**Principles of downstream techniques in Bioprocess – a short course**
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**Lecture – 8**
**Liquid-Liquid Extraction**

Today is the second class on liquid-liquid extraction and it is a very important downstream technique. that is why I am spending more time on this and today we will also look at some problems related to liquid-liquid extraction.

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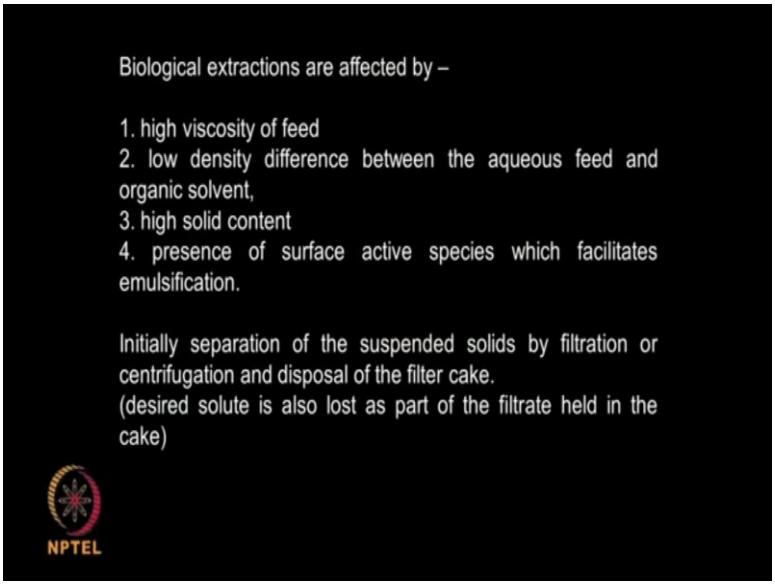


So in bioprocess, liquid-liquid extraction plays a very important role immediately as soon as you filter the solids, you may resort to liquid-liquid extraction to recover your product okay it could be a metabolite, it could be a protein or enzyme and so on. But there are many issues here in biological systems because the feed will be highly viscous, the feed that is coming out of the fermenter broth could be very highly viscous and then there could be low difference in the density between the aqueous feed. That means the fermentation broth and the solvent which you are using that is the problem.

The solid content could be very high because we are talking about dead biomass, live cells, broken cells, precipitated solids because you get lot of solids from salts and media components. Presence of surface active agents like biosurfactants and other material which may be creating froth. So because of all these reasons, you may have problem in extraction, but then extraction is one of the best methods in biological systems. Like I said your proteins, carbohydrates or even enzymes may be very thermally labile and you might not be able to use techniques like distillation or any other high temperature

operations.

Whereas liquid-liquid extraction, you can work in room temperature and even with buffers and sometimes part of your product also may be get lost because presence of solids which may absorb some of your product. Like if you remember, when our early days, early lectures, I did a problem on how some desired product may get adsorbed by the filter K can get lost also.

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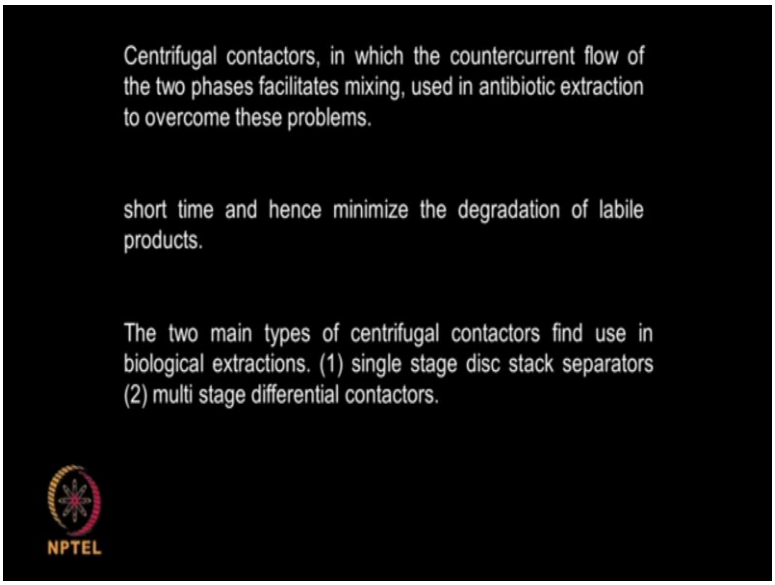


So there are contactors, I think in the previous class, we saw different types of contactors, the mixer-settler type of contactor, then I discussed lot about the column type of contactors. That means vertical columns which occupies less space than centrifugal contactors is another approach, where you can get very high a mass transfer dispersion, so that the transfer of solute from the feed into the solvent becomes very facile actually.

So centrifugal contactors occupies less space and they can be operated in counter-current manner actually and the contact time could also be very very short. So you can minimize even the degradation of labile products using this actually. So you can have disk stack type of contactors, I talked about it and I showed pictures of disk stack centrifuge. So similar disk stack systems can be used for liquid-liquid extraction also or you can have multistage differential contactors or you can have several contactors in many stages.

So centrifugal contactors are becoming quite popular if you are interested in short contact time, if you want to achieve very high mass transfer and very dispersion of these two liquids, when compared to

traditional tower or the normal mixer-settler type of contactors actually.

