Aspects of Biochemical Engineering
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Lecture – 58
Downstream Processing – II

Welcome back to my course ah Aspects of Biochemical Engineering. Now, in the last lecture I started a new chapter that is the downstream processing. And, I told you the downstream processing plays very important role as per chemical and biochemical industry is concerned, because whenever we market any kind of product that should be marketed in a purified form. And for the purification of the product product you we shall to have, that you know pass through the different purification processes.

The in this purification process one most important thing, that we have the solid liquid separation process. In the last ah lecture we try to discuss about the sedimentation process; we require we discuss about the centrifugal process. So, and also we required we discuss about the membrane filtration process.

Now, in this lecture in the today we will be discussing other different aspects of the downstream processing, which is really in practice in the biochemical industries.

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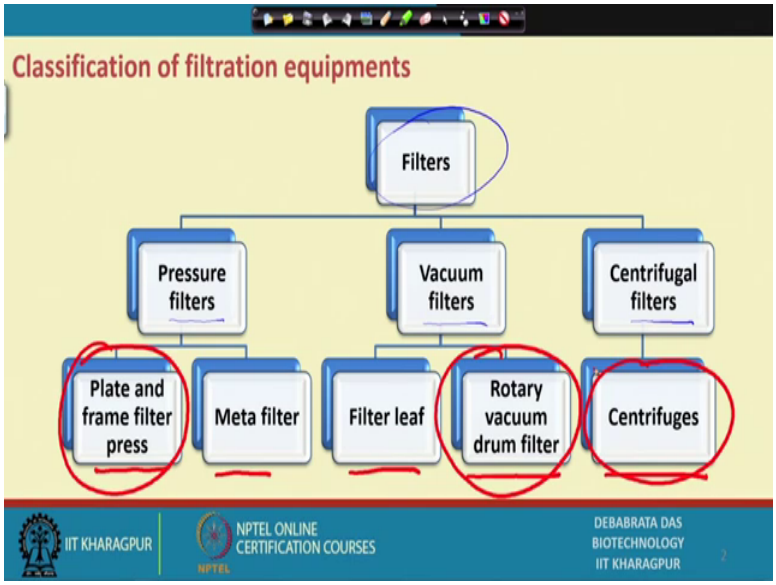

Now, first I want to tell you the classification of the filtration equipment. Now, if you look at the classification that ah the filter this is the filter, that we have and that we have 3 different types; one is called pressure filter, another is vacuum filter another is the centrifugal filter.

Now, if you look at pressure filter, we have plate and frame filter press, we have meta meta filter and vacuum filter, we have the filter leaf rotatory vacuum rotary vacuum filter and centrifugal figure we have centrifuges.

Now, out of that I want to highlight that plate and frame filter plays is largely used in the biochemical industry, rotating rotatory vacuum dry vacuum drum filter, that is also largely used in the biochemical industry and centrifuge also largely used in the biochemical industry.

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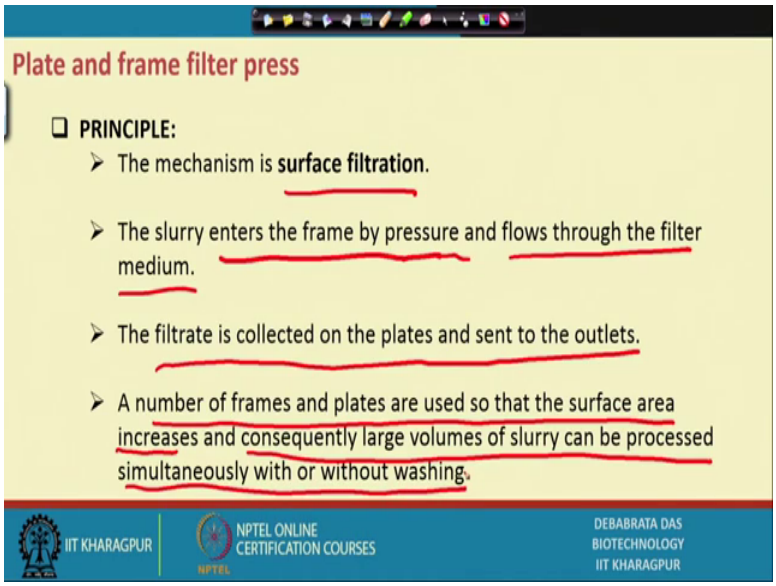

Now, first let us consider that you know plate and frame that filter press that we have. Now, question come how the plate and plate and frame filter press they look, that let me let me let me give you some idea about that.

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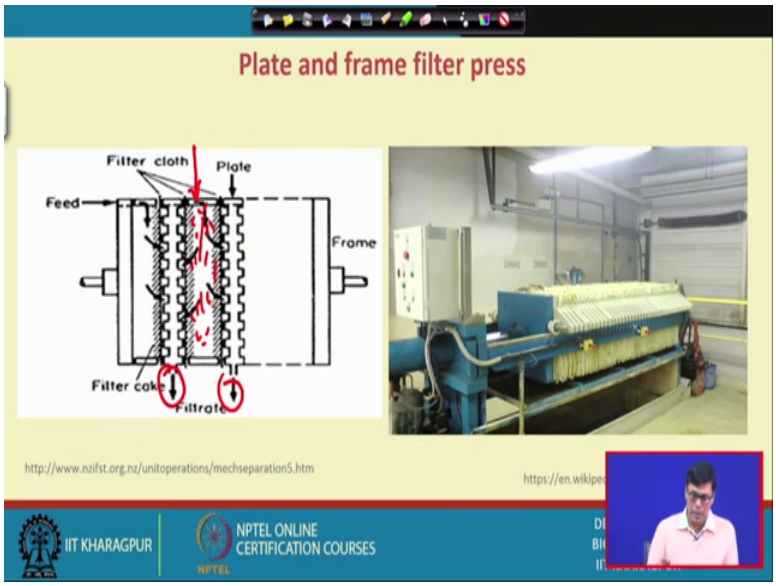

That that you know that if you if you look at this is plate and frame filter press plate and frame, filter press.

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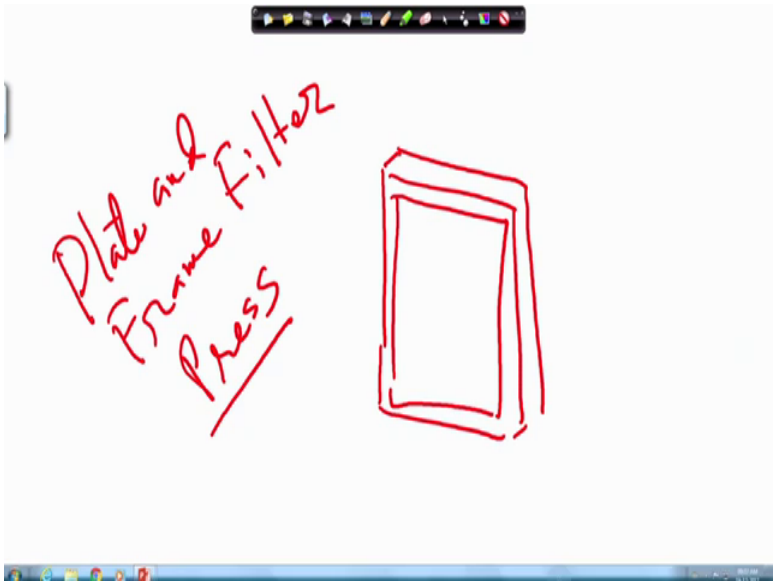

Now, how it looks? Now, we know ah that you know frame wooden frame, that in our house we have window we have we have door that door we have this is the frame that we have am I right.

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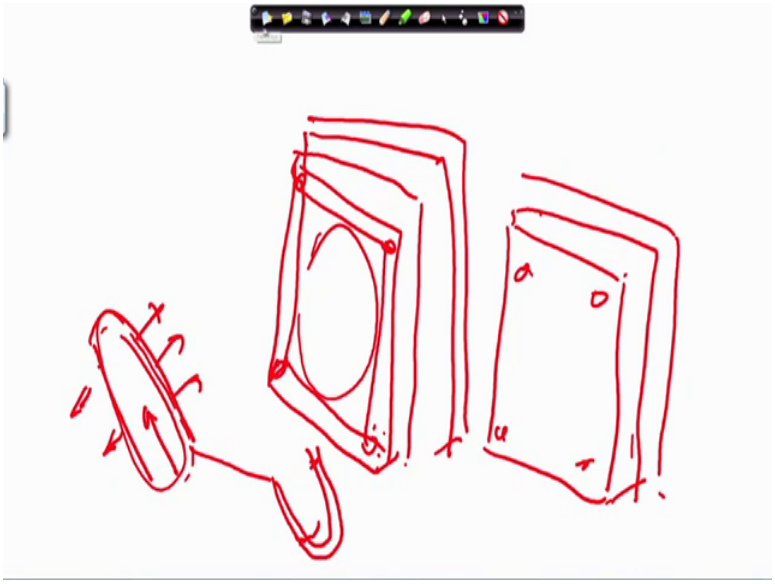

So, this frame we can we can have multiple of this frame, this frame we can have in our different frame we can branch together. That one after another that you know this frame, we can we can have this is the frame that we have and ah this frame we can we can we can have several frames like this like this so, in between the frames so, here this is hollow am I right.

Now, we put some kind of thick cotton pad that in between the frame. And, this since it is made of wooden we can make a whole in it. And, through this whole we can put a some kind of nut and bolt arrangement so, that we can fix that.

So, in between suppose this is a frame and here there is the another frame and there is the another frame like this, you know multiple frame we can we can branch all together like this. And when in between this we have one cotton pad we put inside that and then we pass liquid in between this cotton pad.

So, what is happening the liquid suppose this is a cotton pad is wrapped around and if you pass liquid the liquid will comes out like this, where like this you can collect and yours. So, your solid material will be will be will be will be accumulating inside this filter.

And, when it is totally filled then you can find out that if you connected with kind of nanometer, that you will find the pressure drop will be very high. And as soon we have

high pressure drop, then you can stop the operation and take the solid out this is what is in practice in the baker's yeast industry.

So, this is how it looks this is the, this is you can see this is how the different plates? And this is the this is the cloth you know this is you can see that the how the cloth is looked this is thick cotton pad that we have that is that in between the plates we have and we pass the liquid like that.

So, what is the basic principle of this plate and frame filter press is the surface filtration. And here I want to point out one very important thing, that when we go any kind of filtration process, we design the filtration process on the basis of the size of the particles. Now, we know that in the biochemical industry we use different type of microorganisms. And, different type of microorganisms has difference sizes as for example, bacteria their size is 0.5 to 2 microns am I right. And yeast is about 3 to 7 microns and if you if you look at fungi it is couple of millimeter.

So, naturally kind of filtration arrangement that we have for different type of filtration process will be different. As for example, for bacterial filtration mostly we go for centrifuge, because particle size is very less, but in case of a yeast filtration because baker's yeast fermentation process since the particle size is little bit bigger we go for plate and frame filter press.

