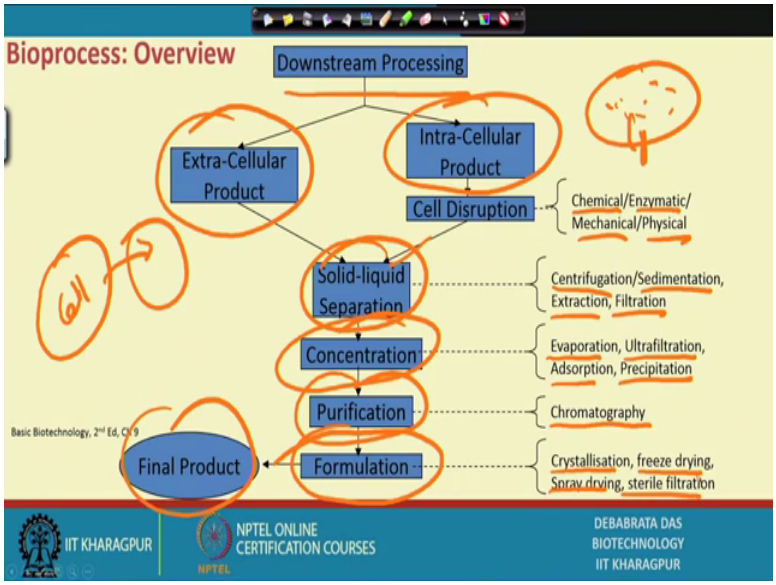
First we separate out the cells then the filtrate we pass through the treat with lime for the separation of in the form of calcium citrate ah, this calcium citrate I separated with the help of some kind of a filtration and then calcium citrate hydrolyze with sulphuric acid to form gypsum and citric acid gypsum again separated out with the help of gypsum filter.

Then again this citric acid you concentrate in the evaporator to concentrate 22 percent to 60 percent then we pull it down and to crystalize this citric acid and crystals of citric acid

separated within the centrifugation machine then this crystals further is dried in the drier drum drier and finally, we pass it through the sieving machine to get the different size of crystals and finally, we packed in the policy impose and sell in the market.

So, what I wanted to point out the difference steps are involved in the downstream processing now let us say let us a this particular presentation I try to discuss.

(Refer Slide Time: 03:30).



How what are the different steps involved in the downstream processing and if you look at the in the biological system we have two type of product, one is extra cellular product, another is intracellular product.

Extra cellular product means suppose this is the cell and if the products comes out of the cell and then it will be easy for separation because we first we separate out the cell then filtrate we purify to get the product, but intra cellular cells is something different the product present inside the cells.

So, we shall have to break the cell wall. So, that the product should comes out from the cell and then we can purify the product thus it is exactly that has been shown here that you know filtrate come this way and this way we then we have solid liquid separation we do the concentration, purification and we do the formulation and then we have the final products.

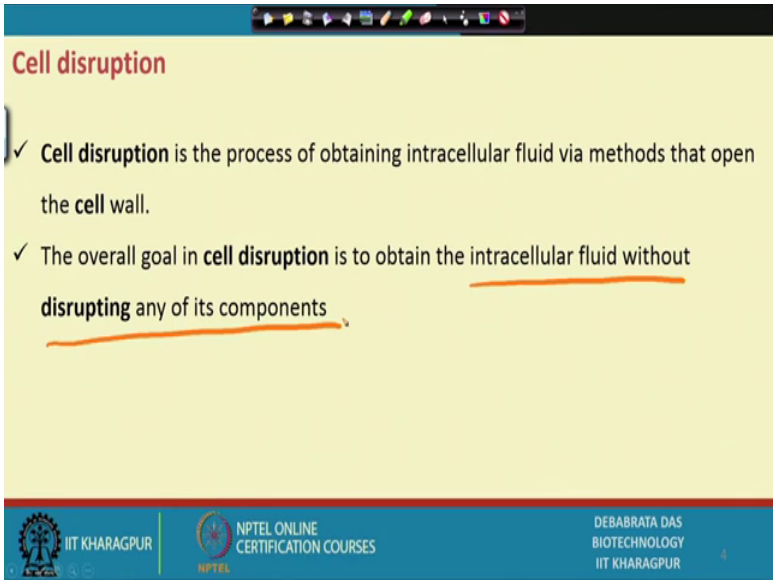
Now, in the different steps we have different type of a downstream processing involved that in case of cell disruption we have chemical enzymatic mechanical and physical processes solid liquid separation we have centrifugation, sedimentation, extraction and filtration in case of concentration we have evaporation, ultrafiltration, adsorption, and precipitation purification. We have chromatography and formulation; we have crystallization, freeze drying, spray drying and the sterile filtration.

(Refer Slide Time: 05:00)

Now, this downstream processing again varies on the different parameters as per example if you with respect to size we have filtration that membrane the separation and centrifugation if you wanted to a separate with respect to density then we have we have centrifugation, sedimentation, and floatation.

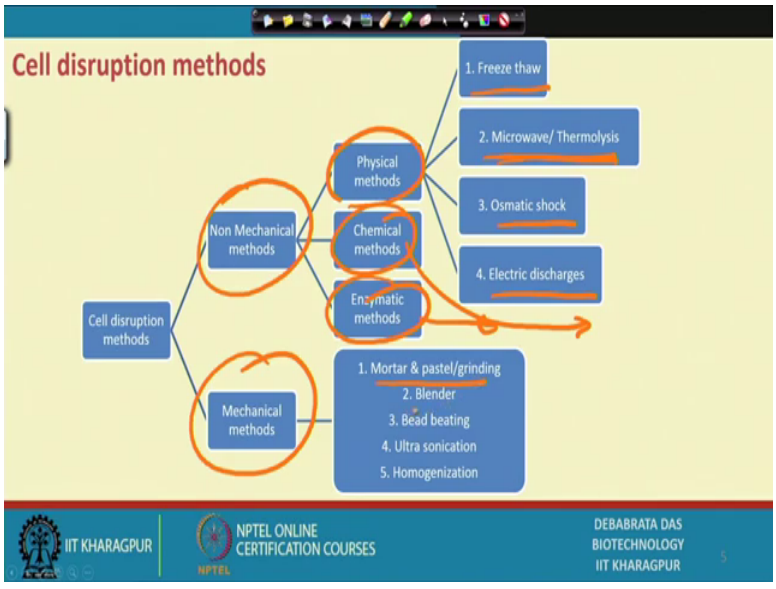
Now, with respect to diffusivity we have membrane separation, with respect to shape we have centrifugation filtration and sedimentation, polarity we have extraction, chromatographic and the adsorption, solubility we have extraction, precipitation, crystallization, electrostatic charge we have adsorption, membrane separation, and electrophoresis and volatility we have distillation, membrane distillation, and pervaporation.

(Refer Slide Time: 05:53)



So, these are the different processes that we involved and the cell disruption if you consider this is I told you that the main goal is to intracellular fluid without disrupting any component present inside the cell that is the main purpose of disruption process.