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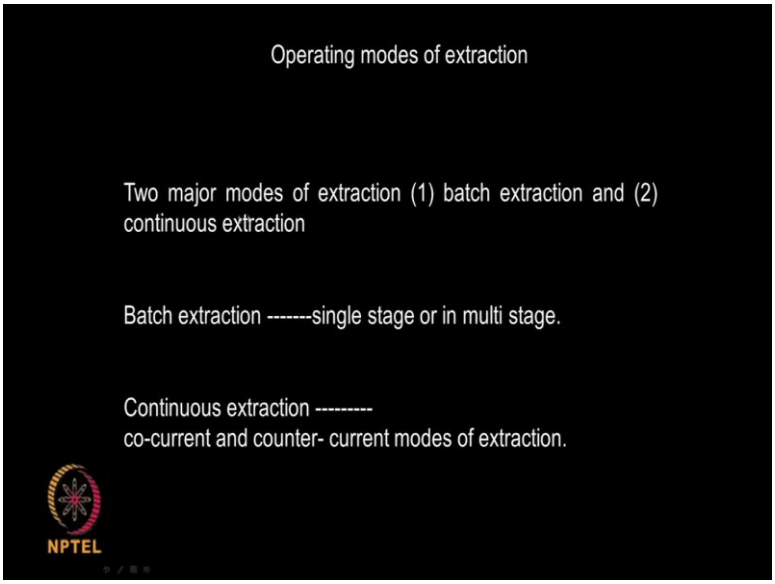


So operating mode of this extraction, it could be a batch or it could be continuous. Okay so the batch extraction could be single stage or multistage, continuous could be co-current or counter-current. Okay the in the single stage you may be adding fresh solvent in the multistage also you may be adding fresh solvent so you will look at each one of them little more in detail.

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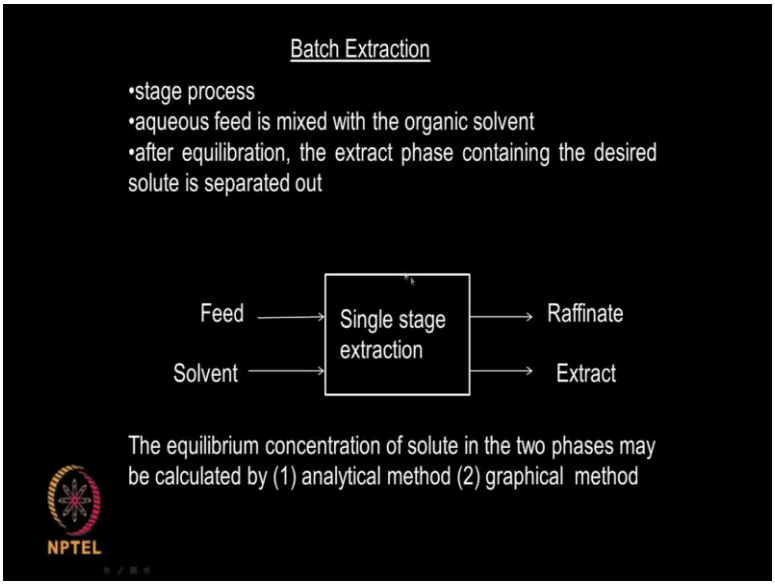


So in the batch extraction, you have the feed and you have the solvent, they get mixed up and there is an equilibrium that takes place. The solute moves from the feed into the solvent because of the high partition or a high affinity. So generally the partition coefficient have to be quite high and the raffinate goes out which will have less amount of solute when compared to the feed and extract that is the

solvent will contain lot of your solute.

Now you can have another stage, another stage and so on actually. Okay now our goal is to find out how much get extracted okay, in order to do that, there are two different methods are there, analytical method and the graphical method, we look at each one of them in more detail.

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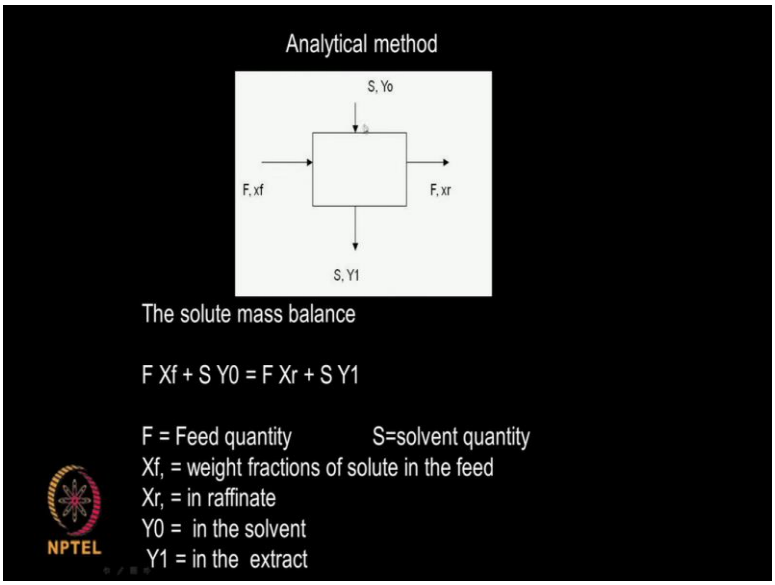


The first step when you are doing a analysis of a single stage extracting unit, we have to do a mass balance. As I said before mass balance is very very important, so you have the feed okay or the broth or the aqua stream it contains Xf the concentration of the solute. Now you have the solvent S, it is coming at S flow rate and the concentration of the solute could be Y0. They get mixed up here and they get separated, so the feed goes out at a flow rate of F and their concentration of the product could be Xr, concentration of your solute could be Xr

Now the solvent has picked up some solute, so the concentration may be Y1 which is greater than Y0. So if you are adding fresh solvent, obviously Y0 will be 0 here, so we can do a mass balance of this particular unit, so 2 input = 2 output so mass balance for the solute F * Xf okay F * Xf + S * Y0 that gives you F * Xr + S*Y1. Correct so if your solvent is fresh that means it will not have any solute. Then Y0 could be 0 then you will have only this term and these 2 terms this will not be there so it is very simple.

