Biochemical Engineering
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Module No. # 01
Lecture No. # 39
Strategies for Biomolecules Separation (Contd.)

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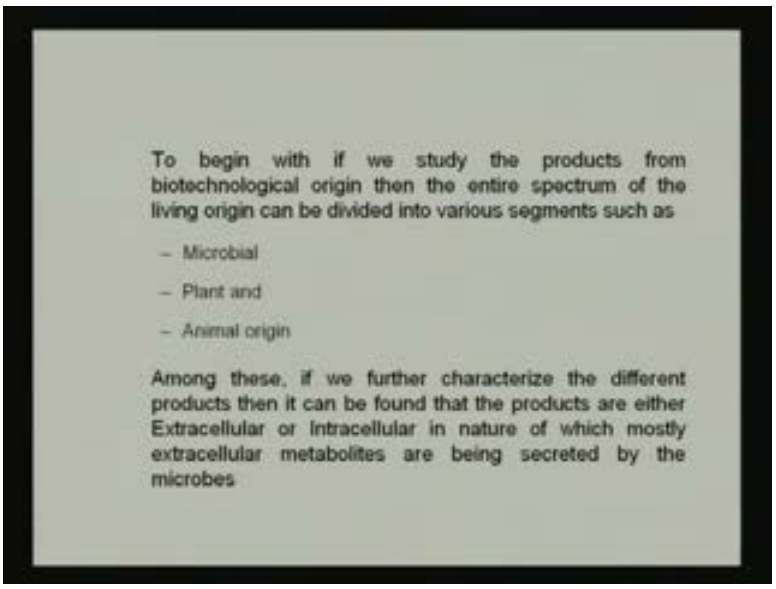

Good morning students. In continuation of my last lecture, where I have taught, told you about the strategies of biomolecule separation, there, I have told you that, there may be the different origin of biomolecule production. So, that may be from the microbial source; it may be the plant, or it may be the animal source. So, if we see the entire metabolites which can be produced by this particular biological means, we can divide the inter metabolites into two major part; one is the intracellular and another group is the extracellular. In my last class, I have also, also mentioned you that, if we are going for any genetically engineered microorganism, then, mostly the metabolites, the targeted metabolites are produced in the form of inclusion bodies.

So, it is then, within the cell structure. So, if we want to take, or if we want to isolate our targeted product, what we have to do? We have to disrupt the cell wall of that particular microbes. So, in one case, and if suppose, in another case, another example, another part

of this product, where we can use the microorganism for production of any metabolites. So, when we are going for such metabolite production, it can be the extracellular also. So, as soon as this extracellular metabolites are produced, that means, that cell is secreting that particular product through the cell wall to the surrounding environment. So, here, the metabolite, which is secreted by the cell, and coming to this surrounding solution, is getting diluted. So, that, that means, in one type of product, though the product are very selective, it may happen that, along with our targeted product some other contaminants are also getting secreted by this particular microbes. But, the number of contaminants are much, much lesser than the intracellular; because when we are taking out, when we are disrupting the cell wall of the microbes, the entire metabolites are coming out. But here, it is we, who are deciding that, how much will be the total volume of the liquid, where we want to extract the targeted metabolite, say for example, x.

So, that volume is maintained by us. So, here, contaminants are huge; n number of contaminants are there, which is contaminating our targeted product, in case of intracellular product. But in case of extracellular product, that number of contaminants are much, much lesser, and dilution problem is one of the biggest problem, in case of extracellular product. Then, what should be the strategies? Then, how we can proceed for purifying our targeted product? So, there should be some strategy. Before starting this purification work, we have to have some knowledge about the metabolites, about the targeted product, about the nature of our final product, what we want to isolate from this mixture. So, when we want to start this purification, we have to have the answer of few questions, before starting this purification work. Now, that is the strategy of purification. So, what are those questions? What we should do that, what we are going to do?

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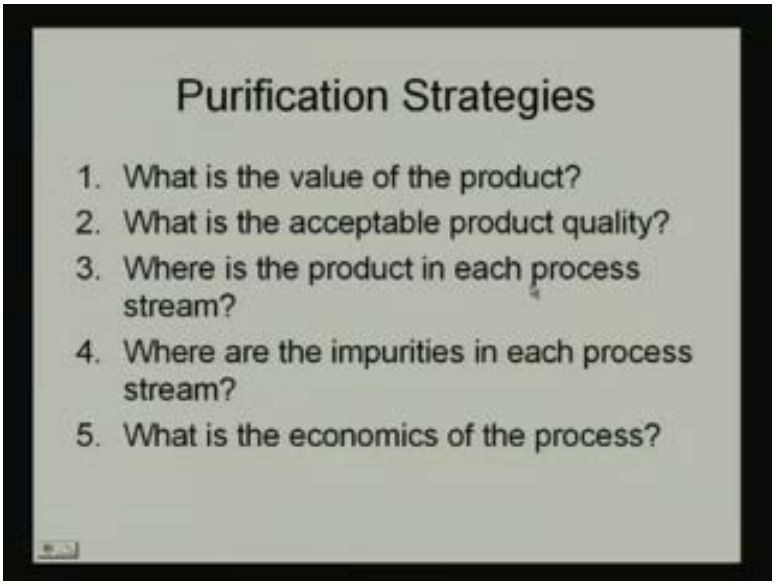

So, first question is, what is the value of the product? So, I have told you that, the metabolites are of two types, and I will be also telling you that, how this low value, high volume product, and low volume, high value products are there. So, we have to have a clear concept of the value of our product. What is the value of, of our targeted product? What is the acceptable product quality? That means, what is the end use of my targeted purified product? Is it for pharmaceutical industry? Or, is it for any other, suppose, detergent industry; suppose for leather industry, leather chemicals and so on, where this percent purification is not that important. But if we are going for the pharmaceutical grade biochemicals purification, we have to have the homogeneous product, end product, and that is the reason, we have to have a clear concept of the acceptable product quality, that end use of our targeted product. The third question what we should know that, where is the product in each process stream; this is very important.

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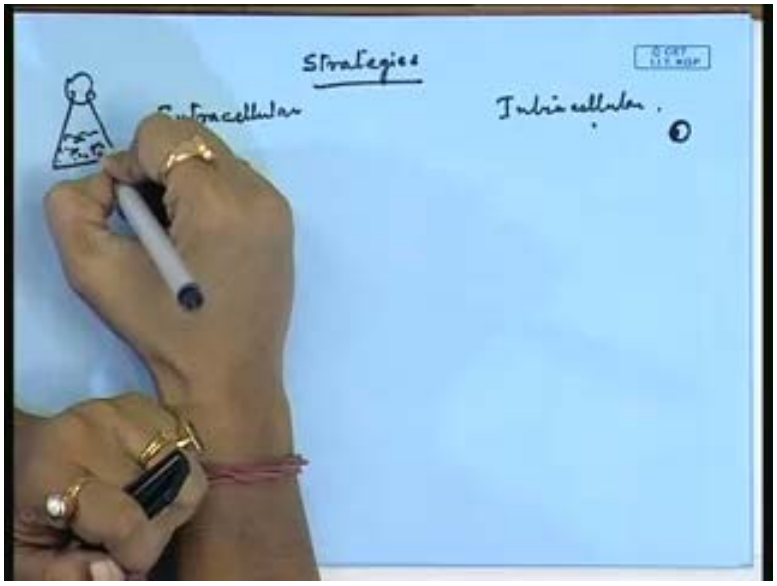

Now, suppose, if we are going for the extracellular product, now, if we are going for this extracellular product, and if we are going for the intracellular product. Now, in case of extracellular product, the product is, the product is already been secreted by the organism to the surrounding liquid; that means, biomass is not our important target. So, we have to remove the biomass, or any other insolubles which are secreted by the microbes. But in case of intracellular product, the cell itself has got this targeted product. So, we cannot discard this cell. Once we are discarding the cell, that means, we are discarding out targeted product. So, we have to have a clear concept that, where, where our product, targeted product lies. So, this concept is very, very important, as far as our targeted product purification is concerned. Where are the impurities in each process stream; that means, we have to have a very important information about the contaminants. What are those contaminant, contaminating proteins, which are mixed up along with our targeted product? That means, we have to have a very clear concept about our enemies, which are there along with our targeted product.