(Refer Slide Time: 06:09)

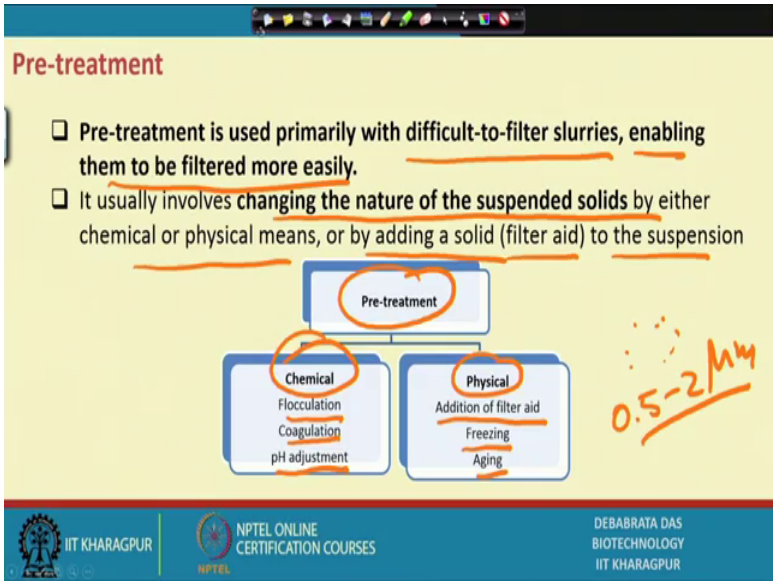


Now, cell disruption process may be divided into two types, we can see that one is non mechanical method, another is mechanical method. Non mechanical method we have physical method chemical method and enzymatic method, now in a physical method we have freeze and thaw and then we have a freeze and freeze and thaw we understand that we frozen the cells then we thaw it; that means, we liquify it.

So, if cells are bus the bus tree in between that then microwave and thermolysis then that osmatic shocks we due to osmatic cell take place electric discharge that also causes the breaking of the cells and then enzymatic.

We have lysozyme we use for breaking the cells, chemicals we have some kind of buffer we use for breaking the cell wall, mechanical we do it physically that is very mortar and pastel or grinding blender bead the beating ultra sonication and the homogenization. So, these are the different technique through which we can do this cell disruption.

(Refer Slide Time: 07:26)



Now, some pretreatment is required is used primarily difficult to filter the slurry enabling them to be filtered more easily. So, we first we separate out the bigger cell. So, the small cell will not pose much of problem to the separation processes, it is usually involves changing the nature of suspended solid by either chemical or physical means or by adding the solid filter aid to the suspension.

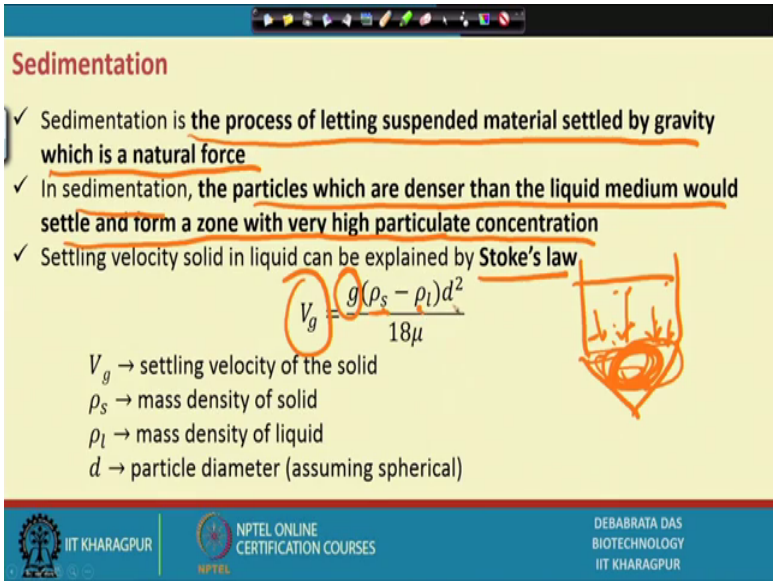
Now, pretreatment we have two type, one is chemical one is chemical, another is physical. Chemical I want to tell you suppose this bacteria cells they are very small in size 0.5 to 2 microns. Now this due to the small size it is very difficult to separate them. So, what is sometimes required some kind of filtrate So, that so, that the accumulation of the cells take place and particle size increase and then it can easily settle down.

So, this is a this is kind of what you call flocculation then coagulation we know we use the alum in the water treatment process to separate the colloidal matter present in the water pH adjustment there is the another technique that we have a physical method we have filter aid addition. I can give you the example of solka floc solka floc.

Basically is illogic material that is used in the chemical and biochemical industry for the separation of activated charcoal activated charcoal is a fine particles and if it present in the product it will looks like black and that is not permissible.

So, (Refer Time: 19:06) is used freezing and aging that is another process that we use. Now solid concentration can be done in two ways; one is sedimentation and another is centrifugation now.

(Refer Slide Time: 19:17)

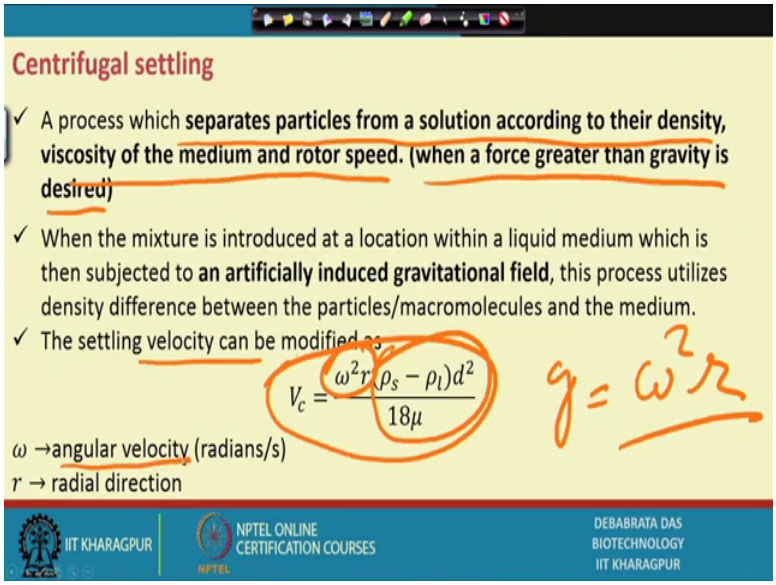


In case of sedimentation I told you that suppose this is the vessel and you have you keep the suspended particle like this. So, what will happen with respect to time you will find this particle will settle down like this am I right.

Now, this settle down of the particle is due to the gravitational force. So, sedimentation is the process letting the suspended particles settled by the gravity which is which is a natural force in sedimentation the particles which are denser then the liquid medium would settle and from a zone heavier a very high particulated concentration.

So, here we will be concentrating of the particularly very high as compared to this and we use the Stoke law for a for explaining the settling velocity of the particle g is the gravitation force and this is acceleration due to gravity this is rho is this is the density of the solid rho l is the density of the liquid d is the diameter of the particle and rho is the viscosity.