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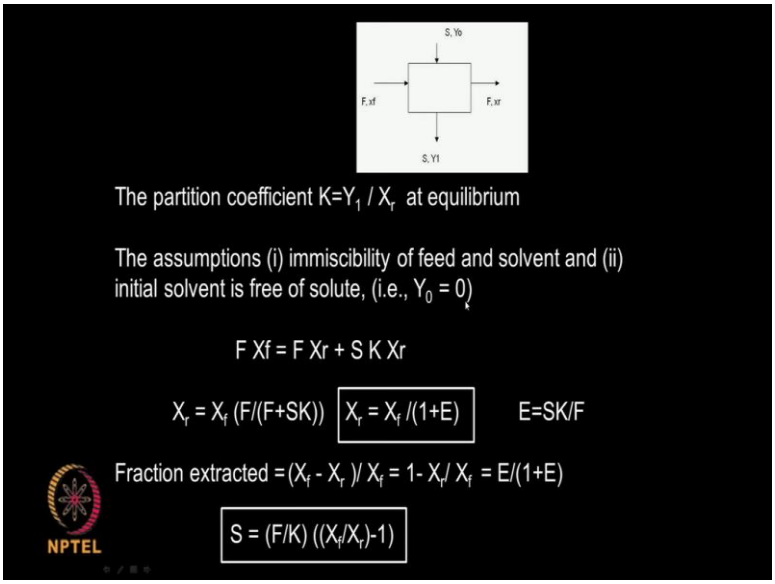


So when you do this mass balance okay and we will assume Y0 = 0 that means the feed solvent that does not contain any solute and these 2 components are invisible. That is very very important okay. They have to be invisible, you may lose solvent, some solvent in the feed that is going out. So you are going to lose solvent. So if I take Y0 = zero you end up with 3 terms here F Xf = F Xr + instead of here, instead of Y1, we are putting this in terms of the partition coefficient * Xr because partition coefficient is Y1 / Xr that is the concentration of the solute in the solvent phase / the concentration of the solute in the raffinate phase Y1 / Xr

So what we are doing instead of Y1 we have replaced it with K into Xr here so you can bring the Xr together so Xr = F multiplied by Xf / F + S into K so if I divide throughout by F what will I have Xr = Xf / 1 + E. E is something like an efficiency factor where E = SK/F. S is the constant the flow rate of the solvent, F is the flow rate of your feed or the flow rate of the broth, K is your partition coefficient okay.

So E and E has to be greater than 0 sorry greater than 1 so Xr = Xf /1 + E so obviously Xr will be less than Xf okay so that is what we accept expect right. Xr has to be less than Xf because the solvent has picked up some of your solute so fraction extractor will be the fraction extractor will be okay Xf that is the feed - Xr / Xf right. Xf is the concentration of the feed Xr is the concentration in the outgoing raffinate / Xf so that = 1 - okay Xr by Xf so if you take this and substitute here you will end up with e by 1+e so fraction extracted is e by 1 + e it is very simple to calculate right.

And E = SK/F and if you want to know how much of solvent, I will require S to extract certain amount.

I just have to rearrange this, so I will get an equation like this S = F/K Xf -Xr – 1. So all these equations are very very important and we are going to do some problems using that equation.

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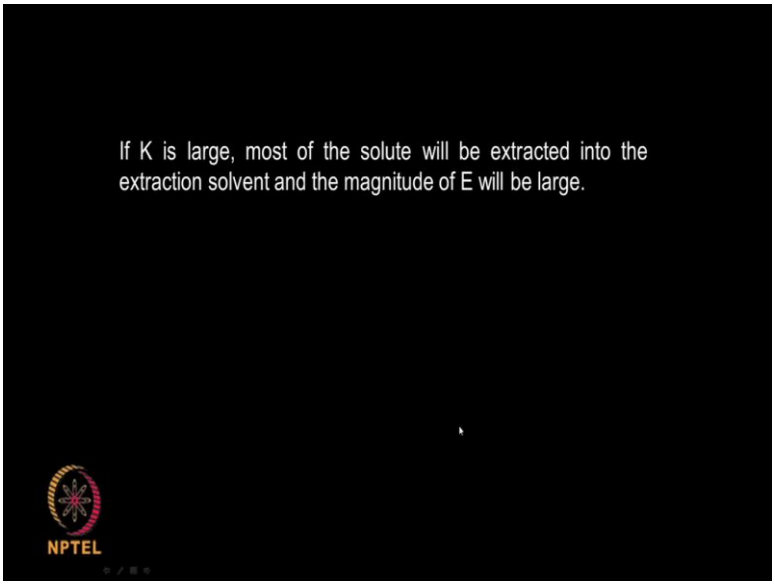


If K is large that means most of the solute will be extracted into the solvent okay. If K is large obviously your E will be large okay so the extraction efficiency will be also very large okay.

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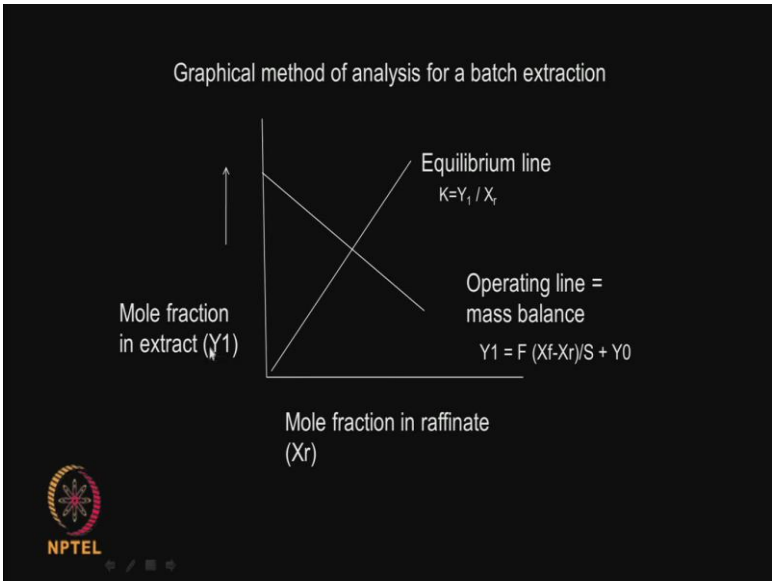


So what you do in graphical method, so in the first method, okay analytical method, if I know these terms like F S and K, I can calculate E and then I can substitute into this and then I can calculate, what will be the Xr so I can get the fraction extractor and so on. In the graphical method for batch extraction, we draw a graph where we have on the x-axis, the mole fraction in the raffinate okay and in the y axis we have the mole fraction in the extract.