Now, in case of fungi, now since the particle size is about couple of millimeter, we go for rotating vacuum filter that I shall discuss. So, here this is mostly used for yeast filtration process, that baker's yeast filtration and other purpose also it can be used. The slurry enters the frame I showed you, by pressure and flow through the filter medium. And filtrate is collected on the plates and sent to the outlet.

The number of frames and plates are used so, that the surface area increases and consequently large volume of slurry can be processed simultaneous with or without washing. This is the main purpose of this particular plate and frame filter press this is how it is look?

So, liquid this is thick cotton pad, that we have and liquid enter like this and liquid comes out here and you drain out like this. So, liquid you can see the liquid drain out through this is the liquid coming out and liquid a this is the in and this is the out.

So, liquid and all the solid material will accumulated in between this. And when your where is total filled up then there will be pressure drop and from the pressure the when you find out large pressure drop we stop the operation open this and take out the solid material, this is how it looks?

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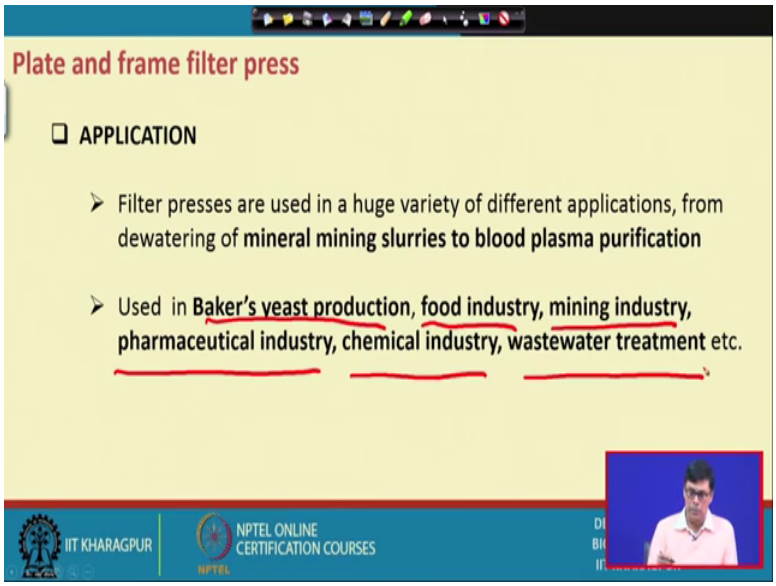

Now, what are the applications we have I told you that, this is used in the baker's yeast fermentation process, then the food industry it is used, mining industry it is used, pharmaceutical industry, chemical industry, and waste water treatment process. So, different we have several applications we have the plate and frame filter press.

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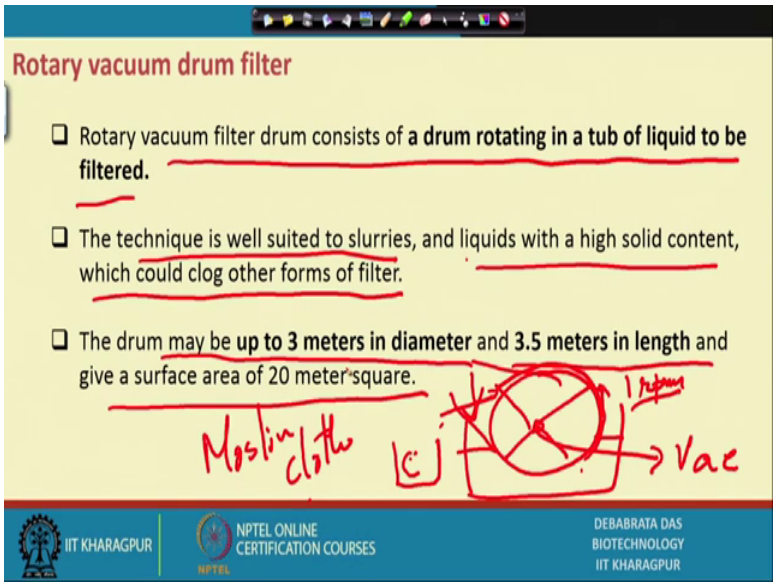

Now, let us let me come to the rotary vacuum filter where, because we know that we have lot of antibiotics fermentation process where we use the fungi I can I can give the example of penicillin fermentation process, where we use the penicillium chrysogenum, which is the fungal strain I work with citric acid industry, where we use the aspergillus Niger that is also fungal strain this size is quite big.

So, there we use rotary vacuum filter rotary vacuum filter consist of drum rotating, in a tub or a liquid to be filtered, the technique is well suited for to slurry and liquid with large solid content and the that could clog other form of filters. The drum made up of 3 meter dimeter, and 3.5 meter length and give a surface area of 20 meter square.

Now, let me explain that that this is suppose this is the drum and this is immerse in the trough this is trough. In the trough we put the fermentation broth like this. And this here we have pipeline this is we have pipeline like this and this drum this is like this drum is like this and this is wrap by the thin moslin cloth, moslin loth. What is moslin cloth? That is the fine cloth this wrap on the surface of the drum.

Now, what do you do here, we applied vacuum? At the middle we this is the pipeline if you applied vacuum what will it is suck the liquid and the cell mass and this rotated very low rpm. May be 1 rpm, 1 rpm means 1 rotation per minute. Now when it rotates like this, that and the solid material the cell mass that will touch on the surface of the thin moslin cloth.

Now, here we have a knife thin knife, that you know this knife tells the moslin cloth and take the solid material out and it drop in this particular may be some kind of screw conveyer is there, that will take the material out and put it in some other vessels or it can it can it can put it in the some wagon.

Though this is how it is how this is in practice.

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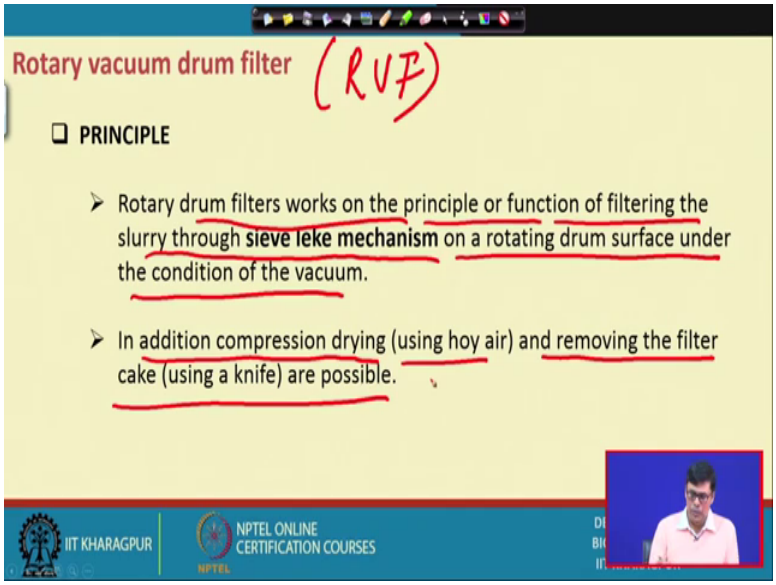

Now, principles of this rotary vacuum filter what do you call RVF this is rotary the in abbreviation form we call it like this. Now, rotary vacuum filter works on the principle or function of filtering slurry through the sleve leke mechanism, on a rotating drum surface under the condition of vacuum.

In addition of compression drying using hot air the removing the filter cake are possible. So, what we let me let me go to the exact photograph of the particular process, it is like this.

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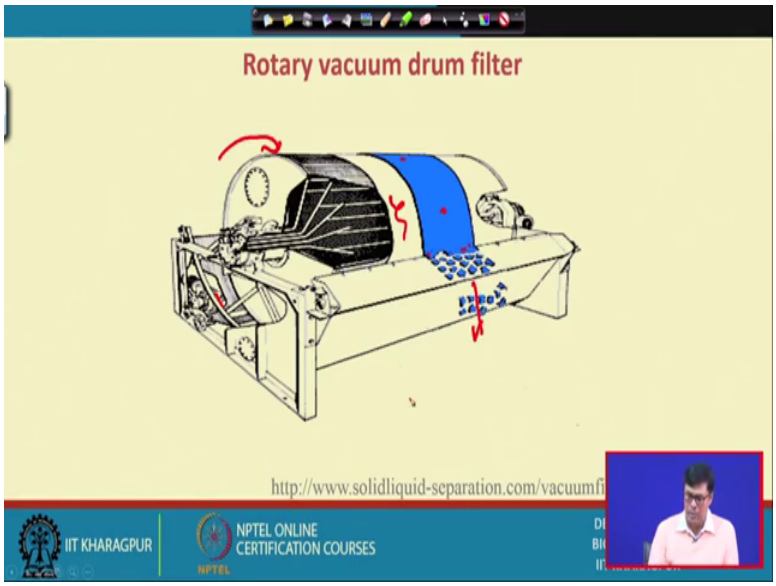

So, you can see this is the drum and this is here you have fine moslin cloth you can see here, it is fine moslin cloth this is the fine moslin cloth. And here when it rotate and this is the trough in which it is immerse when and this trough is rotated at a very low RPM and here there will be fine knife, which touches the surface of the moslin cloth and the solid material the cell mass that attach on the surface of the moslin cloth, that will comes like this it will drop down like this.

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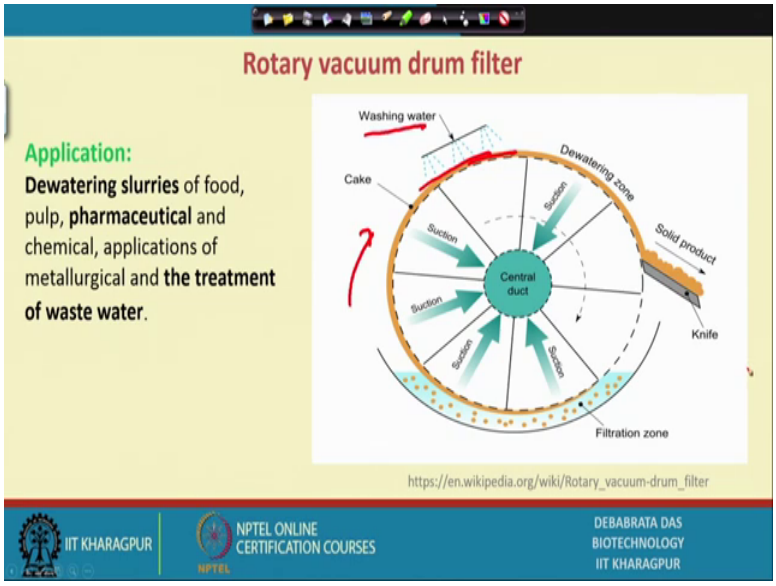

This gives a ah very clear cut picture here, this is the trough where the where we have where we have the fermentation broth and it is rotated at the very low or low rpm suppose here, it is rotated in this direction.

