Principles of downstream techniques in Bioprocess – a short course
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Lecture – 02
Flow Sheet, Mass & Heat Balances

Hello everyone. Today am going to talk about a few flow sheets as an example and I will also tell you how to go about doing mass balance and heat balance for a unit operation.

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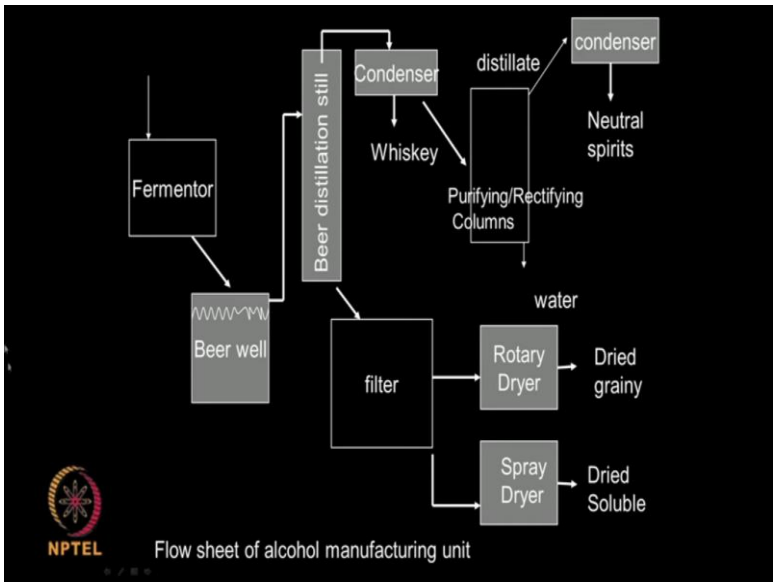

So here, this is an example of a flow sheet of a alcohol manufacturing process okay, as you can see on the left hand side, here the fermenter that is the heart of the entire unit operation, where the fermentation takes place okay. Once the fermentation is over, the product comes down and there is a distillation which happens here okay and the product ethanol is collected here on the top whereas the bottom contains all the solid material which is dried here and then it goes as a dried product.

So there you have a rotary dryer, you have a spray dryer, as you can see this particular downstream process for the fermentation of alcohol contains a distillation unit and you have a filter unit and you have dryers and the down and here the product ethanol is collected here and further distilled to get very very pure spirits here. So this is a very simple downstream process and we do not have many unit operations except for filters dryers and distillation column and as you can see ethanol is thermally stable compound so there is no problem we can use distillation column to remove the ethanol from the top.

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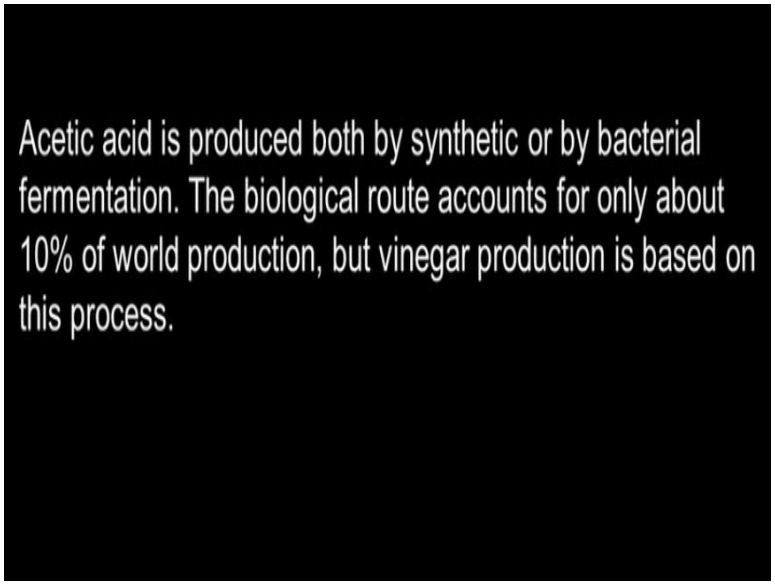

So let us look at another downstream system and this is related to the acetic acid manufacturing. Acetic acid is manufactured both by the synthetic and the bacterial route and the one which is manufactured by the bacterial route is what is used in the food as vinegar okay. 10 % of the worlds production is through this particular route.

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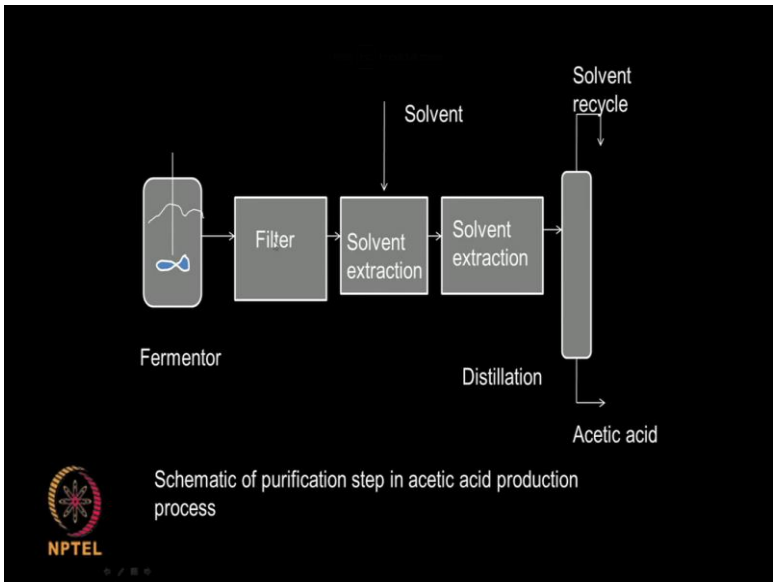

So if you look at here you have again on the left hand side, the fermenter then the filter. If you recall earlier I said that filter is the first downstream process after a fermenter or bioreactor to remove this solids, the biomass and then it goes into the extraction, you have a solvent and you have a dryer or extractor here, where the acetic acid is extracted with the solvent. And then again you have a distillation column where you get the pure acetic acid at the bottom.

And the solvent at the top which is again recycled here for the solvent extraction process. So here the downstream is a filter and a string of solvent extraction units.

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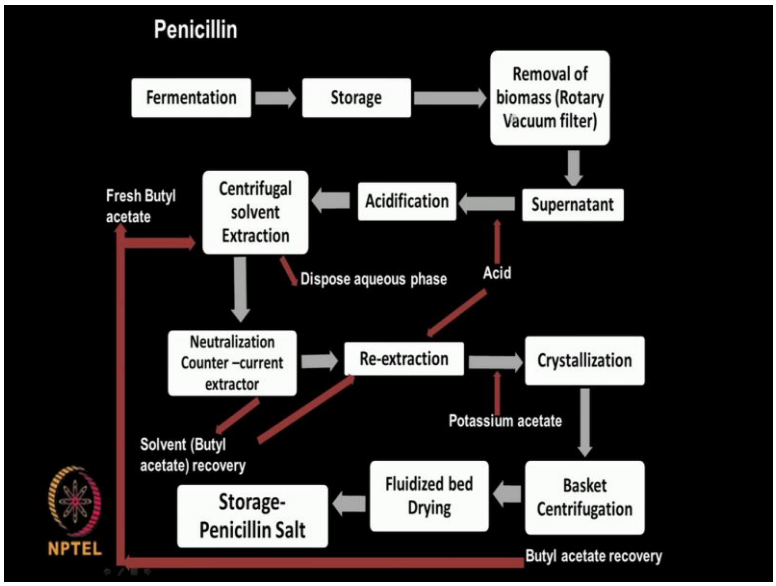

Let us look at penicillin, so one of the important antibiotics and as you can see the downstreams are getting more complicated and complicated so you have the fermentation again here and then removal of biomass using filter and then the supernatant. We are acidifying the supernatant. We are going into a centrifugal process here and the neutralization here. We have the extraction, crystallization, centrifuge finally the product is dried, you get the penicillin salt.