Okay so there are 2 lines that will come. one is the equilibrium line that is K = Y1 /Xr okay that is the equilibrium line and it will be a straight line passing through the origin and the other one will be the operating line which corresponds to the mass balance. If you remember our old mass balance equation, we just have to rearrange little bit here, so you end up having Y1 and Xr. so as the mole fraction in the extract keeps going up that means as the more solute is taken up, more into the solvent you, will have less of the solute in the raffinate and if the mole fraction in the extract is less obviously there will be higher concentration will be raffinate.

So that is why you have a straight line that is going down like this. So these 2 lines will cut at some point and this is the your condition at which you are operating corresponding to both the mole fractions in the raffinate as well as the mole fraction in the extract.

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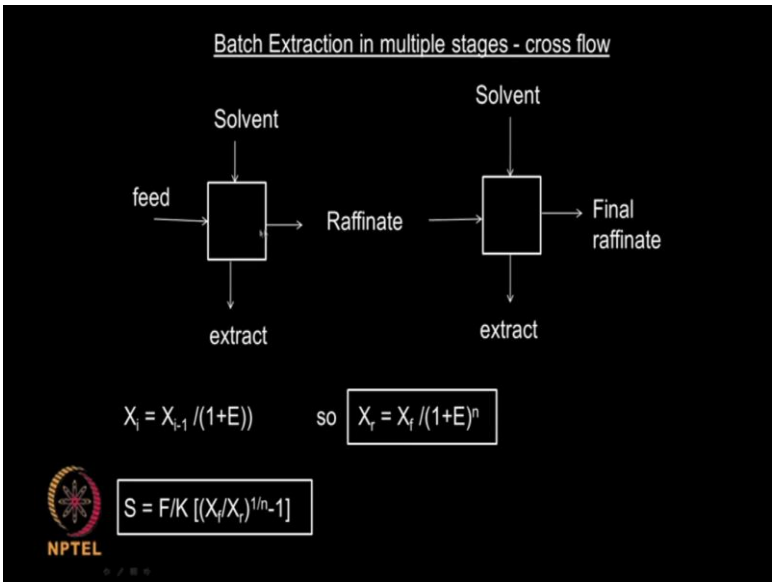


With the help of calculators or computers, we can quickly calculate them. When their computers were not there, then people resorted to this type of graphical method okay. Suppose so you can have multiple stages, so every stage I add some solvent. So you have a feed, you had a solvent and then you are extracting, then the raffinate is going to the next stage, then again you add some solvent again it gets extracted like that it keeps on going.

You may have many number of feeds, many number of stages sorry many number of stages and you may be adding solvents each time. So the final Xr that is final concentration of the solute in the raffinate = Xf that is in the feed / 1 + E raised to the power n. n is the number of stages. Now this is called a cross flow system because your feed is going this direction, solvent is added vertically right,

that is why it is called cross flow. Okay so the final Xr will be = Xf feed / 1 + E raised to the power n.

So when n = 1 will get / 1 + E right so the advantage of this equation is tremendous. So if I want to know how many stages of cross flow, I should have if I have to extract 99% then I can use this equation. If it is 99 % extraction, your Xr will be 0.01 of Xf correct then if I know E, E is nothing but efficiency which we talked about. If you remember this E is nothing but SK/F. So I know S, I know F, I know K and I can calculate E so from there I can get the number of stages.

Okay so this is the very powerful equation for calculating the number of stages required for example to recover 99 % of my solute in n, n stages of cross flow.

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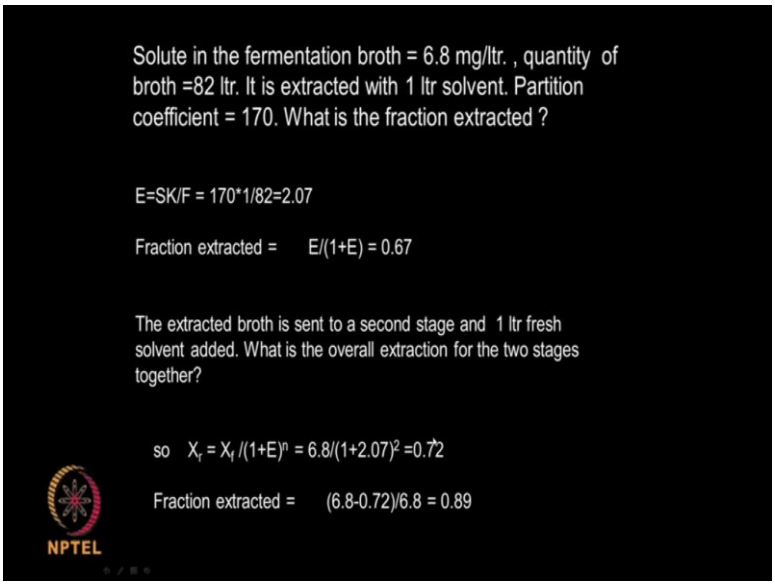


Okay in fact that is what this problem is. so I have a solute in the fermentation broth, 6.8 more milligram per liter. so this is your Xf quantity of the broth is 82 liter this is your F. 1 liter of solvent this is your S. Partition coefficient is 170. so what is the fraction extracted? First I need to calculate Xr I know Xf and then I can calculate fraction extracted okay so what is the formula for fraction extracted? Just recall formula is E/1 + E right so I need to calculate E

E = SK /F, S is given by 1 liter, K is given by 170 and my F is given by 82. So I get E as 2.07, so I told you E is generally greater than 1 then only we will get some extraction done. So I put E / 1 + E that gives 0.6, that is 67 % extracted. Now I am sending it to another stage and again I am adding 1 liter fresh solvent okay. So what do I do? How much they extracted?

Now I have two stages, am adding 1 liter in the first stage and 1 liter so I can use this the previous formula if you remember Xr = Xf /1 + E to the power n. Here n is 2 so I can use that formula right? Xr = Xf/1 + E, 2 I know the E, I know Xf is 6.8. So my Xr becomes 0.72, fraction extracted is 6.8-0.72 /6.8 should becomes 0.89, 89 % so the first stage, I am extracting 67 %, second stage I am extracting overall I am extracting 89 %.

