Downstream Processing
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Lecture - 40
Future Trends, Summary of the Course

For the faster 39 lectures, we have in looking at a recovery purification of the bio-product, be it a pharmaceutical product or be it a protein. We have to go through all these various steps for both a recovery as well as purification. And we look at large number of downstream approaches which can do this job and we also looked at a design of those downstream, one of the issues problems and little bit of costing as well. Now, how does these are future for downstream looks like and we will spend some time on that, how is going to look like? How does the industry look like?

What are the challenges the industries are going to face in a next 5 years or 10 years, and what is the focus currently by the industries, it is very, very important for a researchers in, research labs are as well as faculty, working in the area downstream, because they can focus their entire effort their entire research in that particular direction, rather than looking at various downstream processing step. And so we are going to talk about the future trends, how the future looks like in the area of downstream processing.

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Now, downstream processing is very mandatory for biopharmaceutical drugs, food and flavoring, because the product has to be extremely pure; that means, you need to remove all the unwanted metabolites, toxins and other degrading. So, it said mandatory for all biopharmaceutical drug manufacturing and generally most of the manufacturing in biological processes product is been managed by the contract research organization.

So, the downstream process market is a U S 1.5 billion in 2009 and it is excepted to reach 3.7 billion in 2016. So, you can see almost dubbing of a this particular market in the coming use. Now, if you look at the reagents as you know lot of reagents are being used in downstream solvents, chemicals, acids, buffers and stabilizers, proteins, so many chemical are being used, and the market for that moving from 4300 million in 2009 to almost 6600 million in 2016.

So, that market is also support to grow tremendously, so you see both these markets are going to grow tremendously. So, there is lot of potential for the companies to focus on and try to improve their yields efficiencies, try to reduce the bottle necks or total eliminate bottle necks, find you would strategies; so that they can work better, so they can work more efficiently.

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So, 48 percent of the U S companies and almost 68 percent of the western European companies, they felt the downstream processing is the capacity constrain; that means, I can easily scale up my fermenter, I can easily scale up my bioreactor. There is no

problem, but after the reaction or after the biotransformation or after the fermentation I need to do these recovery and purification and that is going to be the real bottle neck.

That is where the company is feel is going to be a real challenge and this was taken from this particular reference called tracking downstream purification. In downstream is viewed as a capacity constrain now, so, the company is cannot immediately increase their capacity by increasing their fermenter through put or reactor through put, because the downstream purification could be the real bottle neck.

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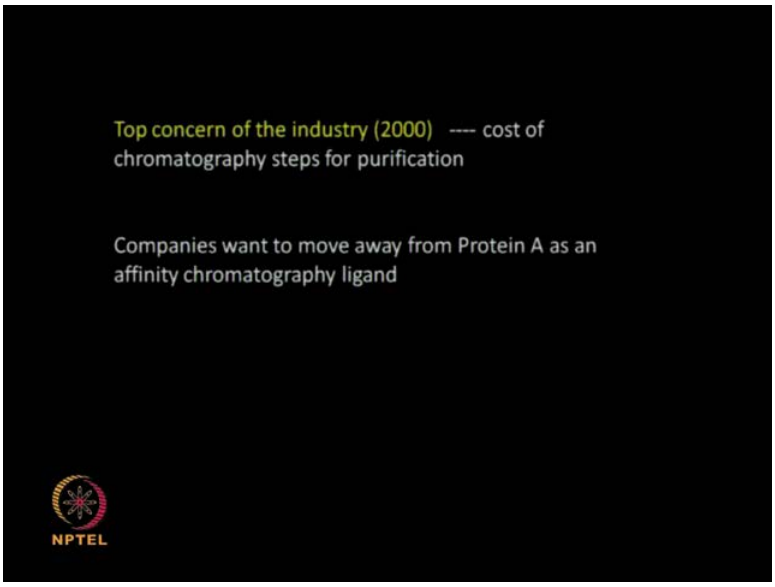

So, the top concern of the industry in 2000, they felt that they chromatography purification is the real constraint that is real concern, because as you know we talk a quite a lot about chromatography, different types of chromatography and the challenges in chromatography, what are the issues and so on. So, you can see that is going to be a real concert for the industrial biochemical engineers as well as industrial managers.

Number 1, number 2 companies want to move away to protein A as an affinity chromatography ligand, I will spend little bit time later on telling you what is protein A, but protein a is protein it is used as a ligand, for purifying a proteins using the affinity technique. So, the company want to move away from protein A, protein A has been there sometime in lot of issues with protein A, so they want to move away from that. So, these are some constraints some worries by the industries in the year 2000.

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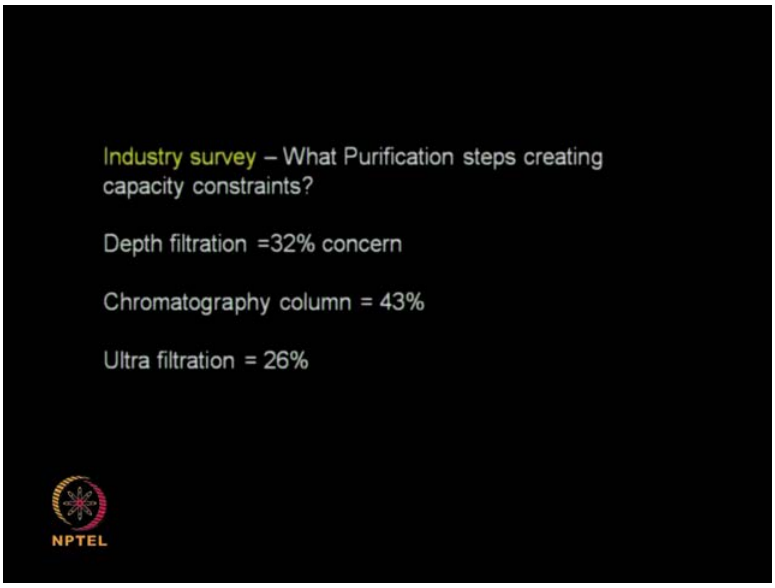

Recently some survey was done and the companies were asked what purification steps are creating capacity constraint; that means, which step is really a bottle neck at they cannot scale it a further. Most of the survey talked about chromatography, so chromatography that is the 43 percent of the survey, the biochemical engineers felt that chromatography column is creating lot of capacity constraint. 32 percent felt the dept filtration is the real constraint, 26 percent felt ultra filtration is the real constraint. So, you see chromatography and filtration are going to be the bottle neck according to them.

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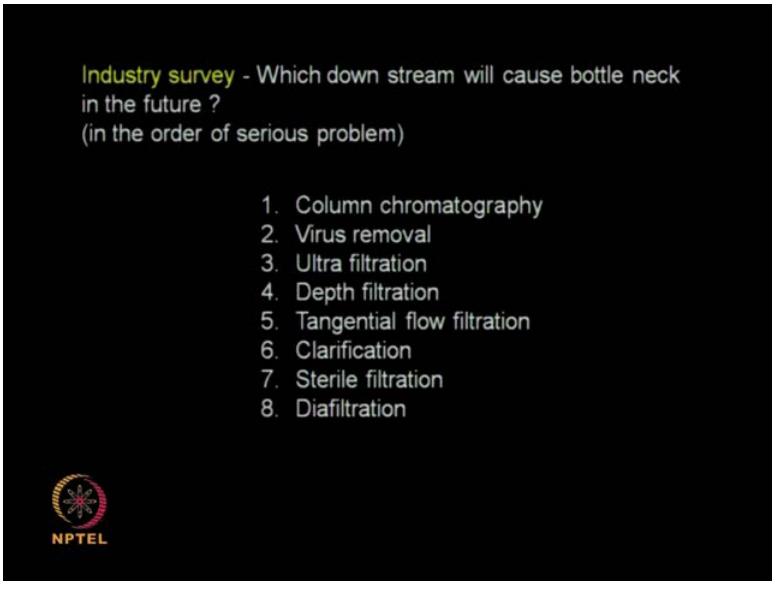

Now, if you rate them or if you rank them according into the seriousness of the problem, which downstream causes bottle neck in the future. Column chromatography comes at the top followed by virus removal, followed by ultra filtration, followed by depth filtration, then the come to tangential flow filtration, clarification, sterile filtration finally, diafiltration.

