Principles of downstream techniques in Bioprocess – A short course
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Lecture-20
Other techniques, Future trends

There are few more techniques which are also called downstream but they are not very adapted in very large scale techniques like absorption, distillation and so on. So I will briefly touch upon that and then later on we look at the future trends or how the research in downstream is going to go towards.

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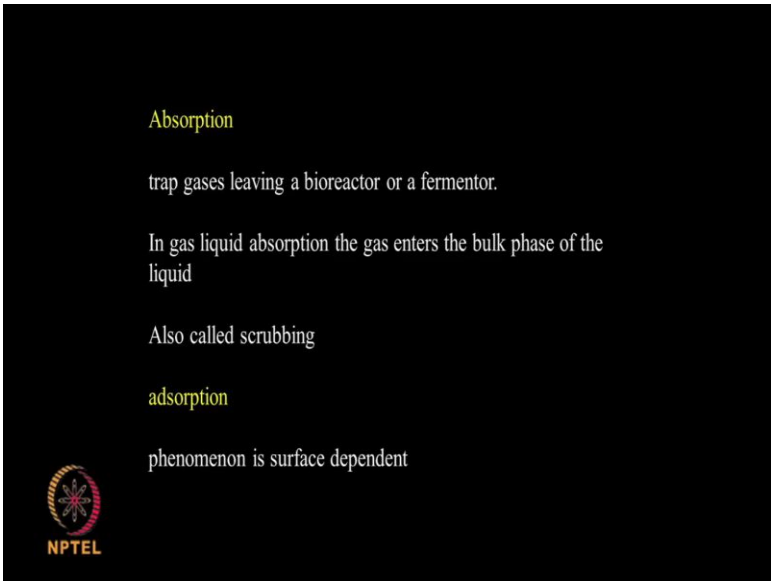

Okay the absorption is the important technique, especially to trap the gases leaving the bioreactor or a fermenter. So they could be carbon monoxide, carbon dioxide, methane and so many other toxic gases also that could be produced during fermentation you do not want it to just enter into the atmosphere. So generally the gases are bubbled through a absorbing liquid, this liquid could be a alkali, this liquid could be water, monical solution and so on actually. So the gas enters the bulk phase of the liquid and this is trapped and this is called absorption.

Sometimes the gas may react with the liquid or sometimes it may be just getting absorbed. This is also called scrubbing. Chemical engineers have been using this term scrubbing for a very long time but in bio we do not use most of that actually. So this is almost similar to adsorption where

the phenomenon is surface dependent so you have a gas or liquid getting adsorbed on a surface of a catalyst or a particle whereas in absorption the gas getting trapped in bulk of the solution so that is the main difference between absorption and adsorption.

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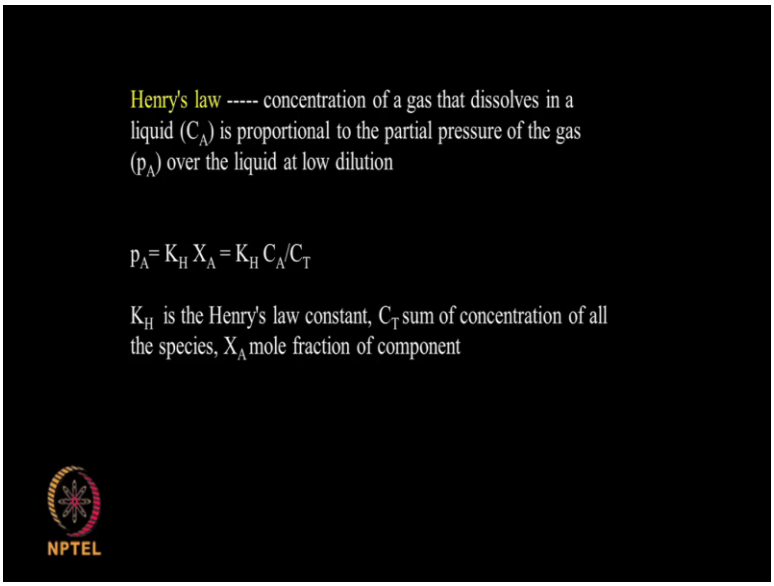

So the most important law here is Henry's law. You must have studied long time back the concentration of the gas that dissolves in a liquid is proportional to the pressure of the gas okay over the liquid at low dilution. So we have a relationship okay which connects the partial pressure of the gas above the liquid and the concentration of the gas okay in the liquid okay and that is given by the Henry's law so PA that is the partial pressure K is the Henry's law constant ka is the mole fraction of that particular component.

That is given by CA by CT so CA is the concentration of the gas that is in the liquid and CT is the sum of concentration of all the species. So you may have many species okay. That is why CA by CT is called the mole fraction and this is valid only in dilute condition and this is called the Henry's law constant okay. It is useful because if I know the partial pressure on that solute gas in the gaseous phase, I will find, I will know what will be it is concentration in the liquid phase

Because both of them will be in equilibrium and that equilibrium constant is called the Henry's law constant. Assuming there is no reaction if there is no reaction, CA will disappear into some product, so there will be more and more of the gas getting absorbed.

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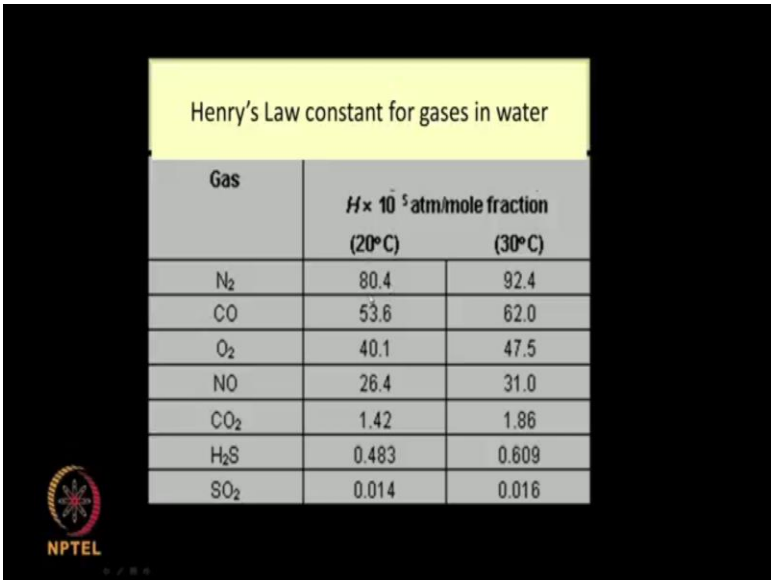

So this is the Henry's law constant for gases in water. For example, you can see at 20 degrees nitrogen, carbon monoxide, oxygen, carbon dioxide and so on actually. Hydrogen sulfide, sulfur dioxide at two different places, so like that you have four different gases with different fluids or liquids.