So here as you can see the product is a solid so obviously you need to go into centrifuge and then we are collecting after drying and then finally as a salt here. So here downstream contains quite a lot of filters and then it also contains dryers as well actually.

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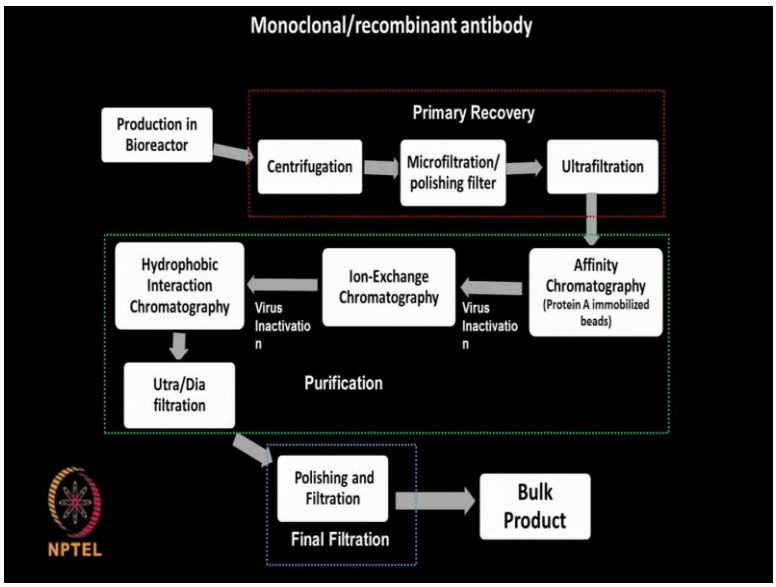

Now let us go further down into complication. Now we are looking at the monoclonal recombinant antibody, so in that as you can see after the bioreactor, we have the primary recovery and then we have the purification and then final filtration so initially again we have a centrifuge and then we

have another filter. Two types of filter as you can see polishing filter as well as the ultra filter. Microfilter and followed by the ultrafiltration. So microfiltration removes larger material, ultra filtration remove smaller material.

Then we go into now affinity chromatography, ion exchange chromatography, hydrophobic interaction chromatography as you can see a string of chromatographies where you are purifying your product and then it goes into a set of filtration and finally you get your product here. So as you can see, you have filtrations and then you have chromatography and then finally you go into again filtration to recover your product.

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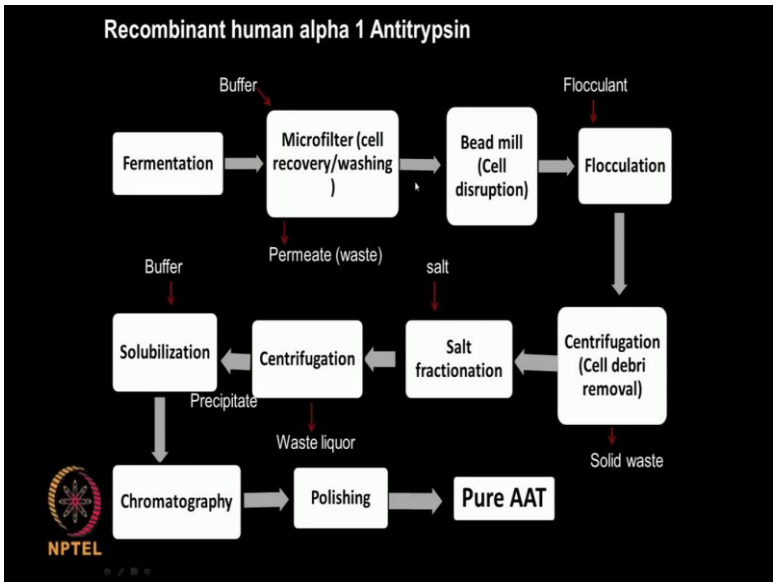

Now let us look at another product, it is called recombinant human alpha 1 antitrypsin. So after the fermentation you can see there is a filtration and now here the product is intracellular so obviously we have to harvest the cells, collect the cells, that means harvest the cells and then disrupt the cells using a bead mill and then we are collecting the product by flocculation, centrifugation. And you are removing it using a salt precipitation again centrifugation then a set of chromatography here and the final washing and polishing and you get the pure recombinant alpha 1 antitrypsin.

So you see, we have introduced new downstream especially a beadmill because the product is intracellular.

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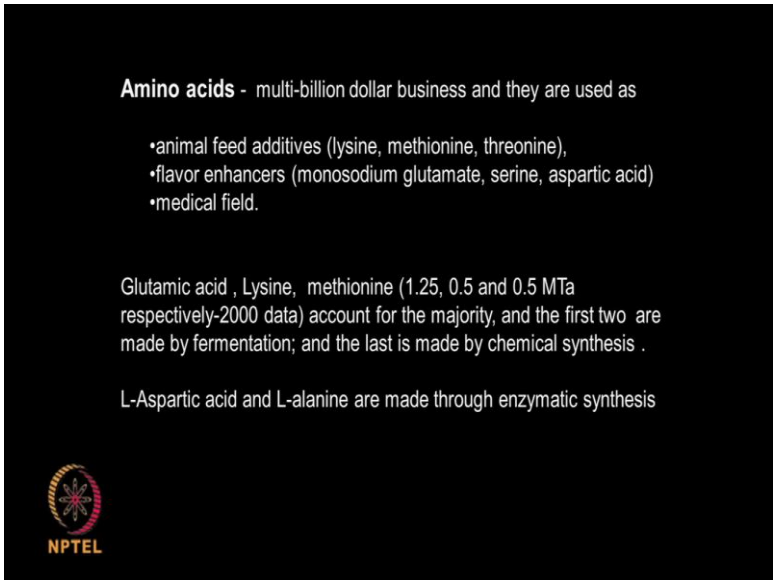

Now let us look at a large number of amino acids. Amino acids are a multi-billion dollar business. They are used in animal feed and it is like lysine, methionine, threonine, they are used in flavour enhancer like aspartine, serine, glutamate, monosodium glutamate, then they are used in medical applications. So large number of amino acids are manufactured using biochemical route products like glutamic acid, lysine, methionine, they are the largest manufacturer as you can see millions of tons are produced actually. Okay, then aspartic acid, alanine also made to enzymatic synthesis so many amino acids are manufactured using biochemical process.