So, most of the concerns or serious problems start from here, and go down, so if the R N D effort by the academics, R N D effort by the industrial, organizations are focused here. Then these bottle necks could be overcome, but this is going to be a very serious problem, so and based on this event the academics could focus or start working on this particular problem.

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So, nearly two-thirds of the biopharmaceutical companies are spending lot of money for acquiring new downstream processes technologies. So, as I said in the beginning of the tough that downstream is going to be a real bottle neck in the next 5 to 10 years, when compare to the manufacturing company. So, many companies are spending lot of money in the downstream technology, so that they could overcome to this particular problem.

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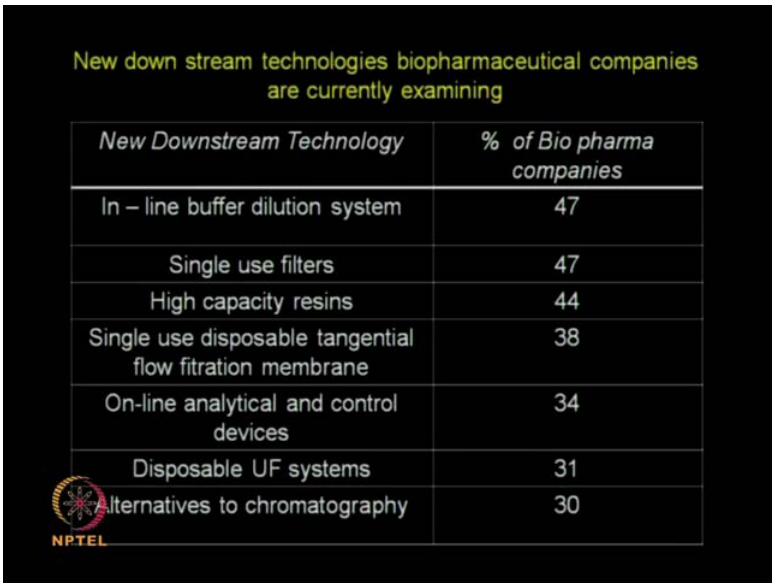

Now, where are the companies thinking about spending their money, in-line buffer dilution system; that means, can a dilute as the process liquid through in-line. Single use filters; that means, we can be filter and then through the filter out, high capacity resins; that means, resins which have very high capacity factors. Single use disposable tangential flow filtration; that means, if I use a single use system, I do not have to worry about sterilizing after use I do not have to worry about contamination, because of the previous batch.

So, single use disposable, on-line analytical and control; that means, as the fluid flows in a manufacturing plant, can a measure the composition, can a measure the various parameters on-line. Disposable ultra filtration systems alternative to chromatography as you know chromatography is extremely in equates, it addresses different aspects of the physicochemical properties of the protein and it can use recover. So, are there any alternatives for chromatography.

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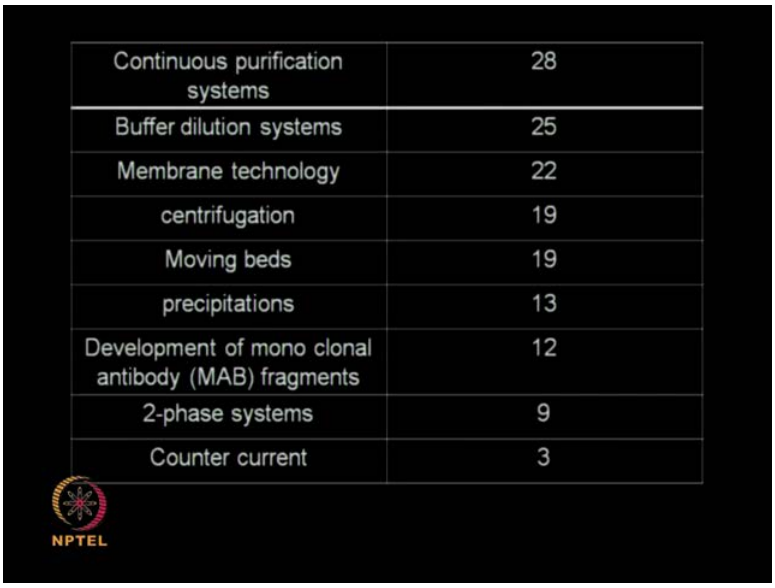

Buffer dilution systems membrane technology, membrane technology is a very big area because there are large number of membranes which are used at are various stages in the filtration and the recovery process. So, looking at membrane technology centrifugation moving bed precipitations and development of mono clonal antibody fragments, 2-phase systems.

That means, we have solvent and buffer or a acquese and organic layers. Counter current systems, current counter current extractions, counter current absorptions and these sort of counter current systems. So, all these are areas in which a companies are thinking about investing and companies are thinking about buying technologies and companies are worried about in the future.

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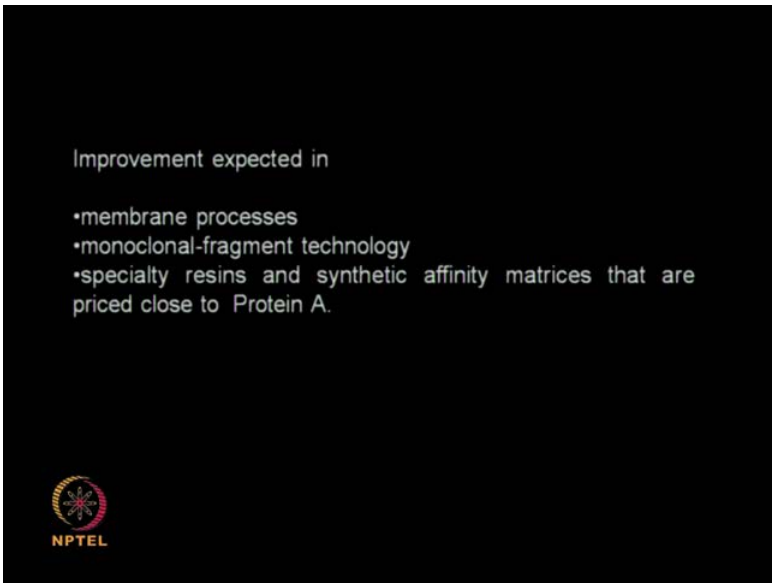

So, as this happens we definitely expect and there is going to be lot of improvement in membrane processes, monoclonal technologies, specialty resigns in chromatographic columns, synthetic affinity matrices. So, they will be very close in cost to protein A, so they are going to be real alternate instead of protein A.

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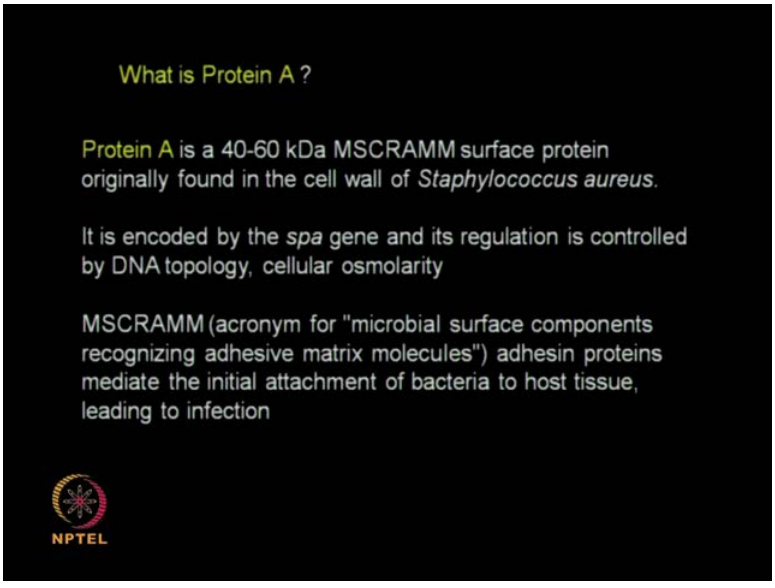

Now, what is protein A? Because, I talk about protein A couple of slides, I talk about this protein A. Protein A is a 40 to 60 kilo dalton microbial surface component recognizing

adhesive matrix molecule, that is MSCRAMM. So, it is a surface protein it was originally found in the cell wall of staphylococcus aureus.