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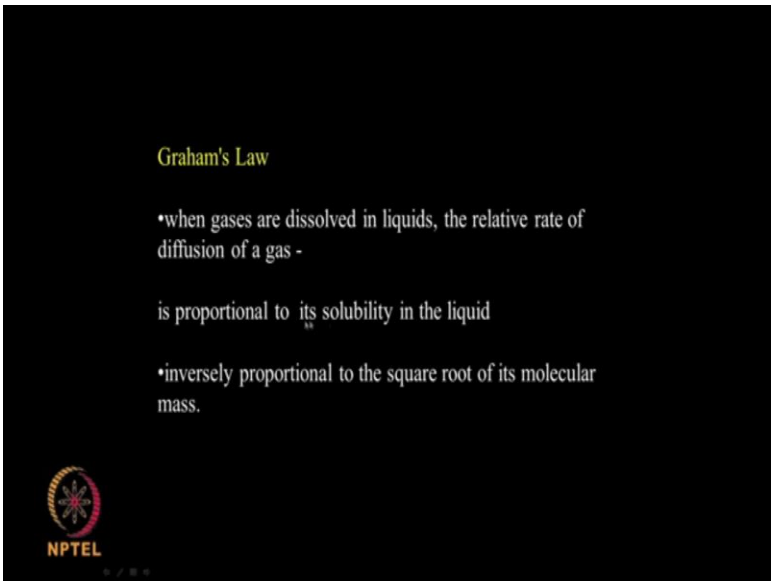

There is another law that is called the Graham's law, when gases are dissolved in liquids, the relative rate of diffusion of gas is proportional to it is solubility in liquid but it is inversely proportional to the square root of it is molecular mass. So larger the mass, the rate of diffusion of

that gas into the liquid is reduced and higher it is solubility, the rate of diffusion increase. So this is called the Graham's law.

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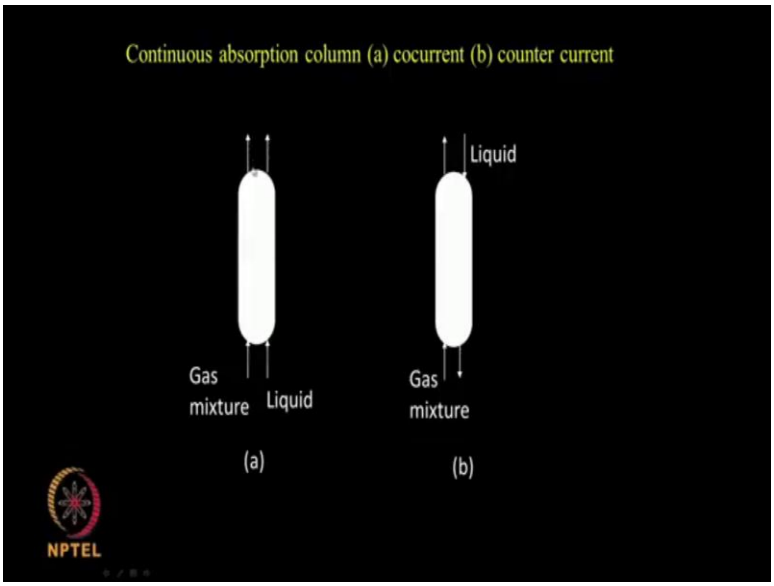

Generally the adsorptions are done in tall tower of columns, okay so you have the gas mixture going in okay and the liquid also going into the column either in co-current or counter-current manner and they to the gas is vented out at the top and the liquid is collected at the bottom. So in the counter-current, the liquid enters the liquid could be water or amenical solution or alkali and A gas that needs to be scrubbed comes from the bottom rises and then it goes out.

So whatever gas you are interested in collecting be carbon dioxide or carbon monoxide is collected. So the gas that is leaving will be divide of that so you will get the liquid at the bottom with that particular gas absorbed okay. So this is very useful technique because from environmental point of view from toxic point of view, you would like to collect A gas leaving out of the fermenter, you do not wanted to be vented out.

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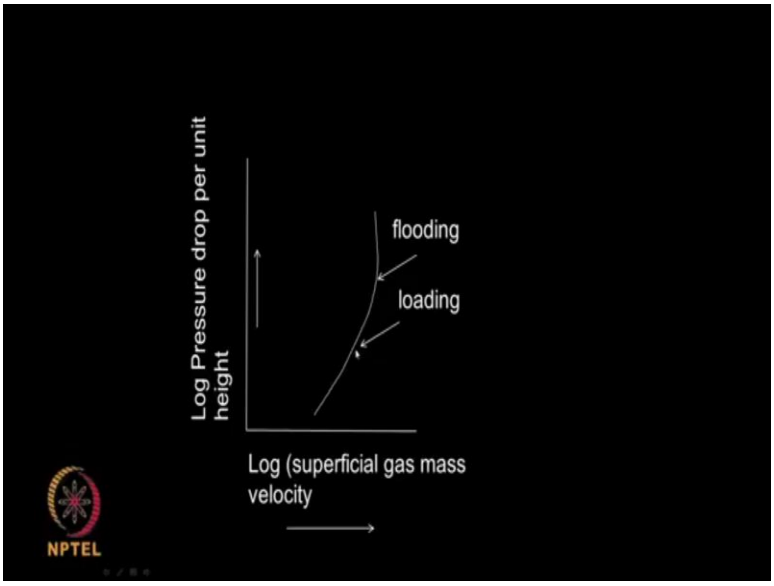

So you can keep on increasing the flow rate of the gas but then you cannot do that too much after sometime the gas velocity will be too high that it will prevent the liquid flowing down and that is called flooding. Okay these are very chemical engineering terminologies. So I need to operate my gas velocity much lower than this flooding velocity. Because if my gas velocity is too high, the liquid will not flow down. So the whole column will come to A standstill, so in the X axis.

We have log of superficial gas velocity and the y axis you have log of pressure drop per unit height. So as the gas velocity is increased the pressure drop increases and certainly just shoots up and this is called the flooding velocity.

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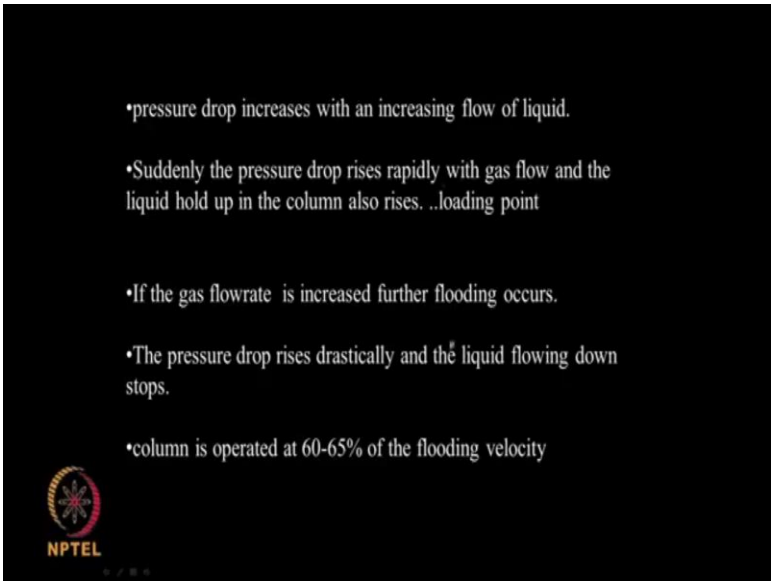

Okay so pressure drop increase with the increase in fluid and suddenly the pressure rises rapidly reaching A loading and then the flooding. Okay. The columns are operated generally 60 to 65% of the flooding velocity.