(Refer Slide Time: 10:22)

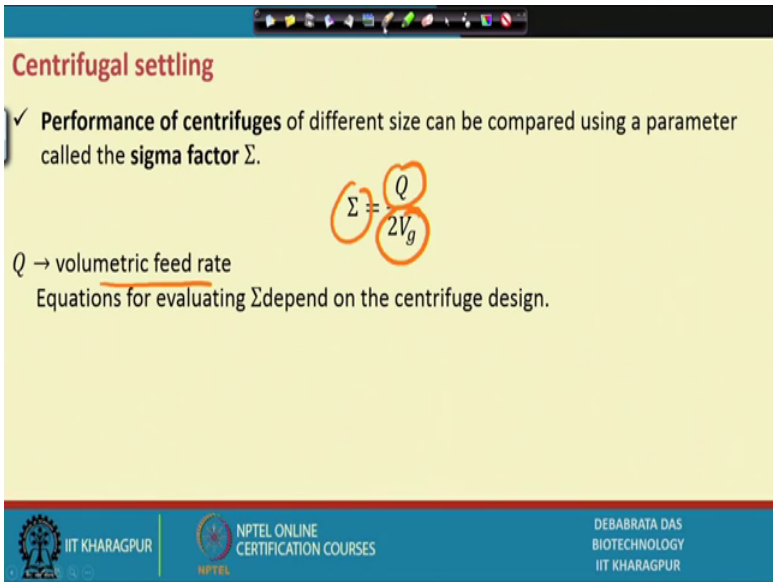


Now, in case of centrifugal force, it is we know in the centrifugal force settling we have 2 type of ah forces, the act on is one is centrifugal and centripetal, centripetal acts that you know towards the center and centrifugal is a towards the periphery.

So, your solid particle is a separated with the help of centrifugal force. Now the process with the separation of the particle solution according to their density and viscosity of the medium and rotor speed and when the force is greater then the gravity is desired.

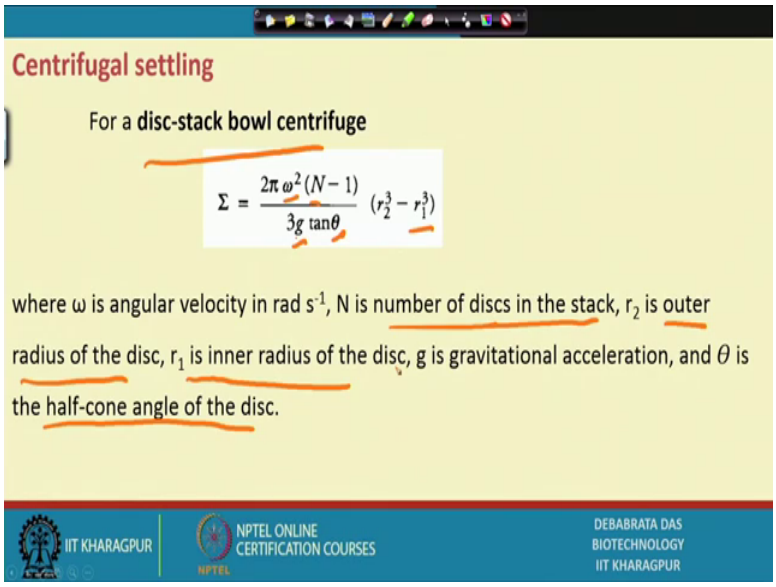
Now, here I can we can say you tell you that settling velocity is defined is the V c equal to omega square r rho s the, if you find this portion is the same as or settling velocity only, the gravitation force is replaced g is replaced by omega square by r am I right. The omega what is omega is the angular velocity this a radian pre second and r is the radials direction.

(Refer Slide Time: 11:32)



Now, in the, we have a sigma factor that we calculate on the basis of Q is the volumetric feed rate and V g that is the is the is the separation velocity that we have it is settling velocity that that can be that that is expressed by the sigma factors.

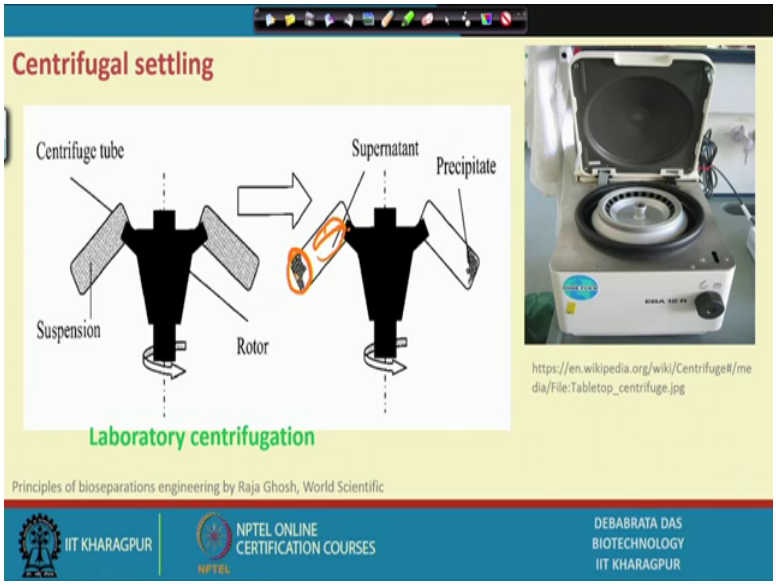
(Refer Slide Time: 11:54)



Now, disc-stack ah bowl centrifuge, we have this kind of equation that the sigma equal to twice pi omega square N minus 1 3 g theta, now omega is the angular velocity, N is the that number of the disc of the stack and g is the gravitation force.

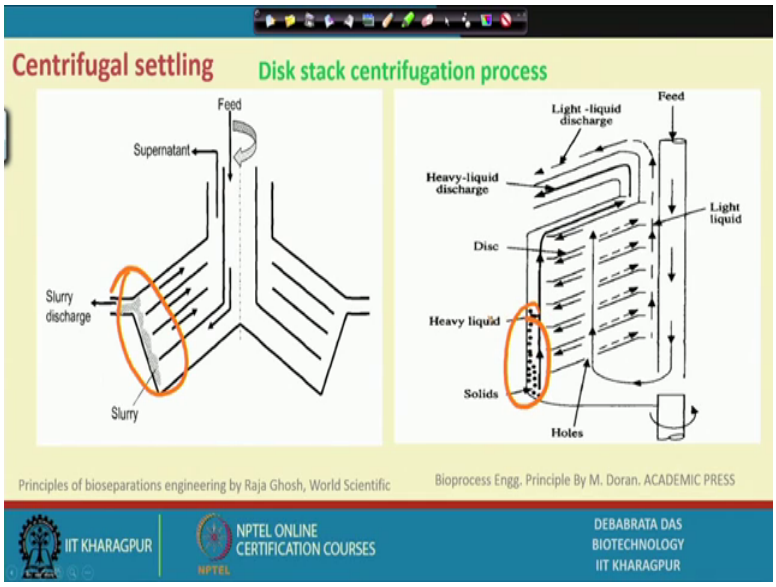
So, the attraction then acceleration due to gravity and theta is the half-cone angle of the disc r 2 square is the outer radius of the disc and r 1 is the inner radius of the disc.