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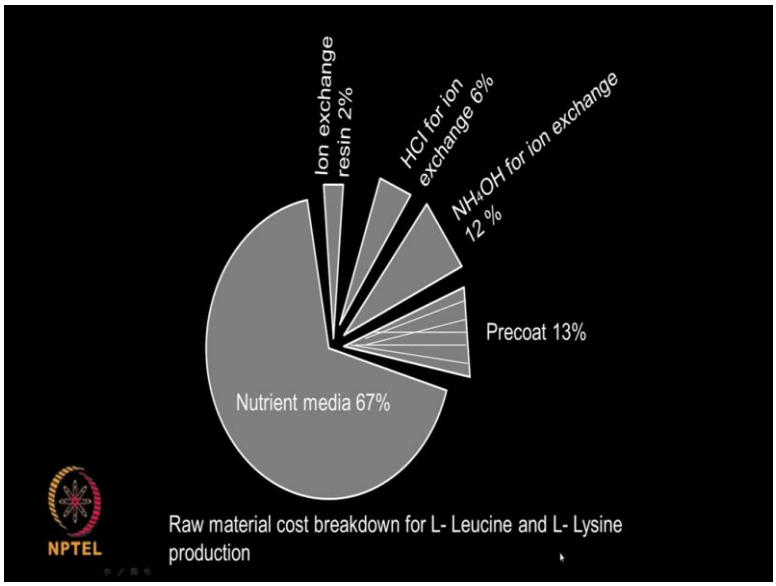

And look at the raw material breakage cost. If you look at the manufacture of leucine and lysine and the breakdown of raw material cost, 67 % is due to nutrient media, about 2 % is about ion exchange chromatography. Okay. Then 12% is because again ion exchange chromatography and again you can see 6 % is because of the ion exchange chromatography of various kinds. So almost 20 % of your raw material cost in the manufacture of leucine and lysine is because of the components used

in the ion exchange chromatography. So if I can reduce the amount of various raw materials used in the chromatography, I will be able to reduce the raw material cost okay.

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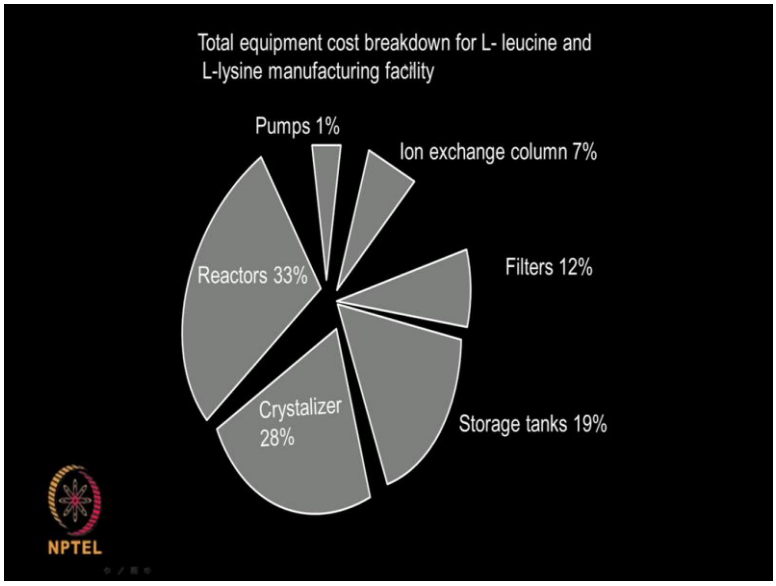

Now let us look at another amino acid like leucine and lysine manufacturer. Again if you look at the equipment cost for manufacture of these two products, you can see only 33 % of the cost is because of the reaction whereas 28% is because of crystallization, 7% is because of ion exchange chromatography, 12 % is because of filter and 1 % is because of the pump and 19% is because of the storage.

So predominantly equipment cost is because of the downstream reactors take only 33 % remaining 67% is because of the various downstream steps, filters, ion exchange columns, storage tanks, crystallizers and so on. So if I can optimize these I will be able to reduce the total equipment cost.

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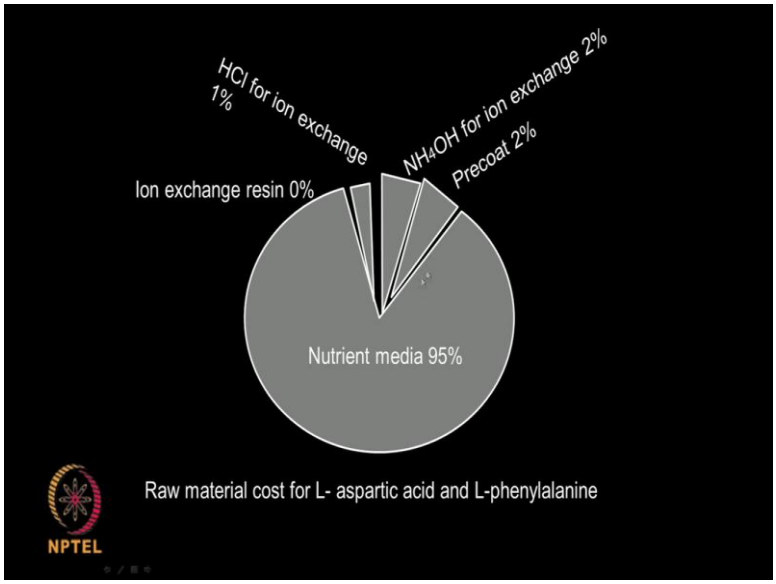

Let us look at again the aspartic acid and phenylalanine but the raw material cost, if you look at the raw material cost here 95% of the raw material cost is because of the nutrient and only the remaining 5% is because of the various raw materials used in the chromatography. So as you can see in the previous case as against here they predominantly the nutrient media takes up most of the cost.

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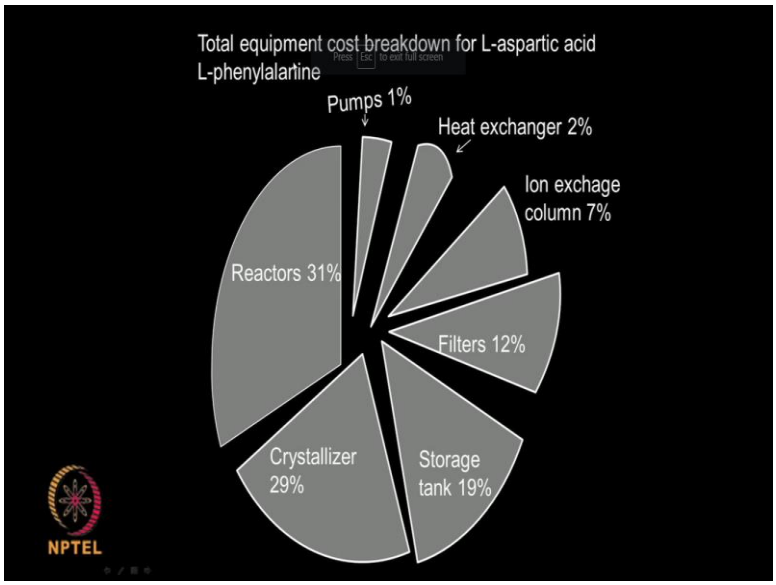

Let us look at the equipment breakdown cost for aspartic and phenylalanine. You can see reactors take only 30 % remaining is because of the downstream; you have the storage tanks, the crystallizers, the filters, and the heat exchangers and exchanges pumps and so on. Almost 69% of your expense is because of those various equipments down of the reactor. So we need to look at how to reduce those number and how to optimize their numbers thereby I can achieve a reduction in the total equipment cost.