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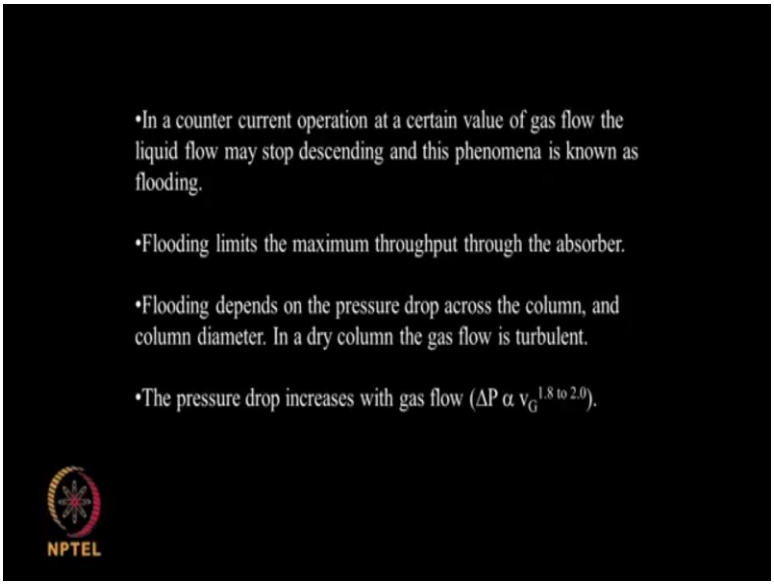

So in a counter-current operation at a certain value of gas flow, the liquid flow may stop descending and that is what is causing the flooding. So the flooding limit is the throughput, so the flooding depends on the pressure drop across the column, column diameter and so on actually so if I have a larger column, I will be able to prevent this type of planning. So the pressure drop

is proportional to the velocity raised to the power 1.8 to 2 actually okay so it is pressure proportional to velocity raised to 1.8 to 2.

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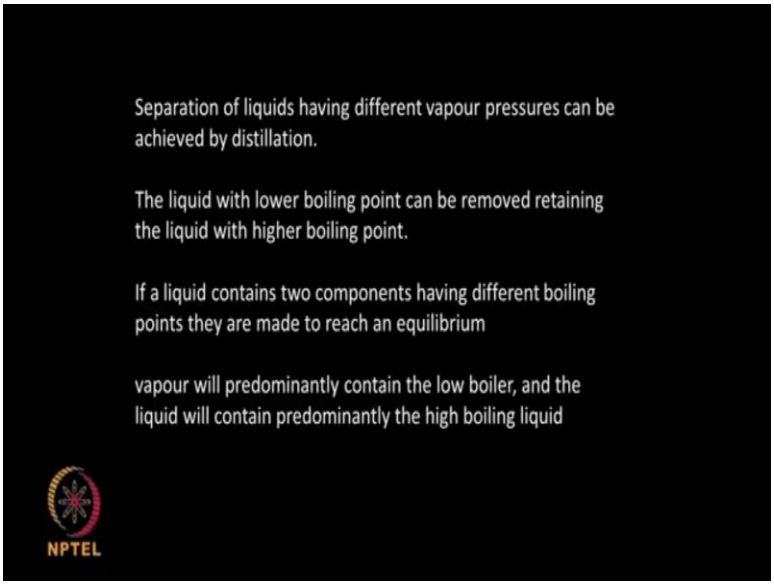

So another downstream it is called distillation. Generally we resort distillation and a chemical processes or where they metabolites or products can withstand high temperature especially in methanol fermentation process, ethanol cant withstand very high temperature acidic acid can withstand. So there we resort to distillation. So distillation is based on the difference in the boiling point of few liquids or difference in pressure so the separation is based on differential pressure or difference in boiling point. So the heavy boiling liquid or higher boiling liquid will be at the bottom, the lower boiling liquid will be at the top.

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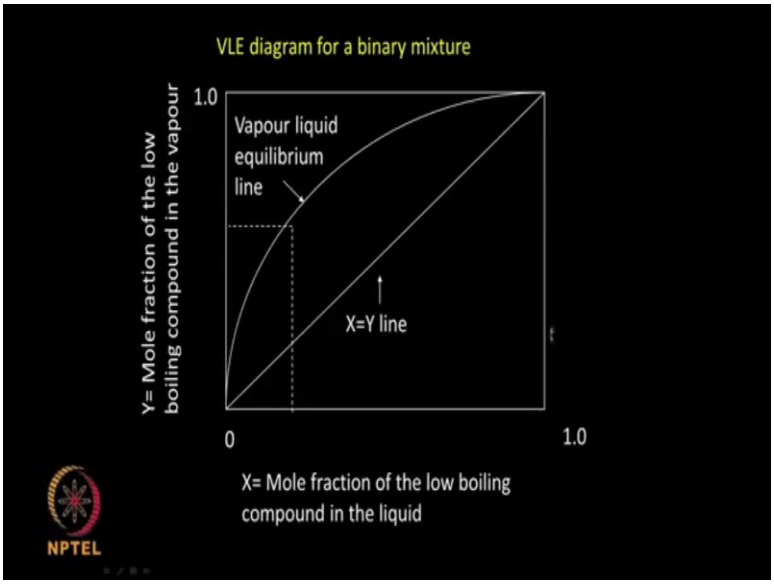

Okay so there is a vapor liquid equilibrium relationship and that is what it is called vapor liquid equilibrium diagram for a binary. Binary is two liquids so the concentration or the mole fraction of 1 liquid on 1 axis 0 to 1 mole fraction of the other liquid on the other axis or the mole fraction of the same in the vapor phase. So if we consider liquid A or component A here, we have the mole fraction of component a in the liquid phase here, we have the mole fraction of component in the vapor phase.

So this is called the vapor liquid equilibrium. So if I take any points here it tells you what is a concentration of that component A in the liquid and what is the concentration of component in the vapor okay so this is what it is. And this is the X-y line here, this is the concentration is same in the liquid as well as in the vapor this is the 5 degree line that is why concentration will be the same in the vapor as well as in the liquid, so generally you will have graphs for the vapor liquid equilibrium in this form, you can also have them this shape also, okay we will look at some of those shapes soon.