So, it is a the this is the central duct where suction takes place and it suck the water and the solid material that the cell mass that adder on the surface, and this is washing water just to if some a product is added on the surface of the cell mass, that also we take care that should also come in the liquid and this is dewatering zone this is remain out. So, that you know little bit drying of the material take place and this is that knife, which touches the surface and take the product out.

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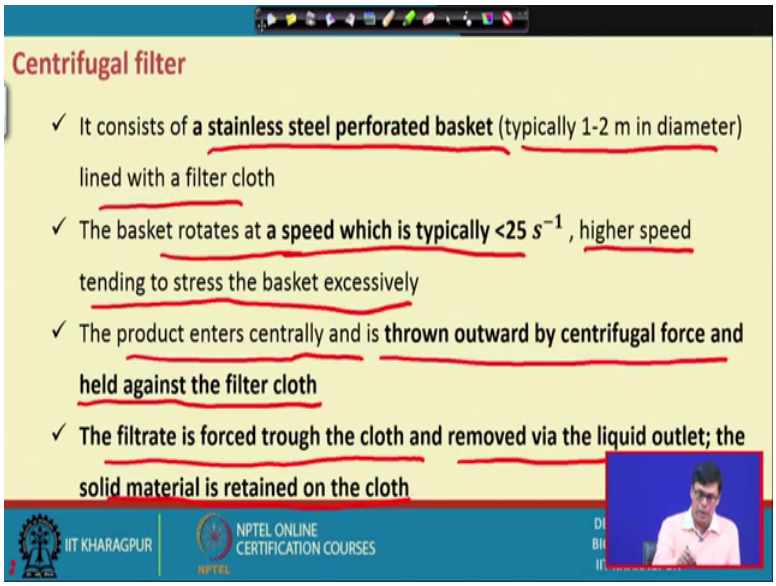

Now, centrifugal filter that is the another type of filter it is constitute of a stainless steel perforated baskets, and you know let me show you the figure then I think it will be very very clear.

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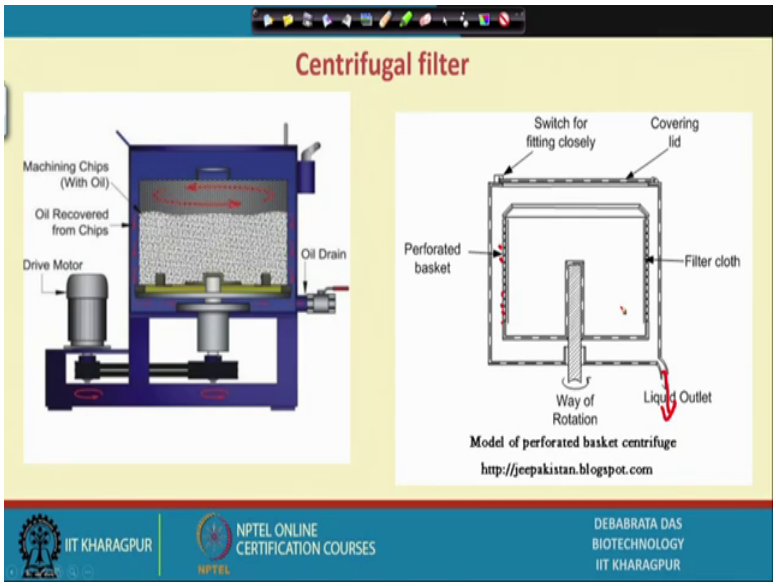

This is this is this is rotated in the high speed and then the material will throw on the surface and here is a here we have here we have perforated disc basket. So, when you throw it the solid material that added on the surface and liquid will goes out am I right. So, liquid we can take it out from this outlet and solid material added on the surface of the cloth.

This is it consists of if you look at it consist of stainless steel perforated basket typically 1 to 2 meter diameter lined with filter cloth. The basket coated rotates rotates at a speed typically 25 per seconds and high higher speed tending to stress of the basket excessively.

The product entered centrally and thrown outwards by the centrifugal force and held against the filter cloth the filter cloth will not allow the solid material to go out. The filtered is forced through the cloth and remove via the liquid outlet and the solid material retain on the cloth.

So, I hope that the principle is very simple.

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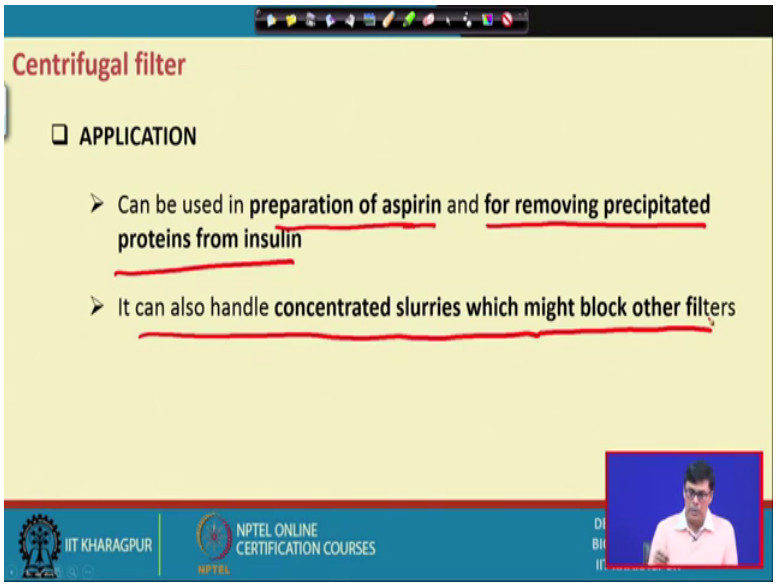

The application of this is the preparation of aspirin, this kind of medicine largely used in the pharmaceutical industry, ah for medicinal purpose this for removing the precipitation of proteins from insulin and it is also handle the concentrated slurry, which might block other filters. So, this is the different purpose where this centrifugal filter may be used.

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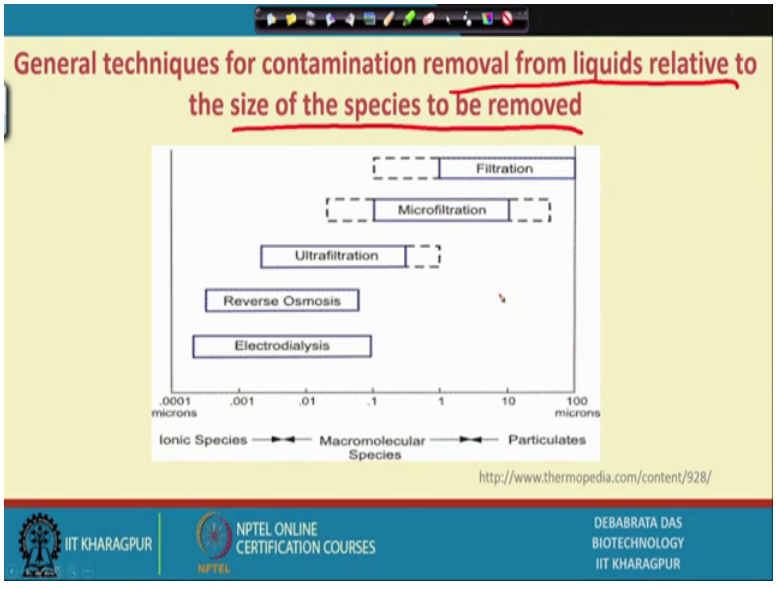

Now, the general technique of for contaminates removal from the liquid relatively to size of the species to be removed. So, we have on the basis of size we can name the different techniques. Like, if the if the size this is the size of the particle this micron and this range

point this 0 0 1 to 100 microns. So, if is very small we have electro dialysis, then little bit more it reverse osmosis then ultrafiltration micro filtration and filtration, we go for the higher sized particles micro filtration usually ranging between in between 0 2 10 microns.

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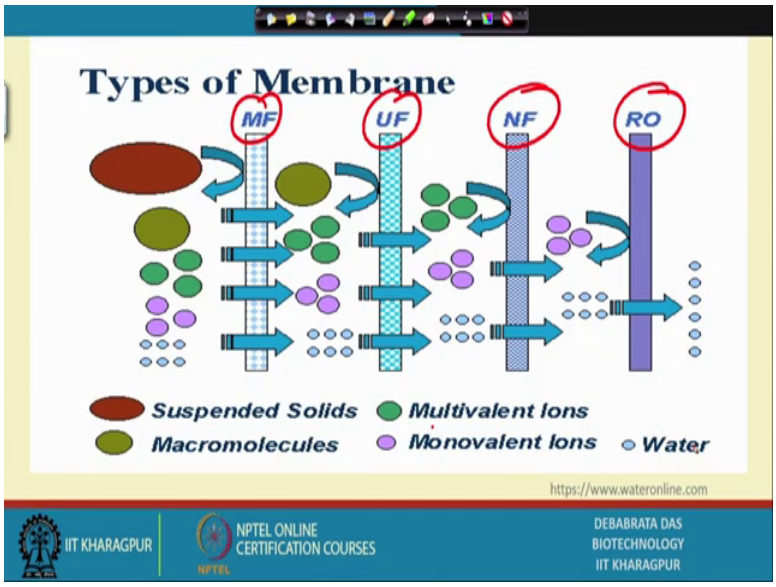

Now, this is this is very clear here, that that this is the membrane filter, and this is the ultra filtration, this is Nano filter, this is ro this is how on the basis of size of the particle, we can differentiate type of membrane that is used for the separation of particles.

So, this is suspended particles that is removed by the membrane filter and then this is the micron macromolecule, that is can be separated by the ultra-filtration and then we have like of multi valent ions that can be used separated by Nano filter and ro can be say used for the for the production of the water, that we use in the day to day life.

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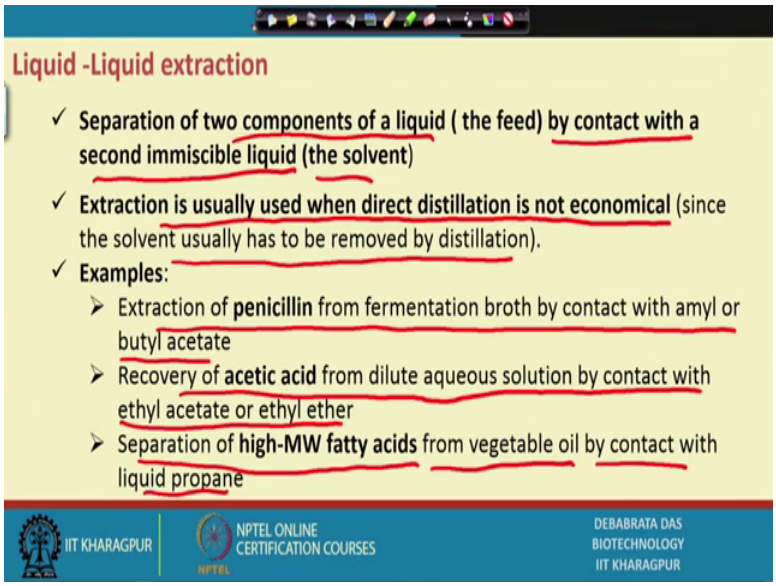

Now, liquid liquid extraction another very important area in the downstream processing, that I can give the example of the penicillin industry, that in the penicillin industry what is happening that that penicillin, that present in the fermentation broth and when you when you reduce the ph of the fermentation broth to 2 then it is a more solubility soluble in the solvent layer that is the amyl acetate or this ah that you know kind of solvent, which is which is not soluble in the water. So, when you when you mix together then the penicillin will come from the aqueous layer to the solvent layer.