So, protein a is 40 to 60 kilo dalton microbial surface components recognizing adhesive matrix molecules, so it can be use as an affinity pelican. It is encoded by the spa gene and it is regulation is controlled by D N A topology, cellular osmolarity and so on. Actually, so it is an adhesin protein, it mediate the initial attachment of bacteria to host tissue leading to infection. So, and this protein A is a surface protein it helps in attachment of the bacteria to host tissue leading to infection.

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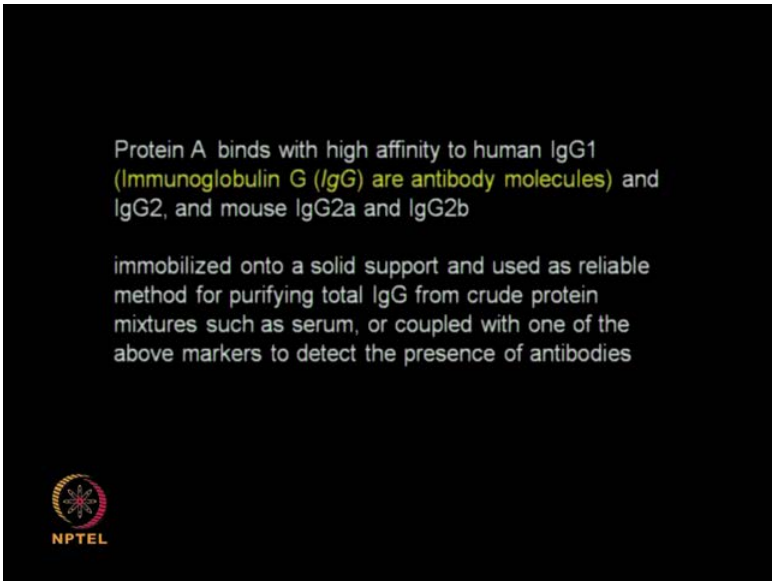

Now, protein A binds with high affinity to human I g G; that means, immunoglobulin g and it is binds very well to I g G 2 and also mouse I g G 2 a as well as I g G 2 b. So, if I immobilized protein A on a solid, support I can use it as a affinity pelican capture I g G human immunoglobulin right. I can use it for purifying immunoglobulins from crude protein mixture, such as serum or coupled with one of the above markers to detect the presence of antibodies.

So it is very, very useful, so I can capture the immunoglobulin from blood serum by having protein A in mobilized on a set on a solid surface. So, protein A act as ligand here. So, protein A technology has been very useful in purifying serums and other crude protein mixtures for getting human I g G 1 human I g two or mouse I g G 2 a or mouse I g G 2 b. So, are there any alternates to protein A, so that the problems that are now

associate with protein A could be over come. And that is what I think the future chromatography ligand based or affinity based chromatography is going to look at.

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So, what is happening in industry currently, there are many things are happening and I am going to show next few slides talking about some of these industries are working on based on their own R N D effort. Single use downstream chromatography, so now, was on is working on single use downstream chromatography; that means, they will do the chromatography and trough it away. So, the problem of sterilization problems cross contaminations are completely overcome. You patented dual affinity polypeptide technology which will replace protein A, but disposable technique; that means, single use approach.

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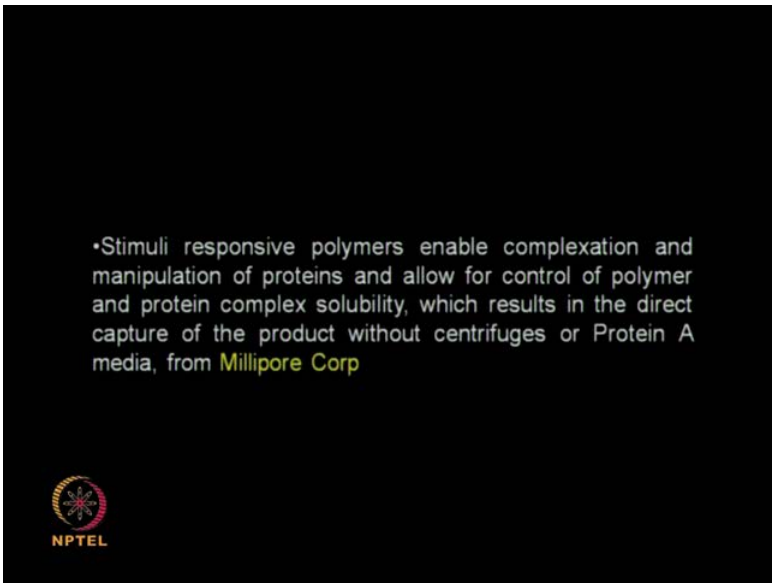

So, now, was on I am working on that, now if you look at another company call Millipore, Millipore has been there in purifications and filtration system. They are looking at stimuli response polymers, these polymers will enable complexation and manipulation of protein, and allows for control of polymer and protein complex solubility. So, they are working in this type of new polymers which will helps in binding serum protein, so this can be used as a direct capture of product without going for centrifuges or protein A type of media.

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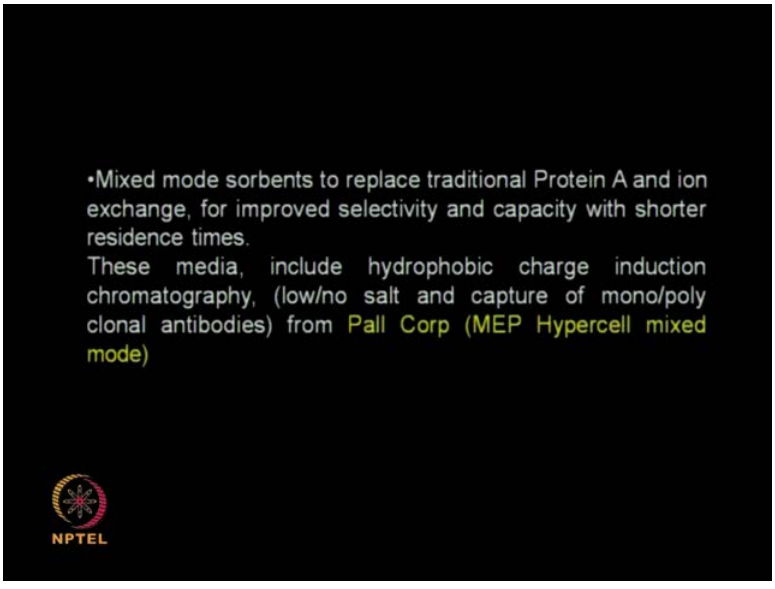

Mixed mode sorbents replace traditional protein A and ion exchange for better selectivity, company like Pall corporation, and they have product called MEP Hypercell mixed mode. So, they are working on certain media which includes hydrophobic charge induction chromatography; that means, it does not have salt and can capture mono or polyclonal antibodies.