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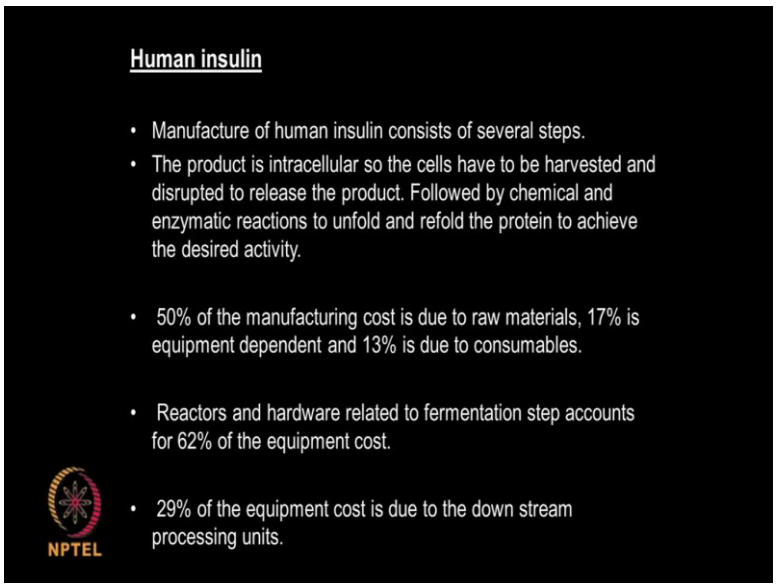

Let us look at another important product that is called human insulin. Okay the human insulin is produced through various steps. The product is an intracellular so that means I have to harvest the cells have to break the cells using some enzymatic approach then I need to unfold and refold again so that the insulin regains its activities. So it is a very large number of steps involved in the manufacture of human insulin and 50% of the manufacturing cost is due to raw materials 17 % is because of equipments and 13% because of consumables okay.

As you can see only 50 % is due to raw materials, rest all is because of the equipment dependant and consumable dependant cost. So I need to play around with that to reduce the total cost of manufacturing of human insulin. Again if you look here 62% of the equipment cost is because of the reactors and 29 % is because of the downstream so quite a considerable junk of expense is because of the downstream steps involved.

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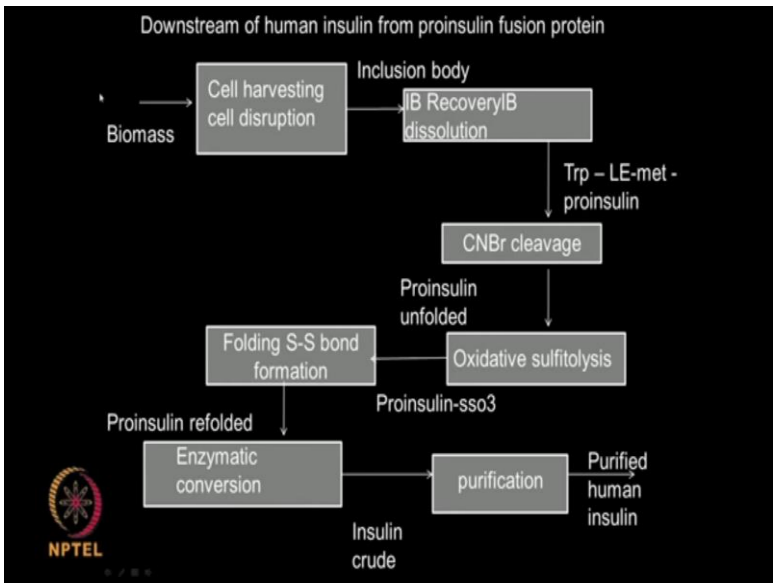

And this is a typical steps involved in the manufacture of human insulin, you are making your

fermentation, biomass cell is harvested and then the inclusion body contains your insulin which is completely lost its activity and you need to unfold and again refold so that it regains its activity. These are the various steps so you have not only the fermentation taking place here, you have the reactions taking place or bio transformation taking place, so that you get your human insulin in the active form.

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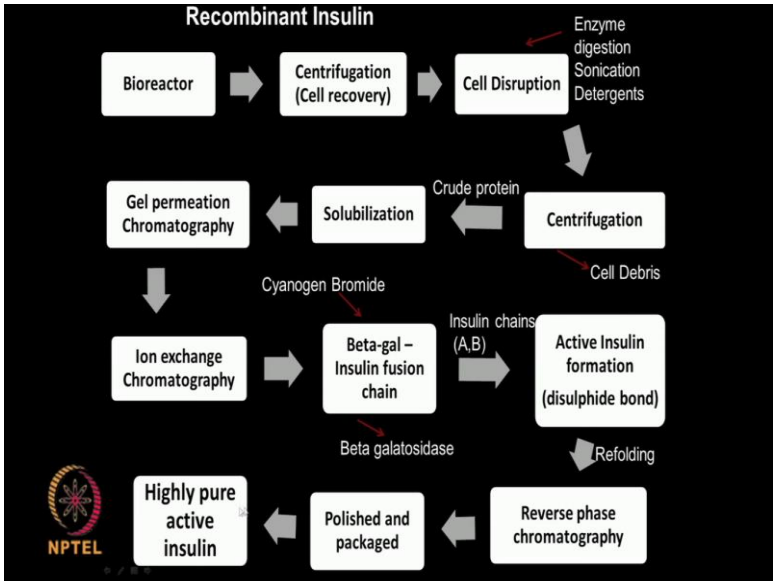

So this is how the entire flow sheet looks like, so you have the cell disruption, you have the centrifugation and then you have the bioreaction, then you have the centrifugation again here, then you have the solubilization, you have the chromatography, two types of chromatography again, you are preparing the folded product, you have the chromatography here, polishing and finally packaging. So you end up with the product here.

So you can see two different biotransformation is taking place. You are seeing lot of chromatography taking place and you are seeing centrifugation as well as the cell disruption taking place here actually.

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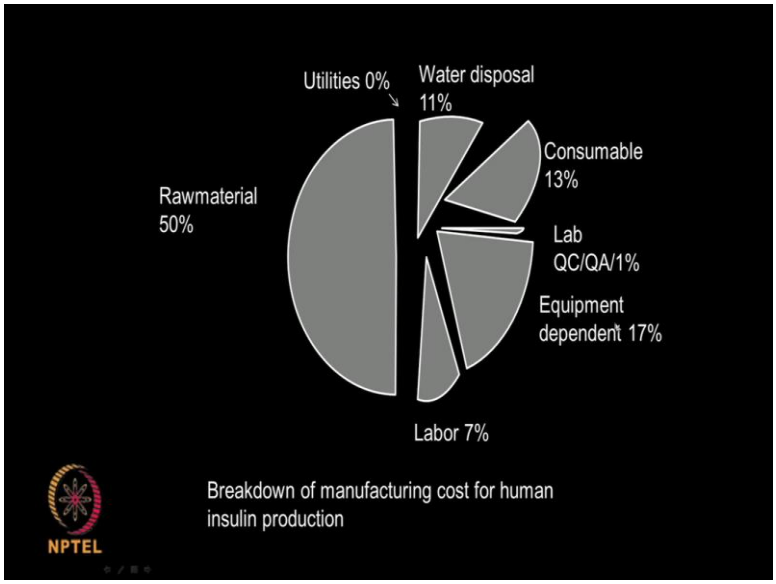

So if you look at the breakdown of cost 50 % is raw material and rest all is related to the equipment related cost which is 17%, consumable is 13% water disposal that means the environmental remediation is 11%, labour is 7% okay.