So it is good to have 2 stages. Okay but of course I have used 1 liter solvent in the first stage and another 1 liter solvent in the second stage.

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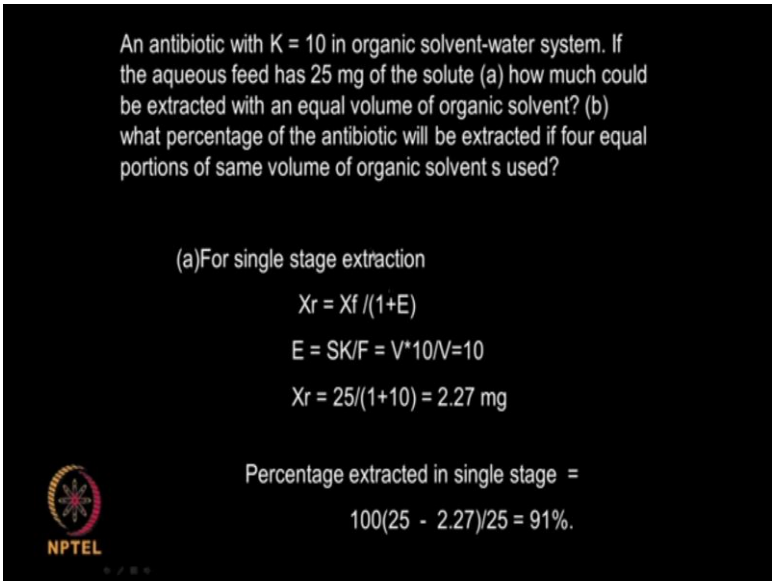


Okay now look at another problem. It is very interesting problem. I have K here, given a feed is 25 mg of solute that is my F, how much could be extracted with the equal amount of organic solvent? that means if I take 25 mg of an that is S solvent, how much will I be able to extract it is very simple. I just calculate E sorry and then E = SK /F okay so that = 10 here and then I substitute I get Xr as 2.27 so percentage extracted, that is I am having 25 initially Xr is 2.7 so I am extracting 91.

Now I am dividing the same 25 into 4 parts okay, 25, 4 that = 25 / 4 is 6.25 and then adding into 4 stage then what will be my extraction efficiency so here my s will become 6.25 K will be same s will be same so my e will come down. But then my equation is different, I will use Xr = Xf /1 + E raised to the power 4 do not forget that okay so my E has come down by 4 times because I have divided but my equation denominator has become 4 so my Xr has become 0.166.

So percentage recovery is 25 - 0.16 / 25 so 99 % very interesting, so if you look at the previous one if I

take 25 mg of solvent and use it in one stage my recovery is 91 % but the same 25.

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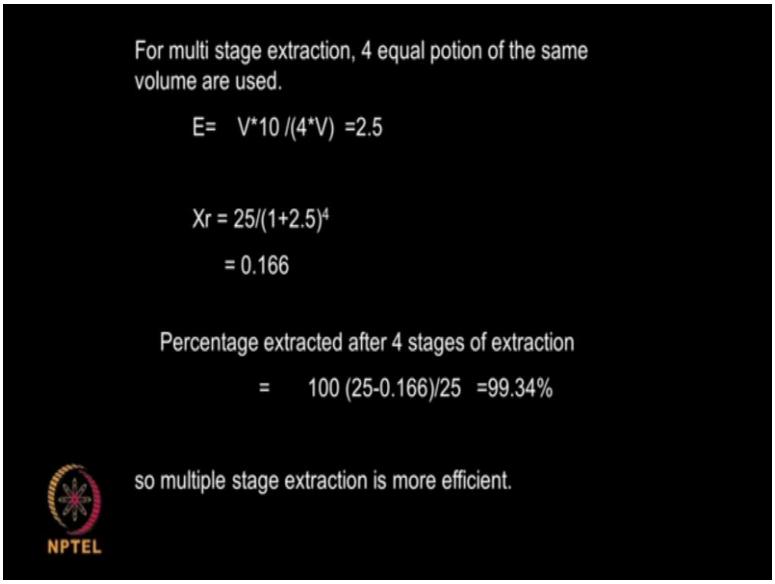


If I divide into 4 parts that is 6.25 milligram and do four times extraction, my efficiency is 99 %. So what does it tell it tells us instead of dumping all the solvent in one stage and trying to recover if I divide it into 2, 3, 4, many stages and use part of this solvent my recovery is going to be better that means multiple stage extraction is more efficient than single stage. So I have used same amount of solvent okay that 25 milligram but when I divide it into 4 parts and use it as 4 stages my recovery efficiency is better okay.

So this is a very important point to keep even when you are doing experiments in your lab so do not dump all the solvent and try to extract. Divide the solvent into may be 2 parts or 4 parts and use little bit extract remove extract remove extract remove extract remove your efficiency will be much higher okay.

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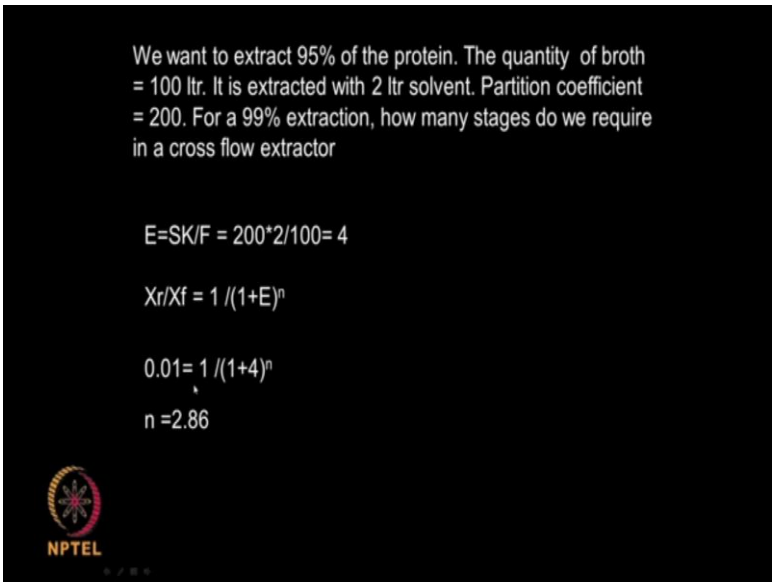


Now another problem let us look at here we are going to calculate the stage you want to extract 95 % of the protein that means my Xr will be 0.05 of the Xf okay the quantity of the broth F is 100 liter 2 liter of solvent. F is 100, S is 2 liter, partition coefficient K is 200, okay so if I want to extract 99 % okay then what happens my Xr = 0.01. Okay so I can use the same equation here, am calculating n okay so 0.01 = 1 by 1 + E raised to the power n. 0.01 = 1 / 1 + e raised to the power n, E = SK/F.