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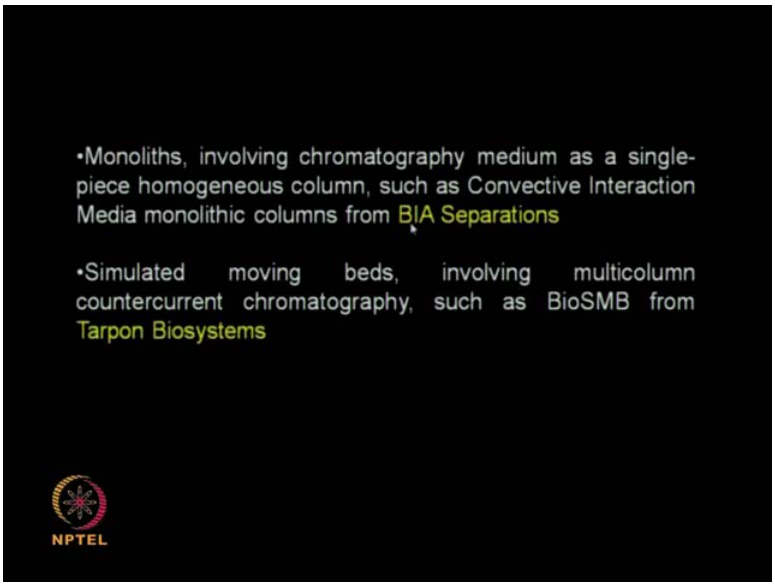

Another company called BIA separations, they are working on monoliths involving chromatography medium as a single piece homogeneous column. Look at another company called Tarpon Biosystems, they are looking at simulated moving belts which involves multiple columns and a counter current chromatography. So, you have many columns and they liquid flows in different in certain pattern into different columns, so that it stimulates the moving that the principle.

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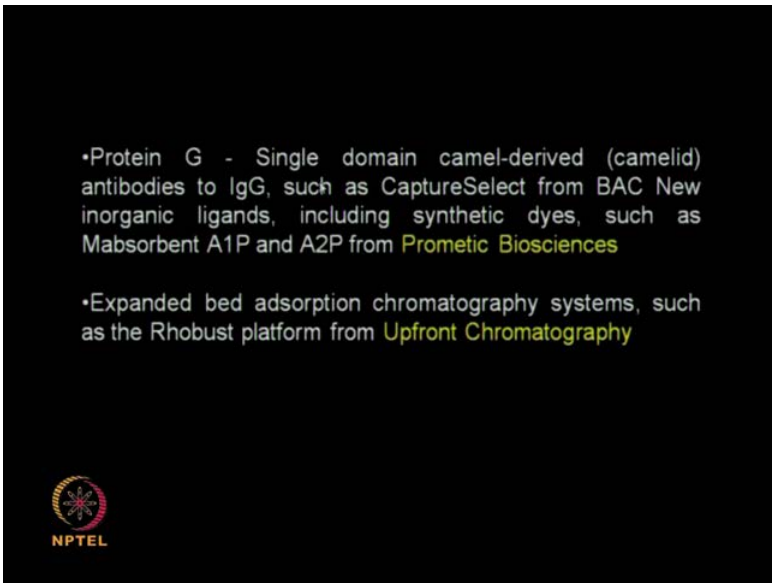

Another approach protein G this is a single domain camel-derived antibodies for I g G that is been looked at by company called a Prometic biosciences. Another company called Upfront Chromatography, they are looking at expand bed adsorption chromatography systems.

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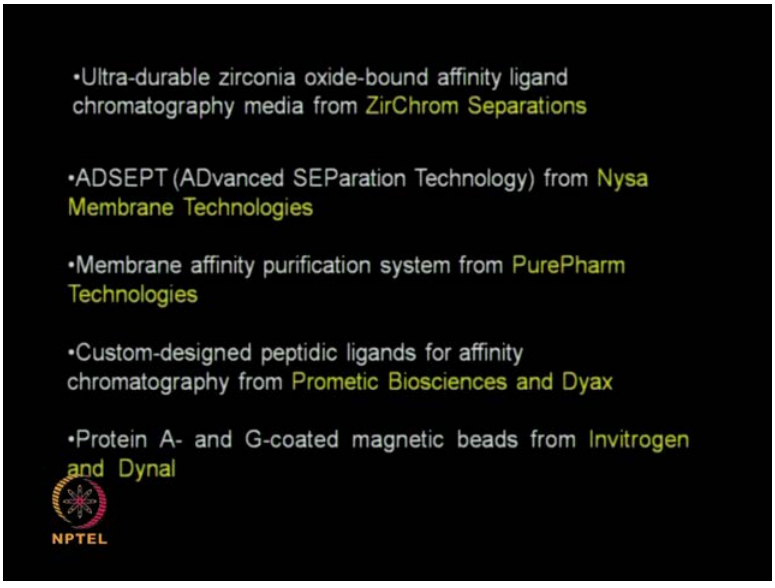

Then there are lot of technologies, there are being looked at by several small and medium sizes companies. For example, company called ZirChrom separation, they are looking at ultra durable zirconia oxide bound affiliate ligant. So, they are looking at in

organic type of a base material, especially for ligant chromatography media. Another company called Nysa Membrane Technology, they are looking at a membranes, alternate membranes, new modified membranes for separations. Another company called PurePharm technologies, they are looking at membrane affinity purification system.

And if you look at Prometic Bioscience and Dyax, they are looking at custom-designed ligands for affinity chromatography. And Invitrogen and Dynal, they are looking at the coating magnetic beads with protein A and protein G. So, the magnetic beads could be manipulated by an external magnetic field, but these magnetic beads will be about by these protein A and protein G affinity ligands. So, these are some ideas that are been looked at technology been looked at and they come in to market next 2 to 5 years.

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And Roche you are looking at affinity purifications method based on expression of proteins, affinity chromatography media, His(tidine) tag media and so on. And then there is lot of works that is been done on protein A alternates, including reverse micelles liposomes, liquid-liquid extraction systems, crystallizations, immobilized metal affinity chromatography, novel membrane chromatography. So, all these are separation techniques and high selectivity techniques which or meant to replace the ligand based affinity chromatography.

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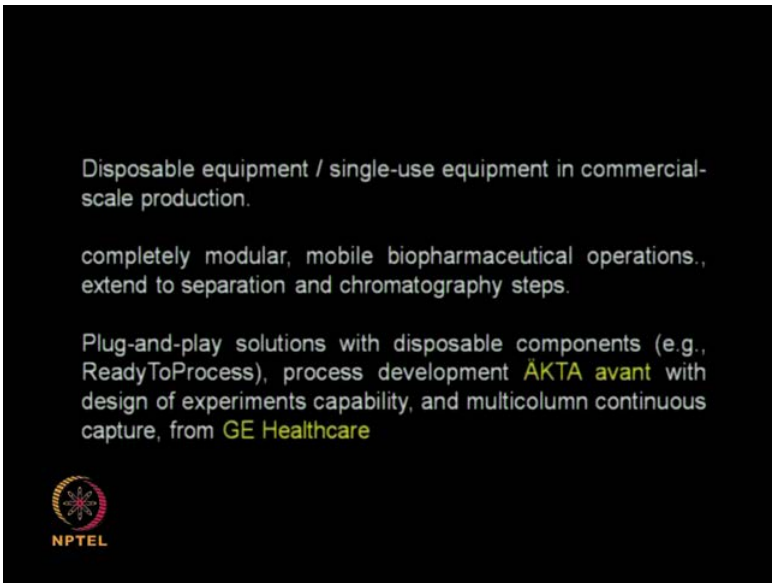

And then disposable equipment that is going to be the future single-use equipment or disposable equipments in commercial scale productions; that means, I mean have 1000 letter disposable bioreactor. I may have a 100 letter disposable system filtration system, so you use it once and through it out. So, you do not have to think about sterilizing this material, we do not have to think about contamination from the previous batch or contamination that will happened to the next subsequent batch and so on actually.