(Refer Slide Time: 12:30)



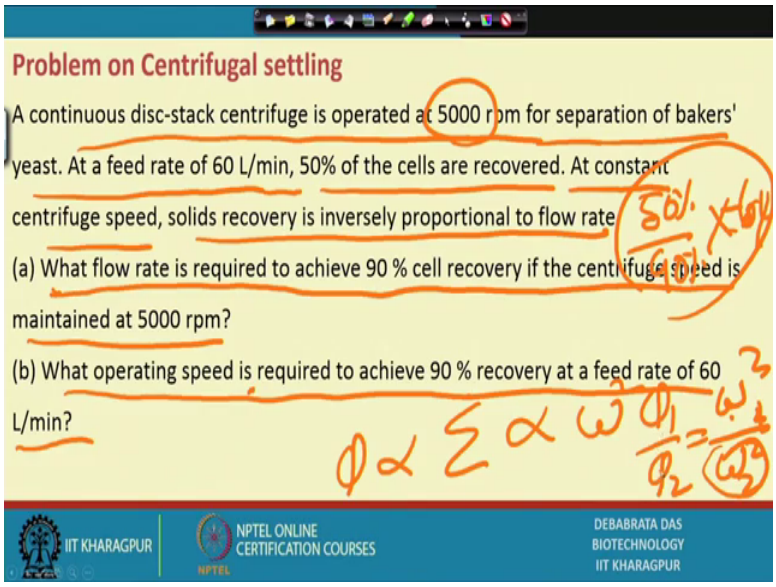
Now, this is how the centrifugal things that works when you centrifuge the liquid will be close to the center and your solid material will be at the periphery the this will be comes out like this how the solid separation take place in the centrifuge and this is the kind of lab centrifuge we using our lab.

(Refer Slide Time: 12:54)



This is the disc stack ah that centrifuge machine we have different discs that we have how the here how the solid material accumulation take place you can see it here that how solid that take place here.

(Refer Slide Time: 13:10)



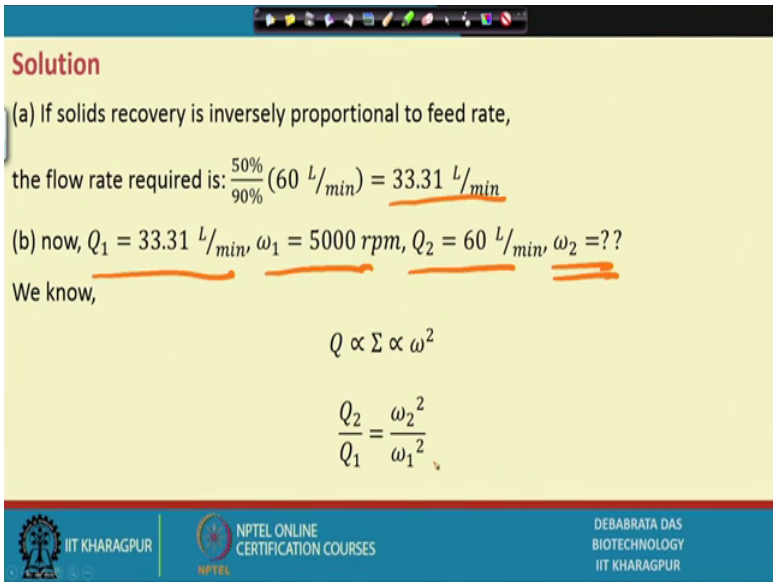
Now, we have a simple problem here a continuous disc stack centrifuge is operated 5000 rpm for separation of bakers is at a at a feed rate 60 liter per minute, 50 percent of the cells was recovered at constant centrifuge speed if the centrifuge speed is constant that is 5000 rpm the solid recovery is inversely proportional to be the. So, what is this, what flow rate is required to achieve the 90 percent cell recovery if the centrifuge speed is maintained 5000 rpm.

So, since it is inversely proportional I can always write 50 percent by 90 percent that it inversely proportional to that; that means, this is to be multiplied by your flow rate that is 60 liter per minute then you can find out the what flow rate is required to get this 90 percent separation of the cells.

Now, next problem is that what operational speed is required to achieve 90 percent recovery at a feed rate of 60 percent. So, here we have seen the sigma that is proportional to omega square and this is again it is proportional to Q. So, we can we can write Q 1 by Q 2 equal to omega square where 21 omega 1 square by omega 2 square am I right.

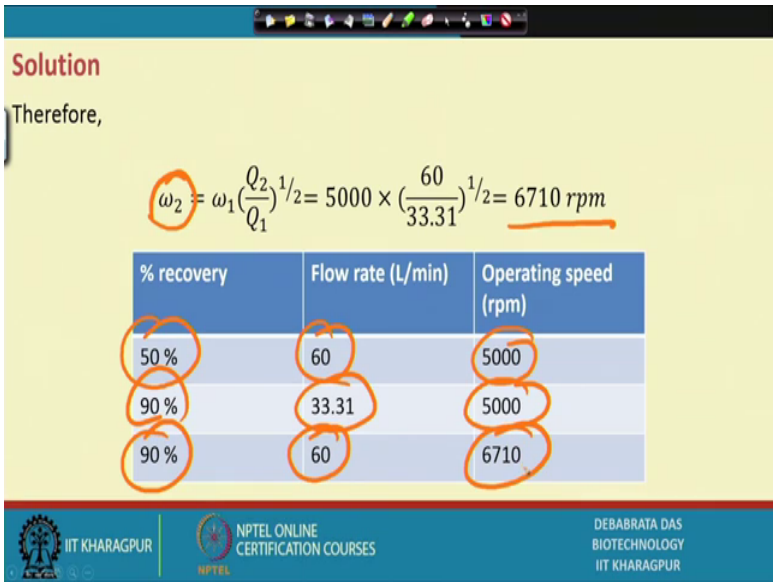
So, here what operating speed is required speed means the; that means, omega 2 you have to find out and omega 1 and the 90 percent recovery omega 1 we know that 90 percent separation we just calculate how much omega one is required Q 1 and Q 2 we know we can easily find out the omega 2.

(Refer Slide Time: 15:00)



Now, let us see how we have done here that ah here exactly what I am saying the 50 percent to 90 percent is 60 liter per minute we can get these 33.31 liter per minute and q 1. So, q 1 is coming like this q omega 1 is 5000 rpm the q 2 is 60 liter because we want to maintain the original flow rate what should be the omega 2 below the this relation we have then we can find out this omega 2 value am I right.

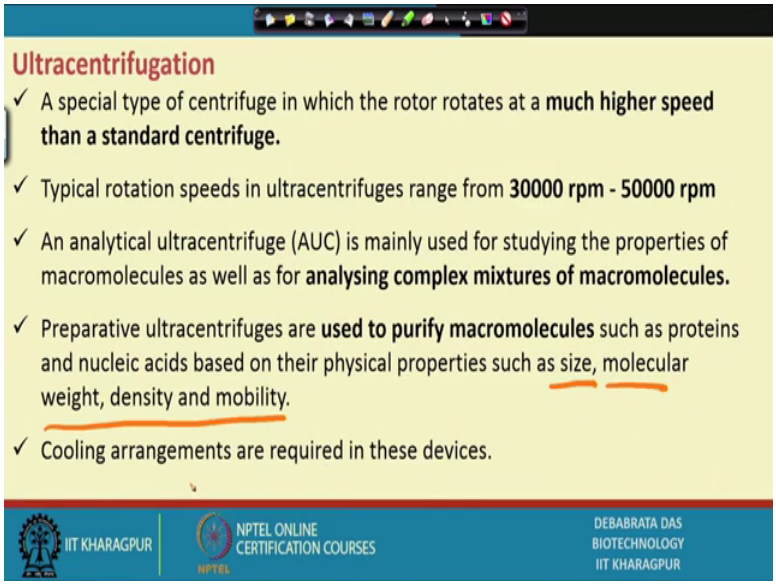
(Refer Slide Time: 15:30)



This omega 2 value we can calculate is coming about 6710 rpm. So, this is summarizing the table that at 50 percent recovery of solid at a flow rate 60 million liter per minute, operating speed is 5000 rpm, but 90 percent here it is solid separation, where flow rate will be should be reduced to 33.31.