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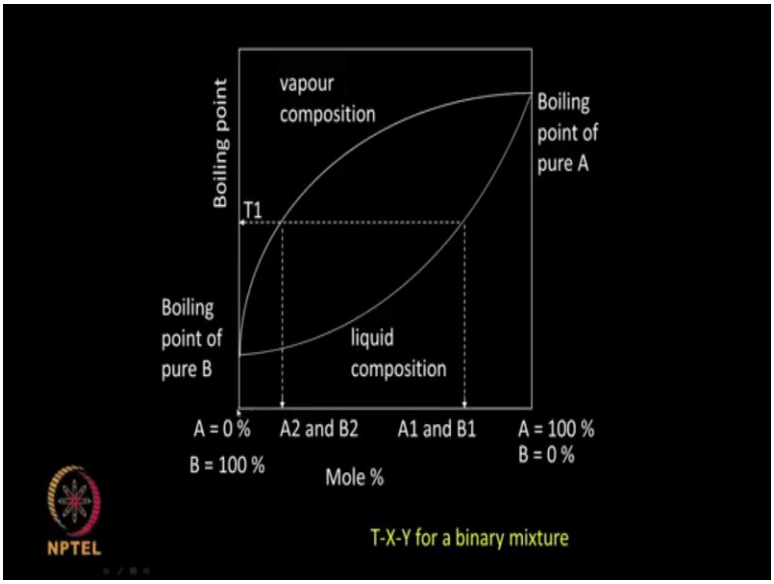

So you can also plot the concentration of A in the X-axis and we can plot the vapor composition, that is the composition of A in the vapor and composition of A in the liquid. So you can have graphs like this so this could be boiling point of pure A and this could be boiling point of pure B so A at 0 % here A at 100 %. So here you have B at 0 % B at 100 % so this is the composition of A in vapor phase this is the composition of B in the liquid phase.

So 100 minus that composition will be give the corresponding composition for the B. So we can take any point here and then if you go to the vapor, that tells you the vapor composition and that tells you the boiling point. So if you extend it here, come here and go down that will tell you the composition of A in the liquid okay. So this will correspond to the composition of A in the liquid and this will correspond to the composition of A in the vapor and this tells you the boiling point okay.

So this is A very useful diagram and of course this is useful only for binary system and the previous graph the vapor liquid equilibrium diagram and the T-X-Y diagram that is called and it is binary why T-X-Y, T is temperature, X is the composition of A in the liquid phase, Y is the composition of A in the vapor phase, that is why it is called T-X-Y diagram.

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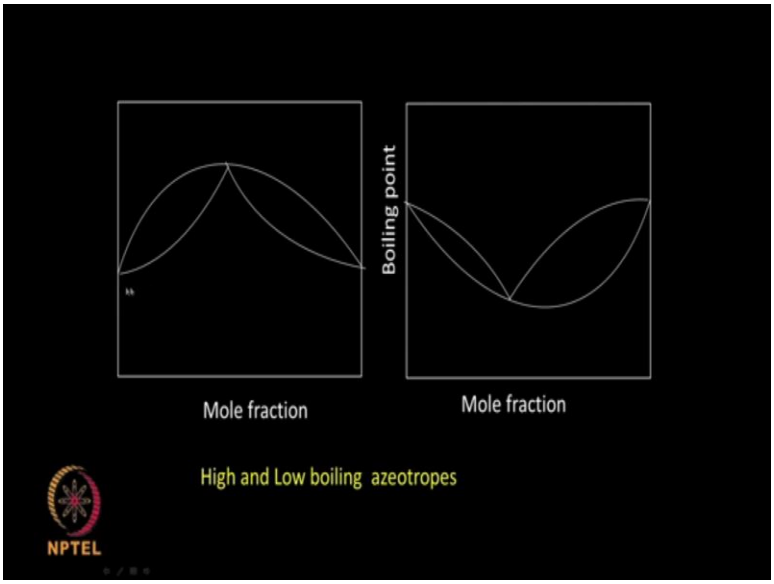

Sometimes these diagram will look like this. Okay instead of this diagram, will look like this. Okay so what does that mean? Okay at this point if you see at this point, if you see the composition of A in the vapor and the liquid are the same. So they are called constant boiling liquids that means composition of the A is solute is same in the liquid and in the vapor phase they are called constant boiling liquid and this is called azeotropes okay. So if A solution has A and B with this composition, you will not be able to separate them at all.

Okay so you have high boiling azeotrope, you have A low boiling azeotrope, so you have two species A and B at this composition. The composition of A is the same in the vapor and the liquid phase the composition of A in the vapor and the liquid is same. This is called low boiling azeotrope because the azeotrope temperature is lower than the pure component temperature of A and B. This is called high boiling azeotrope because azeotrope temperature is above the individual species boiling points.

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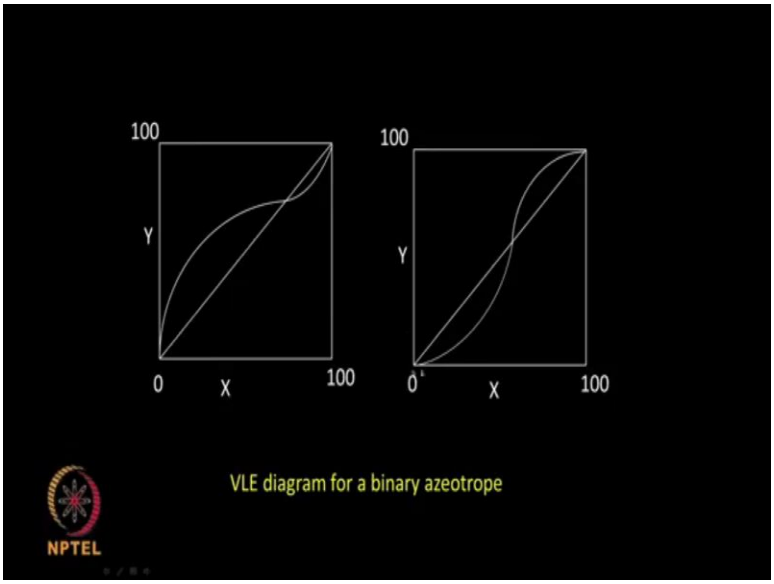

So if you look at the VLE diagrams corresponding VLE diagrams, for azeotrope will look like this. Okay instead of the graph going either up or going down the graph will cross the 45 degrees line. So at this place or at this place the composition of the solute A will be the same into the liquid and in the vapor phase. So if you have this composition, you will not be able to separate by just boiling at all. So they are called constant boiling azeotrope

So there are many examples like in ethanol water, there are certain composition range at which you will not be able to separate them at all. So in such situations we may have to add A third component to break the azeotrope or you may have to change the pressure to break the azeotrope and so on actually. So these are issues when you do distillation, especially if you have two components and they form an azeotrope either high boiling sorry low boiling or high boiling azeotrope, then you are in trouble. You may need to use some other method to separate them completely.

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So the distillation columns can be tray, that can be plate, it can be packed column, even absorption column which I talked about few minutes back, can tray plate and packed. The idea of these is to bring the vapor and liquid into intimate contact so that there is A separation taking place.