So s know okay s is 100 liter K is given here okay K is given as 200, okay so okay sorry solvent is 2 liter, F is 100 liter, K is 200 so when I substitute E comes out to be 4 so from this I get as n = 2.86 right. Sorry so I get n as 2.86 that means I need 3 stages if I want to recover 99 % of extraction okay if you want to extract 99 % if I am using 2 liter of solvent and broth is 100 liter K is 200 liter. So this is very important data in a industrial scale, so I want to extract 90 %, 95 %, 99 % of the material okay

So how many stage I should put in? So in order to understand, I need to calculate E then I substitute into this it is a quite simple equation which I got and I substitute into this equation and I get the result okay.

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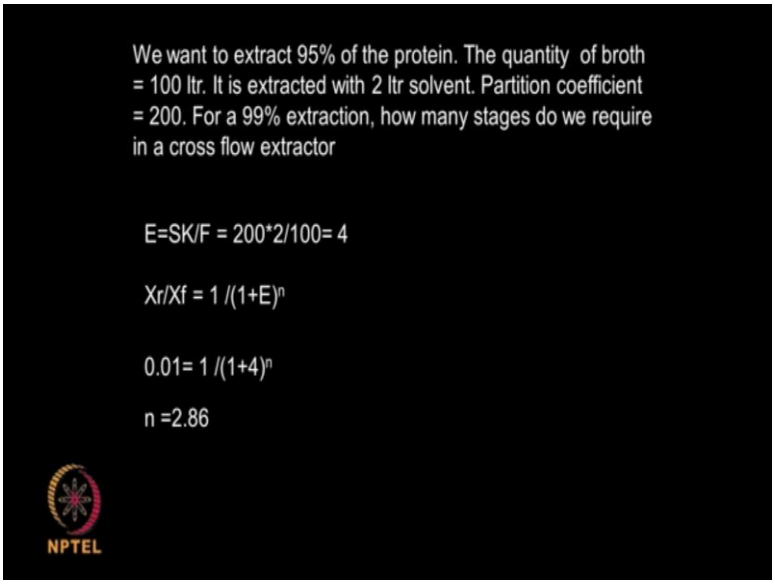


This is called cross flow. cross flow, what do you do? Every time you are adding the solvent, it could be fresh solvent or into each stage okay. So your feed flows stage 1, stage 2, stage 3, stage 4, whereas solvent is entering independently in each of these stages, that is called the cross flow extraction okay.

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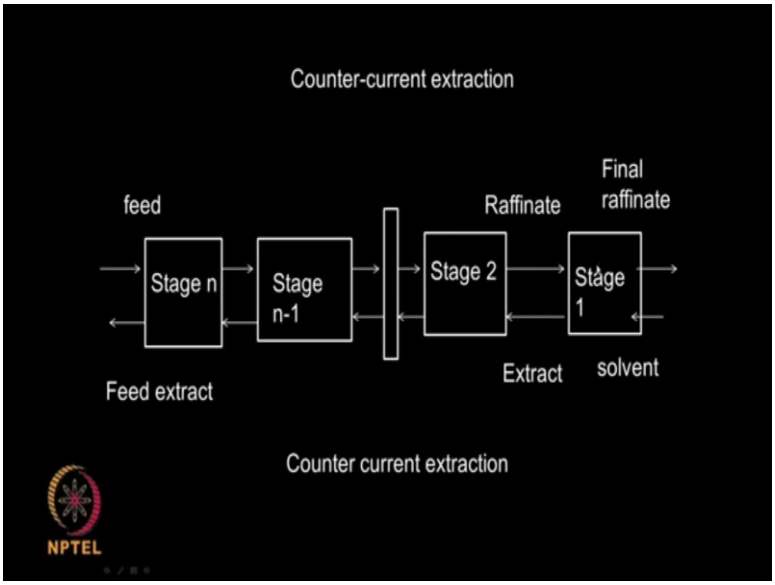


Analogous to cross flow we can also have co-current as well as the counter-current type of operation. This is called a counter-current operation, so you have multiple stages here, your feed may be going from stage here, here, here, here and right down to 1. Your solvent may be coming from stage 1 to 2 to 3 to 4 to 5 up to n so feed is flowing in one direction your solvent is flowing in another direction that is why it is called counter-current. So final raffinate is coming here whereas solvent is coming here.

The solvent here could be very fresh but then solvent coming here will not be fresh. It may be having

some amount of solute because it has picked it up from stage 1 and so on and so the concentration of solute will keep increasing in the extract as you move from the right to left okay . Whereas in this case the concentration of the solute the feed will keep going down as you know from stage n to stage 1.