Completely modular design; that means, you will have bit is and pieces of all the downstream all connected like a leg of piece. Mobile biopharmaceutical operation, so I can move by filter to wherever I want I can move a chromatography wherever I want. There are not fixed and with hard pipes you can have flexible pipes. So, you can know the chromatography before a pre before a downstream operation or you can move it after depending upon the type of a process you are looking at. So, that is called a mobile biopharmaceutical.

And plug-and-play solutions, so we can connect different parts or different aspects of the downstream and complete downstream process technology. So, companies like AKTA avant, G E Healthcare, they are also looking at this type of plug-and-play solutions even for design of experiments. And it is a very important statistical tool for optimizing the processes, looking at multi component, multi column is continuous capture and plug-

and-play solutions. So, these are some more approaches there are being looked at in the their future to come.

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So, according to Robert Blanck bioprocess strategic marketing manager of Millipore in the future direction is going to be integrated disposable process solutions. So, it is going to be integrated and disposable it may have bioreactor, it may have chromatography, it may have tangential flow filtration, it may have virus filtration, sterile filtration, buffer media storage, intermediate handling.

So, you see all these different parts and it is going to be integrated, it is going to be like move around and it could be a disposable in sort of setup. So, you may have a reactor and all of them may be put all arounded and all of them can be through now. And as you said a large scale disposable reactors vessels storage upper media a storage vessels are already coming into market for manufacturing and so the fixed large scale equipments may soon disappear.

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So, what are the features changes in downstream appear to be in, so that is going to be improvements, that is going to be efficiencies, it is going to be modifications in some of these areas. You know affinity chromatography, because that is going to be most important area where we are talking about getting hundred percent pure protein product and for therapeutic applications.

Alternates to protein A new packing materials, virus filtration, because once you complete your cell breakage and recovery protein one of the problem is that could be still is the contamination of the viruses. So, can a completely eliminate the viruses from the therapeutic proteins which are obtained from this viruses. Cross flow membrane filtrations, stimulated moving beds, single use disposable; that means, you do it once and trough it away.

So, you do not have to bother about sterilization and so on, plug and play; that means, you connect two different downstream one after another and then you may at least start using it. So, the commissioning time is reduce to dramatically by this type of a approach of a plug and play. So, these are the areas in which we are going to see lot of changes, because these are the areas which have been giving a considerable problems and during downstream.

And these are the areas which are giving considerable bottle necks, if you want to scale it up further. So, all the research by the industries are being focused in this particular area.

And so, if the academic research also focuses here, it will be very useful for these industries. So, most important as we can see from this list is the chromatography and a filtration. These are the two and (( )) recent advances or the recent concept or recent thinking is in the area of single use disposable; that means, you do it and trough out the equipment. You do not have to think about sterilization and so on.

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So, with that we come to the end of our faster 40 lectures of a downstream processing, it is most important setup operations which help you to recover purify the desired biological product. That means, product which means derived from bacteria or fungus or any other biological source. And so, it is the most important steps that is mandatory, if you are manufacturing a biopharmaceutical product, because the purity of such a product is extremely important.

And downstream process also decides on the cost of the final product, because more number of inefficient downstream steps see you adopt. Obviously, the selling price of the product goes up and you may end up having an economical product entering the market. So, downstream process plays an extremely important role, and in the final cost of your desired product. It determinates the selectivity, it determinates the purity, as well as the cost.

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So, having said that we mention that it is very important for recovery and purification, and biosynthetic product that means products which are made from biological origin. Pharmaceutical product; that means, drugs whether it is chemical based drugs or whether it is biological based drugs. Pharmaceutical food large number of food prevented food and process food or being now being need using biological fermentation approaches. Chemicals bulk chemicals like aesthetic acid especially chemicals and surfactants biopolymers are all being currently being made using biological techniques.

Health neutraceuticals, again health neutraceuticals has become very a important of late and there also being made using a biological origin and through fermentation or through natural products. So, you are going to have downstream at the end of a fermenter or a in same reactor without a downstream. And you cannot have directly use the fermented product for a commercial use.

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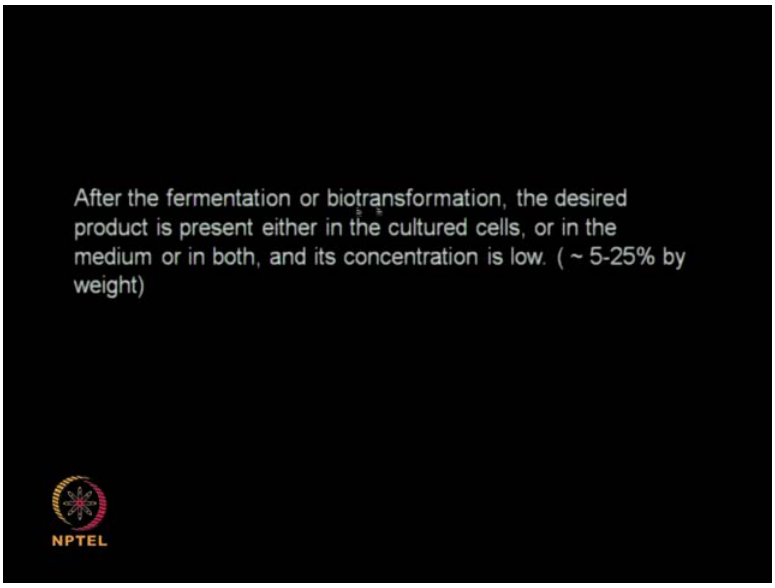

So, after the fermentation or biotransformation the desire product is present either inside the culture or in the medium or in the broth. So, your goal is to recover that and purify that, whether it is inside the culture made or cells outside in the medium. Generally, the concentration is going to be very, very low 5 to may be 25 percent max, but you would like to bring it to 90 95 very, very close to 99.

And if it is a pharmaceutical product you want make it hundred percent and that is way the downstream processing technology is processing approaches which having talking about in the faster in a so many lectures become very, very useful.

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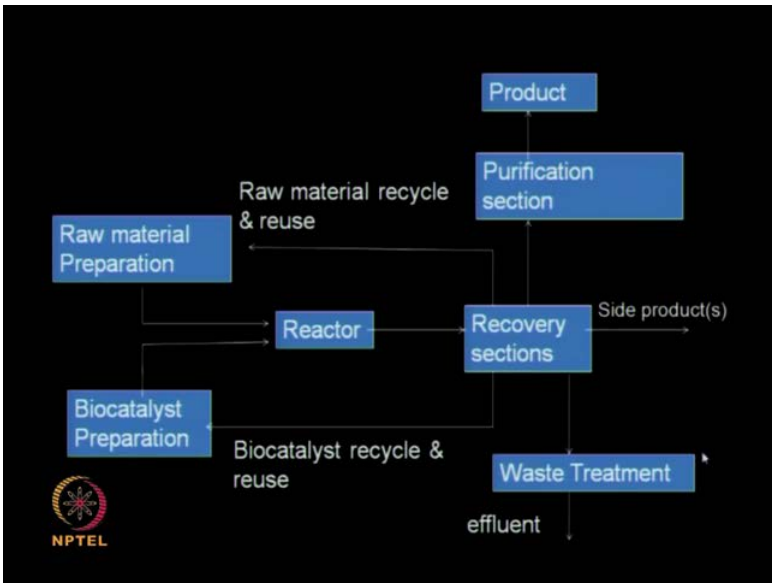

So, this is the slide which I showed almost 40 lectures back remember, I hope you all remember this slide. This is the heart you have the reactor or a fermenter here, and whatever is on the right hand side we call date as the downstream, and whatever on the left hand side we called it upstream. So, you are preparing your biocatalyst you are preparing your media sterilizing your media, media components inoculation little growth of the organism and immobilization of the enzymes all these come under the upstream.