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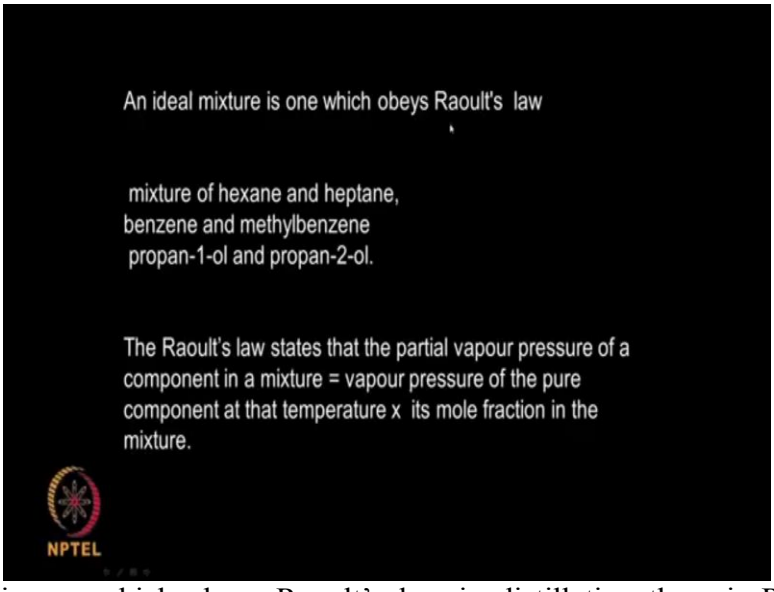

So ideal mixture is one which obeys Raoult's law in distillation there is Raoult's law. Like Henry's law in absorption okay, the Raoult's law states that the partial vapor pressure of A component of A mixture is equal to the vapor pressure of the pure component, at the temperature multiplied by it is mole fraction in the mixture. Okay will come to that so mixtures of hexane and

heptane, benzene and methyl benzene, propan-1-ol and propan-2-ol, they are all called ideal and they obey Raoult's law.

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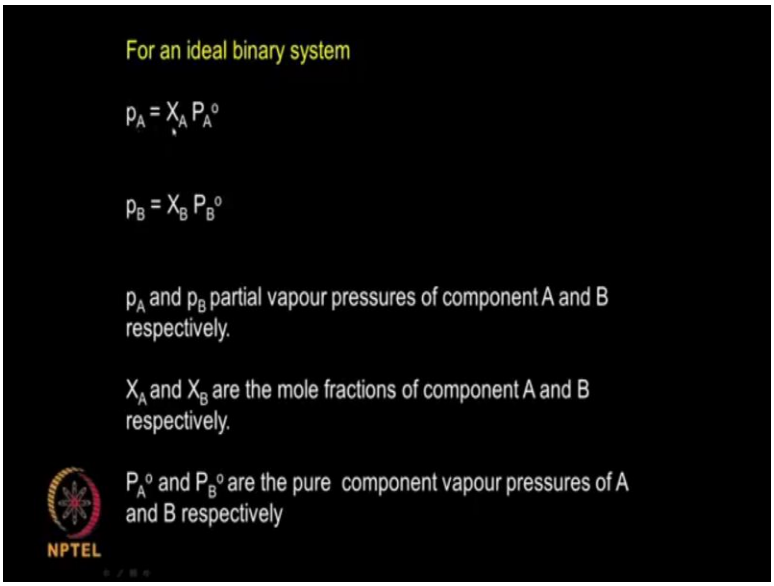

So Raoult's law is partial pressure PA of A is equal to X is the mole fraction of component A multiplied by the PA not that is pure component vapor pressure of A. So we can have PB is equal to XB PB 0 okay. So PA is equal to and there are only 2 species then PA plus PB will be equal to the total pressure and XA plus XB will be equal to 1 because mole fraction if there are 2 species okay so this is Raoult's law. PA partial pressure is equal to XA mole fraction of component A capital P is the acquired component vapor pressure okay.

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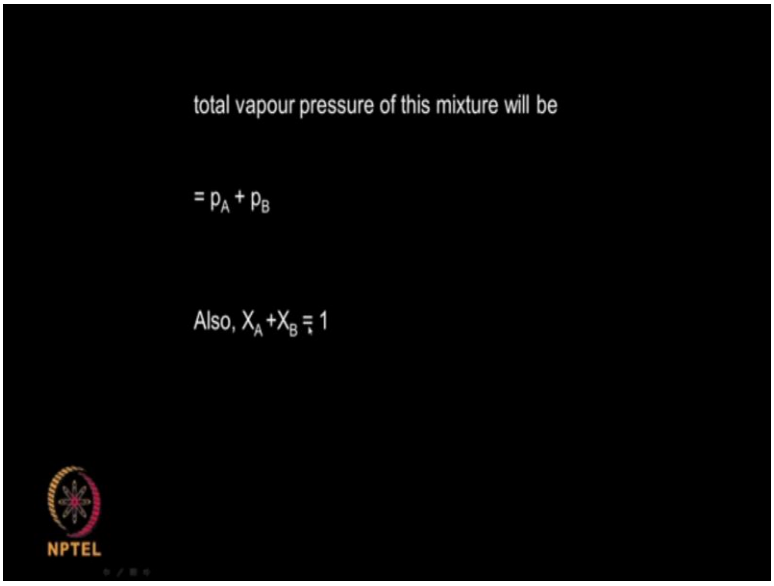

So for A ideal like I said the total pressure, vapor pressure will be PA plus PB and the XA plus XB is equal to 1 okay.

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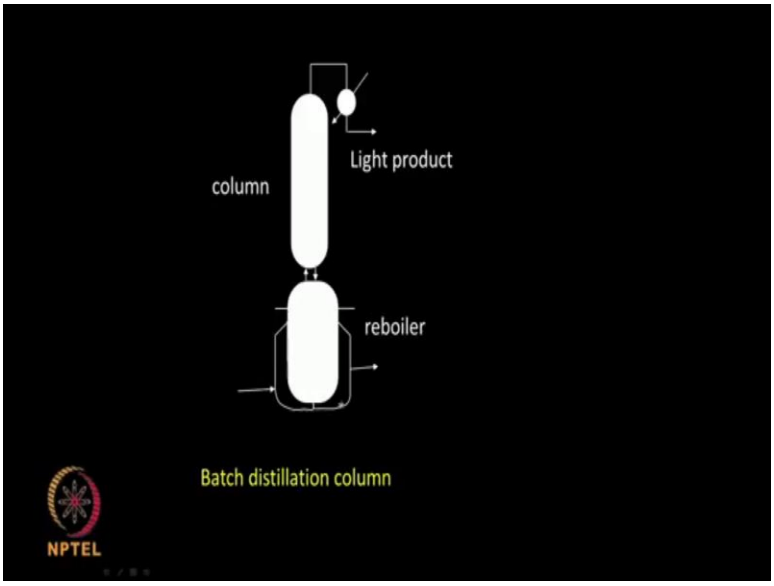

So you can have A batch distillation column. So it could be A big boiler here you put in your mixture here to be separated boil it there is A column on top and this will collect lighter product. This will collect heavier product. So in this column the vapor will flow up the liquid will flow down, so there is an intimate mixing between the vapor and the liquid. So on the top you will collect light product and on the bottom, we will collect heavy product this is A batch distillation.