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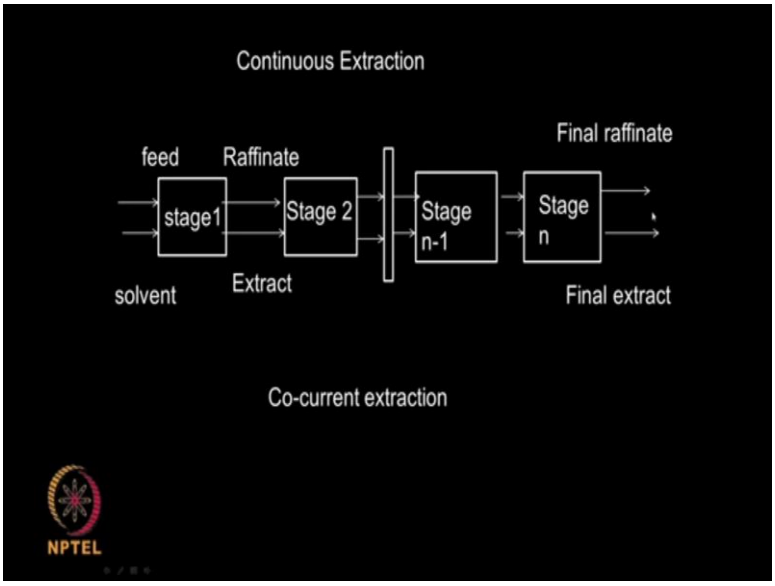


Analogous to counter-current we can also have a co-current that means both are flowing in the same direction okay and like the other one and you can prove that co-current operation is not efficient at all when compared to counter-current. I will leave it to you to prove that that co-current is not efficient compared to co counter-current at all. Okay let us go back to counter-current, so in a counter-current the feed moves in one direction, the solvent moves in another direction.

So we can do mass balance in for each stage and prepare a overall global mass balance when you do that okay when you do that you get a relation.

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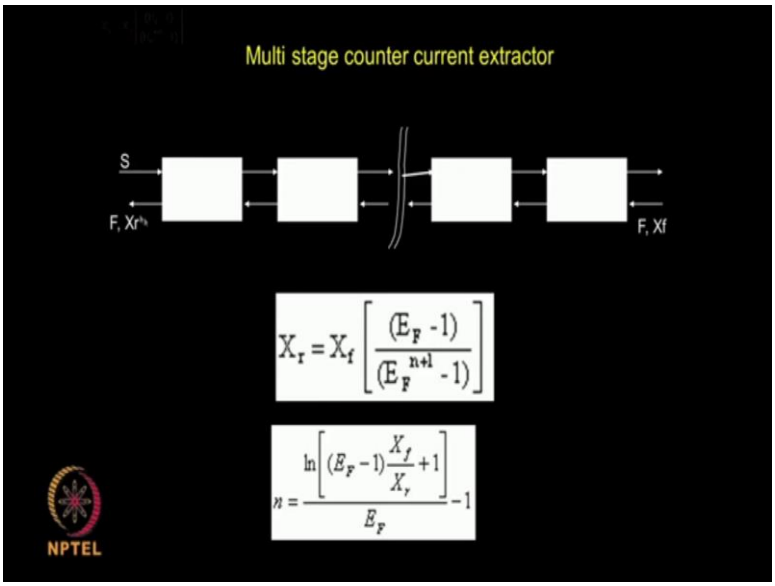


It looks very complicated okay Xr that is the final concentration of the solute in the raffinate Xr, Xf is the concentration of the solute in the feed. Feed is going like this = E - 1 /n + 1 - 1 okay. Now E is again calculated using SK/F formula okay S is your solvent coming from this side to this side, so feed is coming from this side to this side. So solvent keeps picking up material okay. So it is Xr = Xf e - 1 / n + 1 -1 okay.

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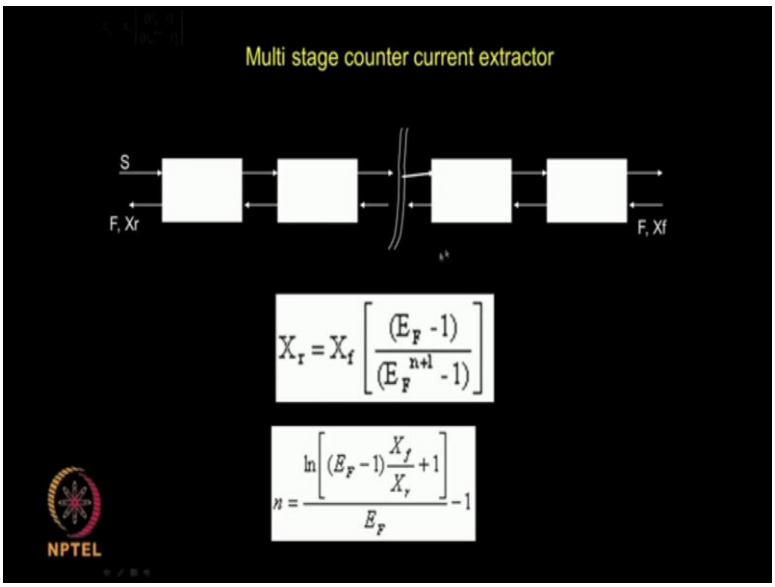


So there is a difference between the previous an this in cross flow we have Xr = Xf / e + 1 or 1 + E raised to the power 1 here the equation is different the assumption here, K is same in each stage that means it does not change irrespective of the concentration, K does not change. so K remains the same in all places okay. So I can adjust these equation to get into relation for n also that means if I want to find out how many stages I can do that also so this equation is very very important.

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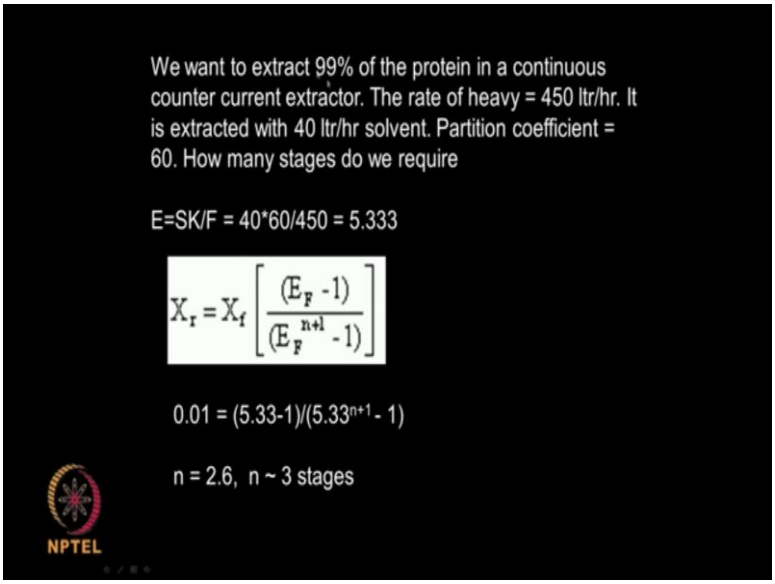


we will just do a problem okay we want extract 99 % of the protein in a continuous counter-current extractor rate of heavy is 450 liters per hour F. It is extracted by a solvent 40 liters per hour s partition coefficient is 60 okay so e = SK/F by F. 40 multiplied by 60 / 50 that comes to 5.33 so I want to know how many stages so I can use this equation I want to extract 99 % means Xr = 0.01 Xf so 0.01 = 5.33 - 1 / 5.33 n + 1 – 1. So all I have to do is calculate n simple so I use this equation and I do that.