So, this particular impurities, nature of impurities, and how similar this particular impurities, is very important to know, as far as the purification is concerned. And, last question is, what is the economics? That means, how costly my product is; that means, in these while fixing, while answering this question, we have to fix our target that, the strategy should be such that, it should not be very, very costly affairs; that means, when we will be selecting our purification strategy, the strategy should be such that, within the

minimum steps, maximum yield of our targeted product can be achieved; that means, this steps, number of steps and the targeted product yield is inversely proportional; number of steps are more, yield is less. So, while answering this question, we have to have a very clear concept about the strategy of purification, how we are going to fix up the steps, so that, within the minimum steps, maximum purity level can be achieved. Then, that means, here the particular thing which is coming that, what is that particular steps which are there.

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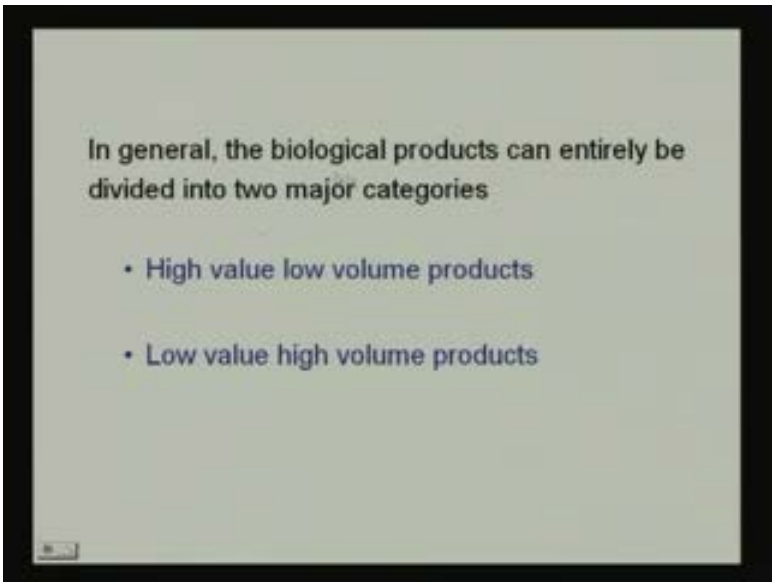

Now, here, so, what I have already discussed is this intracellular and extracellular, and I have already discussed about this high value, low volume product and low value, high volume product.

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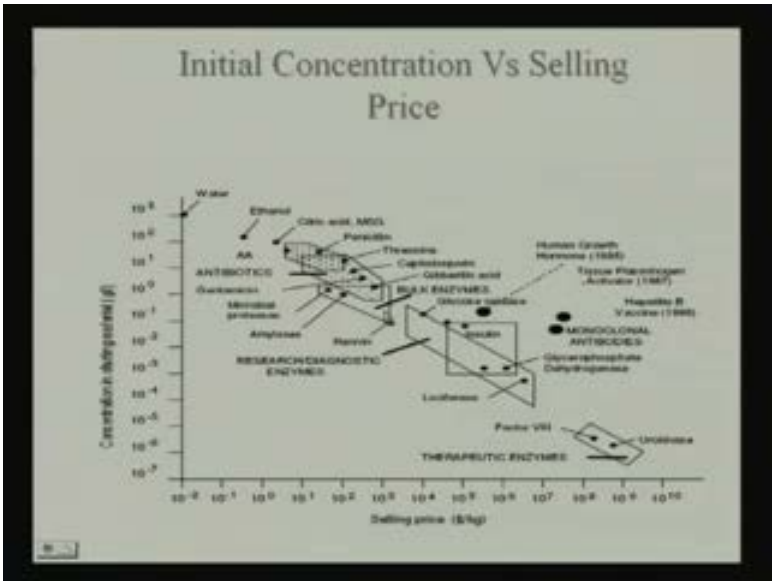

So, here, this is a very interesting graph. If we see this particular graph, we can find that, see, it starts with water; the price is, this is the selling price versus concentration of that particular product. Now, when we are taking this particular water molecule that we are talking about, the bulk molecule, water, ethanol, citric acid, this way this graph is coming; that means, lower the price, volume of that particular chemicals, or biochemical is very high. So, when we are going down to this particular graph, this curve, we can find that, the costliest chemicals are very, very low volume, high value product. So, here, you see the therapeutic enzymes are some of these examples. Then, some other monoclonal antibodies, insulin, some hormones, so, these are some of these products, which the volume-wise is very, very small, but price-wise, selling price-wise, it is very, very costly product. But here, some other group of chemicals, biochemicals, which are this high volume and price-wise it is very, very low. So, these are some of these graph, where we have to have a very clear concept that, where, in this particular graph, where my product is lying, and based on that, we can fix the strategy of purification.

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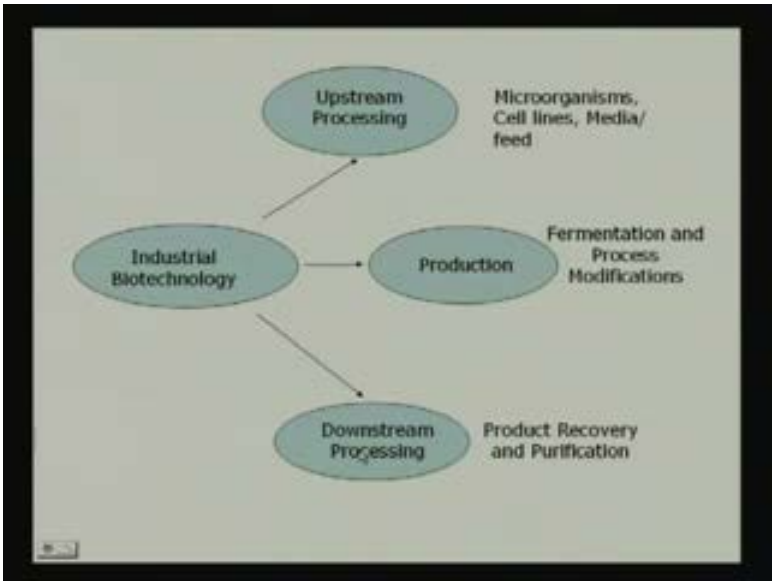

Now, as I have already discussed in my earlier class that, that industrial biotechnology, when we are producing some product, this fermentation, or production of any metabolite, is coming under the transition phase of upstream processing and downstream processing. Now, when we are starting the production, and product is already produced, and it is within the living cell, fermentation is over, production parameters it is already over, downstream processing starts. So, and we are learning that, what are those strategies for downstream processing.

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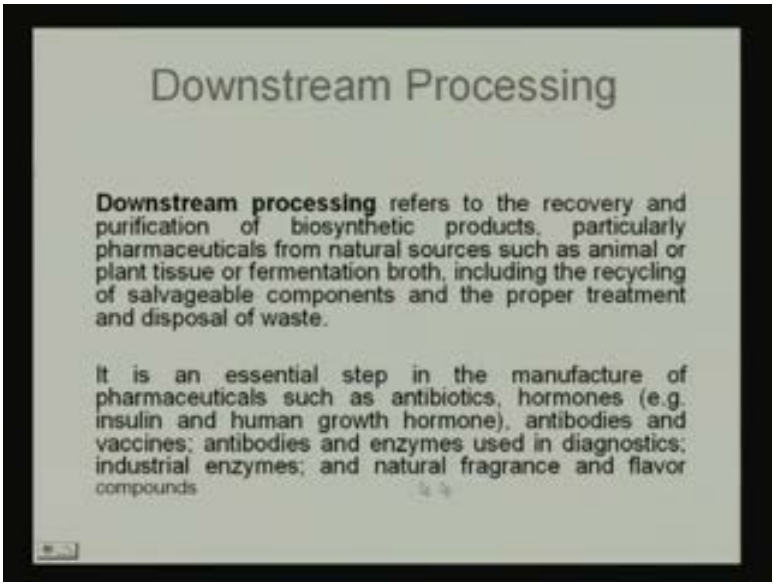

Now, downstream processing refers to the recovery and purification of biosynthetic products, particularly pharmaceuticals from natural sources, such as animals and plant tissues, or by, or through fermentation process, including the recycling of salvageable components and the proper treatment of disposal of waste. It is an essential step in the manufacture of pharmaceuticals, such as antibiotics, hormones, insulin and other growth factor human growth hormones, and so on, antibodies and vaccines, antibodies and enzyme used in diagnostics, industrial enzymes and natural fragrance flavour compounds and so on. So, any, any biological products, which are targeted for production and purification, is coming under this downstream processing.