Now, here you have the downstream, so here we are talking about recovery of your product and then purifying your product and them stabilizing your product and that same time you are going to get some side products. Then you are going to get lot of waste, so I briefly talked about effluent treatment planned here as well, but that itself becomes is the big subject to talk about. But, we just covered very briefly on various effluent treatment be it solids be it liquid or be it sagaciously effluent, so all these come under the area of downstream.

So, in the recovery sometimes you recover raw materials, sometimes you recover enzymes, sometimes you recover active biomass which you send it back for the next batch there by you reduce the cost of the final product. And in the recover section the concentration of the initial product will be very, very little, concentration of the final product will be very, very little. So, you are trying to improve the concentration to larger value and then finally, you are purifying to almost hundred percent. So, this where you

have the types of filtration types of chromatography you have the type of specialization and so on actually.

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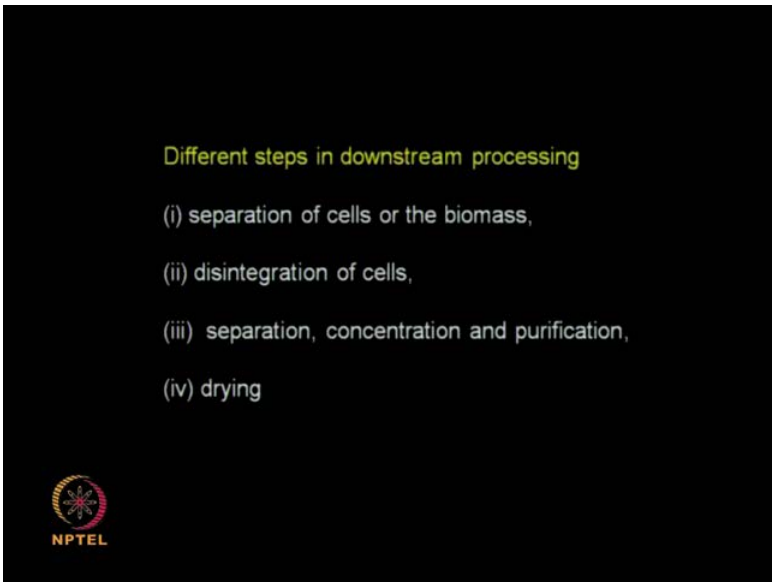

So, here what are the main steps in the downstream, we talked about separation of the cells or the biomass. So, as soon as your liquid broth comes out, the liquid broth contains biomass, cell debris salts and other floating and dissolved a material and so on actually. So, here the first step where you are separating come out, if you are desired product is inside the cells; that means, if your products is a infra cellular or if it is in the periplasmic.

Obviously, you are very much interested in the cells, but if your product is extra cellular, then you not interested in the cells. You recover the cells and through them out, so if you are interested in the intracellular material; obviously, you will be doing a disintegration of the cells. So, we look at large number of techniques, physical based mechanical based, chemical based, enzymatic based techniques for disintegrating the cells and getting your product out in the media.

Once you get this product it is going to a very dilute solution, so if first try to separate it from rest of the material, like the side products metabolites taxing and so on. Then you concentrated and then finally, purify it. So, you purify it to the required degree if it is a bulk chemical purity is not so important, but if it is a pharmaceutical purity very, very, very important. And finally, you going to drying different types of drying we talked

about normal heat base drying lyophilization speed drying a drum drying and so on and then you have the desired product at hand.

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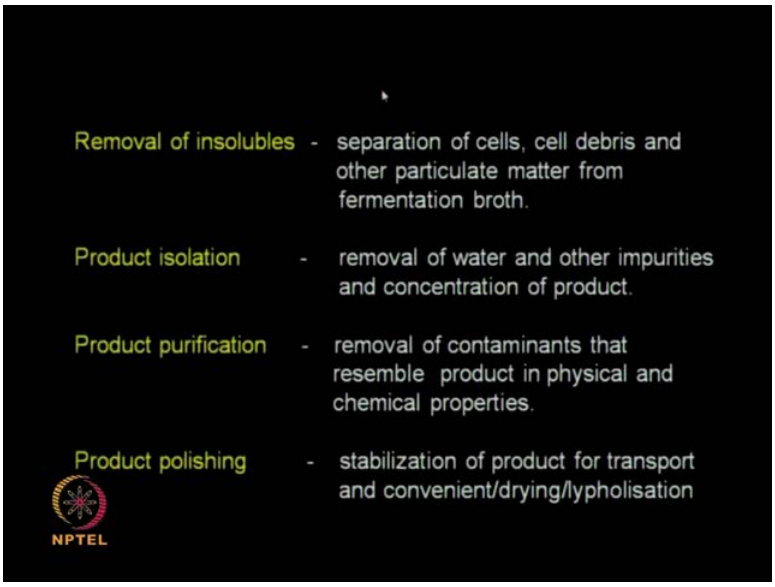

So, these are different steps in the downstream we looked at, so removal of insoluble and separation of cells, cell debris and other particulate matter from fermentation broth. Then the product isolation which said removal water and impurities, then you are concentrate your product. You are talking about extraction and you are talking about different types of membrane filtration, then and them purification here we talked about again some sort of membrane.

Then chromatography different types of chromatographys, then we looked at crystallization, we looked at a lyophilization and finally, the product polishing. You are doing the drying and then you are adding some stabilizers to keep the product from degrading or getting a disintegrator, so that is called the stabilizing. So, all these are the various downstream steps and I hope you remember various techniques, we covered in the course of these lectures. What are the salient features? What are the design parameters? What are the different types of design available and so on.

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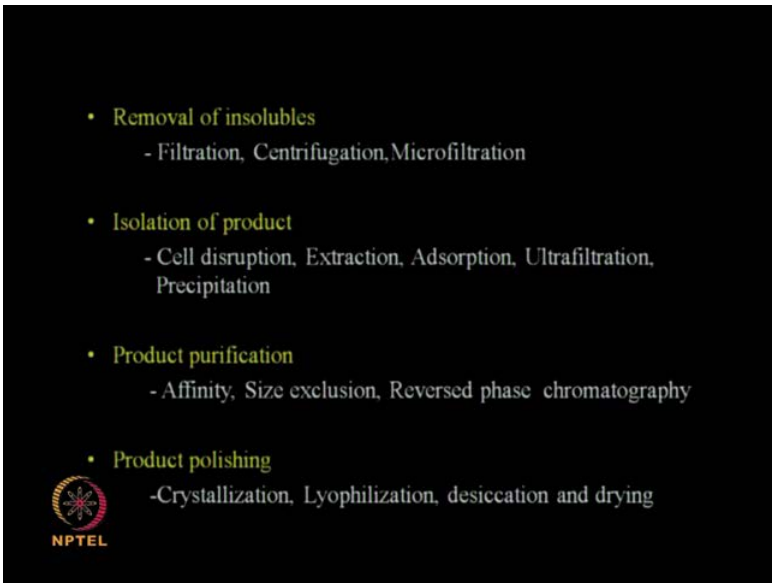

So, removal of insoluble we looked at filtration, centrifugation, microfiltration, nanofiltration, so many different types of filtration we looked at. Even under filtration we looked at centrifuges, different types of centrifuges drum based filtration, leaf filtration plate and frame filtration and so on. Under centrifuge we looked at disk base centrifuge ball base centrifuge, so many different techniques are being used for removing this solid and the liquid.

Now, isolation the product if you look at the isolation of the product, you recovered the solid biomass and you are doing the cell destruction, if it is intra cellular material, and then you are doing extraction. You are using a solvent to extractive your desired product form a large mess then you are using absorption. So, in absorption what we do you are using solid matrix to absorb the desired product, then we can also use ultra filtration. Precipitation is another technique which can just precipitate out your desired product from the broth or the mother liquor.