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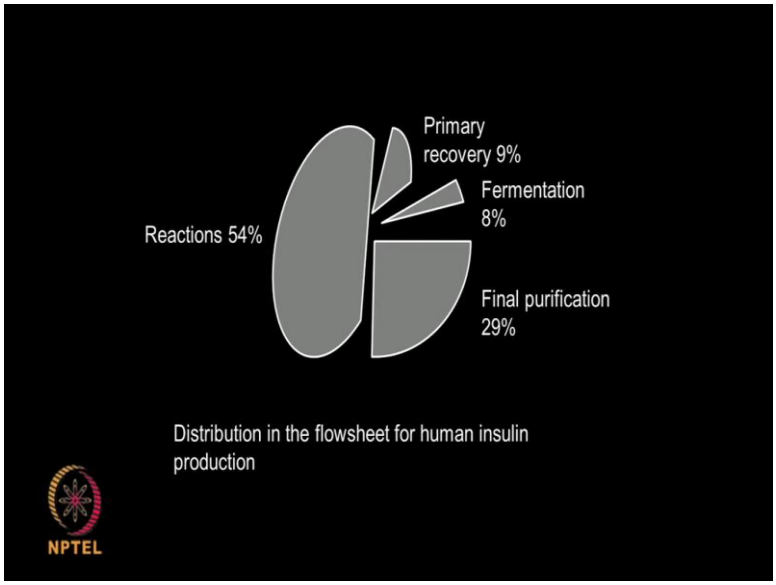

So if you break down the distribution cost the reaction is 54%, primary recovery is 9%, final purification is 29 %, so the downstream primary as well as the final purification will contribute to almost 38% of total cost of your product okay. So what am I trying to tell? I am trying to tell you that downstream plays a very important role in the cost, both in the raw material cost as well as equipment dependant cost. So one needs to really optimize and one need to really minimised, then only one can achieve very competitive product with a good selling price, with the optimum selling price as well.

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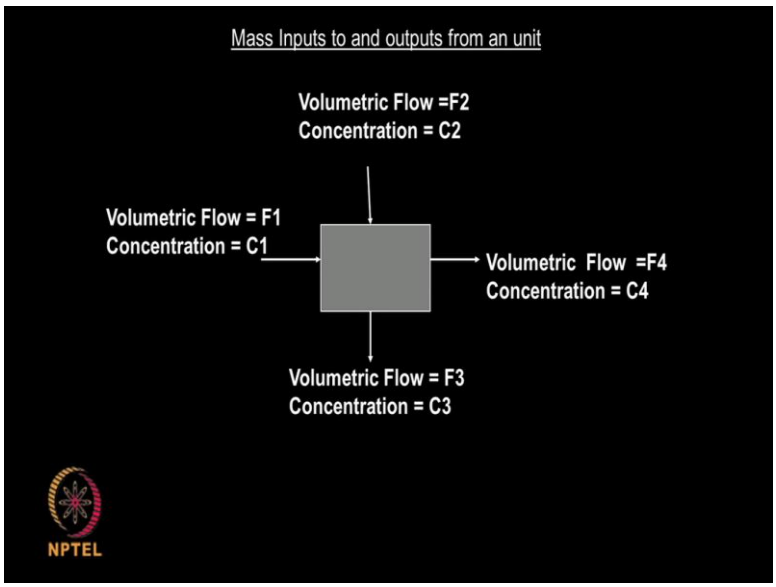

So let us look at some mass and heat balance. So what is mass balance? So if you have assumed two streams entering a unit, it could be anything, it could be a filter, it could be a extractor it could be absorber it could be chromatography, two streams, one streams coming in at a flow rate of F1 with a concentration C1 of a solute, another stream entering with a flow rate of F2 with the concentration of C2. Now those two are leaving because of certain thermo dynamic interaction, the solute gets moved from one stream to another.

So for example if it is extraction, the solute will move from the fermentation broth right into the solvent phase. If its adsorption it may move from the gaseous phase to the gaseous phase to solid phase. If it is an adsorption it may move from the gaseous phase to liquid phase and so on actually. So the two streams are leaving, so you have the volumetric flow rate of F3, concentration of C3. Volumetric flow rate of the fourth stream is the F4, concentration of C4.

So if I want to do a mass balance of this unit what do I do? I consider the inputs equal to outputs that is what I do actually.

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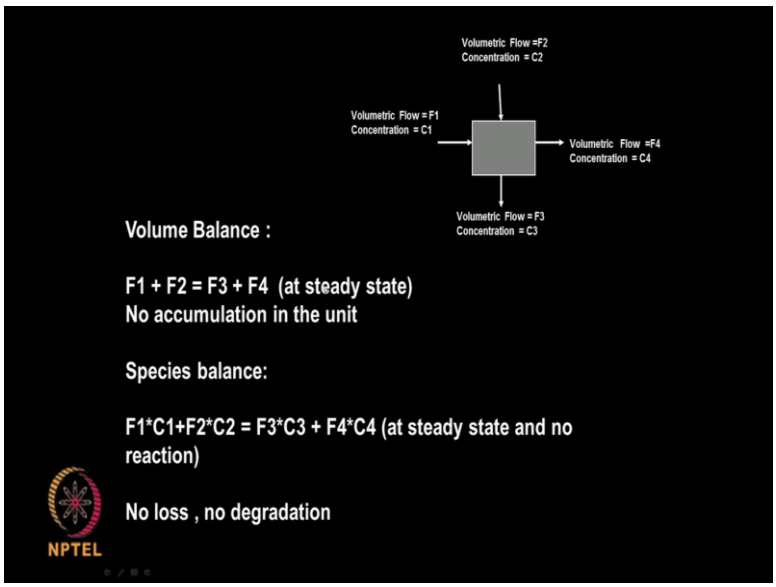

So if you are doing a volume balance F1 and F2 are the streams entering, F3 and F4 are the streams leaving. So F1 + F2 is equal to F3 + F4. So there is no accumulation inside the unit at steady state. Now if we do a solute balance, what do I do? F1 into C1 + F2 into C2 is equal to F3 into C3 + F4 into C4, there is no accumulation this is at steady state. So if I have two solutes then I will do species balance for solute 1, then I will do species balance for solute 2.

So these are very very important set of equations and we will be using these equations for solving mass balance in a adsorber, liquid-liquid extractor, chromatography. So this is the volume balance, that is the volumetric flow rate, this is the balance for the species or the solute. So if you have many species or the solute you will have many equations of this form and please note this is at steady state that means there is no accumulation. We assume there is not reaction, there is no loss, there is no degradation, so we need to consider all these.