Now, when it comes to the solvent layer, then what will happen in a aqueous layer, you have contains lot of un undegraded material or some metabolites. So, for since this the solvent is the and this is invisible in the water. So, you can easily separate aqueous layer from the solvent layer. And, then again you increase the ph to 7, then again you water you then solubility of the penicillin will be more in water it goes to the aqueous layer. Again you decrease the ph then again it goes to the solvent layer.

So, like this we can purified the product and then and then we can we use for different purpose as per as per penicillin is concerned, we know the penicillin can be used in 2 different form either in the form of capsule or in the form of injection fluid, when it when it go for in the form of capsule within a little contamination is there little impurities is there we can we can tolerate, because goes via your stomach, but when it injection fluid no contamination is permitted to the 100 percent sterility is to be maintained.

Now, let us see that how this liquid liquid field extraction system works, separate up 2 components of the liquid by contact with a second in immiscible liquid as the solvent. So, the extraction is usually used when direct distillation is not economical. And since the solvent usually has the removal has to be removed by distillation.

The extraction of penicillin from the fermentation broth by contact with amyl or butyl acetate, that is one of the example recovery of acetic acid from the dilute aqueous solution by contact with ethyl acetate or ethyl ether. And separation of high molecular weight fatty acid from vegetable oils by contacting with liquid propane. This is the different application that we have in the liquid liquid extraction process.

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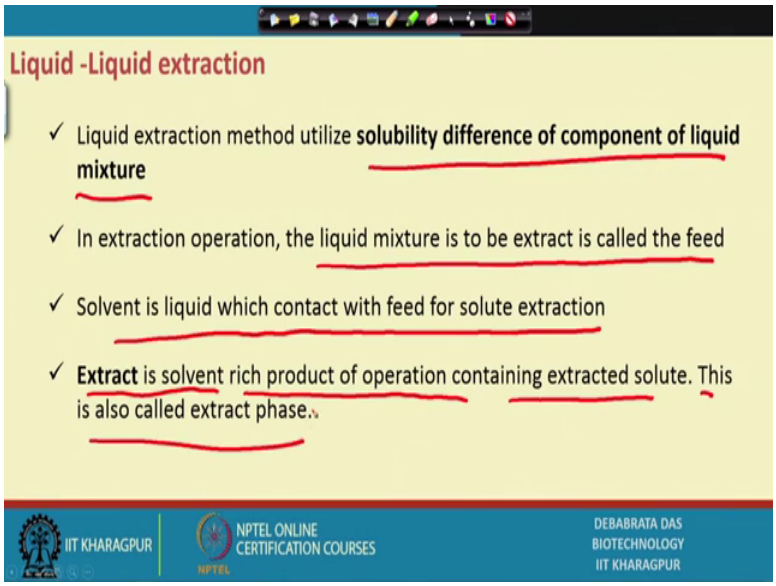

Now, liquid extraction means method is on the basis of solubility difference of the component of the liquid, I was saying telling you that on the basis of solubility, that we this liquid liquid extraction process is used. The extraction operation of the liquid mixture is to extract is called the fed and the solvent is liquid, which contact with the feed for solute extraction. Extract is solvent reach product of operation containing extracted solute and this is called extract phase.

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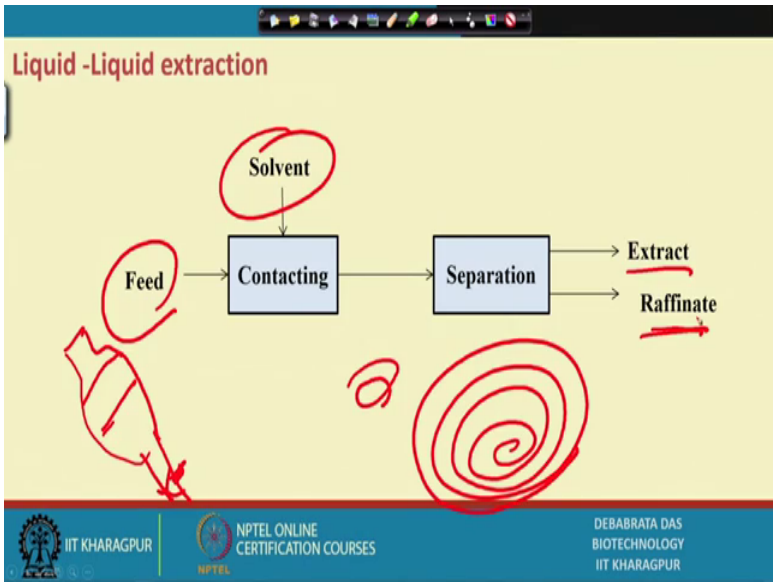

So, we this can be very clear here. So, here see that this is feed and this is solvent. Now, now in the lab we can we know that how we do that we have that supporting funnel like this, that we can put 2 solvent together this is the this kind of stop watch we stop clock we have.

So, here we can take the 2 solvent we can mix together by hand and then we separate out 1 1 layer from other layer, but industry we cannot do it industry what will do that we pass the liquid and you know it is kind of wheel motion you know that we have like this. Now, show that you know that 2 should 2 layer will continuously come in contact with each other and finally, then we dispose in the in the vessel. So, that one then one layer will be separated from other layer we can separate one layer from other.

This is this is this is how it can be done shown like this is the feed, one way and this is the solvent another way, this is the contacting the separation then we do the extract ah. This is the extracts that we have raffinate that remains that we call raffinate that the suppose we use the solvent, solvent we call the extract and the raffinate is aqueous layer.

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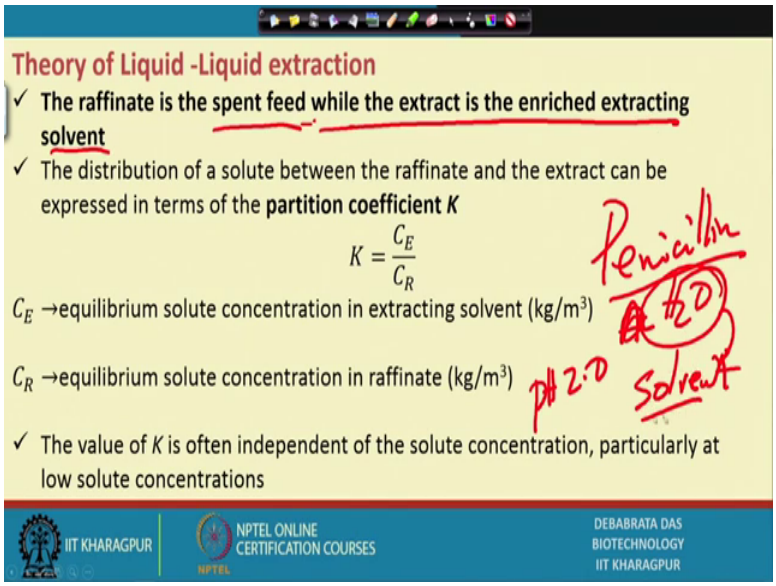

Now, theory of the liquid extraction the raffinate is the spent feed, that while extract is the enrich with extracting solvent as the as the as I told you that penicillin penicillin. So, in case of penicillin it present in the aqueous layer am I right. Aqueous layer means water it contains, another is the solvent solvent what you have amyl acetate butyl acetate that we have solvent that we have.

So, now what you do? When we when we decrease the PH to PH we two then when we mix together, then penicillin will come from the aqueous layer to solvent layer. So, this is on the basis of the raffinate is the spent feed while extracting the, enrich with this solvent the solvent will enrich with the solute.

The distribution of the solute between the raffinate and the extract can be expressed in terms of partition coefficient. The partition coefficient on the basis of partition coefficient actually we can we can find out, that how quickly the material, that can diffuse to the other layer.

So, K is equal to C by C R C is the equilibrium solute concentration of extracting solvent and C R is the equilibrium solute concentration in the raffinate and, if the value of K obtained independent on the solute concentration particularly at low solute concentration.

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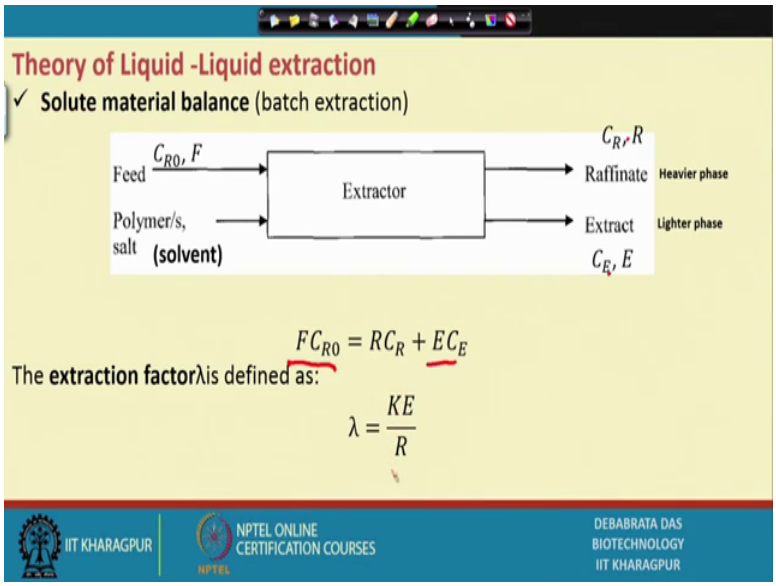

So, this is a, this is the how we can we can do the material balance, suppose this is the feed and this is the feed that we have and this is the polymer salts that we have in the solvent. And, this is raffinate this is heavier phase and this is the lighter phase is the extract and this if you do the material balance material balance through the F F R 0, we can F R 0 plus R R C R, plus E C E, E C C. This is then we can write that extraction coefficient equal to K into E K a K into E divide by R. That is how we can find out this the extraction, that factor the lambda can be defined like this.