So, you would have concentrated your product from may about 58 percent to almost 70 or 60 percent, then you go for product purification; that means, you are increasing it to 90 or much higher than that. There comes your chromatography, size exclusion chromatography hydrophobic interaction chromatography, ionic chromatography, affinity chromatography and so no.

So, all these chromatography in various stages, you use and bring or increase your product purity to here very, very high level. Then finally, coming your polishing; that means, you are using crystallization to get solid beautiful looking crystals. If that is desired buyer costumer some cases product will be in the form of a liquid, but in some cases the product may be in the form of your solids.

Like some proteins could be in the solids, so, we do crystallization, then you do drying which is by heating. Then you do lyophilization different types of approaches by which a you remove the water that is present. So, that the product and become stable and then you sometimes add stabilizers you add stabilizers and so that there is no disintegration not bio degradation of the final product. So, the transportation or self life is increased by this approach.

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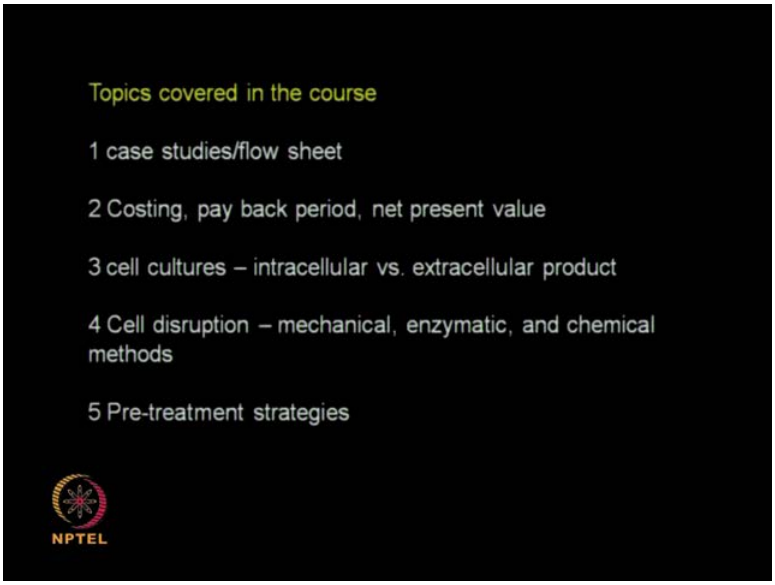

So, what are the topics we covered? We covered wide range of topics starting from what is a basic of a downstream, I mentioned a few case studies, we looked at some flow sheets. And then we went into costing we talked about what is pay back period that present value, what are the cost factors involved in deciding on the land cost and also on the operating cost. This is very, very important you may think why should learned about costing and I am a biochemical engineer, but there is very, very important.

If you are going to select a different types of downstream, should I have a filter or should I have centrifuge should I have extractor or should I go for a chromatography. So, when

you start deciding or when you start thinking, then cost plays a plays a very, very important role that is why we briefly talked about different approaches costing. Then we looked at the cell culture the product is it an intracellular or an extracellular product, different types of cells and what are the issues in involved if you are trying to recover a intracellular product from a bacteria, from a yeast and so on.

So, different disrupting techniques we talked about the mechanical based techniques, then enzymatic techniques and chemical methods. And we also we looked at briefly the advantages, disadvantages which one of them and which one looks good, but the same time which is more expensive then which technique, so we spend some time on that. So, the pre treatment strategies become very, very important should I heat it or should I cool it, so that I can achieve the breakage of the cell membrane and release of my desired product.

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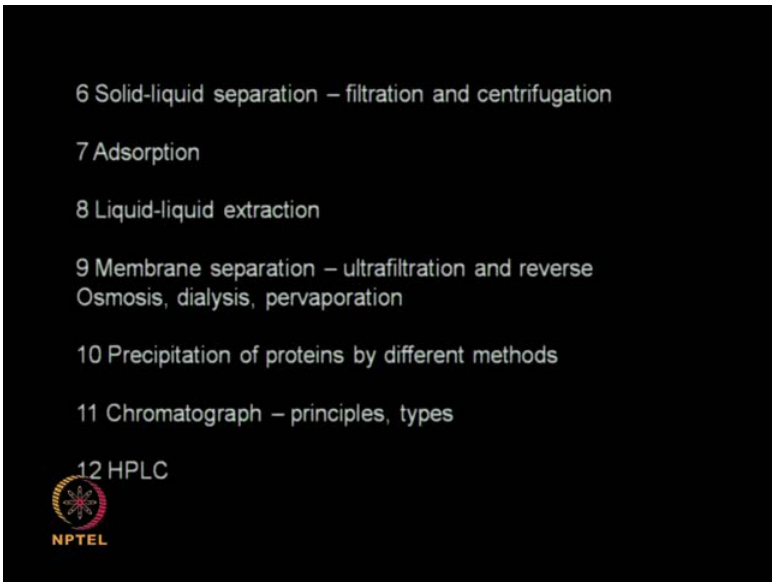

Then we went into the solid liquid separations large number of solid liquid separations based on filtration and centrifugation. Then the important equations, design equations involved in that, then we looked at absorption; that means, absorption of a solute a solid matrix what are the different types of relation mathematical relationships which relate. The solute in the liquid face with the solute in the absorb face and how do you calculate the amount of absorbent required to absorb a particular solute.

Then we went into liquid-liquid extraction this is very, very important downstream processing tool or technology because it is when at room temperature. So, there is no dean activation or dean naturalization of the product. So, we looked at counter current liquid-liquid extraction, we looked at multi stage liquid-liquid extraction, we looked at cross flow type of design and then we did some problems to determinate how many stages are required. To achieve certain separation efficiency or given the number of stages can you tell what is the efficiency of the entire system? So we looked at that.

Then we went into membrane separation different types of membranes starting from ultra-filtration membrane, nano filtration, micro filtration, then we went into reverse osmosis, reverse osmosis is a very important membrane technology. If you want to have potable water, then we went into other membrane operations the dialyses, the electro dialyses. And then finally, we also talked about (( )) is a combination of permeation and vaporization.

So, it is base on size of the particles and filtration, but it is based on the affinity of the solute with the membrane material. So, the technique is very, very different, so you can really concentrate food juices at room temperature or very low temperature. So, that the fruit juices or food and flavoring material that does not loose it is flavoring activity. Then we went into precipitations of proteins various types of a precipitations, because precipitation is one of the methods for product recovery from a big mother liquor.

We talked about using solvencies media of precipitating, we talked about salts talked about polymers electrolytes a precipitating media and mostly precipitation is used in the area of protein purification. Then we went into chromatography, chromatography is the biggest area of our research in downstream different types of chromatography is the principles how interaction between various solutes affect the separation efficiency.

How to calculate the retention time? How to calculate the theoretical plates? How to calculate the efficiency of a chromatography? All these were discussed over several classes and we looked at many problems on that. Then we spent considerable time on the analytical tool HPLC. This is also a chromatography, but we spent some time, HPLC is not use for separating, sorry HPLC is not use separating and recovering like a downstream, but it use in the area of analytical tool for identifying a particular component as well as quantifying a particular component.

Because, HPLC is very, very important we spent couple of lectures on different types of HPLC techniques and what are the factors involved in achieving a good separation? And what does selectivity means? And what does retention time means and so on. So, HPLC is nothing but high performance or high pressure liquid chromatography and we spent some time on the normal phase HPLC as well as the reverse phase HPLC.