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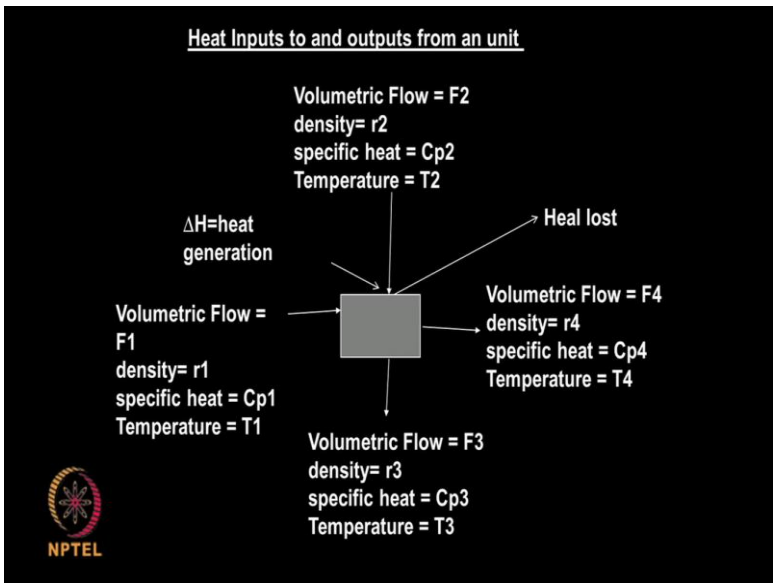

So similarly we can do for heat balance. Heat balance is much more complicated because

sometimes it is generated, when I add one liquid to another liquid because of the thermodynamic mixing, heat is lost to the surroundings, even though you may have a good jacket, you may have a good heat exchanging system, heat may be lost to the surroundings. So you may have to consider that. So here, when you do a heat balance, we need to consider the specific heat of each of the streams.

So not only the flow rate and concentrations, we also have to consider the specific heat of the streams that is entering. Specific heat of the stream that is entering to and specific heat of the stream that are leaving stream 3 and stream 4. So we need to consider all these, then only we can do the heat balance and also we need to consider the heat loss and also heat generated okay. So when we combine all of them heat input equal to heat output assuming there is no accumulation that is what is called heat balance. Okay that is very very important. You do a mass balance first and then later on we do a heat balance later actually.

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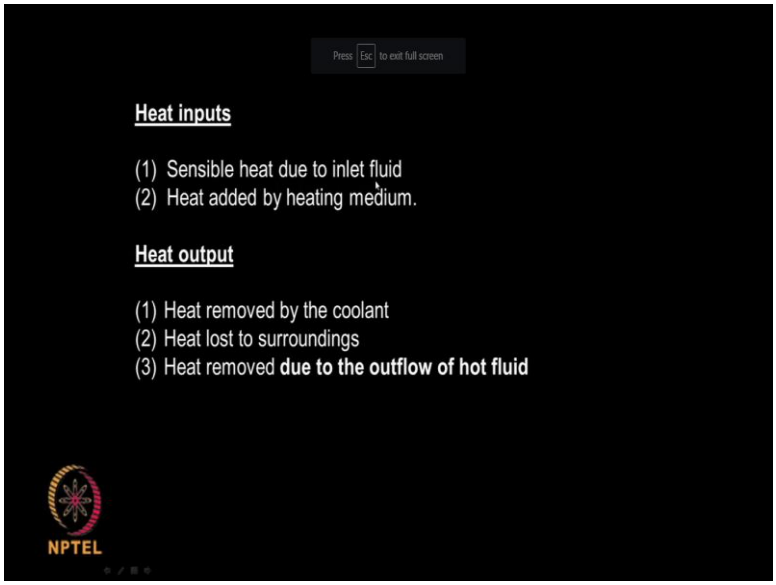

So heat inputs, they are called sensible heats, due to inlet fluid. Heat added by heating medium sometimes we heat the contents of the unit operation because they may be very cold, sometimes we may have to even cool it because there could be a lot of heat generated. For example in a bead mill or in a homogeneous heat is generated so we may have to cool them. Heat output will be heat removed by the coolant, so we may have cooling medium and then heat lost at the surroundings and then heat removed due to the outflow of the liquid.

So we need to consider all these heats and we say heat inputs equal to heat output and that is what we do in a heat balance actually.

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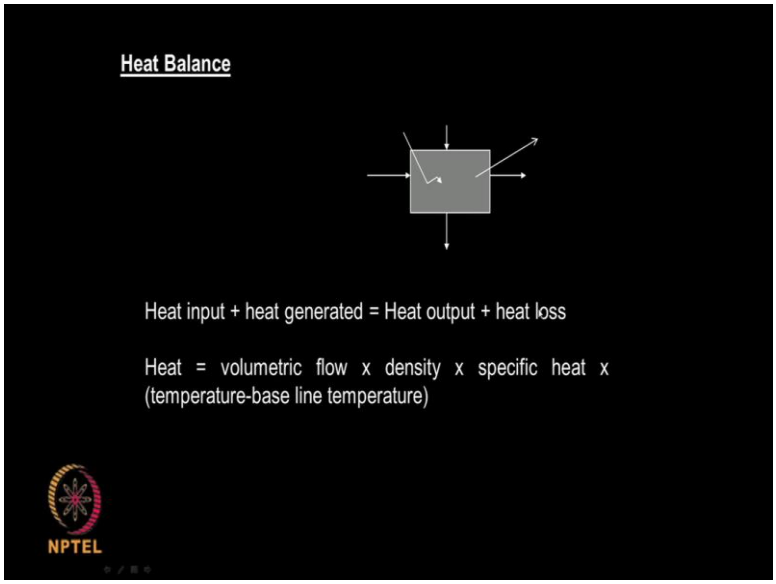

So we say heat input + heat generated is equal to heat output + heat loss. That is what is heat balance is all about. So if you have two streams, there will be two heat inputs. If you have two streams going out, there will be two outputs and if you have ten stream inputs then we will have to calculate the heat input for all the ten streams and if there are ten streams going out you will have to consider the heat output from all the ten streams.

Now this sensible heat how do you calculate? You calculate by taking volumetric flow rate into density into specific heat. That is called CP *delta T. So that is the temperature at which it is entering - some baseline. We have to do a baseline that means generally we take it as 30 degrees or 20 degrees as our baseline then it will be - 20 degrees .So we do that for both the streams that are entering as well as for the streams that are going out.

So the sensible heats are always calculated based on these baseline temperature, so we need to subtract, so if the heat the temperature of the stream that is entering at 30 and if our baseline temperature is 20 we do 30-20 and if the stream is leaving at 40 and if our baseline temperature is 20 then we do 40 - 20, so all the streams and the temperatures are subtracted by the baseline temperature. So you need to remember that. That is very very important.

So that is how heat balance is calculated. Heat balance is slightly more tricky when compared to mass balance because in mass balance we are just calculating the volumetric flow rates, we are calculating the concentrations of the streams entering and equating it to concentration of the stream that is going out okay and so it is quite simple, whereas in heat balance we have to consider specific heat. We have to consider heat lost to the surroundings.

We have to consider the heat that is generated because of mixing of two liquids or three liquids and then we have to consider this baseline temperature which needs to be subtracted so you need remember that. Why do you need to do this heat balance and mass balance? We need to do as you go long, you will do some problems and you will come to know. For example if I want to calculate how much solvent is required to remove a solute from the fermentation broth, if I want to calculate how much adsorber is required to adsorb certain amount of liquid or gas.