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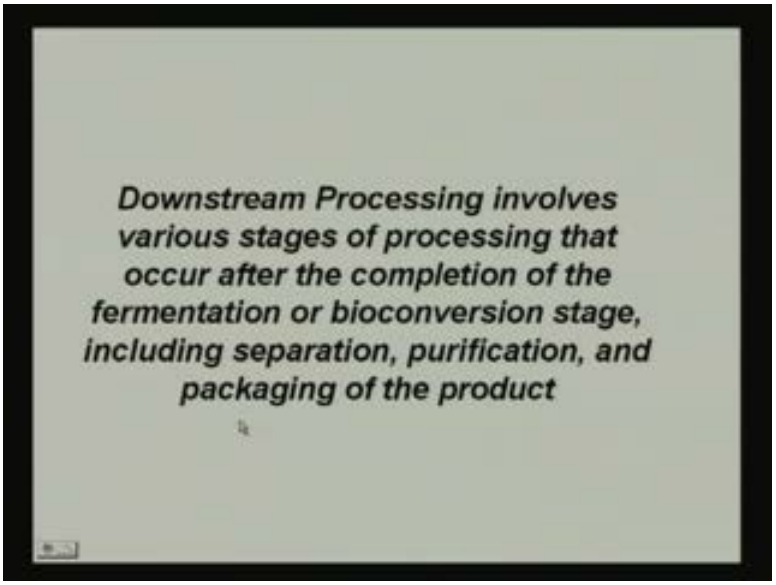

So, downstream processing involves various stages of processing that occur after the completion of the fermentation, or bioconversion stage, including separation, purification and packaging of the product.

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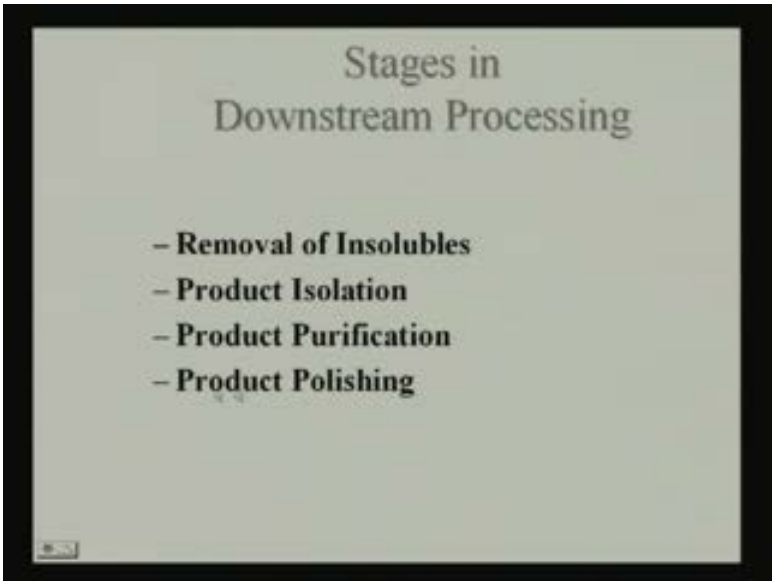

So, if we divide the entire downstream processing, the entire downstream processing can be divided into four major groups. So, first group is the removal of insolubles. Second, is the product isolation; third is the product purification and the last step of this downstream processing is the polishing of the targeted product.

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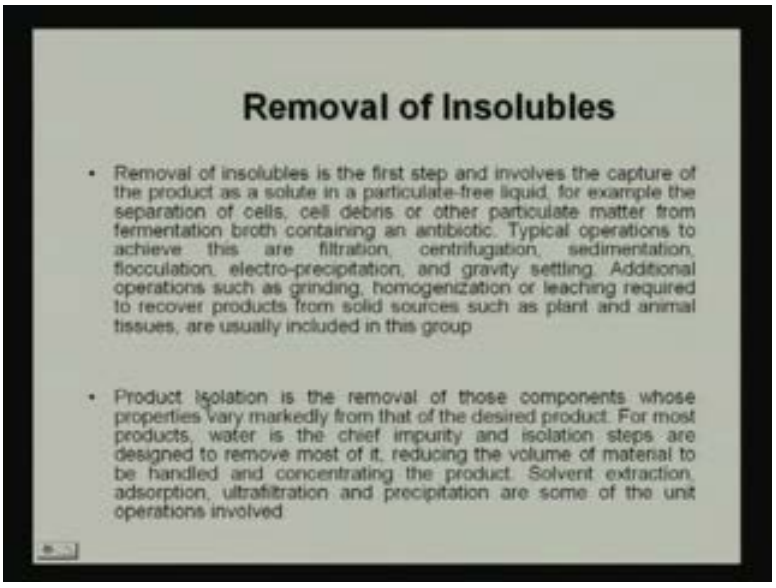

Now, coming to the removal of insolubles. Now, fermentation is over, or production of our targeted metabolite is already over. So, what should we do? Now, first step is the insoluble separation. So, removal of insoluble is the first step of downstream processing.

Removal of insoluble is the first step, and involves the capture of product as a solute in a particulate free liquid; for example, the separation of cells, cell debris, or other particulate matter from the fermentation broth containing an antibiotics, or any other metabolites, like industrial enzymes and so on. Typical operations to achieve this are filtration, centrifugation, sedimentation, flocculation, electro-precipitation and gravity settling. Additional operations, such as grinding, homogenization, or leaching required to recover product from solid sources such as plant and animal tissues, are usually included in this particular group. So, first is the product, that isolation, or the removal of the insolubles.

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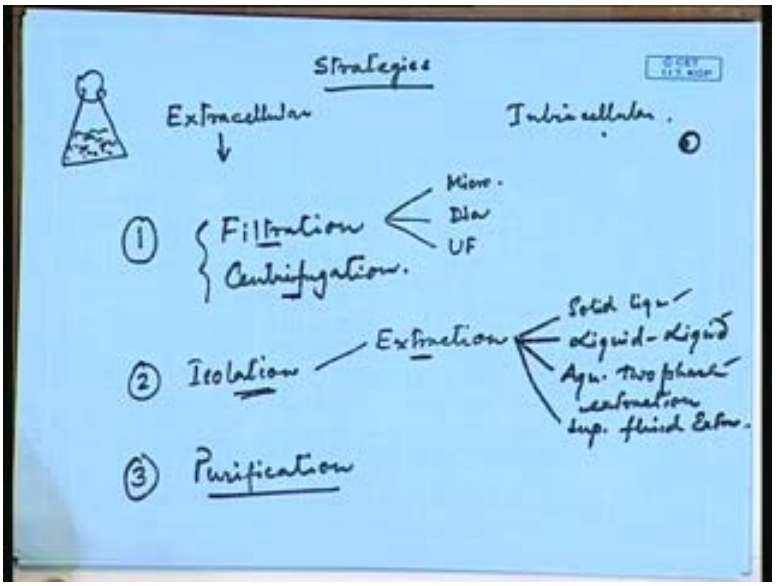

So, what we have learnt? So, from here, we have learnt that, either we can use filtration; we can use centrifugation; we can go for any other precipitating this technique. That filtration may be of different types. It may be micro-filtration; it may be dia-filtration; it may be ultra-filtration and it may be of different types, where we can separate the insolubles from the soluble. Then, alternative to this particular filtration technique, is the centrifugation, through which we can also go for different types of solid liquid separation.

So, this is the first step of downstream processing. In the second step, second step is the isolation of the targeted product. Ihe product isolation is the removal of those components, whose properties vary markedly from that of the desired product. For most

product, water is the chief impurity and isolation steps are designed to remove most of it, reducing the volume of the material to be handled and concentrating the product. Solvent extraction, adsorption, ultra-filtration and precipitation are some of the unit operation which are involved in this product isolation steps. That means, first steps, we have removed the insoluble. Now, whatever product is there it is in the soluble form.