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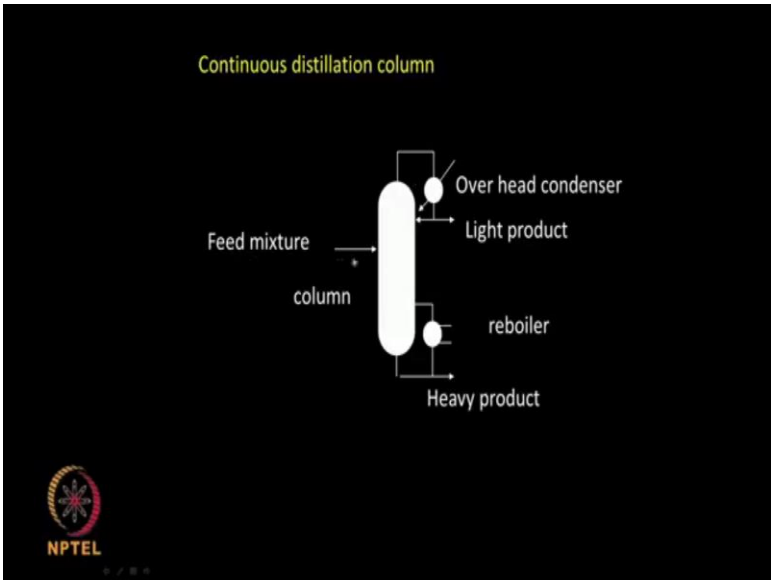

You can have a continuous distillation column, you are continuously introducing feed, the liquid will come down. This liquid will have more heavy product, vapor will rise. The vapor will contain more of light product which is collected here, the heavies are collected here. So continuously you are feedings something continuously, you are collecting light and continuously you are removing heavies this is A continuous distillation column.

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Okay so we looked at a few you commonly used downstream like absorption and distillation. For the sake of completeness, I did introduce them, I did not go too much in details but I talked about briefly what is Henry's law and what is Raoult's law. So we have done in detail, the downstream

operations, the downstream processes that follows subsequently after a year the fermeneter or a bio-reactor.

Now what does the future for downstream appears to be? So there are lot of literature, lot of reports which tells what the experts think about in the future for downstream operations, which are the bottlenecks that one need to focus in the downstream. That is what I will be covering for the next few minutes.

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So downstream process is a mandatory requirement, you cannot avoid downstream especially in biopharmaceutical drug development, even food and so on actually you have to purify your product. If you look at the market in 2009 it is about 1.5billion downstream market which is expected to go up to 3.75 billion and the total reagent down reagents used in downstream so many reagent right? It is about 4.3 million in 2009 which is supposed to go to 6.6 so it is a huge market for downstream process market so it is expected to grow okay.

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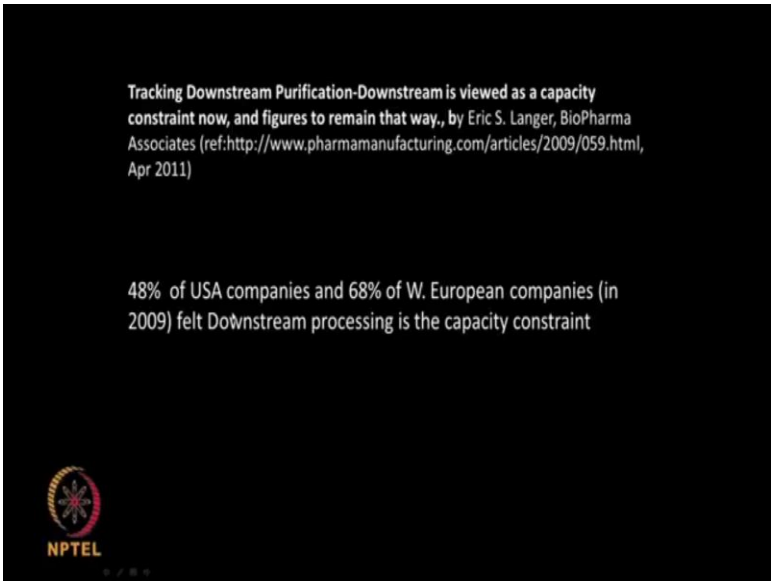

So if you look at some reports, it says 48% of USA companies and 68 % of Western Europe companies feel that downstream processing is the capacity constraint that means it is easy to add more fermenter. It is easy to add one more bio-reactor if I want to expand my process but then the downstream is going to be really constraining and it is not so easy just adding a few chromatography or adding A few separating equipments so that is going to be your real bottleneck okay.

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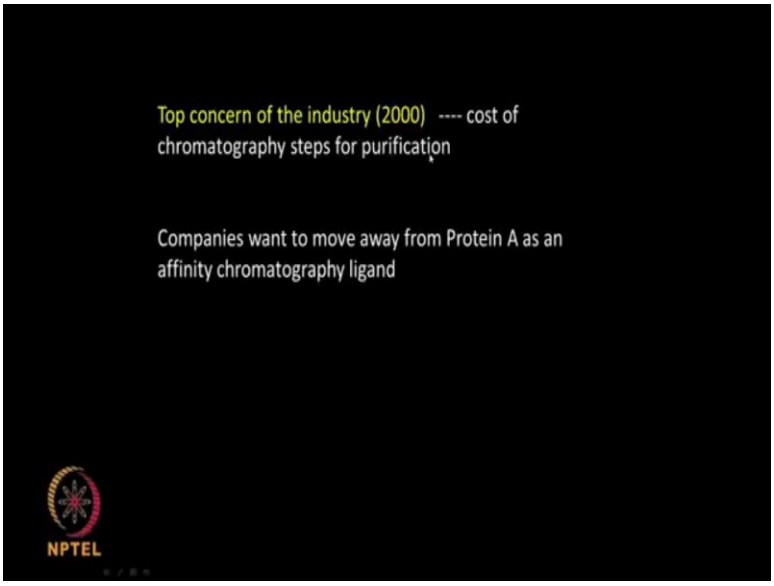

The top concern of industry, the 2000 the day when the survey was done they said the cost of chromatography step in, purification is going to be a big issue and the companies want to move

away from protein A as an affinity chromatography ligands so these 2 concerns in the industry okay.

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When a industry survey was done what purification steps are going to create capacity constraint, depth filtration 32 % worry, chromatography 43 % worry, ultrafiltration 26 % worry, so you see that these are issues going to be in the purification stages which are going to limit your capacity okay.