If you want to maintain the same operational speed, but 90 percent recovery if you want to maintain the same previous flow rate then the rotational speed of the stirrer that if the centrifuge machine that will be increased to 6710.

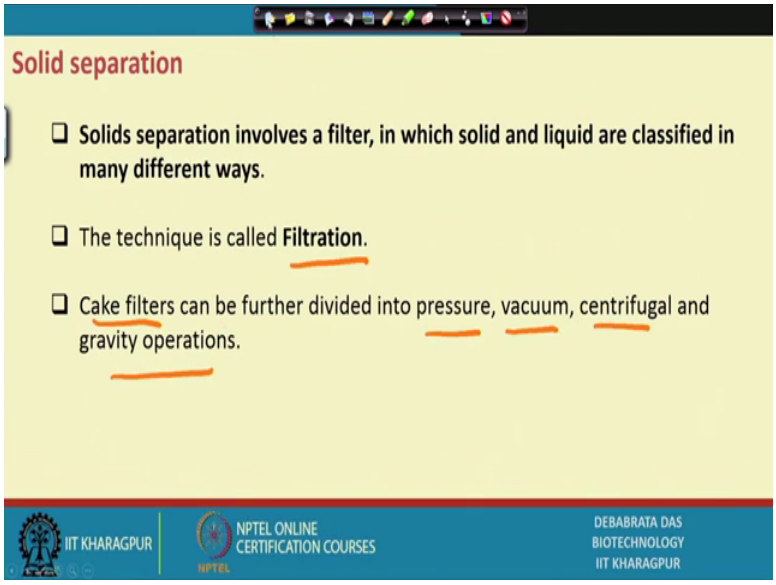
(Refer Slide Time: 16:15)



Now, ultra ultracentrifugation machine is another device where we can use for the separation of the fine particles small particle matter we can use as per example protein RNA DNA those can be separated with the help of this ultras ultracentrifugation machine and that is usually operated at the very high angular velocity the 30000 to 50000 rpm.

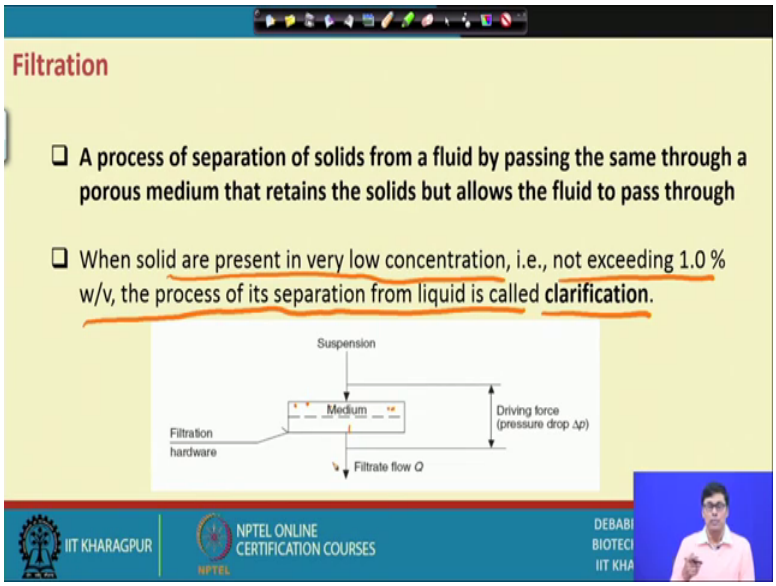
Now, you see that due to this we required lot of lot of heat generation for that cooling device is required. So, this is the main principles behind that is use the size molecular weight density and the mobility of the particles that involve for the separation of the macro molecules present in the in the in the liquid

(Refer Slide Time: 17:05)



Now, solid separation is involved the filter in which the solid and liquid is classified by different ways, one is filtration, another is cake filtered that the cake filter divided by is divided into pressure volume, centrifugal and gravitational operation.

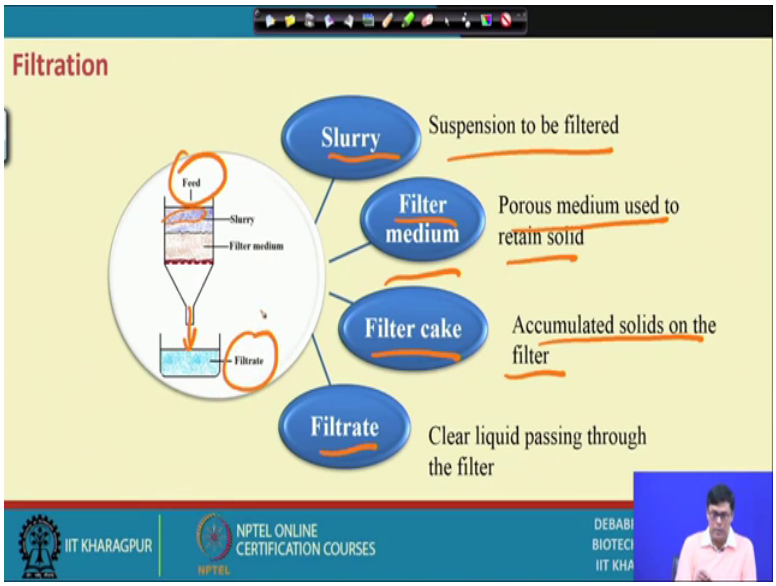
(Refer Slide Time: 17:28)



Now, the filtration technique is very simple this is the filter medium that we have we will filter we have and we put the suspended this is the filter medium, this is the suspension. So, here the suspended material here the suspended material that you know that they accumulated here and filtered with percolate, through this and here we use some time pressure. So, that you know rate of flux of the liquid increases and we will get more filtrate flow.

That ah when the solid is present is very low concentration non exceedingly 1 percent weight by volume the process is separation of the on the liquid is called clarification. So, this clarification basically deals with that separation of the solid from the liquid.

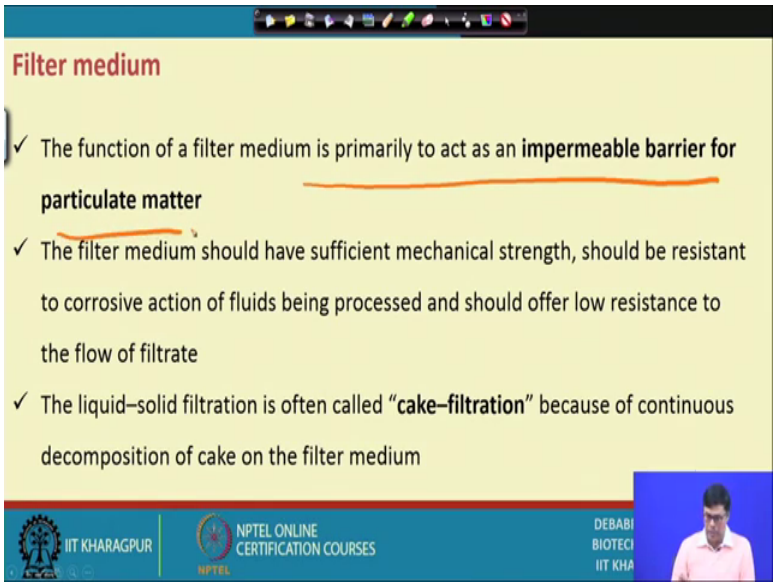
(Refer Slide Time: 18:22).