So, we want to isolate our targeted product. So, in this isolation process, we are mainly going for this extraction. Now, when we are going for this extraction, this extraction can be done by very many techniques; that means, it may be solid-liquid extraction; it may be liquid-liquid extraction; it may be aqueous two phase extraction, or it may be super critical fluid extraction. So, this extraction method is very, very important to isolate the targeted product from the bulk liquid which is present along with the product; that means, water is one of the major contaminant, which is there along with the product. So, what we can do, we can separate, if we know the molecular weight of our targeted product. Then, what we can do, we can use some ultra filtration, a particular molecular weight cut-off membrane, and we can pass the solution through that, where we can extract, we can isolate our product in a much, much concentrated form. We can further go for some extraction process and this extraction can be solid-liquid extraction, liquid-liquid extraction, aqueous two phase extraction, super critical fluid extraction and so on.

So, any type of extraction method will extract our targeted compound from the mixture, where this product is lying. It may be of different types. We can also use the adsorption process. We can use the adsorption to adsorb our targeted product. We can also go for precipitation of our targeted product. So, there are very many techniques to concentrate and isolate our targeted product. Now, what will happen that, if the metabolites are, has got some special characteristics; say for example, I have already told you about the different properties of the macromolecules which are present. Now, suppose, our targeted product is a little bit hydrophobic in nature; that means, water is not at all a very good environment for this targeted product. So, we can go for easily that, solvent extraction method. So, what I have told you here that, say, this solvent extraction is one of such example. But, you have to have a prior idea that, what is your targeted product; how we can proceed to isolate our targeted product from the bulk chemicals. Now, when we are going for the next purification strategies, then, we are going for third one is the purification; that means, we are going for the purification of our targeted product.

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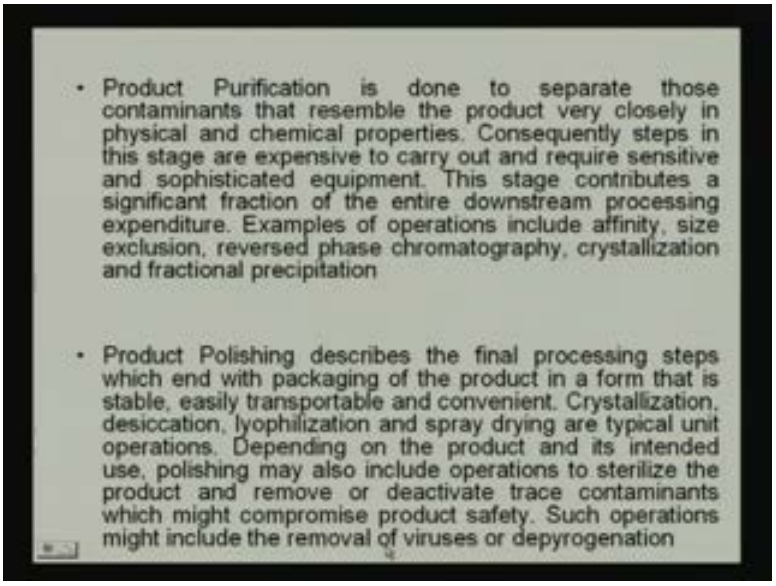

So, this purification is done to separate those contaminants, which, which are present, resemble to the product very closely, in a physical and chemical properties. Consequently, steps in this stage are expensive to carry out and require sensitive and sophisticated equipment. This stage contributes a significant fraction of the entire downstream processing expenditure. Examples of operations include affinity chromatography, size exclusion chromatography, reversed phase chromatography, crystallization and fractionation of precipitate. The last step is the polishing. The product polishing describe the final processing step, which end with the packaging of the product, in the form that is stable, easily transportable and convenient. Crystallization, desiccation, lyophilization and spray drying are typical unit operations, which can be considered to be some of the unit operation process of the product purification. Depending on the product, and its intended use, polishing may also include operations to sterilize the product, and remove, or deactivate trace contaminants, which might compromise product safety. Such operations might include the removal of viruses, or depyrogenations, and so on.

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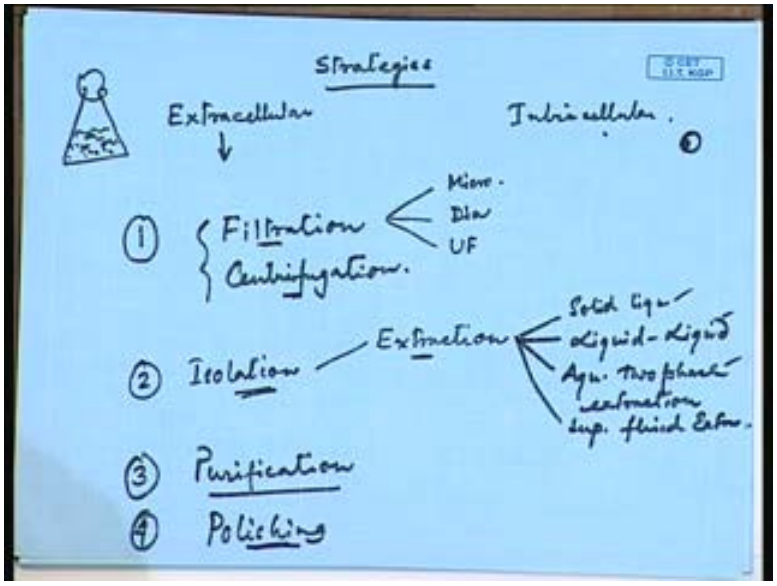

So, these are the four major steps of downstream processing, and polishing is the last step of downstream processing. So, if we see the entire downstream processing, so, if we want to fix the strategy, we have to have a clear concept of downstream processing. So, if we learnt, so, what I have told that, entire downstream processing can be divided into four major operations. One is the removal of insolubles; second is the isolation of the targeted product; third is the purification; that means, removal of contaminants from the mixture, and fourth one is the polishing, that is, the packaging after the purification step, the rest of the steps are coming under the polishing of the biochemicals. So, here, these major activities are these four.

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Now, if we see the downstream processing, and if we fix our goal, then, what is the goal? Goal is our, the improved recovery, very high yield of the recovered product, low or undetectable contaminants; that means, if we want to purify, we want to purify our targeted product; that means, we want to separate, or isolate our targeted product from the contamination, or the from the mixture of contaminants. So, that concentration should be undetectable; that means, it should be a homogenous product. Limited generation of product variants; it should be reproducible; it should be comparable and GMP compliances should be there, with that particular recovered product.

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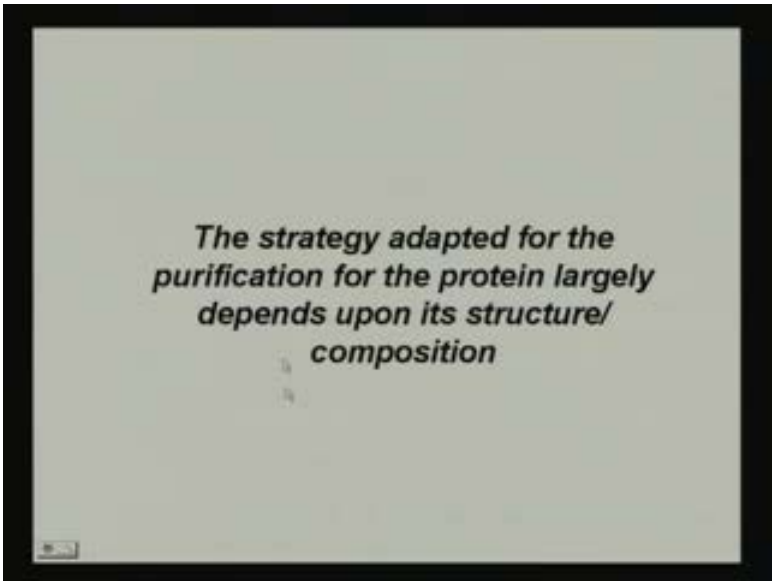

So, if we see the entire downstream processing, the strategy adopted for purification for the proteinaceous material, biomolecule, which largely depends on its structure, or its composition.