So if you want to calculate how much of stationary space in a chromatography required to remove certain amount of solute, from the continuous phase. So in order to do that sort of calculation we need to do this mass balance. Okay that is very very important actually. Let us look at the simple problem which makes use of all these concepts and which is very very useful and very relevant to our particular downstream processing course actually.

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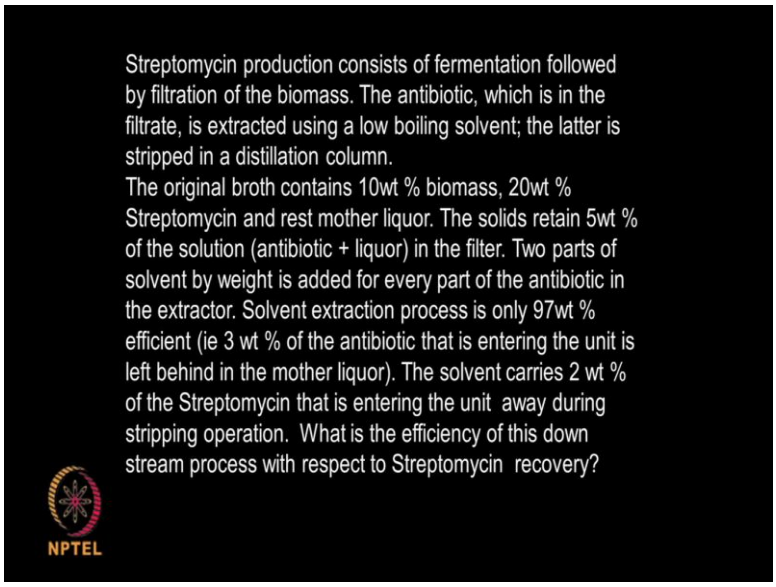

Okay I want to tell you this problem. For example streptomycin is produced in a fermentation. Okay. Then it is filtered to remove the biomass, then after that the antibiotic which is in the filtrate is extracted using a low boiling solvent okay and then there is distillation column where the low boiling solvent is removed at the top and the product is removed at the bottom. Okay so what are the downstreams? You have a downstream of a filtration to remove the biomass.

Then you have a extractor where a solvent mixes up this product and then in the distillation column where your solvent is striped at the top and the product is formed okay. So these are three unit operations so we need to look at mass balance at each one of these three unit operations okay. Now whenever you remove biomass in a filter what happens is, there will be some liquid in the biomass

because the biomass will not be 100% dry, so there will be some liquid.

So when some liquid goes product streptomycin will also go with that. So you will lose some streptomycin okay. Now when we go to an extractor, we are adding a solvent, extraction is never 100 %. Even if it is 98 % what happens is some amount of streptomycin will go away with the parent liquid. It do not come into the solvent. Now when we go to the distillation and when we are distilling your solvent may come at the top, streptomycin will come at the bottom but some streptomycin may also go away with the solvents.

So you will lose streptomycin at three different places. One is in the biomass filtration and one is in the liquid-liquid extraction and one in the distillation. So you are going to lose this in each one of these stages. So there will be total amount of streptomycin lost will be sum of all these three. So we need to do a mass balance for each one of these unit operations that means you do a mass balance for the filtration, when you do mass balance for the extraction, you do a mass balance for the distillation.

Now as we can see the entire paragraph tells you what is happening. Whenever we do a filtration whenever do a extraction, whenever we do a distillation, so that is what its saying the original broth contains 10 weight % of biomass and 20 weight % of streptomycin and rest all mother liquor that means it will contain if we take 100 grams then 10 grams will be your biomass and 20grams will be your streptomycin and remaining 70 grams will be liquid. Okay.

Now when you do a filtration the solids will retain 5 % of the solution. (()) (22:41) so solids are always wet. In a filter we cannot completely dry. So solids are always wet, so when it retains 5 that 5 % of solution will also contain some amount of antibiotic and some amount of the liquid okay. Like in the same ratio the 10 is to 70 ratio sorry 20 is to 70 ratio because original broth, we have 20 streptomycin and 70 mother liquor.

So you are going to lose some amount of streptomycin also in that liquid which is retained by the biomass. Okay now you are adding two parts of solid and to recover the antibiotic by extraction okay, now the solvent extraction is not 100 % efficient. Its only 97% efficient that means three weight % with the antibiotic that is entering the unit is left behind the mother liquor, that means it is able to take out only 97% whatever it is entering only 97% rest of it is gone in the mother liquor.

Now when you are doing this distillation or stripping that is called now 2 weight % of the

streptomycin that is entering is again taken away by this solvent in the distillation column. Okay.
So you have three steps, in each step you are losing some amount of streptomycin, so I want to know how much of streptomycin is totally recovered from the original broth and how much is lost that is what it is.

And this is very important problem because we always come across some issues we would like to recover as much product as possible because the more you lose the more money you are losing the more you recover the more money you are gaining because you can sell them actually okay. If it is a very expensive product each milligram each gram will be very expensive. So you cannot afford to lose any of them. So let us look at the next slide where you are doing a mass balance for each one of these stages.
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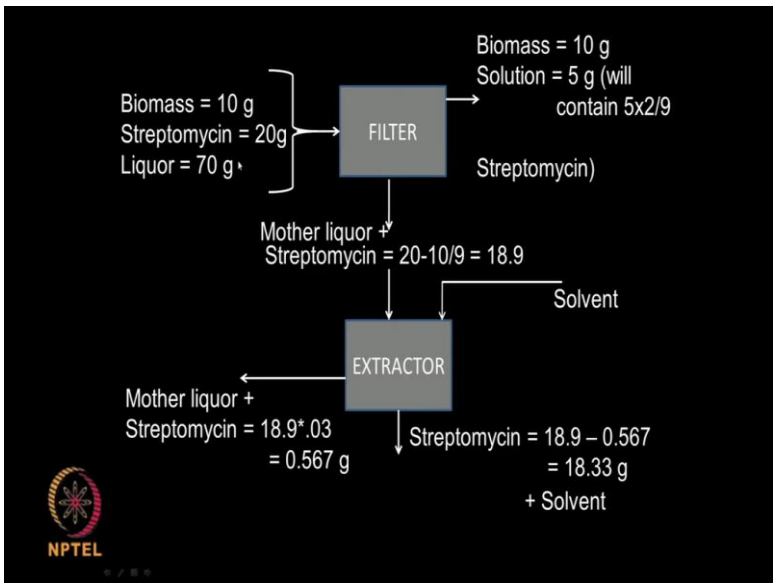

So we are looking at filter, as I said I am having 20 % of streptomycin, so when I take hundred grams, I will call it 70 grams of liquid, 20 grams of streptomycin, 10 grams of biomass. So the biomass captures 5 grams of solution, so 10 grams of biomass is completely removed. So 5 grams of solution is lost now. In this 5 grams I am going to have 2 by 9 amount of streptomycin because as you can see here it is the starting is 2 liquid is 7 so 2 by 7 is the 2 by 9 is the amount of streptomycin here so how do you calculate 5 into 2 by 9 here.