Now, we have this filtration we have different different things involved one is slurry then we have we have filter medium, we have filter cake and filtrate. Now slurry the suspended to be just to be filtered so, this is the feed where slurry is there that comes in the form of slurry and that is to be filtered.

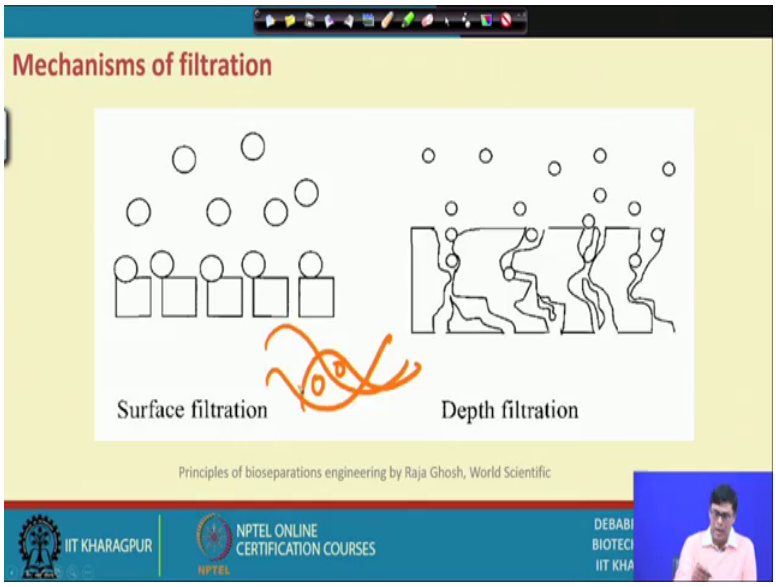
So, we put some kind of filtration medium when the porous medium is used to written the solid then we have filter cake this we can we accumulate of the solid on the filter that you know that cake formation is there and that whatever clear liquid comes out we call it filtrate.

(Refer Slide Time: 19:03)



Now, filter medium that function of filter medium is primarily acts on impermeable barrier to the particulate matter I can I can give the example here that you know that here that that you know that if you look at this.

(Refer Slide Time: 19:18)



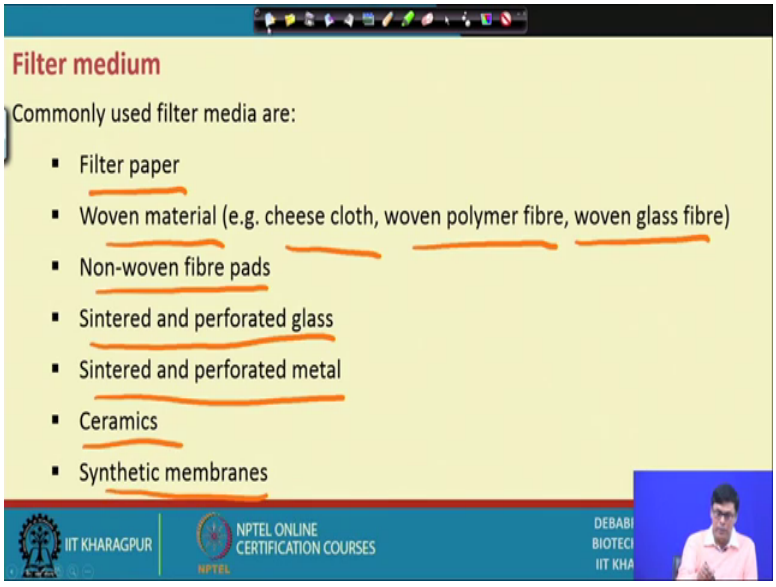
This is the filtration medium and this is how this particular this accumulated on the surface and in case of this is in cases of surface filtration and in case of depth filtration.

The particulate not only accumulated at the surface, but accumulated inside the inside the inside pore also. So, we have this is some balance with your with your filter that you

know fiber filtration also we have shown you the particles how there that that entrap within the fibers.

So, this is the solid liquid separation filtration often the cake filtrate because of continuous decomposition of the cake on the, suppose we have filter and solid particle that accumulated on the. So, this is how the cake development cake filtration take place

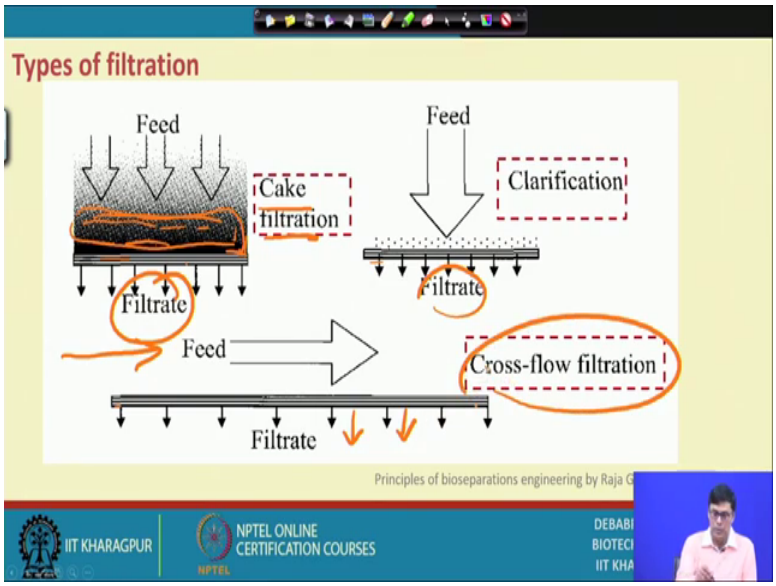
(Refer Slide Time: 20:12)



So, filter medium will be we have different we have filter paper woven filter like cheese cloth, woven polymeric fiber, woven glass fiber, non woven fiber pads, sinter and perforated glass, sinter and perforated metal, ceramics, and synthetic fibers.

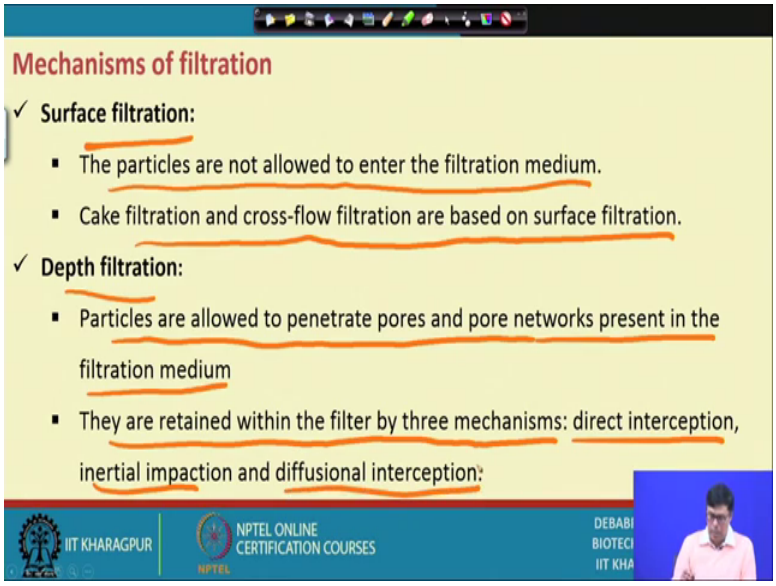
So, different type of filter medium we can use and here type of filtration.