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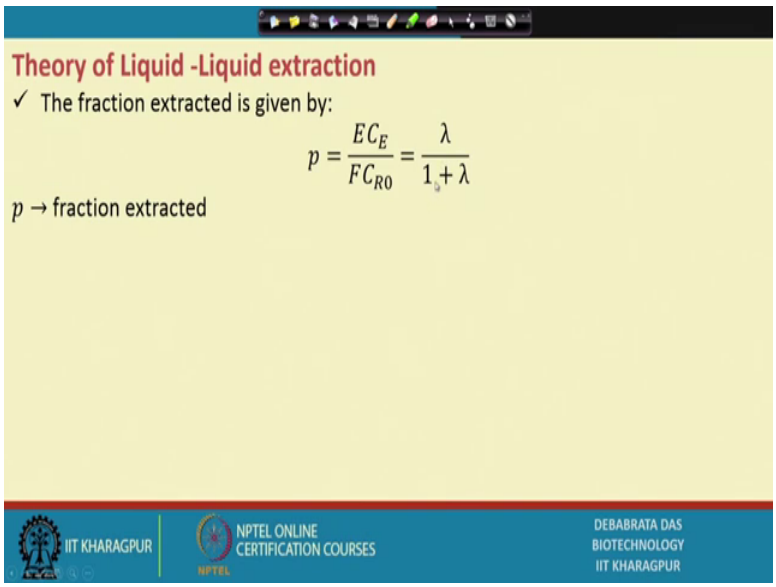

Now, then this equation we can write in this form that this is p equal to E C by F R O, this is lambda we can we can here you can see the lambda equal to K E by R. And this we use here then we can find out these equations.

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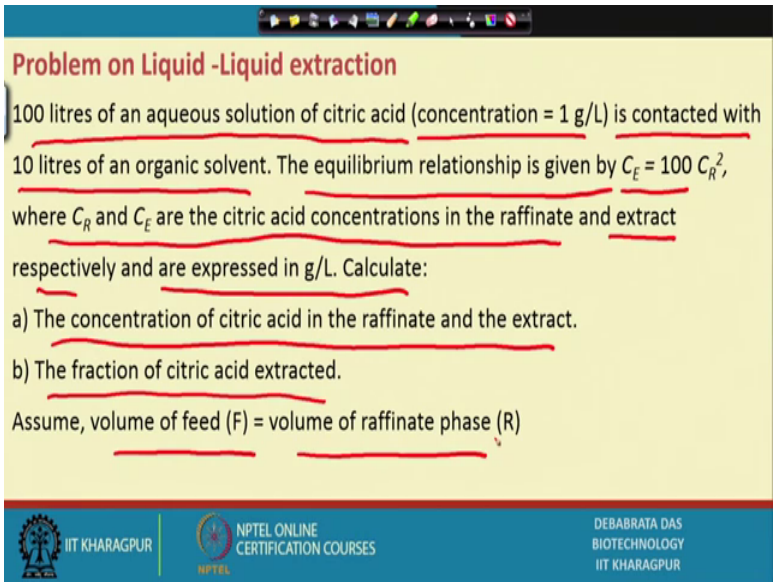

Now, this problem if, we look into that you know that you conception will be little bit clear 100 liters of the aqueous solution of citric acid concentration 1 gram per liter, it contracted with a 10 liter of organic solvent.

The equilibrium relationships C equal to 100 into C R square, where C R and C E is the citric acid concentration raffinate and the and the and the extract respectively and are expressed in gram per liter.

The question is that concentration of citric in the definite and the extract that we shall have to find out and fraction of citric acid extracted. Assume, that volume of the feed equal to volume of the raffinate phase, that is the assumption we made here, what is the in the problem?

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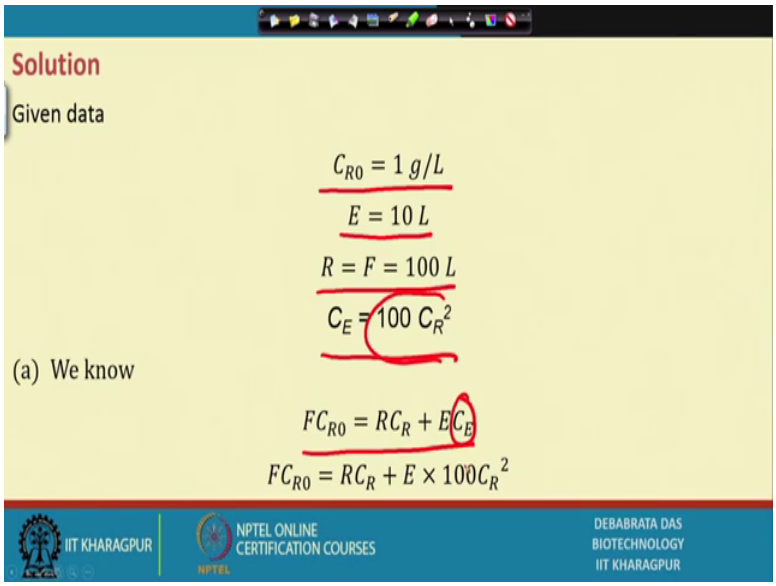

What is given C R 0 is given, that one gram per liter is a is equal to 10 liter, R F equal to R equal to f equal to 100 liters, then equation that is given C equal to 100 C R square.

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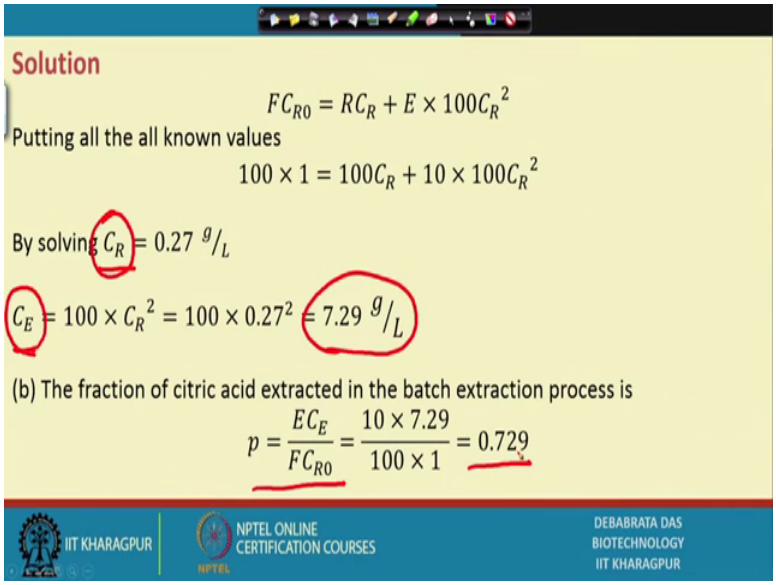

So, this is the material balance equation that we have already find then, we can replace the C E by this equation here, then we can we can put the value of different values then we find out the value of R C R we can find out. And C since C equal to 100 into C R square you can easily find out the value of. So, one is the raffinate what is the concentration? What is the concentrated extract citric acid that we can find it out?

The fraction of citric acid extracted in the batch extractor p can be that equation already given before, that we just put the value here we can find out the what fraction that can be extracted? So, this is how we can solve this problem?

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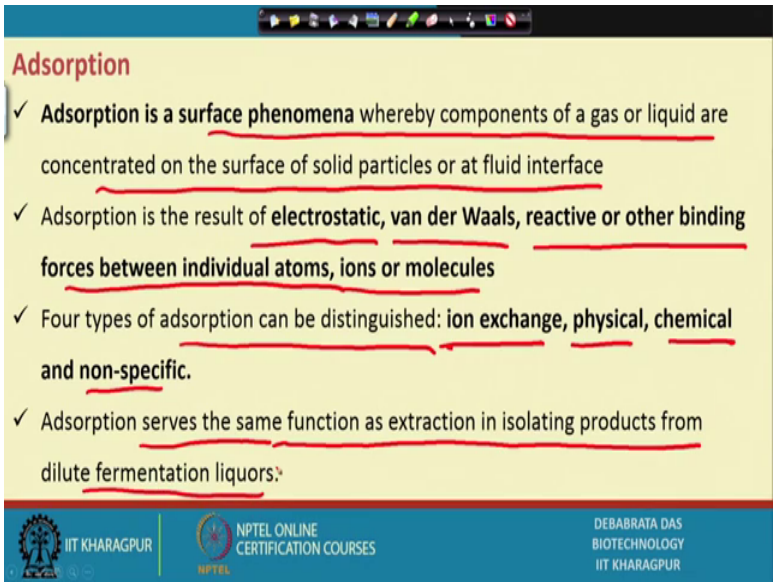

Next is the adsorption phenomena, that is another technique that is largely used in the downstream processing, adsorption is the surface phenomena whereby component of the gas liquid, are concentrated on the surface of the solid particle adsorption results from electrostatic Van Der Waals, reactive with other binding forces between the individual atoms ions and molecules, four types of adsorption distinguish the from each other one is ion, ion exchange, physical, chemical, and nonspecific.

Adsorption serve the same function as a extraction isolated isolating the products from the dilute fermentation liquid.

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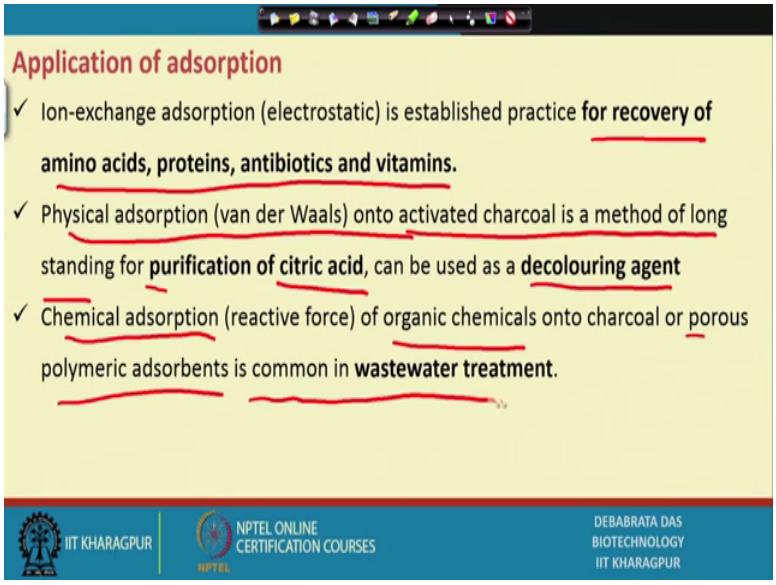

Now, let me let me show you this that Ion-exchange, adsorption that you know that is established in practice for the recovery of amino, acid protein antibiotics and vitamin. Physical adsorption due to Van Der Waals force on the activated charcoal is a method long standing for purification of citric acid particularly, for the removal of color and chemical adsorption of organic chemicals on to the charcoal or porous polymeric adsorption is commonly used for the waste water treatment process.