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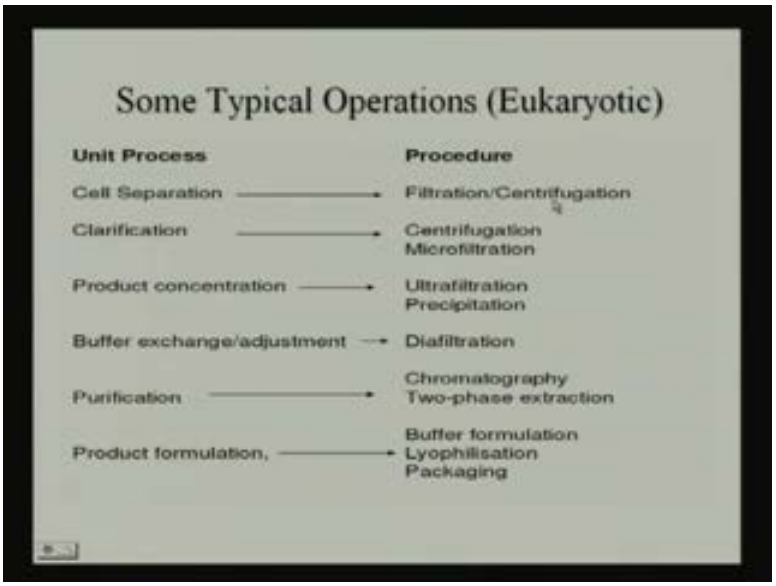

So, if we see the entire strategy of downstream processing, then, we can find that, we can, that unit operations can be fixed, based on some of the basic criteria. Now, if the unit processes are that cell separation…Fermentation is over; we want to remove the cells from the fermented broth. So, if this is the step, we can adopt this processes, which include filtration and centrifugation; that means, I am talking about the first step, removal of insolubles; not necessarily that, every time that, this will have to separate the cells. Suppose, some of the components are insoluble; some of the carbon sources are insoluble in nature; we do not want, after fermentation, we do not want this type of contaminants in that product.

So, what we can do, we can remove that and we can adopt any of this particular step, either filtration or centrifugation; not necessarily that, every time we have to adopt this process for separation of cells; any insoluble, which is present inside this fermentation broth can be separated. If we, this unit process is clarification, we can go for centrifugation; we can go for microfiltration. If the unit operation is product concentration, we can use a definite molecular weight membrane for ultra filtration, or

we can go for this precipitation; and, this precipitation may be the salt precipitation; if this precipitation may be solvent precipitation.

So, depending upon the nature of the product, and the targeted, the characteristics of the targeted product, we have to fix this process, whether we will be adopting ultra filtration, or we will be going for precipitation process. If unit operation is buffer exchange, or adjustment, then, we can go for the diafiltration process. Once we are going for this purification process, we are mainly adopting the chromatographic technique. We can also go for the extraction process. If suppose, the product is a secondary metabolite, insoluble in nature, and has got some peculiar characteristics, we can exploit that particular character of that particular compound, and we can go for, we can fix the strategy of purification, whether we will go for chromatographic technique, or we will go for this extraction process. We can also, if the unit operation is the product formulation, then, we can go for this buffer formulation, lyophilisation and ultimately, we have to go for the packaging of this particular biomolecule. Now, coming to this purification process, where I am talking about the chromatographic techniques. So, chromatographic technique is coming under the novel separation technique, which is practised, now, it is, for improved productivity of the targeted product.

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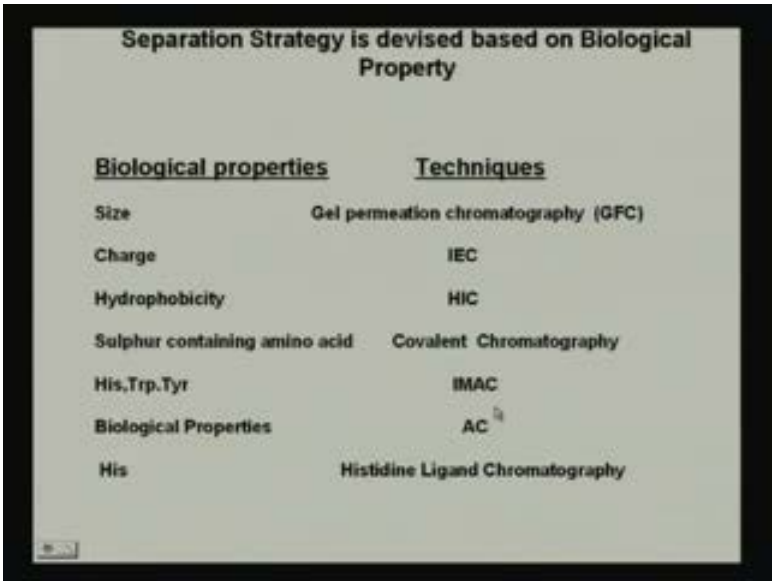

Now, when we are going for this strategy, we are, we are going for the purification strategy, we have to have some biological characteristics, and based on these behavioural

properties of the targeted molecule, we can fix the steps of purification. That means, what should be the different strategies, that purification techniques, chromatographic techniques, we will be adopting for removal of contaminants, from our targeted molecule.

Now, as I have told you, while talking about this proteinaceous molecule, it has got a definite molecular weight. So, if we know that, my targeted protein, say for example, the industrial enzymes. Now, suppose, we are producing this industrial enzyme. It is extracellular in nature; so, number of contaminants are much, much lesser. Now, we know that, our microorganism has secreted it extracellularly. Now, if we know that, it is a proteinaceous in nature, it has got a definite molecular weight. So, based on the size of the particular molecule, we can select the chromatographic technique, which is called gel permeation chromatography or GFC, gel filtration chromatography. So, based on the size of the molecule, we can separate our targeted molecule from the mixture of contaminant. Sometimes, the biological molecules are charged. I told you that, proteins are charged molecule; and, if we are going for the separation of this biological molecule based on its charge, we can select the ion exchange chromatography. Biologicals are a very specific characteristic, biologicals has got a definite characteristics. And, while discussing this protein molecules, or any other macromolecules, we have already learnt that, it has got very peculiar characteristics. Hydrophobicity is one of such characteristics, where we can separate the biologicals based on its hydrophobic nature; hydrophobicity of the biological macro molecules, we can separate the targeted protein from the mixture of contaminant. And, the process which we are adopting is called hydrophobic interaction chromatography.

Based on the sulphur containing amino acids, we are going for the covalent chromatography; that means, if any sulphur containing molecules; so, where we have seen sulphur containing amino acids? Sulphur containing biomolecules in amino acid, one is the methionine; and other is the cysteine. So, if any protein contains methionine or cysteine like molecules, we can easily select this covalent chromatography, if our targeted molecule is a protein. Based on the other amino acids like histidine, tryptophan, tyrosine, etcetera, this amino acids, if that, these amino acids are present in the protein molecule; that means, histidine has got imidizolium group; tryptophan and tyrosines are the aromatic amino acids.

So, based on their particular characteristics, we can go for immobilised metal chelate affinity chromatography. Based on the other biological properties, we can go for, we can select the affinity chromatography. Based on these histidine, histidine is one of the very important amino acids, which can be exploited maximum and we can go for histidine ligand chromatography.

So, if we summarize the entire chromatographic technique, we can go, we can conclude this particular table like this; that, if we are going for the separation of our targeted molecule based on its size, we can go for gel filtration chromatography, gel permeation chromatography. Based on charge, you can go for ion exchange chromatography; based on their biological properties, we can adopt the affinity chromatography, the affinity of one molecule to other. So, it is the exploitation of the inherent biological characteristics of the biomolecule and based on that, we can separate the targeted molecule, based on the affinity chromatography. And, if we see, the affinity chromatography is a big umbrella, then, we can find that, hydrophobic interaction, covalent chromatography, IMAC, histidine ligand, pseudo-biospecific affinity chromatography, and so on, are coming under the big umbrella of affinity chromatography; that means, the chromatographic techniques can be based on the size of the macromolecule, based on the charge of the macromolecule, and based on the biological characteristics of the macromolecule. So, let us learn one by one, this, what is the separation, or what is the ion exchange chromatography, where we are separating the molecule, targeted molecules based on its charge.