(Refer Slide Time: 20:35).



We can see this is the suspended particle the when its settle down, this is the filter, filter that filter medium and a this is the how the solid particles that deposited on the surface of the that filter medium and this is called cake filtration and then it is the it is the kind of filter that where this feed is going and you will get the filtrate here also you get the filtrate and a here suppose you have a fixed filter medium and your flow is like this and then filtration take place in this direction we call is cross-flow filtration.

(Refer Slide Time: 21:17)

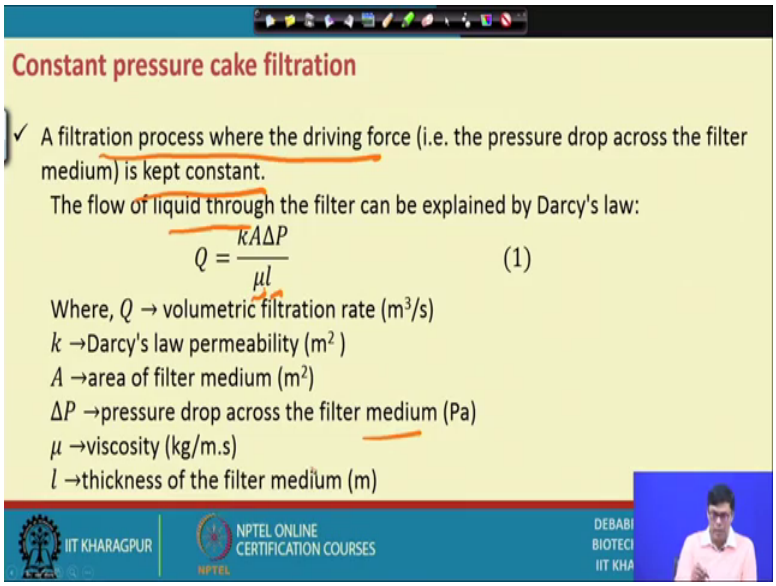


Now, mechanism of filtration we have different we have surface filtration, we have depth filtration in case of surface filtration particles are not allowed to enter into the filter medium that I have shown you I shall I have already shown you and cake filtration or cross filtration are based on surface filtration.

But in case of depth filtration particles are allowed to penetrate in the pore and pore network present in the filtration medium they are retained within filter by three mechanism : Direct interception, inertial impaction and diffusion interception because this all principles I explained during the air, air filtration process.

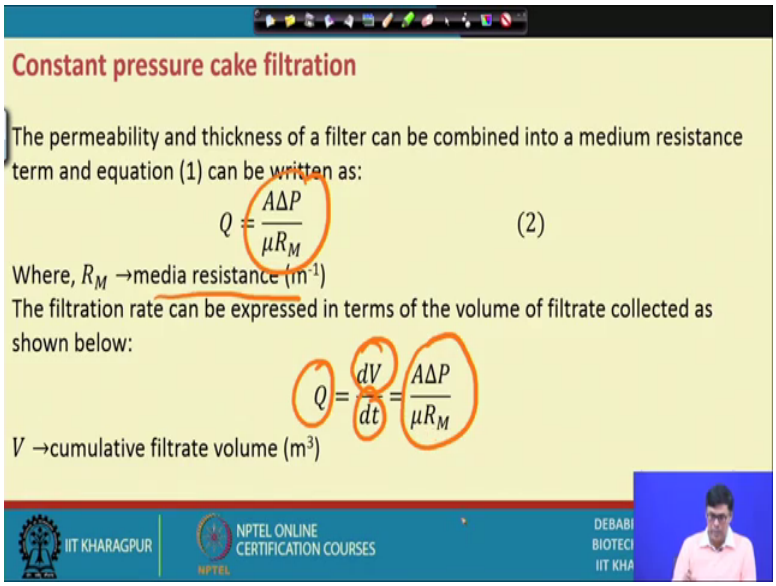
Now this I have already explained the sum material entrapped in a inside that in like your ah that you know he has sterilization process this occurred in case of surface filtration the particle remain on the surface of the filtration medium.

(Refer Slide Time: 22:14)



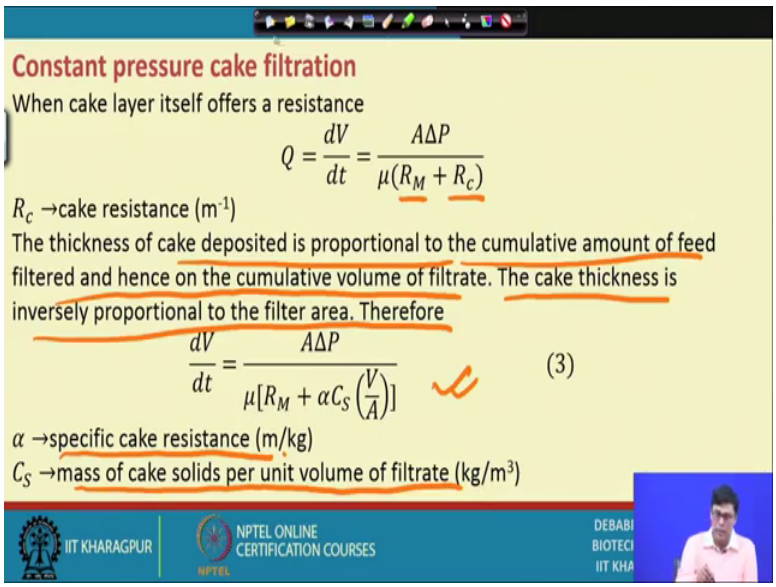
Now, the constant pressure filter cake that you know filtration process that we the where the filtration where the driving force is kept constant, the flow of liquid through the filter is depends on k into n into del P rho by l where k is the Darcy law of permeability is the area and of the filter del P is the pressure difference across the across the filter medium rho is the viscosity l is the thickness of the filter medium.

(Refer Slide Time: 22:48)



Now, Q is the kind of flow rate is the passing through the filter medium is again Q equal to A into del P mu into r m R M is considered as the that medium resistance and ah. So, Q is the flow rate this can be express at d V by d t and this is this is again will be equal to this same formula that we can put it here.

(Refer Slide Time: 23:14).

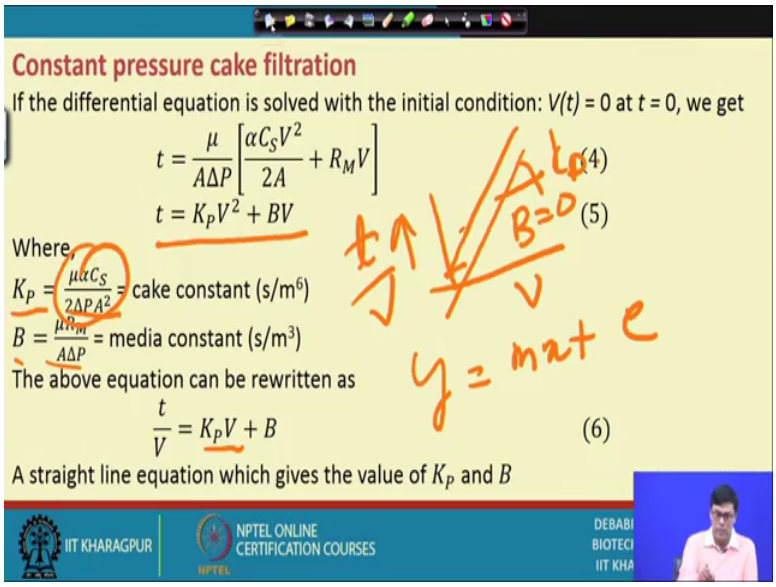


Now, finally, their constant pressure cake we have this equation d V d t del by d m and d a R M by R c what is R c is the cake resistance. So, the thickness of the cake deposited is proportional to cumulative amount of feed filtered and hence the cumulative volume of

filtrate and the cake thickness is inversely proportional to the filtered area and it can be written in this form.