So when I do that I will get three stages okay so I need 3 stages of counter-current liquid-liquid extraction to recover 99 % of it is solute when the e is 5.33 very useful equation okay.

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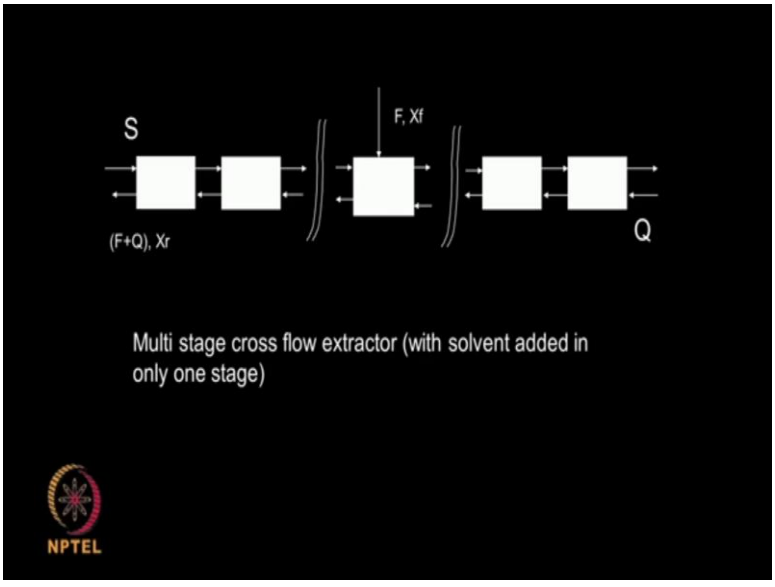


Similarly just like counter-current, we can have also something called multistage cross flow extractor

okay I want you to look at how this looks like as against the this one. So here you have only 2 streams okay so you have the feed or the heavies or the aqueous goes like this Xf is here, Xr is here, we have the solvent coming here fresh solvent entering and going all the way down okay and going there okay. So that is how both of them look like.

Okay now whereas the next one next setup is called multistage cross flow extractor. So we have the feed entering from the middle F Xf so we have a stream aqueous stream it is called Q solvent is coming from here okay. So here after this the quantity increases F+Q okay because feed is coming with the flow rate of F and you are having another stream of heavy Q. So Q does not contain any solute please note that that is why we have not put any term here Q does not contain any solute okay.

So after this stage you are going to have F + Q that is the total amount of the heavies or the aqueous stream or the raffinate flowing like this okay. The solvent goes like this and then goes like this and finally you get the extracted solute, so this is called a multistage cross flow extractor. Solvent is added only in one stage, okay the normal cross flow, solvent is added in several each of the stages whereas here it is added only in one stage. So you also have an equation for this type of setup also combining Xr was is Xf was is E and please note you are going to have two types of E here.

One E for this side of it because normally when we calculate SK/F okay here we have the heavy flow is only Q and when you come here this side the heavies has 2 flows F + Q so you will have one E and you will have another E so we can call one E and another one as E dash so that is because your F changes from here to here. That is F means when I mean F is the amount of the heavies or the amount of the aqueous amount of the feeds changes here so you also will change so for this portion of it as well as for this protein of it.

So there will those portions will come into the equation so still you can derive but all these derivations are based on mass balance only so it is very simple to you have good mass balances, you have to combine all the mass balances to arrive at a convenient equation which relates the Xr that is the concentration of the solute that is entering the extractor okay. So I think with that we complete what we want to talk about on liquid-liquid extraction liquid-liquid extraction is very powerful setup okay.
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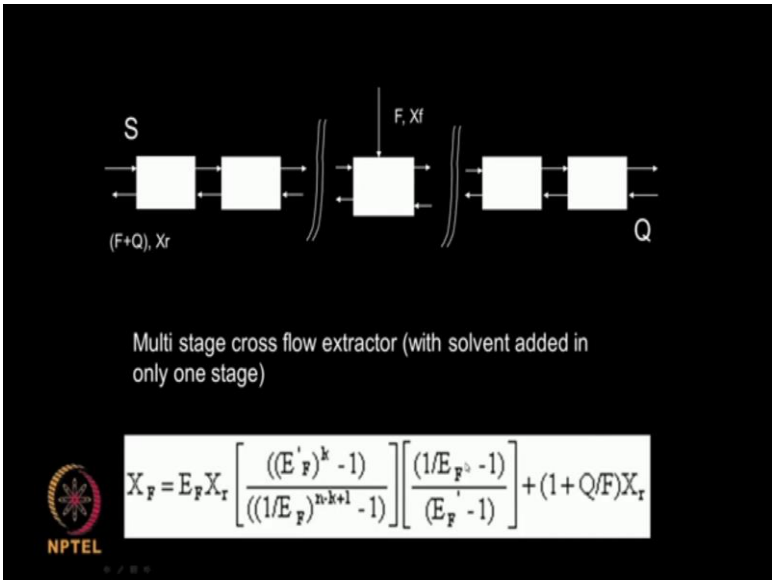


Look this is the equation it looks very complicated and as I mentioned there are 2 types of there E and E dash one E relating to Q flow rate another E relating to F + K okay.

Thank you very much!!