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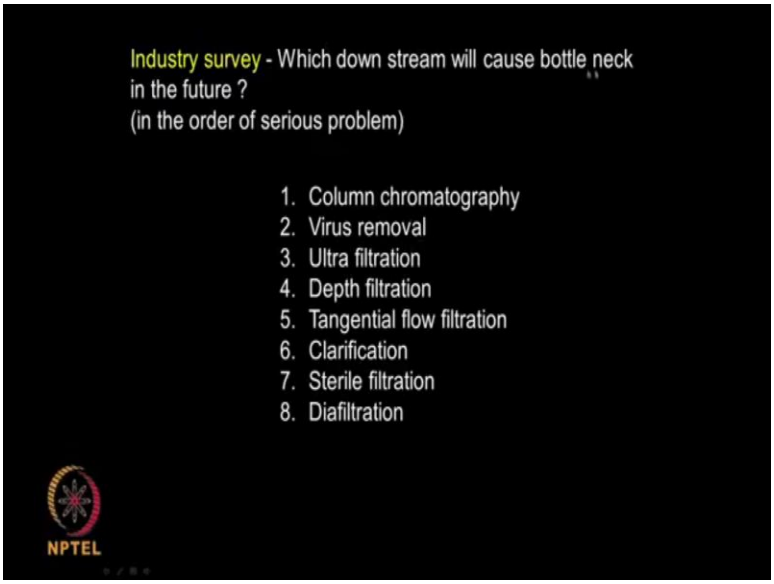

When a industry survey was done which will cause bottleneck in the future in order of serious problem column chromatography virus removal because if you are collecting antibiotic through fermentation. You have to be very very sure that bacteria or the yeast that is producing these antibiotics is completely removed because antibiotic is a drug. Virus removal is a concern. Ultrafiltration, Depth filtration, Tangential flow filtration, Clarification sterile filtration, diafiltration.

So we see lot of filtration issues that are going to be your worry in the future. So one needs to put in lot of effort in process improvement lot of effort in research and development to address these types of downstream steps okay. Nearly two-third of biopharmaceutical companies are spending for acquiring new downstream processing technologies.

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So they are still looking around what type of technologies are available for two thirds that is a big number of companies who are worried about their downstream and how to acquire new technologies okay.

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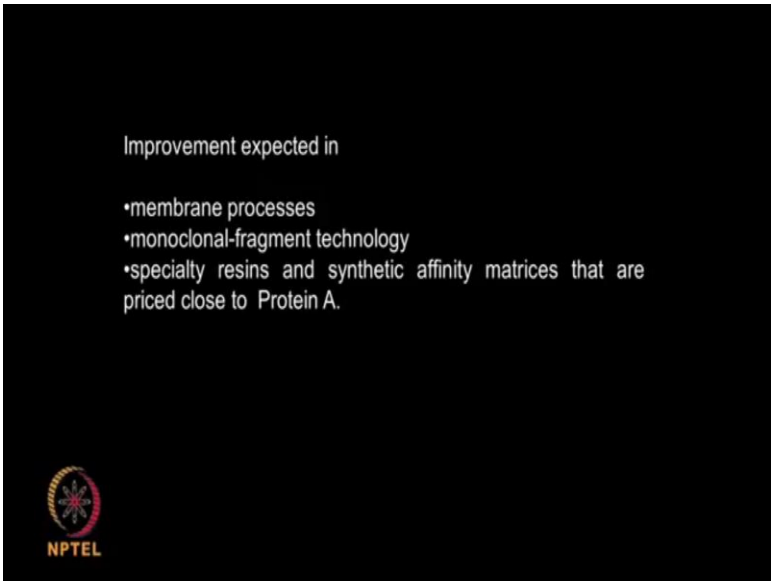

So what is going to happen? Improvement expected to be in membrane processes okay so the membrane as I talked about quite a lot is going to be improved further and further. Monoclonal fragment technology, new resins, new affinity matrices, that will be looked at better pricing protein a so the pricing of these specialty resins, synthetic resins, synthetic affinity matrices is going to be an issues which is going to be improved further and further.

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Okay then comes disposable equipment and single use equipment. I think industries are also moving towards that can I do it one use and then throw them because then I do not have to think about cleaning them. I do not have to think about whether there is going to be cross contamination. So disposable reactors disposable separating columns ah single use equipments in commercial scale okay completely modular mobile biopharmaceutical operations plug-and-play solutions with disposable so ready to process.

So they are going to take over soon and they are going to have lot of advantages because I do not have to think about it every time sterilizing, I don't have to think about am I getting cross-contamination so use once and throw them. But the cost is still high, so the cost of those disposable equipments needs to be reduced further and further and that is where the future is going to happen modular mobile, so these are going to be some keywords in downstream okay.

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So according to Robert Blanck is a bioprocess strategic marketing manager at Millipore, he says the future direction is integrated disposable process solutions, you may have bioreactors chromatography, tangential flow filter, virus filtration, sterile filtration, buffer media storage and intermediate handling. So all these are going to be the future direction in downstream. so you need to address all these okay.

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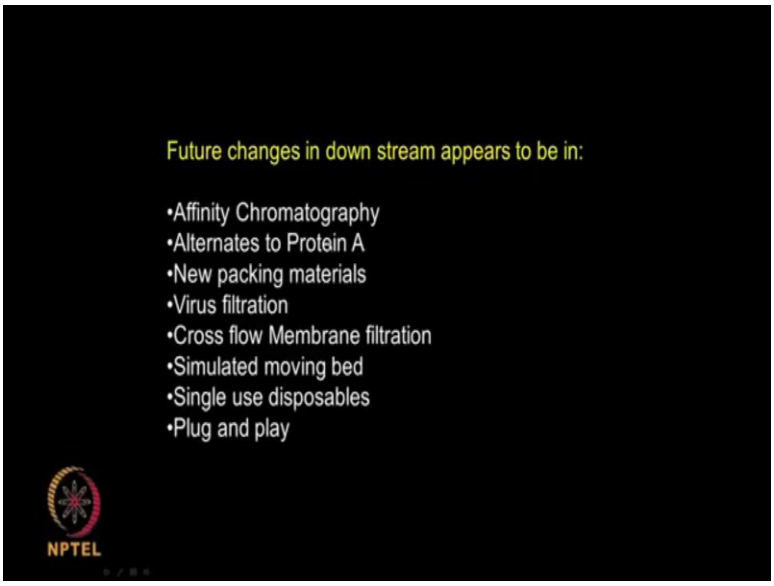

Future changes in downstream okay you are going to see big changes in affinity chromatography alternate to protein A new packing materials virus filtration, cross flow membranes, simulated

moving bed, single use disposable plug-and-play. So the research and development and scale-up and industry use is going to be in these directions okay.

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So few slides we looked at and it tells you that this is where the future is going to be. So if you are if you are a researcher in a industrial R and D if you are a researcher in the fundamental institutes, then you need to focus in these areas of downstream process because that is the concern for the industrial users. So we have spent about 20 lectures, looking at the various principles involved in downstream processing. We look we did quite a lot of problems these problems helps you to understand the concepts and downstream processing.

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And as you know downstream process is recovery from a fermentation broth, intermediate purification and final purification. So they are very useful for a bio synthetic products, for pharmaceutical products, food, neutraceuticals, bulk chemical, specialty chemicals, health care products, a wide number of products, you need to have a downstream purification. The level of purification could be going up from 85% right up to 99.9% depending upon it is a biopharmaceutical product or whether it is a bulk chemical okay.