Okay that is 10 by 9 that amount of streptomycin is lost here. So what is left behind in the mother liquor? I will have 20 - because 20 is the amount of streptomycin coming here. So 20 - 10 by 9 will be 18.9 grams. Now I am adding solvent in the extractor and I have told that 3 % of streptomycin that is entering is lost in the extraction, your extraction is only 97 %. So what do I do? 18.9 is what is entering 3 % is 0.03 that is 0.5675 grams is lost that means remaining is 18.9 - 0.567 that is 18.33

grams okay. So now the amount of streptomycin as you can see has come down from 20 to 18.9 to 18.33.

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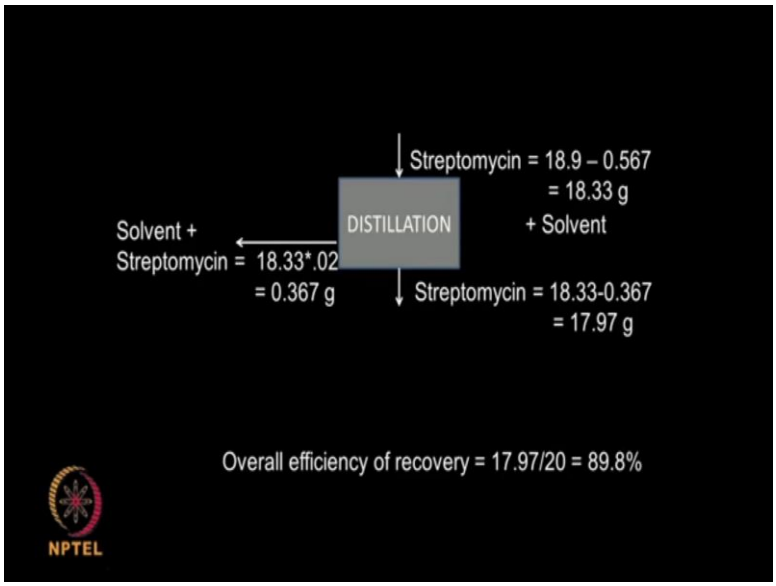

Now let us go to distillation column, the stripping column as it is called. Now the problem says 2 % that is entering is lost in the distillation, so what is 2 % of 18.33 18.33*0.02 that is 0.367. So what do we do? We say 18.33-0.367 that is 17.97. So you started with 20 grams of streptomycin in the original liquid with the biomass okay.

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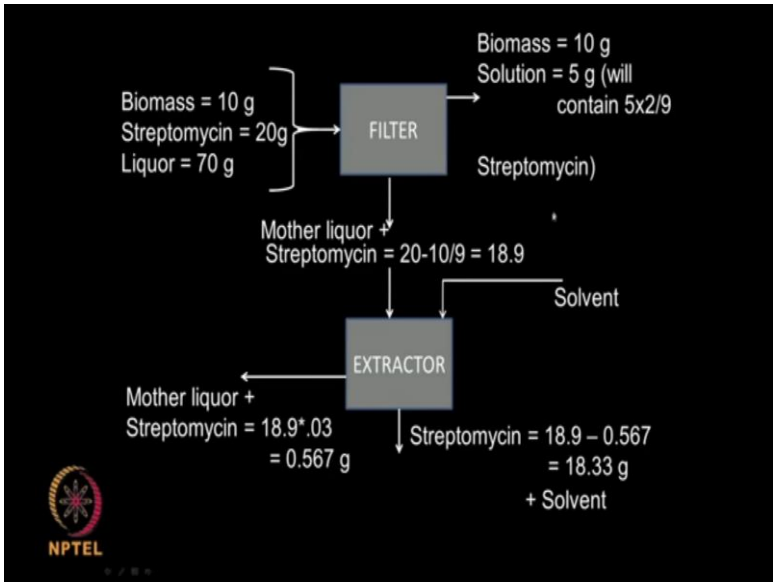

Then you have the filtration where lost some streptomycin we ended with 18.9 grams, then we went into an extraction. We lost some streptomycin, we ended with 18.33 grams and then we came down to the distillation, where we lost some streptomycin we came down to 17.97 grams.

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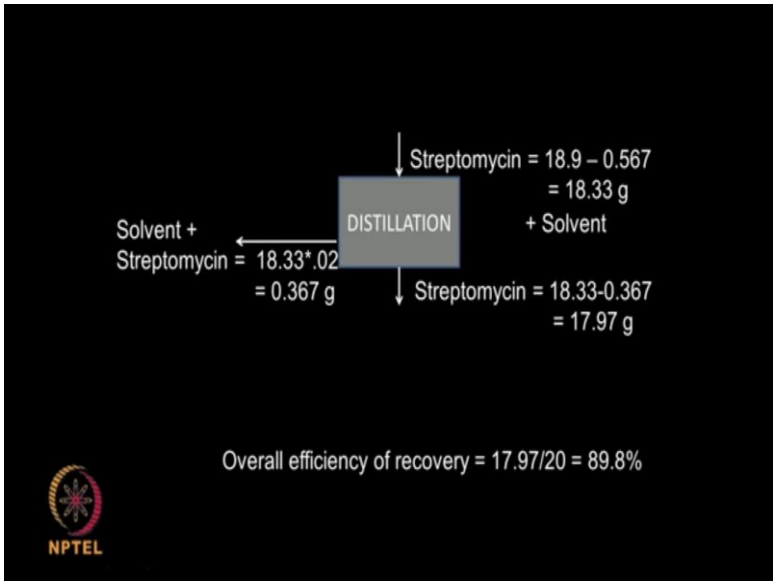

So the overall efficiency will be 17.97 by 20 into 100. So we end up with 89.8 %. So what does it mean? I have lost 10.2 % of streptomycin in these three unit operations that is quite a lot of streptomycin. So what do I do? I need to improve on the efficiencies of each one of these. How can I do that? I can make my filtration more efficient so that when a filter I will not allow too much liquid to stay in the bio mass that is the solid I will try to make it dry as much as possible, so that all the liquid goes down into the filtrate.

I can improve the efficiency of my downstream so that instead of 97 %, I can make 98 or 99. I can improve the efficiency of the distillation so instead of losing 2 % I can make it less may be 1 % or so by doing each one of these I can improve the overall efficiency of the entire process. So I may be able to move up to may be 95 96 % efficiency, I may not be able to achieve 100 % but I may be able to go much higher. So this is a very simple problem but it tells you lot of information how to use mass balance, a very simple technique to understand which unit operation is very inefficient.

So I can focus on that particular unit operation and try to improve I can also determine how much will be the overall efficiency of the recovery. That is very very important because as a manufacturing scientist I would like to enhance my overall efficiency of each as much as possible towards 100 because higher the efficiency higher will be the product recovery and I will be able to achieve better profit into the entire process actually.

So this particular lecture which I have been talking about, gave you a very brief rundown on various unit operations which are used in the manufacture of antibiotic and several other biochemical process and people use sometimes filtration, sometimes chromatography, sometimes different types of membrane processes. Finally if it is a solid you use a crystallizer and then you use a dryer and

then depending upon the purity requirement of the product.

You may have a series of chromatographic steps as we saw in the poor manufacture of insulin and other antibiotic as well actually. So now in the next course of lecture I will be spend more time on various unit operations which are show in these flow sheets. Thank you very much!!!