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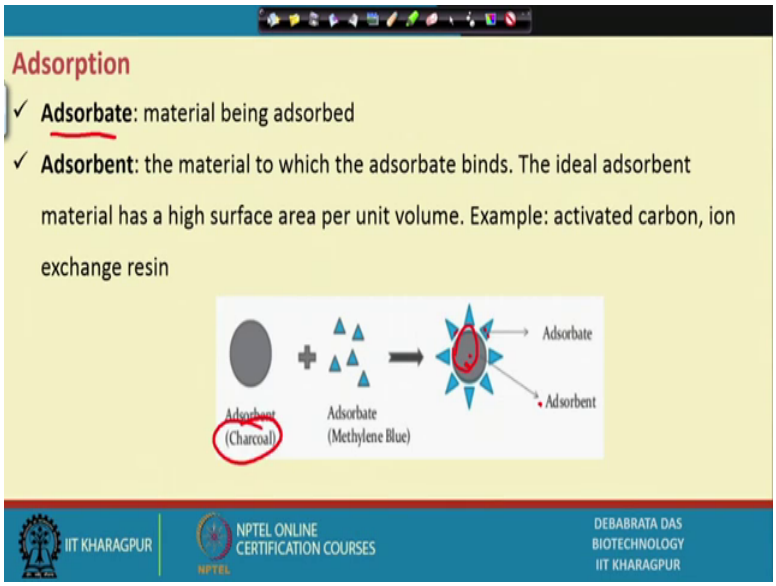

This is how adsorption can be explained this we can the adsorbent is the material on which the adsorption. So, this is the material suppose we are talking about charcoal and this is the adsorbent suppose methylene blue. So, this is how it adhered on the surface of

the material. This is the adsorbent and this is this is what you call this is the adsorbent and this is this is adsorbent this is the adsorbate and this is the adsorbent. Just to adhere on the surface of the solid matrix.

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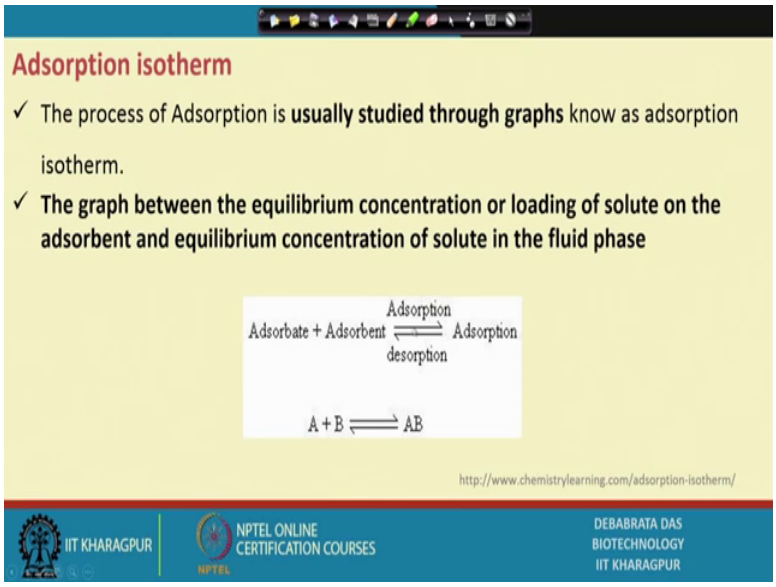

Now, this is the equation that we have adsorbent plus adsorbate equal to this adsorption and desorption, if you desorption then the materials will comes out and adsorption with the header on the surface of the solid matrix.

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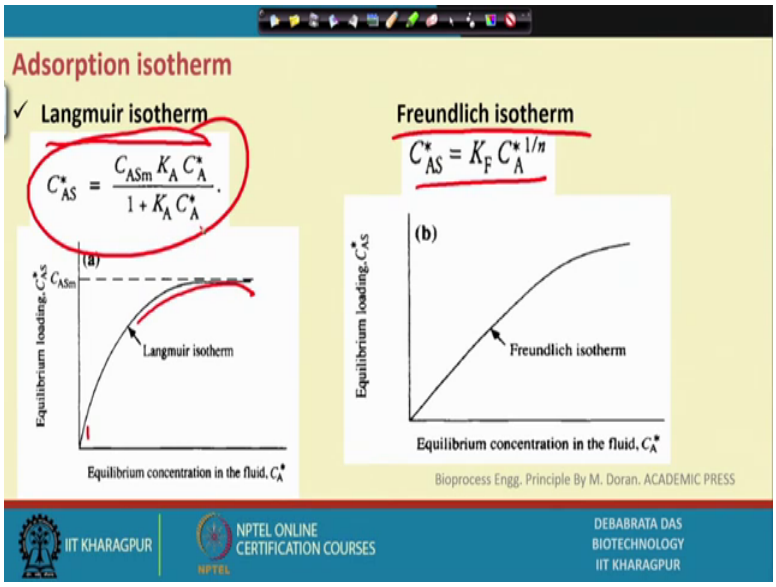

Now, this principle we can express by 2 different isotherm one is Langmuir isotherm, another there freundlich isotherm, that Langmuir isotherm this is the equation that we have and kind of correlation that we have with like this. And freundlich the isotherm it is little bit ah the flatted type of thing and the correlation is like this.

So, we have 2 different type of correlation for different type of two different type of isotherm. Depending on the adsorption characteristics, we can find out which is following in the adsorption process.

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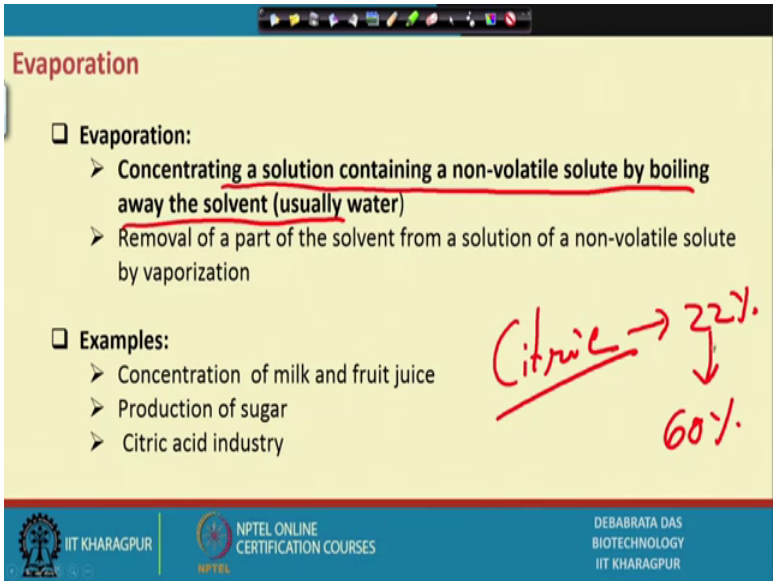

Now, next is the evaporation, now if you look at evaporation main purpose is the concentration a liquid, through a nonvolatile solute by boiling away the solvent that is the water.

I can tell you that when citric acid, we because after ah I told you after hydrolysis of calcium citrate, we get citric acid and gypsum and this citric acid concentration is 22 percent and this we have to increase to 60 percent.

So, (Refer Time: 29:26) naturally you have to remove water from that and how you do it? We do it very easily suppose.

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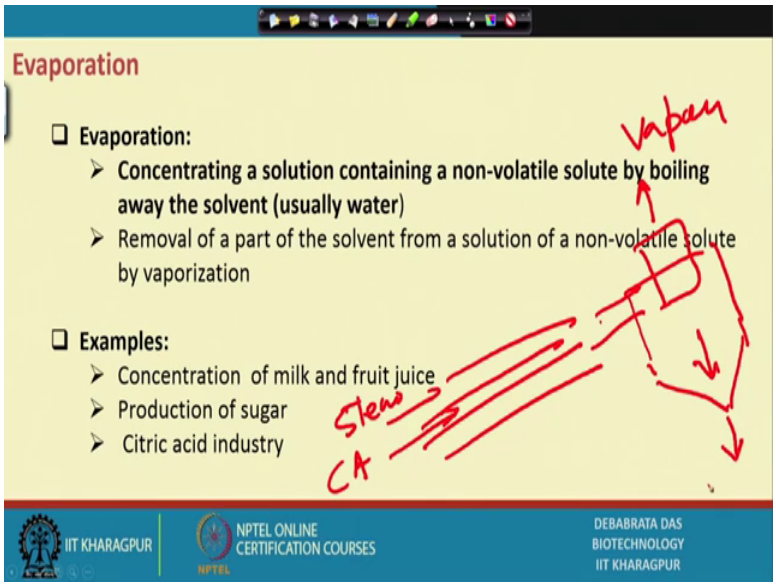

This is the this is a pipeline and here we pass the citric acid, and this is a stream we pass and then we heat it to high temperature then, we put it in the big like you know we cycle separated type of things, tangentially is comes like this and then the vapor will go out this is vapor will go out and concentrated liquid, will go down. So, you will get the concentrated liquid like this.

So, this how it is removable part of the solvent of the solution from the nonvolatile solute by because, because we know citric acid is a nonvolatile solvent. This is for concentration of milk fruit juice production of sugar citric acid industry it is largely used.

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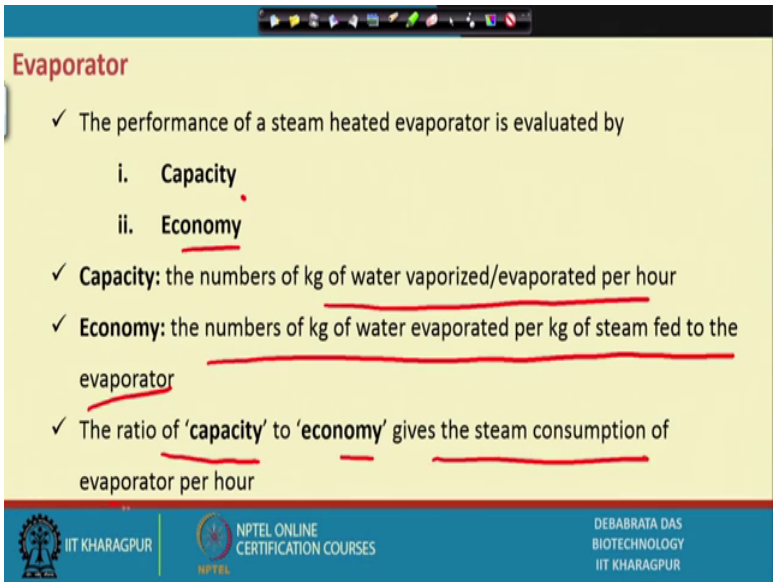

Now, the performance the it depends on the capacity and economy, the capacity is the number of kg of water vaporize or evaporated per hour, economy the number of kg of water evaporated per kg of steam fed to the evaporation and ratio of capacity to economy give the steam consumption of the evaporated per hour.