Whereas, alpha is the specific cake a cake a resistance meter per kg and Cs is the mass of cake solid per unit volume of the filtrate express as kg per cubic meter. Now, this equation we can write in the form of t that if you solve in the form of t then we can write t equal to this equation and this we can simplify.

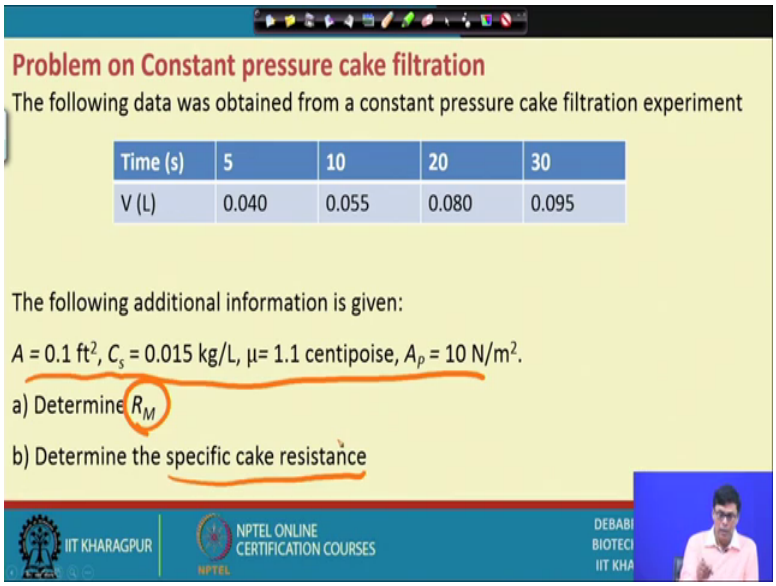
(Refer Slide Time: 24:05)



In the form in the form this K equal to K p V V square B into V and K p equal to this one and V beta equal to this one and finally, t by V equal to K p V into B and this equation is something similar to y m x plus c.

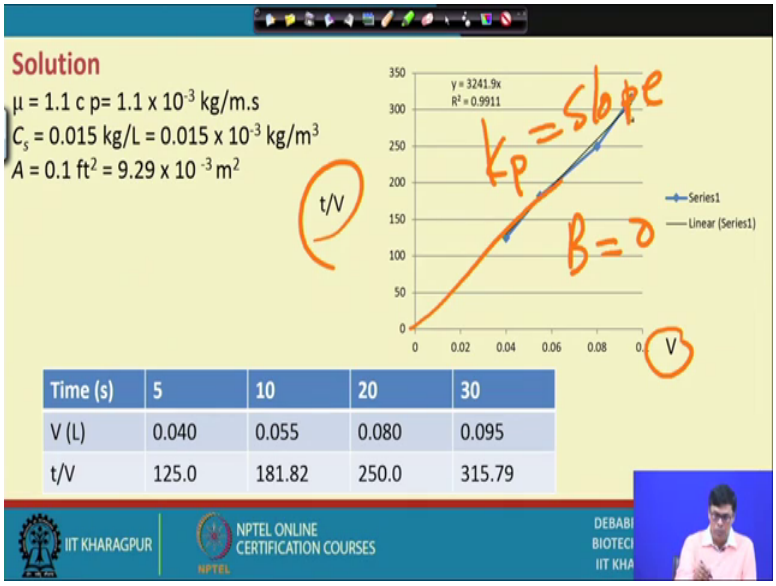
Now, if we plot t by V versus V then we will get this straight line if you pass through here then we have some B value, but if you pass through origin then beta will be B equal to 0 then the slope will be equal to K p and from K p we can K p is equal to again equal to this it is so, we can find out the different other parameters if you want to calculate.

(Refer Slide Time: 24:50).



Now, this is the problem that we have the following data's are obtained for a constant pressure cake filtration experiment the time is 5 second, 10 second, 20 second and 30 second. The volume is a increased like this. So, volume of the cake that is increased like this. So, A equal to this parameters are given and what you have to find out the R M value and specific this the resistance of the cake and this specific resistance of the cake both we have to calculate.

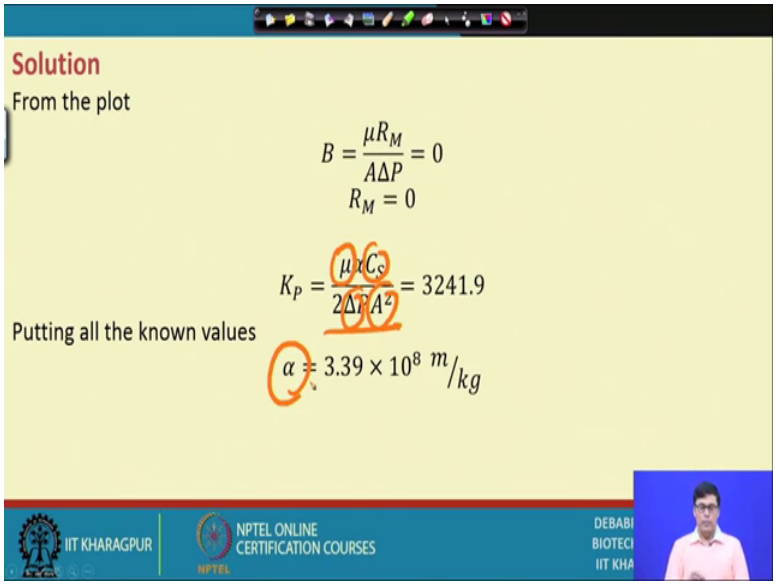
(Refer Slide Time: 25:23)



Then let us see how you can calculate because I told you that we shall have to plot this t by V versus V and we will get and if you look at the straight line is goes almost though the origin. So, we can assume B equal to 0.

So, we have K p value K p we can easily find out from the slope due to the slope and this K p once we find out the K p value this is beta equal to 0. So, R M equal to 0 and then we can write K p equal to this one and we know mu mu value here C s value del p value a value you know so we can find out the specific resistance of the cake.

(Refer Slide Time: 25:48)



So, in this particular lecture I try to point out that how you can the how the downstream processing that important in the biochemical industry that one that is the steps that is common to all chemical and biochemical industry is the separation of the particles.

Now separation of the two particles can be done by different means one we with respect to gravity by using sedimentation technique with respect to force separation by using the centrifugal force into by using centrifuge.

So, we have I discuss the membrane filtration we discuss the cake filtration and cross flow of a filtration process and we try to find out the worship the cake specific resistance over the resistance of the cake, how the how the angular velocity of the centrifuge that flow is proportional with the So, percentage separation of the solid particle.

Thank you very much.