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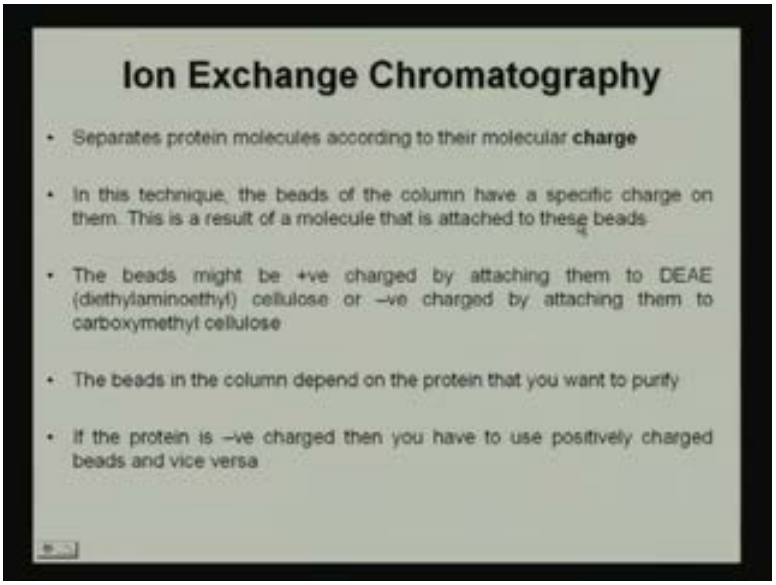

So, separation of protein molecules, according to their molecular charge is coming under the ion exchange chromatography. This is the technique. The beads of the column have a specific charge on the, on them, and this is the, this is the result of a molecule that is attached to these beads.

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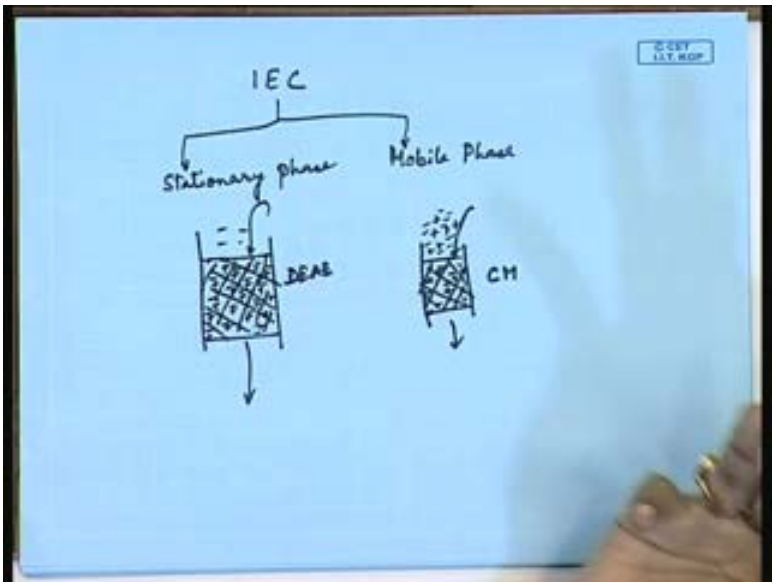

That means, if we see that, this ion exchange chromatography, we can divide this ion exchange chromatography into two; one is called the stationary phase; another is the mobile phase. So, that means, here, what…In this technique, the beads of the column

have a specific charge; that means, something in a column, some materials are getting packed, which has got certain charge within it; that means, either it will be positively charged, or it will be negatively charged, which is loaded inside the column. The beads might be positively charged by attaching them to the DEAE, that is, di ethyl amino ethyl; that means, here, this DEAE group is there; or, if it is a negatively charged group; that means, column, that matrix is negatively charged means, here, all negative groups, that means, in this particular matrix, that carboxymethyl group is attached; that means, here the negatives, C O O H group is present, and DEAE means di ethyl amino ethyl; that means, amino group is there; that means, N H 2 is there in this particular group; that is the reason, why it is positively charged; and here, it is the carboxyl group is there; that is the reason, why it is negatively charged group.

The beads in the column depends on the protein that we are interested to purify. If the protein is negatively charged, we can use the positively charged bead and if the protein, our targeted protein is positively charged, we can use the negatively charged; that means, we know that, positively charged proteins, that charge is getting attracted towards the negatively charged. So, in this way, the binding is taking place inside the column. Now, when binding is taking place, that means, this is positively charged matrix; when this negatively charged protein molecules are coming in contact with this, the negatively charged protein molecules are retained within this matrix, and the similar type of charges are going out of this column; they are coming out of the column, because positive positive does not have any attraction. So, the positively charged protein molecules are coming out of the column. Negatively charged proteins are retained within this particular bed. Similarly, if it is a negatively charged and our targeted molecule is positively charged…So, this positively charged protein will retain inside the column, and if any, these mixture, any negatively charged proteins are also there, it will just come out, because of the lack of attraction. So, this is the basis of separation; that means, based on the charge, we are targeting our molecule.

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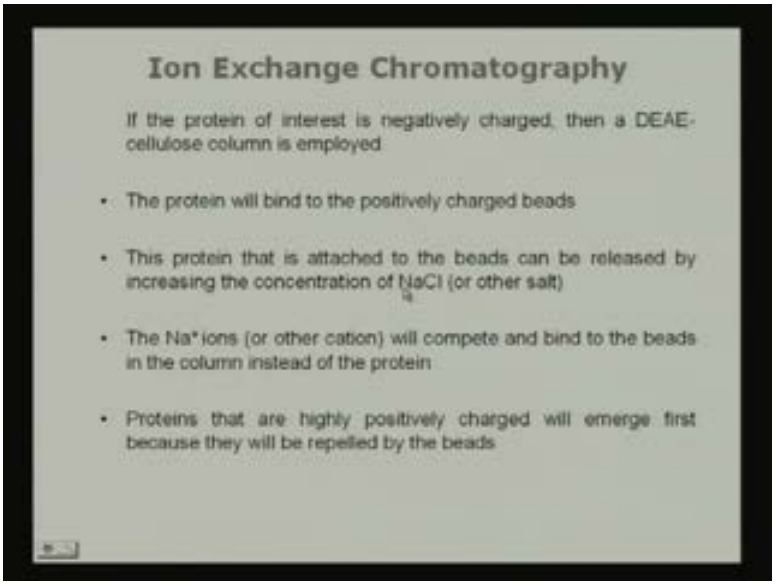

If the protein of interest is negatively charged, then DEAE cellulose column is employed. The protein will bind to the positively charged beads. The protein that is attached to the bead can be released, by increasing the concentration of Na Cl.

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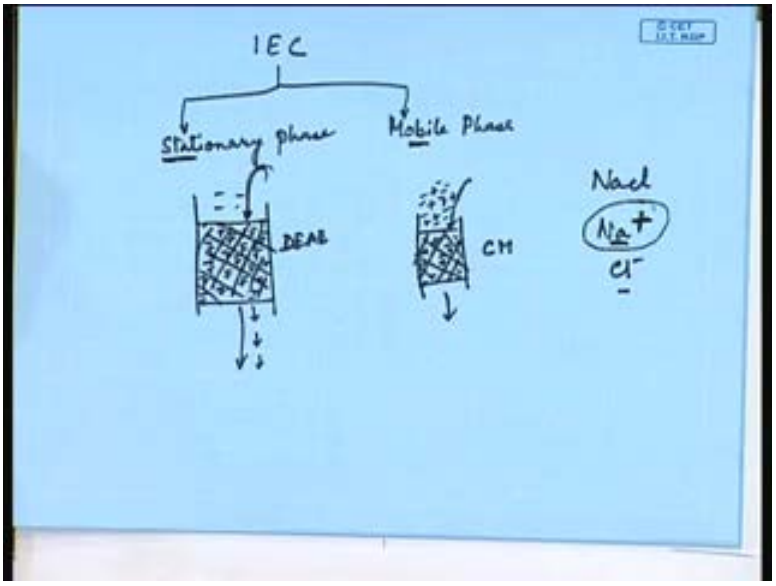

So, as I have told you that, see, here, I have mentioned you that, entire chromatography, any adsorption chromatography is divided into two phases; one is the stationary phase another is the mobile phase. Stationary phase is that phase, where adsorption is taking place; that means, we are loading our column with some material, which can adsorb

some of the protein, or some of the biological molecules, based on its characteristics. So, if it is ion exchange chromatography, so, based on the net charge of the protein, the binding is taking place. Now, when this binding is taking place, then, this protein is now retained inside this bead. We have to get our targeted protein. How will we getting, until and unless, we are dragging something, we are putting something inside, and it can drag our molecule out of this bead, to the outside of this column, we cannot get our targeted protein.