So, this is very important the industrial point of view, because how much what is the money involvement is there

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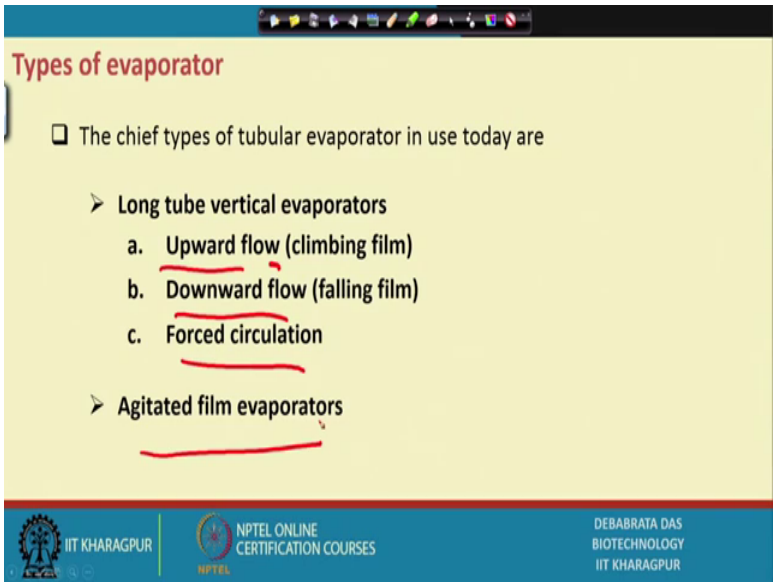

Now, different type of evaporator that we have upward upward flow, downward flow, and we have upward flow, this is the one is the upward flow, then downward flow and force circulation and agitated flame evaporator. The different type of evaporator is used by the industry.

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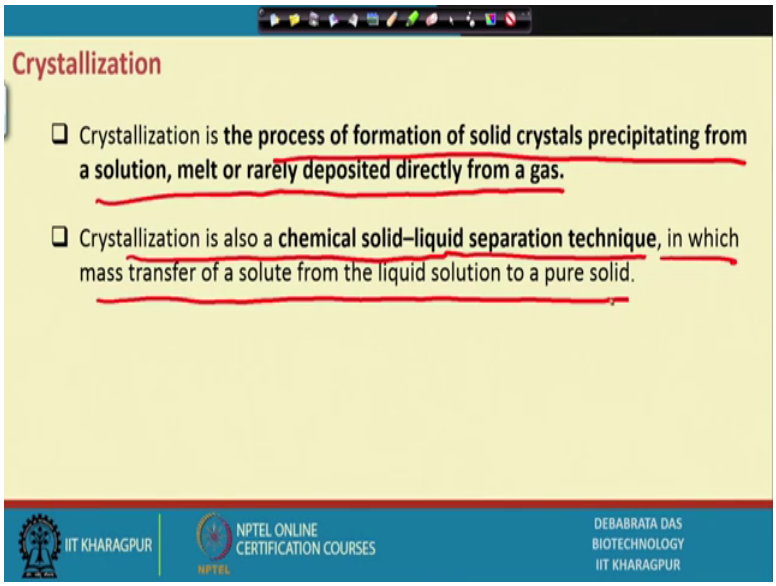

The, next is the crystallization process when we get the concentrated citric acid, then I told you that we reduce the temperature to about 10 10 10 to 20 degree centigrade, then put it in a crystallizer, where the crystals of citric acid separated out.

So, crystallization is the process of formation of solid crystal, precipitating from the solution melt, rarely deposited directly from the gas and crystallization is also chemicals solid liquid separation technique, in which the mass separation of the solute from the liquid solution to a pure solid.

So, I can give another simple example of sugar at high concentration at we reduce the temperature sugar crystals will be separated out from the liquid. It is just we pass through the centrifuge we can separate the crystals of a sugar from the liquid. And liquid we call a cane molasses and sugar; we can sell it in the market as sugar crystals.

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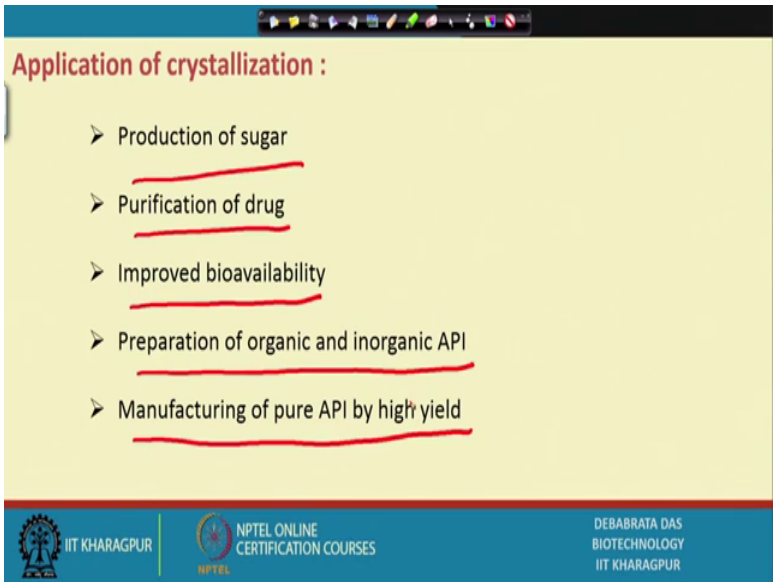

We have several applications, production of sugar, purification of drug improved, that bio availability preparation of organic and inorganic API and manufacturing pure API, by high yield this is the different purpose you can use.

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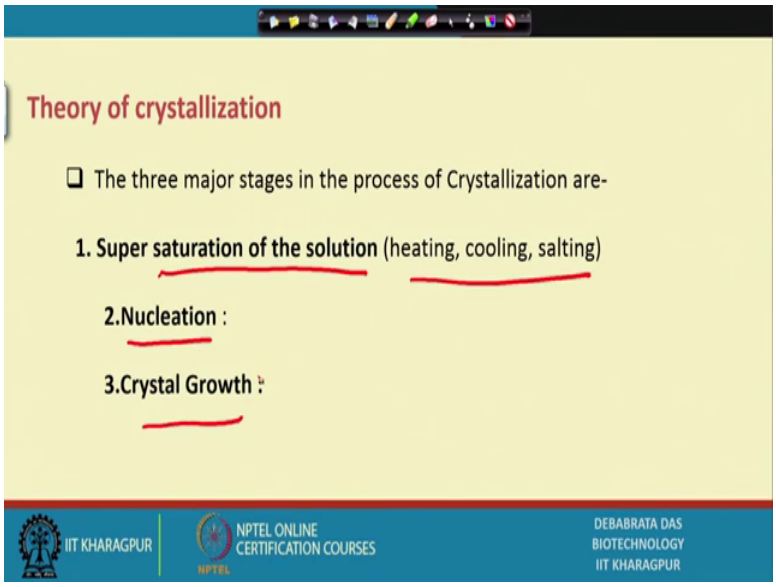

And theory of crystallization first is the super saturation of the solution; this is to be done heating cooling and salting and then nucleation then the crystal growth.

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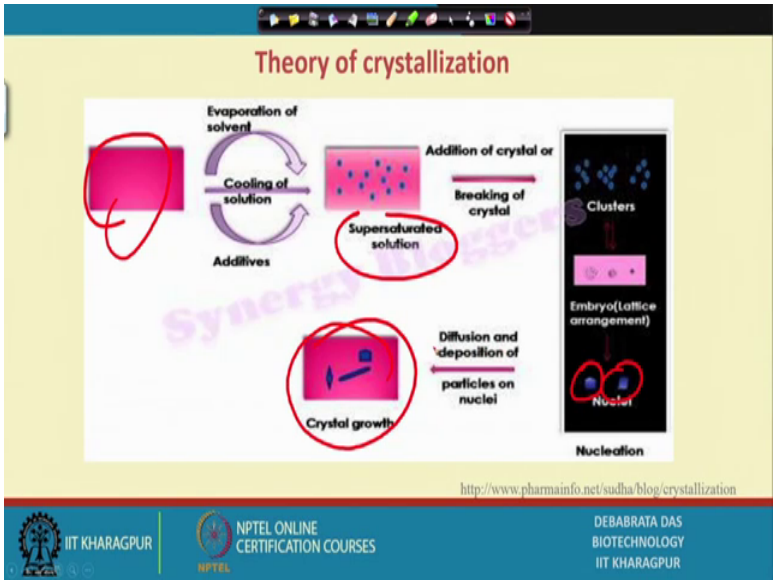

It can be it can be explained like this, that if you look at this is we concentrated the liquid, this is the dilute, dilute dilution of the liquid, then when concentrated the particles are very close to each other, then it coming here, then nucleus formation take place in a when the nucleus formation take place, then the crystal growth take place. This is how the crystal formation, that take place.

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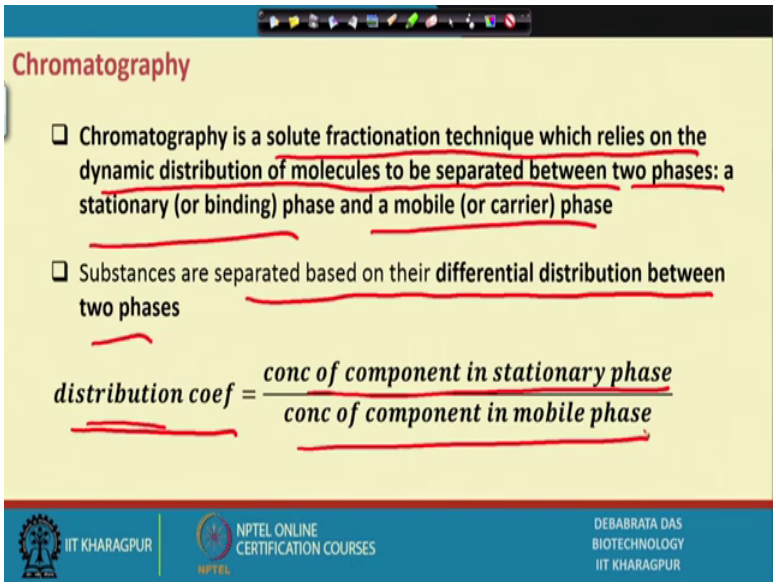

Now, last I want to discuss about the chromatography. Chromatography technique is largely used for the separation of different component present in the reaction mixture and chromatography is a solute fractionation techniques, which relies on the dynamic

distribution of the molecules to be separated between the 2 phases a stationary phase and the mobile phase.

The substance are separated and differential ah that distribution between the 2 phases, the differential coefficient you get distribution coefficient will be concentration of component in the stationary phase, concentration of component in the mobile phase, this is how we can express that.