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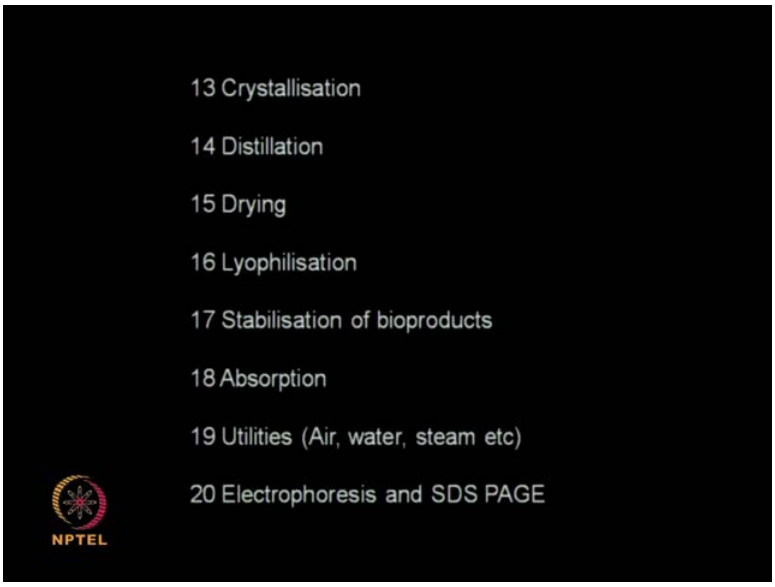

Then we went into crystallization what are the approaches by which one we gets solid crystal, and what are the factors that affect crystallization and so on. Then we come to the recovery, product recovery, product stabilization and product flashing techniques. The distillation is used if you want to recover the solvents that are used in a extractor or you want to recover the solvent that are used in the chromatography, You cannot trough the solvent.

So; obviously, we need to recover them and there may be more than one solvent or metabolite present. So, distillation is a tool is a technique used for boiling out the desired solvents from a mixture. Then we went into drying a drying is used for the removal of moisture from a solid product, drying is not used the pure protein or bio product is temperature sensitive. So, we have to very careful a different types of dryers we looked at and also looked at some equation which are involved which characterize this drying process.

So, if your product is temperature sensitive, then the best technique to go for this lyophilisation that is a you cool it down, convert the water into solid ice and then you remove the solid ice as the vapor. There by you are avoiding the liquid water stage that is called lyophilisation and it is very good, if you want to really remove water from blood plasma or other highly temperature level bioproducts.

So, lyiophilisation is another technique which we spent some time on, and then we briefly talked about stabilization of bioproducts. So, how do you stabilize a bioproduct, so that there is no degradation, there is no degeneration of the product or there is no bacterial growth or fugal growth, because all these bioproducts need to be kept for considerable amount of time before they are sold in the market. So, the product should be stable in the manufacturing side.

So, stabilization of bioproduct is also important downstream which needs to be consider. Then we talked about absorption although it is not a direct downstream and the gases that are living a fermentor cannot be directly went into the atmosphere, because it may contain talk sake vapors or dangerous gases which cannot be expose to the flora fauna. For example, carbon monoxide or S O 2 or a S O 3 or fluorine, so absorption is a technique which is adopted to recover or capture all the gases before, the inner gases are went into the atmosphere

So, absorption is based on a bulk technique, so we use either alkali, mild alkali, you may use water or you may use ethanolamine. These types of chemicals will absorb the gases which should not be wented out in the atmosphere and then very briefly talked about some a fluent treatment techniques. And what are the various stages in the fluent treatment?

Then in order to run a downstream manufacturing plan you need lot of utilities, you need air, you need water, you need a steam, high pressure steam, low pressure steam, you need chilling water, you need cold water and so on. So, utilities are very, very important number 1, number 2 utilities add to the selling price of the product, because utility is add to the working capital.

So, managing the utilities is also very, very important if you want to keep your cost of the downstream step low. So, utilities are very, very important and depending upon the complexity of the downstream the number of utilities may be very high. So, downstream

process may be having just water as utility, where are in some other places you may have child water cold water steam and air present. So, utilities add to the overall selling price.

And we also spent some time on another analytical tool that is called the electrophoresis and SDS PAGE. Although, this is not used in the downstream manufacturing, it used an as an analytical tool for a separating proteins and also for identifying proteins. Based on the charge on the proteins, based on the iso electric P h of the proteins based on the size. So, we did spend may be a class on this particular analytical tool, that is the electrophoresis and the SDS PAGE.

Although, they do not come directly and as the downstream, so we spends some time little bit time on tool analytical tools. One is the HPLC which is extremely important and separating identifying and quantifying proteins or biomolecules and electrophoresis, and SDS PAGE which again can be use for separating proteins, identifying proteins, based on the charge, based on the mass. So, these are the two analytical tools which are very, very important which we talked about in the past 39 lectures.

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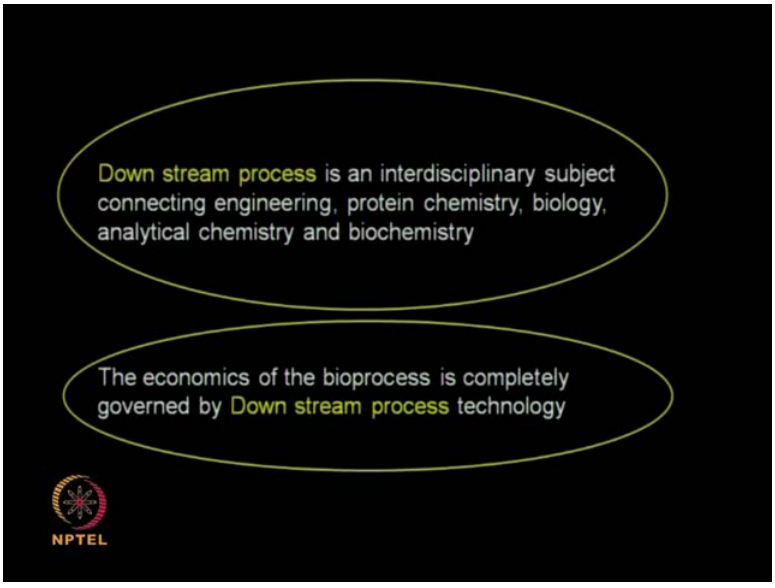

So, to conclude would say downstream process it is an highly interdisciplinary subject, now it connects engineering as you can see. And the biochemical engineers, chemical engineers are need a connects engineering with protein chemistry, lot of proteins are being looked at chromatography, separation, SDS PAGE, HPLC all these are involves protein. It connect biologist which should because you have lot of biological tools and

techniques, it connects analytical chemistry, because we are talking about analytical tools like a H P L Gs and electrophoresis.

And the biochemistry, because there are lot of biochemical acids involved in downstream processing. So, which are highly inter disciplinary subject it a connects a large number of a principles starting from engineering to chemistry to biology. So, I do not think one expert will be able to manage a large planed of so many different techniques and it may require a team of biologist or a protein chemist or engineers to solve the problems that faced actually.

And economics of the entire bioprocess is completely governed by the downstream processing technology, as I said the cost of the product is highly dependent on how complicated is your downstream, because I showed you many flow sheets where in some flow sheets you have so many chromatography's and so many filtrations that the downstream contributes almost 50 60 percent of a cost.

So, the entire cost of a product depends on the downstream process technology. So, you can spend lot of effort in improving the process efficiency, improving the selectivity, so that cost comes down. Usage the solvents can be brought down, usage of the large scale equipments can be brought down; that means, trying to use smaller equipments, usage of the utilities like a steam or water can be brought down by doing all these you are reducing the working capital as well as the overall final selling price of the product.

So, this completes the course on the topic downstream process in bioprocess technology, I hope you enjoy the pastor 40 lectures. It was it was in some area some times it was given as a very broad spectrum in some places event quite deep into the fundamental is look it at the physical principles involved in arranging at a certain separation techniques. And as you can see it appears to be a combination of some the chemical engineering step, like distillation or absorption or extraction with the biochemical steps like chromatography. So, it is a mixture of both the chemical engineering as well as the biochemical engineering and that is what is downstream process is made up of and that is what a makes it very challenging for engineers as well as the biochemist protein chemist as well as the biologist.