So, what we are doing, we are using the mobile phase. This mobile phase is dragging our molecule out. How it will be dragging our molecule out? We are using the Na Cl as one of the salt. So, in ionised form, we are getting Na plus and Cl minus. So, this is also this ionised. Now, when it is coming, it has got now competition with the protein molecule, which is already occupied some of the charges. So, first, what is happening that, Na Cl, this is coming and saturating any free charges if present, available, inside the column matrix; and then, when this saturation is taken place, then, there is some competition with the binding force of the protein molecule along with this charge particles, and this Na and Cl. So, here, this competition is going on and when this, and if this affinity of Na is more, then, it starts replacing this particular binded protein; that means, hydrophobicity of that matrix is getting increased, and because, this, and then, it is, this protein start gradually coming out. So, what is the nature of coming out of the protein?

So, those protein, which has got more strongly binded, this, those protein which bound, which is attached to these stationery phase more strongly, will come last; the protein which very feebly binded with the matrix, will start coming first. So, based on this strength of binding, the replacement of this protein along with this Na Cl is taking place, and we are getting the targeted protein, along with some other contaminants. So, why this contaminants? It may happen that, our targeted protein is positively charged, sorry, negatively charged and there may be some other negatively charged proteins present inside the mixture. So, those proteins also got binded along with our targeted protein, and it will, all will start coming, when we are giving this Na Cl solution. The Na plus ion, or other cation will compete and bind to the beads in the column, instead of the protein, as I have already mentioned; protein that are highly positively charged, will emerge first, because, they will be repelled by the beads. So, in this way, this particular elution of the targeted protein is taking place.

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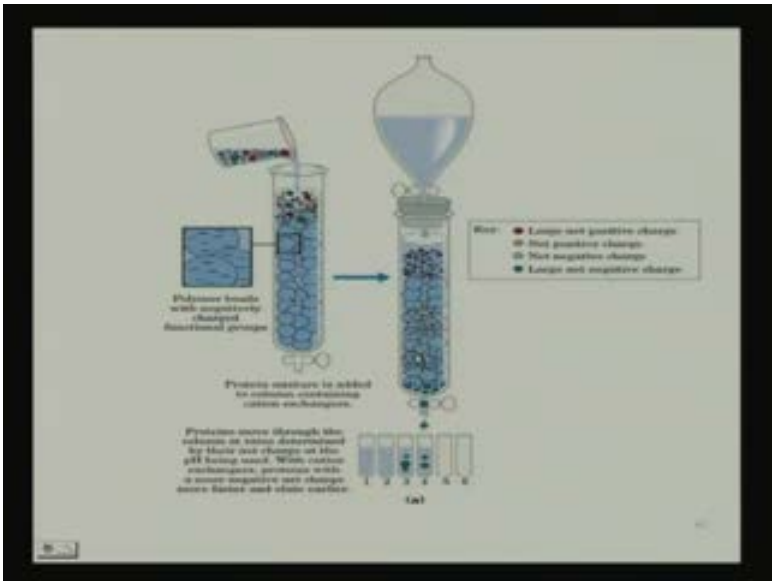

Now, here, in this picture, you see, this is a coloured picture, so that, you will be understanding; see, this is the polymer of this base, and here, we are adding this mixture of this protein. And then, based on this charge, this separation is taking place and when we are just starting this elution, this elution, this proteins, targeted proteins are coming out of the bead. And, this way, this proteins are, this targeted proteins which are, which are there on the bead, starts coming out from this column; and this is the basic principle of separation.

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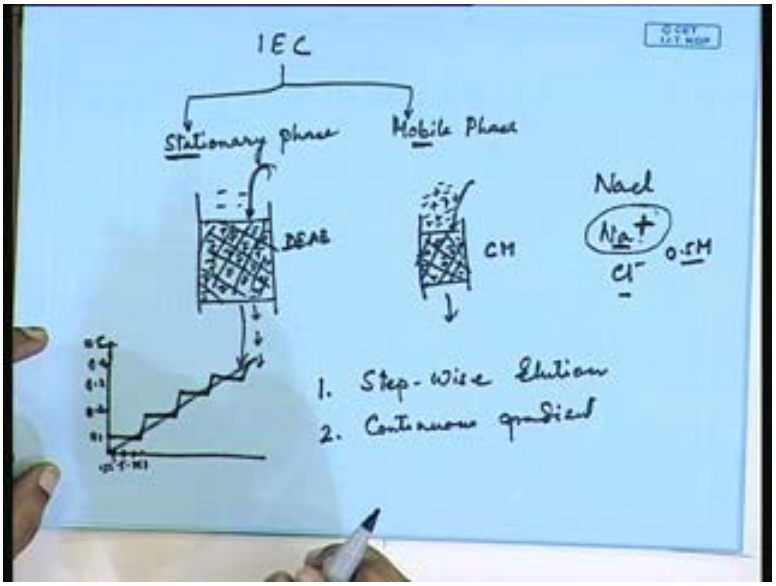

Now, this elution can be done in different way. One of the technique is called step wise elution. Another, another type of elution is called continuous gradient. Now, in case of step wise elution, what is happening? Suppose, we are separating the protein, this binded protein, we want to separate. Now, here, we want to…It is we, who are deciding that, what will be the total elution volume. Suppose, we are interested to isolate our targeted protein in 10 different fraction, and each fraction will have 1 millilitre of volume, total volume. So, suppose, here, we want to use that, up to 0.5 molar of Na Cl concentration. So, initially, we will start with 0.1 molar N a C l. So, in, in, in one ml fraction. So, if this is, suppose, we are taking 0.25, 0.5, 0.75 and 1 ml, and in this way, if we are going for this separation…So, in this way. So, this is 0.1; this is 0.2; this is 0.3; this is 0.4; this is 0.5, and in this way, it is going on. So, this is called step-wise elution. When we are going for this continuous gradient, that means, from here, we are just continuously increasing the gradient.

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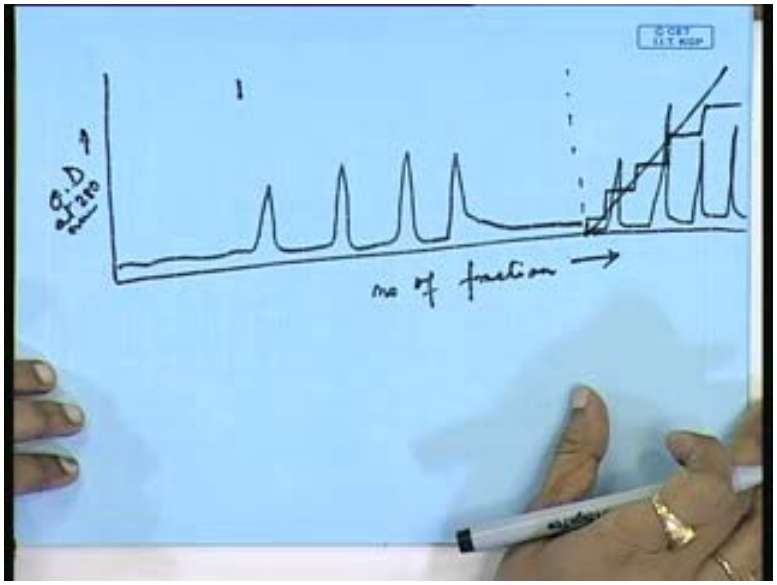

So, if we see the graphical representation, then, how we can tell this ion exchange chromatography? That, see, initially, initially we have, initially we have loaded our targeted protein to this, and here some molecules which are of oppositely charged retain and same charged molecule came out. So, how, if we are plotting this graph, what we will be getting? We will be getting suppose, same charges protein which came out. So, after this, when no other unbound protein is present inside our, this column, then, we start placing the gradient. So, there, either we are going for this step wise gradient, or we

can go for the continuous gradient of isolation of our protein. And, in this way, we are getting the, in this way, we are getting number of fraction. And here, it is the O D at 280 nanometre. So, this is the purification profile of ion exchange chromatography.