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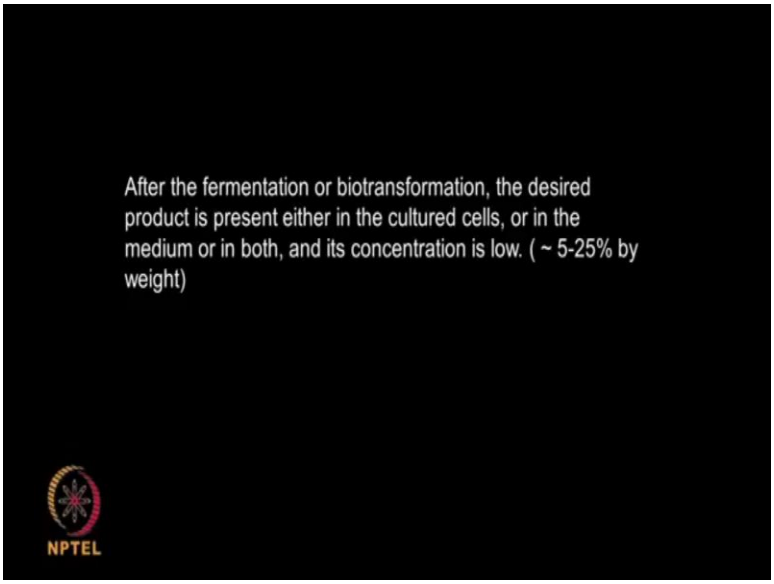

So after the fermentation, biotransformation, the desired product is in the cultured cells so if it is inside the cells, we need to harvest the cells, break the cells and then recover the product. If it is in the extracellular medium, we can do quite a lot of downstream purification. The concentration generally will be very very low 5% 10%. So we need to bring it up right up to 99%. So the downstream is the heart of any product of development and that downstream decides the cost of the or the final selling price of your product okay. So the downstream is very very important.

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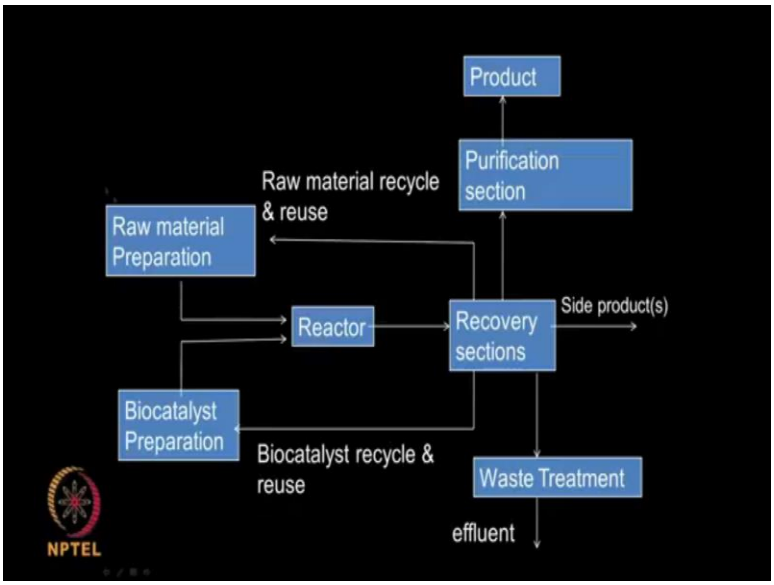

That is what I have been talking about in the past so many lectures. So we have the reactors we have the fermenter here this side, I said is the upstream where are preparing your raw materials,

you are making your media, you are sterilizing your media here, you are having your biocatalyst, your microorganism, it is taken from the stock and initial growth of the microorganism. So all these happen in the upstream and here we have the downstream, we have the recovery section.

We have the purification section, then you have the product and of course you also have the side products here and also the waste disposable going to effluent so your (())(25:57) of the raw material may be getting some of your biocatalyst, getting recycled especially if it is an enzyme you cannot just throw them out you need to recycle them for better cost effectiveness. So the entire block diagram is called the downstream. Of course I dint spend much time on effluent and effluent treatment strategies but these we did spend lot of time okay.

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So we have removal of insoluble, which may include filtration, centrifugation, microfiltration, some membrane operation then we have the isolation of product, cell disruption, adsorption, ultra filtration, precipitation. Then we have the product purification chromatography, affinity chromatography, size exclusion chromatography, reversed phase chromatography, then finally product polishing it could be crystallization, lyophilization, desiccation, so the product is drying.

So if the product is solid, you end up with dry solid sometimes we will have a solid, liquid product then you may have to add some sort of a stabilizing material there, which will help to increase the shelf life of the product, which will help to prevent oxidation of the product. So these are the various downstream steps and we looked at most of them. Some of them in very

detail, some of them superficially and we did some problems, which would have helped you to understand the basic principles.

These problems are also very useful as you can see to understand the concept of cycle, the cycle time and the concept of scale-up as well. And in the very early lectures, I talked about costing and how cost plays a very important role and what are the types of cost involved when you are designing a plant because ultimately it is the cost that breaks or succeeds especially in the area of downstream processes.

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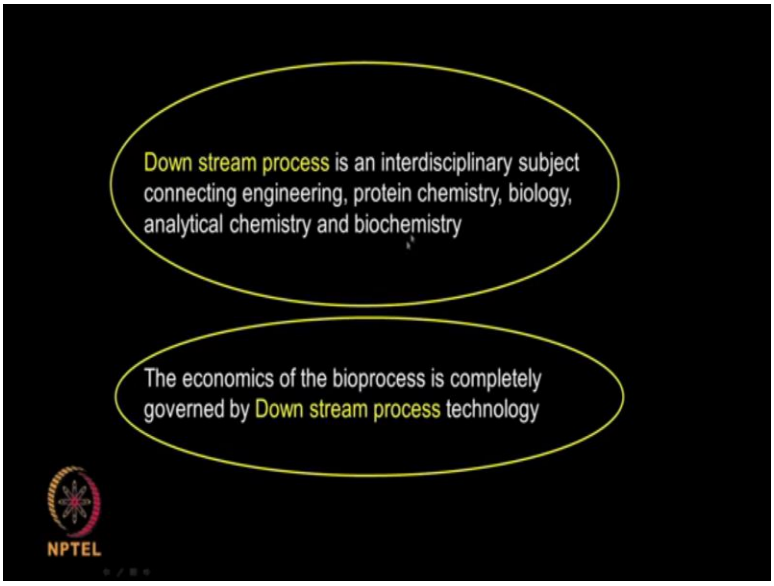

So downstream is an interdisciplinary subject, as you can see lot of engineering, there is protein, chemistry, biology, analytical chemistry and biochemistry and the economics of the bio process is completely governed by downstream process technology. So for a bulk chemical, downstream has to be very simple. For a high expensive pharmaceutical product, you can have a very complicated downstream because it can take up the cost because here pharmaceutical products are a high value added products will be very expensive whereas a bulk chemical will be a very low cost product.

So the total expense involved in manufacturer should be very low okay. Thank you very much for your time. I hope you enjoyed these 20 lectures and I hope you gained from this lectures as well as I hope you solved some of the problems which I had given as assignments, which will

sort of help you to identify important points related to this downstream and thank you very much for your time I hope you gained some knowledge from this short course.

Thank You.