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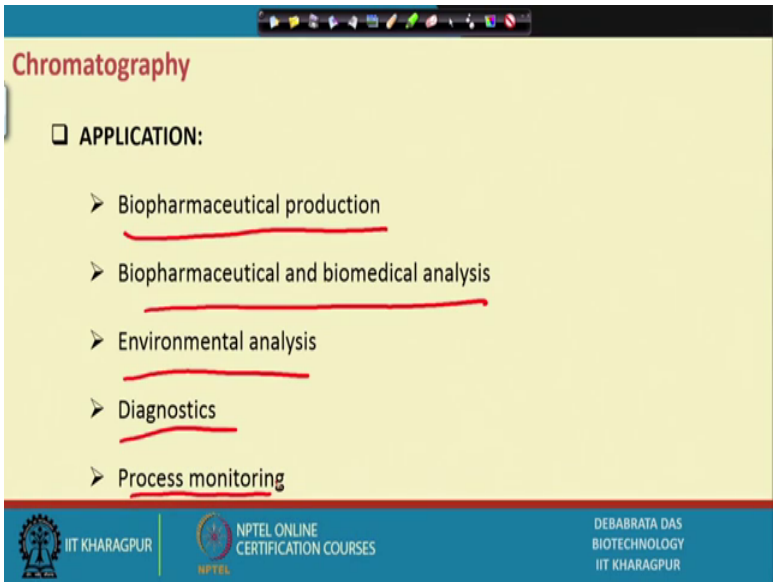

Now, application we have several we have biopharmaceutical production, biopharmaceutical and biomedical analysis, environmental analysis, diagnosis, and the process monitoring. The different purpose it is used we have different type of chromatography.

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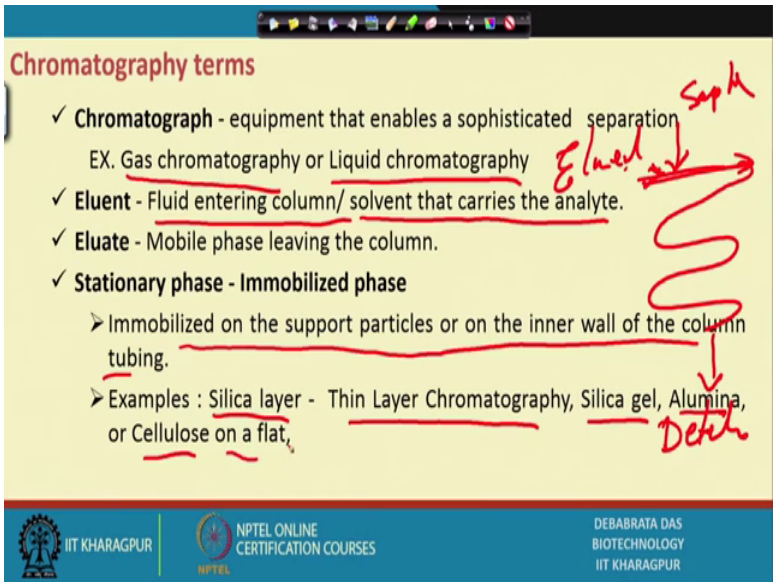

We have gas chromatography, we have liquid chromatography. Liquid gas chromatography is the element is the gas and liquid chromatography element is the liquid.

Now, the element that in a fluid enters in the column are solvent that carries the analyte. So, suppose this is you inject the sample, which and this is this is this is the element. So, you know that take the sample to the column this is this is the column, where the separation take place and then we have detected where that detection of the molecules take place.

So, this is the stationary phase the immobilized of the support material inner wall of the column of the tubing, example is the silica layer thin layer chromatography silica gel, alumina and cellulose on a flat. This is different type of material that is used in the chromatography.

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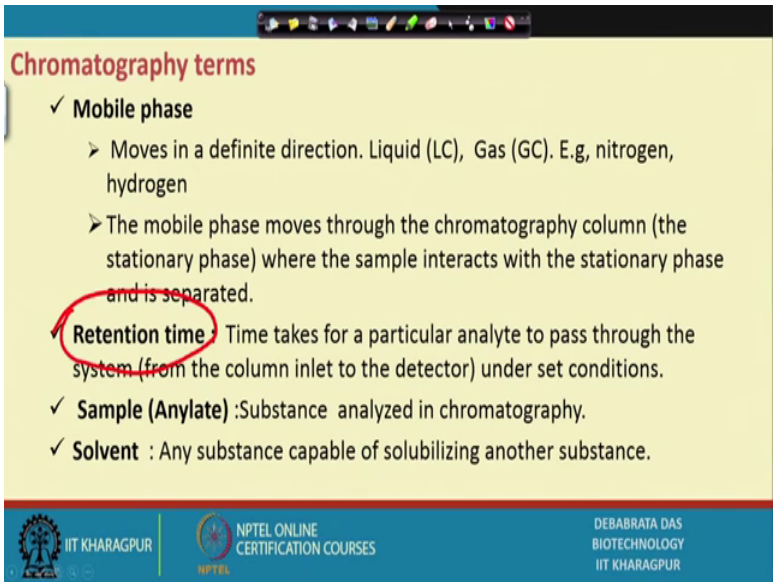

So, we have mobile phase that main the basis on which the particle that we identify that is the retention time, the after how much time that particular components separated in the by the adsorption column, that we can easily find out that first we inject the standard sample. We find out what is the retention time then we injected our sample and find out at that particular retention time, whether we are getting any peak or not and on the basis of we can we can find out this that concept that, the quality the qualitative analysis we can find out.

But, if you if you want to do the quantitative analysis then we shall have to inject the definite amount of the sample.

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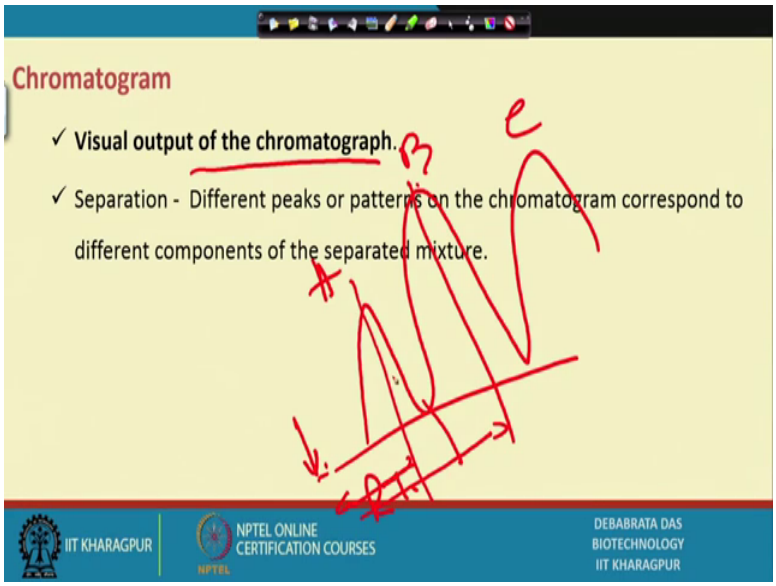

And on the basis of that we can correlate what is the amount of that you know that component present in the sample.

The visual output, because if you of the chromatograph we can we can get the peak as I told you this is peak we will get like this. And suppose you inject the sample here. So, you know what is the time here this is the retention what you call retention time and this is another retention time that we have this is this is another retention time.

So, you if this is for A B C. So, if a the more peak you get; that means, then this indicate that more sample that component present in the sample. The different peaks pattern of the corresponds to the different components of the separate of the separated mixture.

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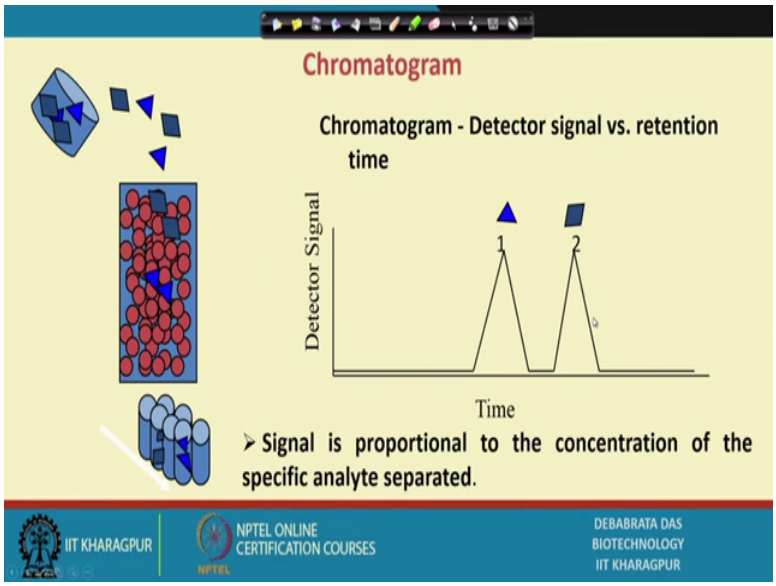

So, this can be represented like this is a where you inject the sample and this you will get the peak like this.

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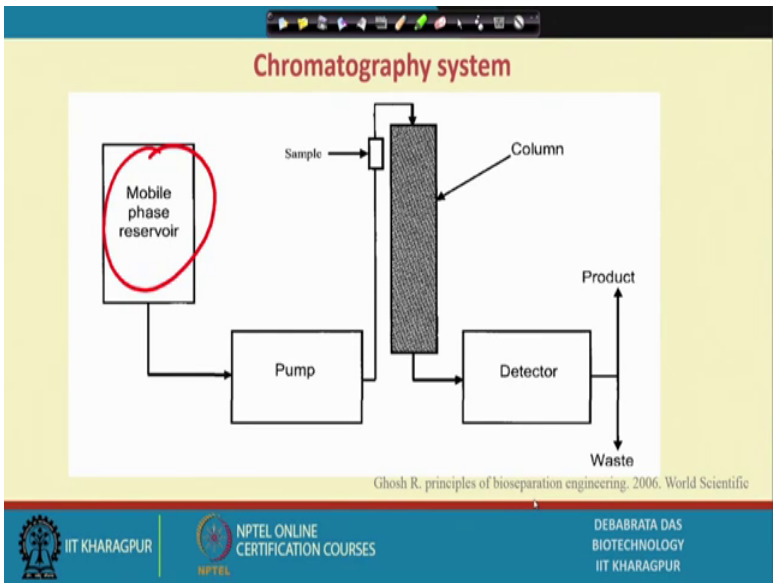

So, this is this is ah this is like this is the mobile phase and this is the pump and it pass through this column and this is the detection from, where we detect the sample and did detection mostly either potentiometric or amperometric.

So, in this particular lecture I try to discuss different type of downstream processing, which largely use both chemical and biochemical industry I try to pick up some example

of the downstream processing, which is mostly used in the biochemical industry. I have given the example of plate and frame filter phase, which is used for baker's yeast industry I have given the example of rotated rotating ah rotary vacuum filter, which is used for most of the penicillin industry where we use the fungi I have given the example of penicillin, where we use the penicillin chrysogenum.

Then, we there we centrifuge we use for the separation of different particulate matters and then there are other techniques that is also for the separation of the particles, we have adsorption particularly color adsorption we do by using activated charcoal. Then finally, I discuss about the chromatography where we can find out the we with this is mostly used for the analytical purpose, where we can also for separation of macro molecules we can do that, that that there where different type of chromatographic technique we have we have gas chromatography we will the high performance liquid chromatography technique, one case we give keep the gas as a mobile phase another case we keep the liquid as a mobile phase.

Thank you very much.