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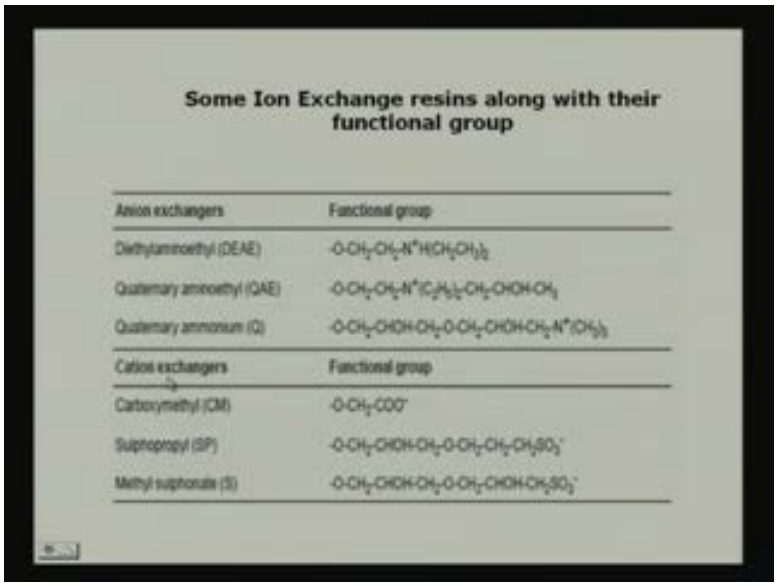

Now, here, if we see this anion exchanger, if we can find that, here this amino group is present in this as a functional group; see, here, DEAE, QAE, Q are the anionic exchanger. Similarly, cationic exchanger means C O O H; it may be sulfopropyl, methyl sulfonate are the some of the common example of ion exchange chromatography.

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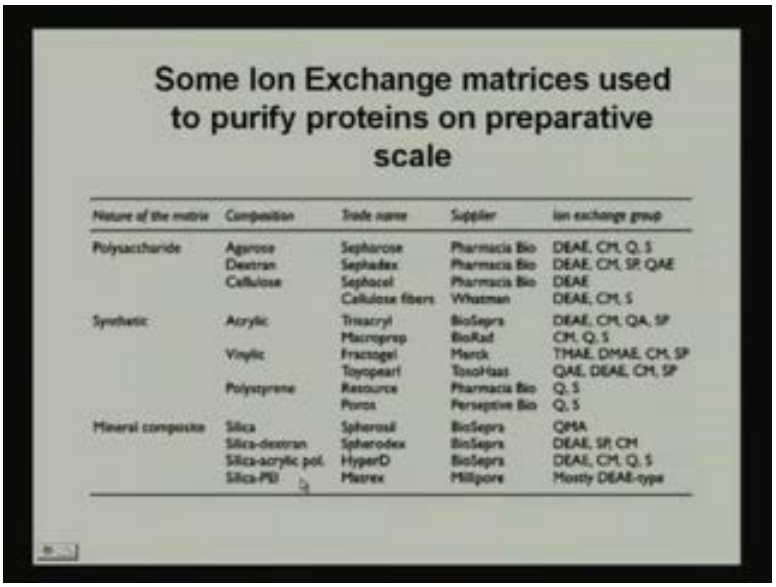

Now, these are some of these ion exchangers which can be considered. That means, some of the natural polysaccharide like agarose, dextran, cellulose, and so on. Some of these synthetic like acrylic acid, vinylic acid, polystyrene beads are some of the example of this synthetic resin ion exchangers. And, some of the mineral composite like silica, silica dextran, silica acrylic polymers, silica based matrix are coming under the different types of polymers.

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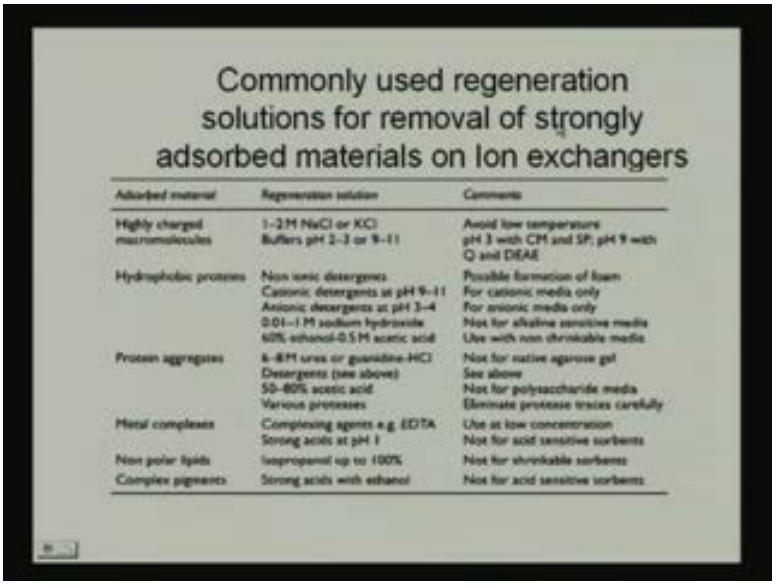

Now, when we are talking about the commonly used regeneration of this ion exchangers, we can find that, it is, some of the adsorbed materials are highly charged macromolecules; some are hydrophobic proteins; some are the protein aggregates, metal complexes, non polar lipids, complex pigments, etcetera. And here, the different strategies of the regeneration of the solution can be adopted for this commonly used regeneration solution, for a removal of strongly adsorbed materials on the ion exchanger matrix. So, what is the problem with the very strongly bound compound, with this ion exchanger matrix is that, we have to have some drastic element to pass, to drag the targeted molecule out. And, as you know, the biological molecules are very sensitive in nature, sometimes, we are getting the targeted product, but in the denatured form. So, under no circumstances, our strategies should be very strongly bound reaction compound, within the mixture. And, this way, we are going for this separation techniques and separation technology for this separation of any targeted macromolecules.

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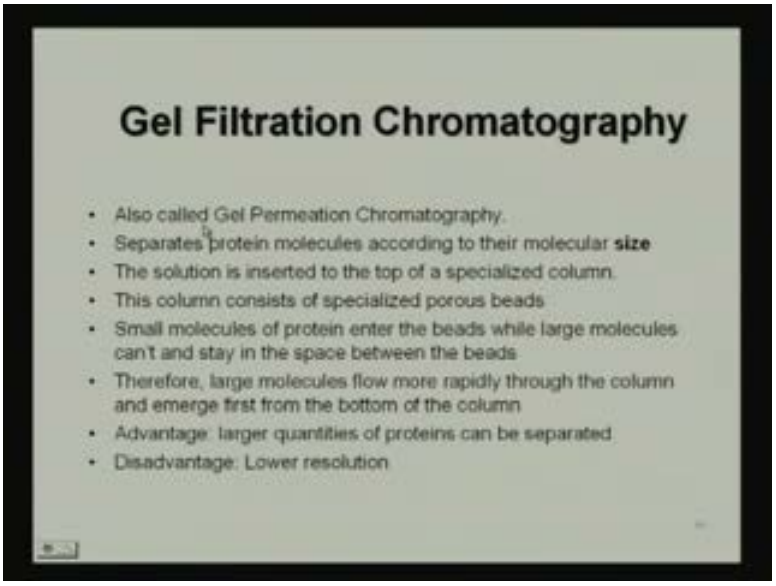

Now, when we are going for this other chromatographic techniques, we are going for the gel filtration chromatographic techniques and there, this separation is taking place, based on the size of the macromolecule. So, in the subsequent class, I will be going through the separation of different, that separation of macromolecule, targeted protein, based on the size of the biologicals. So, gel filtration is one of such technique. Now, ion exchange is based on the charge; besides that, if any secondary metabolites are there, we can go for the extraction process, and solid-liquid separation is a very, very traditional technique of separation of any biological macromolecules. So, we have learnt today, the strategies of biological macromolecules separation, and different processes, which we should adopt, for the better yield, or better recovery of the targeted molecules. So, in the next class, I will be once again continuing the, this purification strategies; and I will be telling you that, what are those different techniques which we can adopt, for the separation of biological molecules. Thank you very much.